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Highlights

- Classification of Elements
- Mathematical Objects in Chemistry
- Compounds of Endemic Nepeta
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Discovering Thoughts, Inventing Future

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Charting Atomic Characteristics: A Pathway to Element Classification

By Ousmane BARRY, Mamadou Yaya BALDE, Souleymane BALDE, Lamine KABA & Aboubacar Safie SYLLA

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Abstract- The classification of elements serves as a rich source of knowledge in chemistry. This article explores the atomic model based on wave mechanics, represented by a graph characterizing the atom, to elucidate both periodic and ordered classifications of elements. Three types of classifications emerge from this graph: periodic, ordered, and hybrid classifications, with the latter resembling periodic tables but incorporating elements of the ordered form. The periodic table stands out as one of the most profound and unifying concepts in modern science. New illustration methods, such as condensed tables with orders and periods, are introduced. The results underscore the conclusiveness of the findings, revealing that classifications extend beyond periodicity, encompassing ordered types of tables as well. This research sheds light on the diverse approaches to classifying elements and opens avenues for further exploration in the field of chemistry.

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Charting Atomic Characteristics: A Pathway to Element Classification

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I. Introduction

In any chemistry class or laboratory, there is almost always a periodic table hanging on the wall. This table, which includes all the known elements, provides a lot of information on each. As one progresses in the study of chemistry, the usefulness of the periodic table becomes more and more obvious[1].

Atoms were first suggested by the Greek philosophers Democritus and Leucippus around 400 BC, the concept was primarily based on a hunch. In fact, for several centuries thereafter, no convincing experimental evidence was available to support the existence of atoms. The first real scientific data were collected by Lavoisier and others from quantitative measurements of chemical reactions. Thus around 1790 the scientist was already able to identify 33 chemical elements. The results of these stoichiometric experiments allowed John Dalton to propose the first systematic atomic theory. This one, although crude, has stood the test of time extremely well.Once atoms were admitted, logically a number of questions arose such as: What is the nature of an atom? How is it composed? What are the constituent parts, their properties etc.?

The most striking phenomenon in chemistry was very early the discovery of an analogy and the periodic repetition of the properties of the elements with each other. Several groups of elements with great similarities in their chemical behavior were identified. This is how the need to classify the elements arose. During the first 30 years of the 20th century, the appearance of a new theory, quantum mechanics, made it possible to explain the behavior of atoms under the effect of light. This so-called modern theory made it possible to develop the electronic structure of atoms. This confirmed the periodic behavior of chemical elements in terms of electronic configuration and then the properties of atoms.

In 1817 Döbereiner succeeded in relating the atomic mass of certain elements to their properties. He noticed the existence of similarities between elements grouped in threes which he called "triads". He highlighted the fact that the mass of one of the three elements of the triad was the intermediate (the average) of the other two. In 1850, we could count more than twenty triads to arrive at a first coherent classification.

In 1862 Chancourtois, a French geologist, highlighted a certain periodicity between the properties of the elements of the table. Later the English chemist Newlands announced that "the eighth element which follows a given element resembles the first as the eighth note of the octave resembles the first". But this law could not apply to elements beyond calcium: “The notions of transition elements were unknown”. This classification therefore remained insufficient, but the periodic table was beginning to take shape.

In 1869 and 1870 respectively, Dmitri Mendeleev and Lothar Meyer claimed that the properties of elements could be represented as periodic functions of their atomic weight and presented their ideas in the form of a periodic table. As new elements have been discovered, the original form of the periodic table has been significantly modified and it is now recognized that periodicity is a consequence of variation in ground state electronic configurations. A modern periodic table emphasizes blocks of 2, 6, 10, and 14 elements that result from filling the s, p, d, and f atomic orbitals, respectively. An exception is He, which for chemical reasons is placed in the rare group [2].

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The most famous of the periodic classifications was that of MENDELEIEV in 1869. It is to him that the merit goes to having presented the first, a well-structured and coherent classification of all the elements known at his time. He then realized the periodic trend in the properties of these placed in increasing order of their atomic masses [3]. This table had 6 columns with short periods with empty boxes according to his manuscript. This intuition of the scientist was confirmed by the later discovery of these new tenants corresponding to his prediction. More than a century after the death of the main founder of the periodic system, different types of periodic tables have emerged. It is time to revisit the origins, the precursors and even the status of this classification which has had a profound influence on the development of chemistry and modern physics. Scientists today are still debating the best possible presentation of the periodic table [4].

The American chemist Glenn T. Seaborg, Lawrence Berkeley National Laboratory, Berkely, California, United States of America whose only address is composed of five chemical elements (Sg, Lr, Bk, Cf, and Am respectively seaborgium, lawrencium, berkelium, californium and americium) had the merit of completing MENDELEF's periodic table of elements with the addition of Actinides in 1945 [5]. From Lavoisier in 1790 to Glenn T. Seaborg, nearly two centuries of scientific exchange and research have led to several types of classifications, all periodic. It only differs in the mode of presentation.

In 1990 FERNANDO Dufour proposed a three-dimensional classification which he called “element tree” or “tree of elements” also called “periodic tree”.

In 1995 PIERRE Demers of the University of Montreal proposed a classification system called “Québécium”, a pyramid with 4 faces and 4 levels; the upper level made up only of 2 elements of the “s” block (1st period); the next is composed of the elements of the “s and p” blocks including 4 of “s” and 12 of “p” (the 2nd and 3rd periods), the penultimate level includes the elements of the “s, p and d” blocks » including respectively 4, 12 and 20 elements (periods 4 and 5) and the last level the base containing the elements of the four blocks “s, p, d and f” respectively 4, 12, 20 and 28 components of periods 6 and 7. The atomic number values Z provide an undeniable if not sufficient ordering principle. A classification called modern, somewhat analogous to the Quebec system but more explicit, is also presented as a pyramid of 7 levels, each corresponding to a period. From the top “the 1st period” to the base “the 7th period” of the pyramid, lines evolve from top to bottom materializing the identity of the valence electrons, that is to say the families (columns) of the periodic table of elements [6].

Eric SCERRI, for the sake of greater regularity, proposes a table in which the hydrogen and helium of the 1st period are brought back to the 2nd period respectively at the level of halogens and rare gases with the columns np^5 and np^6 of block p cut and moved to the right, this leads to a disrupted periodic table of elements, with a regularity of the type 8 8 18 18 32 32 2 as opposed to 2 8 8 18 18 32 32 [7].

All these classifications present a certain discontinuity from one period to the next according to certain researchers, this seems to mark a disagreement with the logical sequence of the usual ordination of natural numbers in which the atomic numbers Z of the atoms would be found (Z ranging from 1 ……118).

This reason indicated above (mentioned) aroused concern and caught the attention of other researchers and served as a reference for them to promote new types of classification. Thus were born the spiral classifications. Professors PHILIP Steaward and JAN Scholten each proposed a spiral classification model, periodic spiral systems or “periodic tables” which according to them respect the natural ordination of atomic numbers. Théodor BENFEY's snail system from the 1960s and others are also of the spiral model. It should be noted that the difference between these presentation models is the simple notion of parallel and spiral linear chains. Periodicity is the only criterion for all these classifications.The period is an instantaneous phenomenon, it is the time that a phenomenon takes to complete the phases of its duration, it is the time interval at the end of which a phenomenon reproduces under the same conditions, such as the properties of the elements chemical; In chemistry periodicity is examined in terms of the number of elements with which identical properties recur from one group to another. It is this phenomenon that the English chemist Newlands wanted to explain with his famous quote: “the eighth element which follows a given element resembles the first as the eighth note of the octave resembles the first”.

In 1989 Ouahès, R. made a proposal for a new periodic table of elements. This other table is still called periodic of 8 periods: He explains his table in these terms: “This means, in terms of atomic structure, that each period corresponds to the filling of shells and subshells according to $n + l = k$ (k: constant) where n and $l$are the principal and azimuthal quantum numbers. The constant k is the rank of the period. The results are given in table 1. The number of periods is 8 instead of 7.”

This article will answer questions such as:

- Can we explain the periodic classification using a more practical vision than that, entirely empirical, of Mendeleev?
- How does the distribution of electrons in an atom help explain periodic properties?
- Is this other table periodic?
- Is the number of periods greater than 7?

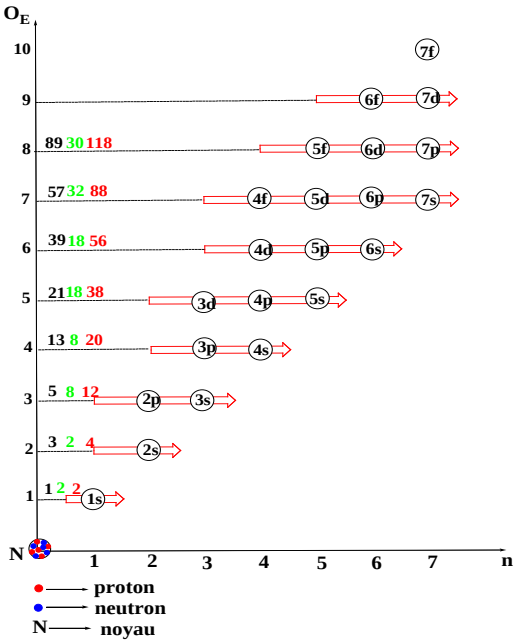
In general, the electronic structure gives all the information about an element, mainly its coordinates in

the different tables. Its physical and chemical properties also depend on it. Valence electrons provide the main concepts of chemical language. They open the way to forms of classification of elements, hence the different methods of illustrating the classification [8].Obtaining specific graphs from the characteristic graph of the atom was explained in the methodology section. The types of classification of chemical elements were illustrated and discussed and a conclusion was drawn.

II. Methodology

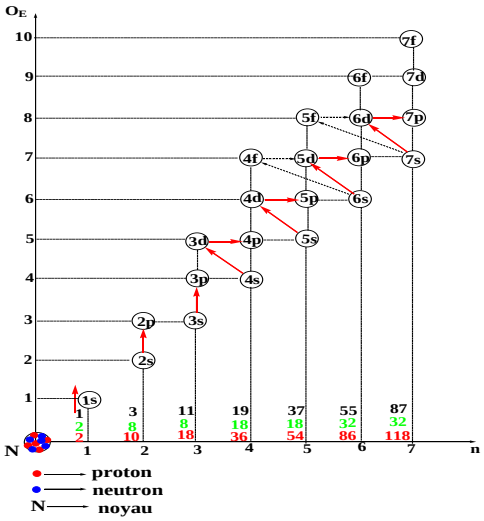
The specific graphs describing the orders and periods concretely explain the principles of stability, aufbau, and in general the Kletchkovky rule [9]. The distribution of the electrons of the different atoms along the axes of these graphs (abscissa and ordinate) by the periods and the orders respectively make it possible to explain each classification model in the literature and even to propose new methods of illustrating the classifications of the elements.A new criterion for classification of chemical elements becomes the order of the energy level "O_E", absolutely different from the period "*n*", has even emerged, it leads to a new classification called the ordered classification of the elements [10].

- Electronic structure according to order (y-axis)



Graphe 1: Electronic structure of elements according to order

- Electronic structure along the period axis



Graphe 2: Electronic structure of elements according to period

The ordered tables are obtained according to *graph 1.* The classification is made according to the ordinate axis "O_E" which becomes the new classification criterion leading to tables of a new type called ordered tables, the set of ordered tables is summarized by a so-called condensed order table. This goes without saying for all periodic classifications, the only classic classification criterion of which is the period "n" according to *graph 2* and are all also summarized by a condensed period table. These two condensed tables are analogous but different, the energy order "O_E" is different from the period "n". See below the respective condensed forms which are effectively new methods of illustrating the classifications.

Table 1: Condensed table to order

Order	1	2	3	4	5	6	7	8
Underlays	1s	2s	2p3s	3p4s	3d4p5s	4d5p6s	4f5d6p7s	5f6d7p…
Z	1- 2	3- 4	5- 12	13- 20	21- 38	39- 56	57- 88	89-118…
Type of tables	2	2	8	8	18	18	32	30……

Table 2: Condensed period table

Periods	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Underlays	*1s*	*2s2p*	*3s3p*	*4s3d4p*	*5s4d5p*	*6s4f5d6p*	*7s5f6d7p…*
Z	*1- 2*	*3- 10*	*11- 18*	*19- 36*	*37- 54*	*55- 86*	*87-118…*
Type of tables	*2*	*8*	*8*	*18*	*18*	*32*	*32….*

III. Results

To make the classification, whether periodic or ordered, it is enough to arrange vertically the stacks of the different sub-layers in each case. Give each block the number of columns corresponding to its electronic capacity: 2 for block "s", 6 for "p", 10 for "d" and 14 for "f". The classifications are either short, medium and long depending on the stacks chosen as a basis. The main stacks are "sp / ps" for tables with 8 columns "sdp /dps" for those with 18 columns and finally "sfdp/fdps" for long classifications with 32 columns.

The vertical arrangements of the sublayers by stacking the periods and/or order offer the appearance of tables: the periodic tables are fairly well-known emblematic figures which adorn quite a few scientific circles today. Ordered tables constitute a completely new classification model and appear in the form of stairs with regular steps. The longest ordered arrays are made up of four steps corresponding to the four blocks of elements. These stairs start from block "f" via those of "d, p" and end with that of "s", from bottom to top respectively.

a) Periodic

n				
1	1s			
2	2s			2p
3	3s			3p
4	4s		3d	4p
5	5s		4d	5p
6	6s	4f	5d	6p
7	7s	5f	6d	7p

b) Ordered

				O_E
			1s	1
			2s	2
		2p	3s	3
		3p	4s	4
	3d	4p	5s	5
	4d	5p	6s	6
4f	5d	6p	7s	7
5f	6d	7p		8

Comparing these two classifications, it appears that the order is obtained by the transfer of the "s" block from the left of Mendeleev's periodic table to its right with a shift of one level upwards; which allows you to go from 7 periods to 8 orders. Orders always end with "s" while periods begin with "s" and are composed respectively as follows:*(n-3) f (n-2) d (n-1) p ns and ns (n-2) f (n-1) d np*.

The ordered classification puts the transitional elements, blocks f and d, of group "B" before the non-transitional elements, blocks s and p, of group "A". This leads to a more regular, coherent presentation, compared to the periodic system which inserts transition elements between normal and non-normal ones. In the

periodic system the elements “d” start with IIIB progress to VIIIB then follow groups IB and IIB marking the end of the “d” block. The ordered system puts the normal elements “s and p” of the large group “A” in the same arrangement as those of the transition. The “p” elements start with IIIA progress to VIIIA and follow the IA and IIA of the “s” block.

In an ordered array the transitional elements are at the beginning and the non-transitional ones at the end. This can justify the reason which divides the four blocks of elements into two large groups A (normal elements) and B (transition elements). By the amplitude of the stacks or the types of tables of orders and/or periods, the methods of illustrating the classifications are summarized by series with values corresponding to the number of elements by order “O_E” and/or by period « n »:

- Order series « O_E » : 2 2 8 8 18 18 32 30
- Series with period « n » : 2 8 8 18 18 32 32

IV. Discussion

Knowing that for the block “s” the order is identical to the period and for the three other blocks “p, d, f” the period is less than the order of one unit, it is possible to go from one classification ordered to a periodic system. This is possible by tilting the “s” block of the ordered system one level down to obtain a periodic system like the ordered one, thus we go from 8 orders to 7 periods corresponding to the periodic and ordered system. This hybrid classification conveys the characteristics of both types of classifications.

a) Ordered

O_E				
1				1s
2				2s
3			2p	3s
4			3p	4s
5		3d	4p	5s
6		4d	5p	6s
7	4f	5d	6p	7s
8	5f	6d	7p	

b) Periodic like the ordered one

					n
				1s	1
			2p	2s	2
			3p	3s	3
		3d	4p	4s	4
		4d	5p	5s	5
	4f	5d	6p	6s	6
	5f	6d	7p	7s	7

The transformation of the ordered system into the periodic classification in its image is a stepped but periodic system. It has all the form of the ordered table but it is also periodic. It also comes in 8, 18, and 32 columns as in the case of Mendeleev's periodic table.

This is how its 18-column classification would look like:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
GROUPE B										GROUPE A								
III	IV	V	VI	VII	VIII			I	II	III	IV	V	VI	VII	VIII	I	II	
3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	1	2	
(BlocKs "f & d)										(BlocKs "s & p")					2	1	2	1
										5	6	7	8	9	10	3	4	2
										13	14	15	16	17	18	11	12	3
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	19	20	4
39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	37	38	5
57*	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	55	56	6
89*	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	87	88	7

*	58	59	60	61	62	63	64	65	66	67	68	69	70	71
*	90	91	92	93	94	95	96	97	98	99	100	101	102	103

Graphs 1 and 2 depict the composition of the electron cloud elements in order and period respectively. The arrangement of the sublayers from 1s to 7p is practically done according to the increasing order of the energy level either by the ordinate "O_E" or by the abscissa "*n*" in the usual ordination of natural numbers.

The structure of an order according to the accepted values is *(n-3) f (n-2) d (n-1) p ns* and that of the period is *ns (n-2) f (n-1) d np*, it is remarkable that the order ends with the sublayer "ns" while it can start with any sublayer even with ns if the order is short as for the cases: n = 1 and 2. Practically the orders go from 1 to 8. For the periods, they start with "ns" and end with "np", the coefficients of the intermediate sublayers are counted from "n" to the limit values. The order is then different from the period, the periods go from 1 to 7. The coefficients of the sublayers in each case are linked to O_E and/or n, knowing that for the block "s" O_E = n. Thus, it is enough to be able to count from 1 to 7 and/or from 1 to 8 to develop the rule of stability.

Table 3: Group of sublayers according to the order of energy *(Graph 1)*

Orders « O_E »	1	2	3	4	5	6	7	8
Underlays	1s	2s	2p 3s	3p 4s	3d 4p 5s	4d 5p 6s	4f 5d 6p 7s	5f 6d 7p …

Table 4: Group of sub-layers according to the period *(Graph 2)*

Periods « n »	1	2	3	4	5	6	7
Underlays	1s	2s 2p	3s 3p	4s 3d 4p	5s 4d 5p	6s 4f 5d 6p	7s 5f 6d 7p

These two tables sufficiently express the difference between an "O_E" order and a "n" period, but these two criteria are well linked.

Table 5: Relationship between period and order *(Graphs 1 & 2)*

Periods « n »	1	2		3		4		5		6		7	
Underlays	*1s*	*2s*	*2p*	*3s*	*3p*	*4s*	*3d4p*	*5s*	*4d5p*	*6s*	*4f5d6p*	*7s*	*5f6d7p…*
Orders « O_E »	1	2	3		4		5		6		7		8

These two very similar tables are different. For block "s", O_E = n, the order is identical to the period and for the others the orders are greater than the period by one unit. The condensed period form confirms all current periodic classifications of the elements. It is the summary of all the classic period processes developed according to the abscissa axis "n" whatever the two or three dimensional model, spiral or not.

The condensed ordered table is also another classification process leading to entirely new forms of tables which are far from being periodic. They are ordered or ordered tables of the elements, obtained along the "O_E" ordinate axis. These resulting paintings are presented in the form of a staircase also with 8, 18 and 32 columns. If for the periodic system the classification criterion is the period "n", the ordered tables are carried out around a new classification criterion, namely the order "O_E". Except that they are not periodic.The word "period" or its adjective "periodic" was not accidentally used by the founding father of the classification system. This choice expressed the behavior of the properties of the groups of elements which were born, evolved then diminished and canceled after a certain number of elements which make up said period. An order is a combination of the elements of two successive periods.

V. Conclusion

This new classification conforms to the electronic structures of the elements according to order and period. It can have historical and educational interest, because it is based on simple to understand concepts.

This work demonstrated that all classifications in the literature are not only periodic. The characteristic graph of the atom made it possible to confirm the existence of the two classification criteria leading to fairly classic periodic tables built around the period "n going from 1 to 7" and completely new ordered or ordered tables which carry out around the order of the increasing level of energy order of the sublayers "O_E starting from 1 to 8" following the ordinate of the graph. The ordered table corresponds to the transfer of the "s" block from the left of the periodic table of elements to its right with the tilting of the latter one level upwards from 7 stacks to 8 others.A third classification intermediate to the other two was envisaged by the reduction of the block "s" of the ordered form by one level downwards from 8 stacks to 7 others, hence a periodic classification in the image of the ordered form. A new illustration of the classification of elements.

We hope to soon provide a rigorous proof of atomistic terminology using the equation of the line,

such as the deduction of valence electrons, the electronic transition and the relationship between the nucleus and the electron cloud of an atom.

References Références Referencias

1. ZUMDAHL, Steven S., (1988), *chimie générale*, 2ème édition De Boeck Université.
2. Cathérine E. Housecroft and Alan G. Sharpe, (2005), Inorganic Chemistry 2nd édition Pearson Education Limited.
3. DURUPTHY, André, et *al*., (2012), *Tout-en-un chimie PCSI-1ère Année*.
4. DE HEMPTINNE, Gwendoline, Guillaume LEIBENGUTH, (2004), *Une incursion dans le monde des particules,* Mars.
5. DEMERS, Pierre (2004) *Système du Québécium, La nouvelle classification des éléments*, ISBN 2-9802454-7-X, Éditions PUM, 2004.
6. PIVETEAU, Jean-Baptiste, (2011), *Histoire de la classification périodique*.
7. Ouahès, R. (1989) *A Proposal for a New Periodic Table of the Elements. Journal of Islamic Academy of Sciences, 2, 1-2*. https://jag.journalagent.com/ias/pdfs/IAS_2_1_1_2.pdf
8. GRENIER, Eva. (1991), *En quête des propriétés et de la structure*, Laval, HRW.
9. Barry, O. and al. (2023)Electronic Structure of Chemical Elements Described by the Characteristic Graph of the Atom, *Global Journal of Science Frontier Research: B Chemistry*, Volume 23 Issue 1 Version 1.0 Year 2023, Online ISSN: 2249-4626 & Print ISSN: 0975-5896.
10. Balde, S., Barry, O. and Sylla, A. (2022) New Overview of the Energy Classification of Underlays. *Journal of Applied Mathematics and Physics*, 10, 828-836. doi: 10.4236/jamp.2022.103056.
11. Baldé, S. (2017) Atomistic Study Described by an Affine Equation: Hypothesis of a New Table. *Mémoire de Master, Université Gamal Abdel Nasser de Conakry, Conakry, Guinée*.

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Screening of Therapeutic Potential and Compounds of Endemic *Nepeta pilinux* P.H. Davis in Kew Bull. from Şanlıurfa

By Fatma Seker, Gulsum Yildiz, Emine Akyüz Turumtay, Adem Demir, Nimet Baltaş Vagif Atamov & Mine Kurkcuoglu

Van Yuzuncu Yil University

Abstract- *Nepeta pilinux* P.H. Davis in Kew Bull. was recently recorded as an endemic species in Birecik, Şanlıurfa. The essential oils were obtained from air-dried aerial parts by hydrodistillation and their composition was investigated using GC-FID and GC/MS. Determination of antioxidant capacity, and urease and xanthine oxidase inhibitions of the methanolic extracts were performed with HPLC-DAD and spectrophotometry. 34 compounds were identified constituting 89.2% of the total essential oil compounds. The major components were determined as T-cadinol (31.1%), γ-muurolene (14.4%) and 14-nor-cadin-5-en-4-one isomer A (11.0%) in the oil. Mainly rosmarinic acid, chlorogenic acid, and caffeic acid derivatives were quantified together with apigenin, luteolin and tangeretin derivatives in the extracts by HPLC-DAD. The total phenolics of the extract from leaf and flower parts, 50.81 mg GAE.g-1, was higher than the extract from stem part, and the radical scavenging activity of this extract was also stronger. While, the leaf and flower extract had significant urease and xanthine oxidase inhibitory activities (62.47 and 48.48 μg.mL-1), stem extract had low inhibition on both enzymes.

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Strictly as per the compliance and regulations of:

Screening of Therapeutic Potential and Compounds of Endemic *Nepeta pilinux* P.H. Davis in Kew Bull. from Şanlıurfa

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Abstract- ***Nepeta pilinux*** **P.H. Davis in Kew Bull. was recently recorded as an endemic species in Birecik, Şanlıurfa. The essential oils were obtained from air-dried aerial parts by hydrodistillation and their composition was investigated using GC-FID and GC/MS. Determination of antioxidant capacity, and urease and xanthine oxidase inhibitions of the methanolic extracts were performed with HPLC-DAD and spectrophotometry. 34 compounds were identified constituting 89.2% of the total essential oil compounds. The major components were determined as T-cadinol (31.1%), γ-muurolene (14.4%) and 14-nor-cadin-5-en-4-one isomer A (11.0%) in the oil. Mainly rosmarinic acid, chlorogenic acid, and caffeic acid derivatives were quantified together with apigenin, luteolin and tangeretin derivatives in the extracts by HPLC-DAD. The total phenolics of the extract from leaf and flower parts, 50.81 mg GAE.g^{-1}, was higher than the extract from stem part, and the radical scavenging activity of this extract was also stronger. While, the leaf and flower extract had significant urease and xanthine oxidase inhibitory activities (62.47 and 48.48 μg.mL^{-1}), stem extract had low inhibition on both enzymes.**

Keywords: nepeta pilinux, essential oil, rosmarinic acid, GC-FID, GC/MS, HPLC-DAD.

I. Introduction

The genus *Nepeta* is distributed over a large part of Central and Southern Europe, and West, Central, and Southern Asia as a multi-regional genus of the Lamiaceae (labiatae or mint) family, consisting of approximately 300 taxa (1, 2). 34 species (40 taxa) of the genus Nepeta were recorded in the Flora of Turkey (3, 4). According to the revision of this genus, it is represented by 39 species (46 taxa) in Turkey with recent studies. Endemism rate on the basis of this species is 44% (5). *Nepeta* species are usually named as catmint or catnip due to the sedative effects on cats and they are commonly used as diuretic, spasmolytic, diaphoretic, bronchodilator, antitussive, anti-asthmatic and sedative agents in Turkey. Due to their antiseptic properties, they are used topically in the treatment of children with skin rashes, and in snake and scorpion bites as well. *Nepeta caes area,* an endemic species in Turkey, has folkloric uses in southern Anatolia and is used as a herbal tea to treat gastric disorders (6,7). Some *Nepeta* species have been known their feline attractant activity since they have nepetalactone and its derivatives which are responsible for attractant properties (1,7). Nepetalactones have been reported to effect on insects compare to DEET (N, N-diethyl-m-toluamide) (8-10). Nepetoideae is essential oil-rich genera of the Lamiaceae, therefore has potential economic interest (11).

According to the various studies on the essential oil composition of *Nepeta* species the essential oil composition depends on the species, place of cultivation, climatic conditions and method of analysis (12-26). The most comprehensive study on 22 *Nepeta* species was performed by Baser et al. (2000). They were classified into two groups according to composition of essential oil of these species; nepetalactone-containing and nepetalactone-less. Nepetalactone-containing species have 4aα-7α-7aα nepetalactone as the most frequently contained nepetalactone. *Nepeta cadmea* Boiss., *Nepeta cataria* L., *Nepeta caesarea* Boiss. and *Nepeta pilinux* P.H. Davis contained 4aα-7α-7aα nepetalactone while *Nepeta racemosa* Lam. contained 4aα-7α-7a*β*-nepetalactone as major compound in their oils. Caryophyllene oxide or 1,8-cineole/linalool were identified in the essential oils of Nepetalactone-less species as the major components. As main compounds, *β*-pinene, *a*-terpineol, germacrene-D, and spathulenol were respectively determined in the oil of *Nepeta phyllochlamys* P.H. Davis, *Nepeta viscida* Boiss., *Nepeta sorgerae* Hedge et Lamond, and *Nepeta trachonitica* Post which are out of the two groups (1).

Being a type of the most popular antioxidant secondary metabolites, phenolic compounds were investigated in the *Nepeta* species as well. Rosmarinic

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acid, epicatechin, chlorogenic acid, caffeic acids, quercetin, rutin, ellagic acid, thymusin, luteolin, and apigenin which are well-known as antioxidant compounds were found in the extracts of *Nepeta cadmea* Boiss., *Nepeta nuda* subsp. *albiflora, Nepeta asterotricha* Rech., *Nepeta rtanjensis* Diklic & Milojević (27-30).

Ureases, a nickel-dependent metalloenzymes, are synthesized by plants, some bacteria, algae and fungi. The jack bean urease (urea amidohydrolase EC 3.3.1.5) catalyzes the hydrolysis of urea to form ammonia and carbon dioxide (31). *Helicobacter pylori*, a gram-negative microaerophilic pathogen survivable in a limited pH (4.0–8.2) range. This pathogen can successfully colonize and persist in the mucous layer of the human stomach since its urease activity which produces ammonia to reduce stomach acidity. Since antibiotic-resistant strains of H. pylori can emerge against antibiotics, it is believed that plant-derived urease inhibitors would be more beneficial against gastroduodenal disease associated with this pathogen (32, 33).

Xanthine oxidase (EC 1.2.3.2.) produces hydrogen peroxide and superoxide anion, which are reactive oxygen species (ROS) during the oxidation of hypoxanthine and xanthine to uric acid. Under physiological conditions, ROS is kept at a low level by the antioxidant system. Disruption of the balance of ROS and antioxidants due to some diseases causes tissue and DNA damage due to the increase in metabolism of ROS. Inhibition of xanthine oxidase reduces the amount of uric acid and ROS in the bloodstream to prevent both hyperuricemia and oxidation stress (34). Inhibitory effects of some flavonoids such as diosmetin, luteolin, chrysoeriol, apigenin, kaempferol on xanthine oxidase have been reported in various in vitro studies (35-37).

Nepeta pilinux P. H. Davis in Kew Bull. is an endemic species of *Nepeta* genus growing in the Southwestern Anatolia (Antalya: Alanya) (3). However, in recent flora research, *Nepeta pilinux* was encountered in Şanlıurfa, Birecik district and was recorded as new endemic species for Şanlıurfa flora. *Nepeta pilinux* is named as 'top pisik otu'. In Şanlıurfa, the fresh aerial parts of the plant are used to heal mouth sores (38).

There is no knowledge about the essential oil and phenolic composition of *Nepeta pilinux* endemic species from Şanlıurfa. Chemical compositions of the polar and apolar extracts from *Nepeta pilinux* were determined by HPLC-DAD and GC/MS respectively for the first time in this study. Radical scavenging activities against DPPH and ABTS radicals and enzyme inhibition activities on urease and xanthine oxidase were investigated in vitro to elucidate the bioactivities which may have developed by this species depending on its chemical composition.

II. Experimental

a) Materials

Nepeta pilinux was collected from Şanlıurfa: Birecik, Kelaynak area in Turkey in 5 May 2018. The voucher specimen has been deposited at the Herbarium in the Recep Tayyip Erdoğan University (RTEUB 6079), Rize, Turkey (Voucher specimen no: FABAK 1702). The plant material was identified by Prof. Dr. Vagif ATAMOV (Recep Tayyip Erdogan University, Faculty of Science and Literature, Department of Biology, Rize, Turkey). All standards of phenolic compounds were obtained from Sigma-Aldrich (St. Louis, MO, USA) but quercetin from Fluka Chemie GmbH (Switzerland). Na_2CO_3 and $K_2S_2O_8$ were provided from Sigma-Aldrich (St. Louis, MO, USA. HPLC grade acetonitrile, methanol, acetic acid, Folin ciocalteau, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were supplied by Merck (Darmstadt, Germany).

b) Isolation of the Essential Oil

Aerial parts of the plant were water distilled for 3 h using a Clevenger-type apparatus. The essential oil was stored at 4°C in the dark until analyzed.

c) Extraction of Phenolic Compounds

The aerial parts of the plant were divided into two parts. The leaves and flowers were combined in one part and the stems were separated into the other part. The part consisting of leaves and flowers was called NP-LF for short, and the part consisting of stem is called NP-S. These parts were finely ground and 0.5 grams of each were defatted by using 10 mL of hexane. The plant residues were dried at 40 °C for 30 min after removing the hexane extract. 20 mL methanol were added to these residues for the extraction of phenolic compounds. Extraction was continued overnight at 37 °C in the dry thermo-shaker cabinet at 352 rpm, then the extracts were centrifuged at 5000 rpm and supernatants transferred into the falcon tube. This procedure was repeated by adding 10 mL of methanol in the residue. All extract was concentrated until 5 mL by using rotary evaporator at 35 °C. 500 and 1500 µL of these extracts were stored at -20 °C for HPLC-DAD analysis, antioxidant and enzyme inhibition tests. Remaining 3 mL of extract was evaporated to calculate the concentration of the extracts. NP-LF and NP-S extracts concentration were 23.17 and 23.50 $mg.mL^{-1}$ respectively.

d) Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC/MS) analysis of essential oil

The oil was analyzed by capillary GC and GC/MS using an Agilent GC-MSD system.

i. GC/MS analysis Conditions of Essential Oils

The oil was analyzed by capillary GC/MS using an Agilent GC-MSD system (Agilent Technologies Inc.,

Santa Clara, CA). HP-Innowax FSC column (Hewlett-Packard-HP, U.S.A.) (60 m × 0.25 mm i.d., with 0.25 μm film thickness) was used for separation of components in the oil and helium as a carrier gas (0.8 mL/min). The GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. The split flow was adjusted at 40 mL min^{-1} with 40:1 split ratio. The injector temperature was set at 250 °C. Mass spectra were taken at 70 eV with the mass range *m/z* 35-450.

ii. *GC Analysis Conditions of Essential Oils*

The GC analysis was done with Agilent 6890N GC system fitted with a FID detector set at a temperature of 300°C. To obtain the same elution order with GC/MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatogram.

iii. *Identification of Essential Oils*

Identification of essential oil components were performed by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library (39-41) and confirmed by comparison of their retention indices. A homologous series of *n*-alkanes were used as the reference points in calculation of relative retention indices (RRI) (42). The relative percentages of the separated compounds were calculated from FID chromatograms. The analysis results are expressed as mean percentage as listed in Table 1.

e) HPLC-DAD Analysis Conditions of Methanol Extracts

The chromatographic analyses were performed using a Dionex (Thermo scientific, Germering, Germany) Ultimate 3000 high performance liquid chromatography (HPLC) system equipped with an Ultimate 3000 diode array detector (DAD).

A Thermo acclaim C30 column (150mm. 3mm id. 3μm pd) was used with Macherey Nagel (3mm id) guard column. Gradient elution was used with mobil phases; A: 2% acetic acid in water and B: 70%acetonitrile-30% water. Flow rate was 0.37 mL/min and injection volume was 10 μL. Column temperature was 25°C. Following 24 phenolic standards were used to calibration and validation of HPLC-DAD analysis method: Gallic acid, protocatechuic acid, *p*-hydroxy benzoic acid (*p*-OH benzoic acid), vanillic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, vanillin, epigallocatechin gallate (EGCG), epicatechin, *p*-coumaric acid, ferulic acid, chicoric acid, rutin, luteolin-7-glycoside, hesperidin, apigenin-7-glycoside, rosmarinic acid, luteolin, quercetin, hesperetin, apigenin, and tangeretin. They were diluted from their stock solution into nine different concentration at 0.3125; 0.625; 1.25; 5.0; 10.0; 25.0; 40.0 $mg.L^{-1}$ in 1:1 methanol-water solution. External calibration method was used and their regression coefficient were found at least 0.999. Repeatability of the retention time and peak areas were measured as coefficient of variation (CV) which was under 0.93 for retention times and 6.02 for areas of the peaks. Limit of detection and quantification values of the peaks were under 0.11 and 0.37 $\mu g.mL^{-1}$ for all standards. Chromatograms were processed at 254, 280, 315, and 370 nm with DAD which operated 200-400 nm. The identification of the peaks was carried out by comparing the retention times and UV spectra with those of standard phenolic compounds. Some peaks had the same or very similar UV spectra as some standards, but with different retention times. They were defined as derivatives of standards with similar UV spectrum and quantified as equivalent of those standards.

f) Determination of Total Phenolic Content

Total phenolic content was determined by using the yellow colored Folin–Ciocalteu's phenol reagent, which was reduced to its blue complex in the presence of reducing agent such as phenolic compounds (43). Gallic acid and quercetin were used as phenolic standards to generate standard curves in a range of 0.0156 and 0.500 mg/mL at 6 concentration levels (r^2 = 0.998). The optical density of the extracts with phenol reagent in the alkaline solution was measured at 760 nm with a UV–Vis detector (Thermo Scientific Multiskan Go, USA). The results were expressed in mg of gallic acid (GAE) and quercetin equivalent (QE) per gram of extracts. All concentration point of the extracts was analyzed in triplicate.

g) DPPH Free-Radical Scavenging Activity Assay

The free-radical scavenging activity was determined based on the reduction of the purple colored 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical to the yellow colored DPPH-H form by the effect of an antioxidant species such as phenolic compounds in the extracts. It was spectrophometrically performed at 517 nm (44). Briefly, 0.15 mL of plant extract was mixed with 0.15 mL 0.1 mM daily prepared DPPH in methanol and incubated for 30 min in the dark. Gallic acid and quercetin were used as standards to compare with the methanol extracts. Results are reported as SC_{50} values, demonstrating the concentration of extract (μg extract per mL methanol) necessary to scavenge 50% of DPPH•. All concentration point of the extracts was analyzed in triplicate.

h) ABTS Radical Scavenging Assay

7 mM of ABTS solution and 2.4 mM of potassium persulfate solution were mixed in equal quantities and allowing to oxidation reaction of ABTS by $K_2S_2O_8$ for 18 h at room temperature in the dark to form

the $ABTS^{\bullet+}$ radical. Obtained radical solution was then diluted with methanol 25 times to obtain an $ABTS^{\bullet+}$ solution has optical density of 0.700 ± 0.01 at 734 nm (45). 50 µL plant extracts were allowed to react with 250 µL of the $ABTS^{\bullet+}$ radical solution and the absorbance was measured at 734 nm after 30 min using a spectrophotometer. The $ABTS^{\bullet+}$ scavenging capacity of the extracts were compared with that of gallic acid and quercetin and reported with SC_{50} values (µg extract/ mL methanol). All concentration point of the extracts was analyzed in triplicate.

i) Urease Inhibitory Assay

Urease inhibition of the extracts were performed according to the phenol-hypochlorite method developed by Weatherburn (1967) (46). Jack bean urease was used as a model enzyme. Optical density of the resulting blue-navy colored mixture at 625 nm were recorded on a spectrophotometer (1601UV-Shimadzu, Australia). To calculate the IC_{50} values of the polar extracts, different concentrations of the extracts or inhibitory compounds were prepared. Acetohydroxamic acid, well-known inhibitor of urease, were used as positive control.

j) In Vitro Anti-Xanthine Oxidase Assay

The inhibition of xanthine oxidase was measured by UV spectroscopy technique at 295 nm which is attributed to the released uric acid from xanthine. The inhibitory activity of the extract was determined using a slight modification of the reference methods (34). Briefly, the reaction mixture consisted of 500 mL of the extract solution, (diluted in DMSO), 770 mL of phosphate buffer (pH 7.8) and 70 mL of bovine milk xanthine oxidase (0.4 U/mL, Sigma Aldrich, St. Louis, USA) was prepared. The reaction was initiated by the addition 660 mL of xanthine solution (0.4 mM) into the mixture after incubation at 25°C for 15 min. The assay mixture was incubated at 25°C for 15 min again. The reaction was stopped by adding 200 mL of 0.5 N HCl and the absorbance was measured at 295 nm using UV/vis spectrophotometer (1601UVShimadzu, Australia). A well-known XO inhibitor (XOI), allopurinol (Sigma Aldrich, St. Louis, USA) was used as a positive control. XO activity was expressed as percent inhibition of xanthine oxidase, calculate as (1-B/A) x 100, where A is the change in absorbance of the assay without the test samples. (Δ abs with enzyme - Δ abs without enzyme), and B is the change in absorbance of the assay with the test sample (Δ abs with enzyme - Δ abs without enzyme). The assay was done in triplicate. The IC_{50} value was determined as the concentration of the extract that gave 50% inhibition of maximal activity.

III. Results and Discussion

a) GC And GC/MS Analysis of Essential Oils

Essential oil yield in the sample was calculated as 0.26%. Thirty-four compounds comprising about 89.2% of the essential oil were identified. Identified essential oil components were compared with literature polar column retention times. The major components were determined as T-cadinol (31.1%), γ-muurolene (14.4%) and 14-nor-cadin-5-en-4-one isomer A (11.0%) in the oil. *Nepeta pilinux* essential oil has oxygenated sesquiterpenes (44.6%), sesquiterpenes hydrocarbons (17.3%), oxygenated monoterpenes (10.1%), monoterpene hydrocarbons (2.3%) and others (14.9%). The analysis results were shown in Table 1.

Our results were not similar to the classification of *Nepeta* species reported by Baser et al. (2000) (1). *Nepeta pilinux* which was collected from Antalya, Alanya district was in the group containing nepetalactone, an iridoid monoterpene according to their report. Although 89.2% of the essential oil was determined in *Nepeta pilinux* from Şanlıurfa, nepetalactone was not detected. The determination of different essential oil compositions from *Nepeta pilinux* from different regions may have resulted from the difference in locality.

The major constituent of water-distilled essential oils of *Nepeta heliotropifolia* and *Nepeta congesta* subsp. *cryptantha* was determined by GC/MS and GC-FID and found to be germacrene D (36.7% and 38.5%, respectively). Their main aroma component was determined as eucalyptol (48.0% and 24.7%, respectively) (47). Although the major essential oil and aroma compounds of these two species were quite different from the main essential oils of *Nepeta pilinux,* the compositions of these three species had lots of common compounds such as α and β-pinene, γ−muurolene, myrtenal, pinocarveol, caryophyllene oxide, cubenol, T and α-cadinol ect. The presence of more or less components may be due to differences between species, as well as environmental conditions and the harvesting time of the plant.

Table 1: Composition of the Essential Oil of *Nepeta Pilinux*

RRI[a]	RRI[b]	Components	%	IM
1032	1032[c]	α-Pinene	2.3	t_R, MS
1118	1118[c]	β-Pinene	tr	t_R, MS
1213	1213[d]	1,8-Cineole	tr	t_R, MS
1376		*trans*-Muurola-3,5-diene	0.3	MS
1499	1499[c]	α-Campholenal	0.7	MS
1535	1535[e]	Pinocamphone	0.1	MS

1553	1553[d]	Linalool	2.3	t_R, MS
1577	1577[e,g]	α-Cedrene	0.3	MS
1586	1586[c]	Pinocarvone	0.7	MS
1617	1613[g]	β-Cedrene	tr	MS
1648	1648[e]	Myrtenal	0.6	MS
1670	1670[d]	*trans*-Pinocarveol	1.3	t_R, MS
1684	1684[e]	*trans*-Verbenol	4.4	MS
1694	1693[g]	β-Acoradiene	tr	MS
1704	1704[c,d]	γ-Muurolene	**14.4**	MS
1706	1706[d]	α-Terpineol	tr	t_R, MS
1726	1725[e]	Verbenone	tr	t_R, MS
1747	1740[e]	*p*-Mentha-1,5-dien-8-ol	tr	MS
1751	1751[d]	Carvone	tr	t_R, MS
1797	1804[d]	Myrtenol	tr	MS
1845	1845[d]	*trans*-Carveol	tr	t_R, MS
1853	1849[d]	*cis*-Calamenene	1.8	MS
2008	2008[c]	Caryophyllene oxide	0.6	t_R, MS
2050	2050[e]	(E)-Nerolidol	0.4	t_R, MS
2080	2080[d]	Cubenol	3.7	MS
2089		6-Methyl-5 (3-methyl phenyl)-2-heptanone	0.8	MS
2187	2187[d]	**T-Cadinol**	**31.3**	MS
2256		*epi*-α-Bisabolol	0.3	t_R, MS
2257	2233[f] 2256[e]	Cadalene	0.5	MS
2258	2219[c] 2255[d]	α-Cadinol	1.4	t_R, MS
2264	2264[e]	4,7-dimethyl-1-tetralone	0.8	MS
2320	2324[c]	**14-Nor-cadin-5-en-4-one** isomer A	**11.0**	MS
2349	2349[e]	Cadina-4, 10 (15)-dien-3-one	6.1	MS
2931	2931[c] 2913[f]	Hexadecanoic acid	3.1	MS
		Monoterpene hydrocarbons	2.3	
		Oxygenated monoterpenes	10.1	
		Sesquiterpenes hydrocarbons	17.3	
		Oxygenated sesquiterpenes	44.6	
		Others	14.9	
		Total %	**89.2**	

RRI[a]: RRI Relative retention indices experimentally calculated against *n*-alkanes; RRI[b]: RRI from literature [c (48); d (49); e (50); f (51); g (52)] for polar column values; % calculated from FID data; tr; Trace (<0.1 %); Identification Method (IM): t_R, Identification based on comparison with co-injected with standards on a HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the libraries.

b) HPLC-DAD Analysis of Phenolic Compounds in the Methanol Extracts

The list of standard phenolic compounds has a wide range of phenolic standards such as 5 benzoic acids (gallic, protocatechuic, *p*-OH benzoic, vanilic, and syringic acid), 1 hydroxybenzaldehyde (vanillin), 5 cinnamic acids (caffeic, *p*-coumaric, ferulic, chicoric and chlorogenic acid), 3 flavanols (catechin EGCG, epicatechin), 1 flavonol with its 1 glycoside (quercetin and rutin), 3 flavones with 2 sugar attached derivatives, (luteolin, apigenin, tangeretin, luteolin-7-glucoside, apigenin-7-glucoside), 1 flavanone with its 1 glycoside (hesperidin and hesperetin), and rosmarinic acid which is a caffeic acid ester *(supp. Table 1)*.

A total 6.9 g and 2.1 g of phenolic compounds per 100 g of NP-LF and NP-S, respectively, were quantified by HPLC-DAD *(Table 2).* The stem part of the plant had at least 3 times less amount of phenolic compounds than the leaf and flower parts (NP-LF). Rosmarinic acid (RA) was found to be the major compound at 3.1 g per 100 g of NP-LF (3.1%) as expected from Lamiaceae family members. The peaks which had same UV spectrum as RA but eluted earlier were identified as RA derivatives (der.) and quantified as equivalent of RA *(Fig. 1).* They could be the sugar or other functional groups attached to the RA causing this early elution. Total ratio of RA derivatives in the extracts

were 4.3% for NP-LF and 0.3% for NP-S *(Table 2)*. Caffeic acid and derivatives including RA covered 4.5% of the NP-LF.

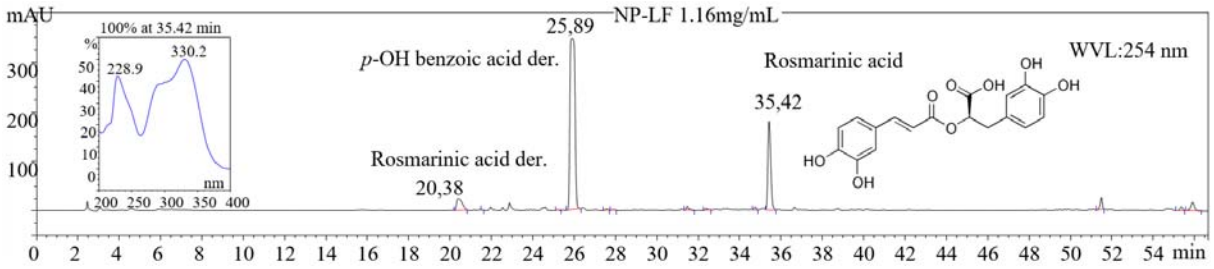

Figure 1: HPLC-DAD chromatogram of the extract from leaf and flower parts of *Nepeta pilinux*

Protocatechuic, chlorogenic, caffeic, *p*-coumaric, chicoric acids and luteolin-7-glycoside were also determined in the extracts by comparing the retention time and UV spectrum of the peaks with those of the standard phenolics. There was extremely large peak among others in the chromatograms at 254 nm which was eluted at around 25.9 min and had overlaying two compounds with 243 and 257 nm maximum wavelengths. These peaks were identified as *p*-OH benzoic acid der. and quantified as its equivalent *(Fig. 1 and Table 2)*. The peak eluting at 21.4 min. and having max. absorbance at 293 nm was identified as *p*-OH benzaldehyde (4-hydroxybenzaldehyde) since its elution order and UV spectrum were consistent with this phenolic aldehyde (53). The regression equation of vanillin which is the 4-hydroxy-3-methoxybenzaldehyde was used to quantified *p*-OH-benzaldehyde due to their structural and spectral similarity. Four tangeretin derivatives were identified tentatively by comparing UV spectrum of these peaks with that of tangeretin. Since tangeretin has 5 metoxy groups without hydroxyl group, it is eluting in the last part of the chromatogram from

Table 2: The amount and spectral details of phenolic compounds determined in the extracts by HPLC-DAD

	RT		Maximum Wavelength		mg/100g extract	
	NP-LF	NP-S	λ max	Compounds	NP-LF	NP-S
1	ND	8.6	259-292	protocatechuic acid	ND	5.5
2	15.8	15.3	232-284	catechin der.	83.8	57.7
3	20.4	20.3	330-300sh-232	rosmarinic acid der.	1055.9	164.9
4	21.4	21.2	293	*p*-OH benzaldehyde	2.8	16.2
6	22.0	21.7	326-295sh-237	chlorogenic acid	86.9	6.7
7	22.9	ND	328-295-242	caffeic acid	75.9	ND
8	ND	22.9	315-230	*p*-coumaric acid der.	ND	14.2
9	23.7	ND	333-243	chicoric acid der.	2.8	ND
10	ND	23.9	281-229	syringic acid der.	ND	5.4
11	24.5	24.3	329-302sh-249	rosmarinic acid der.	84.3	31.9
12	25.2	25.0	330-300sh-243	rosmarinic acid der.	29.0	6.7
13	25.9	25.7	243 and 257 mix	*p*-OH benzoic acid equivalent	2204.7	1605.3
14	26.8	26.6	229-310	*p*-coumaric acid	3.7	2.4
15	27.1	26.8	352-255	luteolin-7-gycoside der.	12.7	5.7
16	28.9	ND	312-295-228	p-coumaric acid der.	11.6	ND
17	29.2	29.0	329-305sh-243	chicoric acid	3.7	2.1
18	ND	30.8	329-233	rosmarinic acid der.	ND	3.7
19	31.5	31.2	338-253-225	apigenin-glycoside der.	54.4	3.9
20	ND	31.4	342-246-226	luteolin-glycoside der.	ND	2.5
21	32.4	ND	327-228 and 347-270	ferulic acid der. and luteolin-7-glycoside	mix	ND
22	ND	32.4	320-244	caffeic acid der.	ND	1.2
23	33.2	33.0	228-278	syringic acid der.	31.4	20.1

24	33.4	33.2	228-278	syringic acid der.	17.8	17.3
25	ND	33.4	331-275	apigenin-glycoside der.	ND	3.2
26	ND	34.1	338-275	apigenin-glycoside der.	ND	5.6
27	35.4	35.3	330-290sh-229	rosmarinic acid	3099.7	59.1
28	ND	35.7	314-230	*p*-coumaric acid der.	ND	2.7
29	36.6	ND	328-300sh-234	caffeic acid der.	21.6	ND
30	ND	38.3	319-230-295sh	ferulic acid der.	ND	6.8
31	38.7	38.6	328-277-226	tangeretine der.	10.7	43.6
32	39.4	39.2	329-277	chicoric acid der.	6.3	1.6
33	ND	39.4	338-276	apigenin der.	ND	5.6
34	39.8	ND	327-300sh-239	chlorogenic acid der.	8.4	ND
35	ND	41.6	267-340	apigenin der.	ND	6.4
36	ND	42.0	324-270	tangeretin der.	ND	1.6
38	ND	45.1	328-277-214	tangeretin der.	ND	1.4
39	ND	49.8	327-275-223	tangeretin der.	ND	7.4
40	ND	50.3	316	*p*-coumaric acid der.	ND	16.2
41	50.4	50.6	315-214	*p*-coumaric acid der.	11.5	0.9
				total	6919.7	2135.2
				total rosmarinic acids	4269.0	266.3

Since *Nepeta pilinux* is an endemic species and newly recorded in Şanlıurfa flora, there is not any scientific report for its phenoreverse phase chromatography *(Table 2).* Tangeretin derivatives may differ from each other in the number of hydroxyl and methoxyl groups and the presence of sugar or other functional groups. Thymusin which is the 5,6,4'-Trihydroxy-7,8-dimethoxyflavone (5,6,4' demethyl-tangeretin) and isothymusin were determined in *Nepeta asterotricha* Rech. (29). Therefore, two of the tangeretin der. could be thymusin and isothymusin. Apigenin and luteolin derivatives, determined in the extracts based on their UV spectra, were identified and quantified as apigenin, apigenin glycoside der. or luteolin glycoside der. based on their elution orders.

lic composition. On the other hand, there are some reports for chemical composition of the other species of *Nepeta* genus to compare with. For instance, epicatechin, caffeic acids, chlorogenic acid, quercetin and ellagic acid, well-known as antioxidant compounds were found in the ethanol extract of *Nepeta cadmea* Boiss. by HPLC-DAD (27). They couldn't have determined RA because it was not in their standard compound list. Unlike their results, epicatechin, quercetin and ellagic acid were not detected in *Nepeta pilinux* extracts *(Table 2).* Observation of similar but different phenolic compounds in species shows that differences between species also cause differences in metabolic product synthesis. In addition, different derivatives of phenolic compounds can be observed in samples of the same species grown in different geographical conditions. RA, apigenin, and quercetin were determined as major compounds in the ethanol extract of *Nepeta nuda* subsp. *albiflora* (28). Isolation of some iridoid glycosides such as nepetamoside, nepetaracemoside B, nepetonic acid and some polyphenol and flavonoid components such as RA and its methyle ester, thymusin, luteolin and apigenin from *Nepeta asterotricha* Rech. were performed by Goldansaz et al. (2019) (29). Methanolic extracts from *Nepeta rtanjensis* Diklic & Milojević which is an endemic perennial plant, in a very limited area in Southeast Serbia were investigated in a scientific study (30). The presence of high levels of chlorogenic acid, RA and rutin in these extracts was thought to be the reason for their antigenotoxicity.

RA, which is the main component in these extracts, is synthesized by the phenylpropanoid pathway starting with L-phenylalanine and L-tyrosine. From L-phenylalanine, *t*-cinnamic acid, *p*-coumaric acid and *p*-coumaroyl-CoA are successively produced, while p-hydroxyphenylpyruvic acid, p-hydroxyphenyllactic acid are successively produced from L-tyrosine. Then, the production of p-coumaroyl-p-hydroxy-phenyllactic acid by condensation of p-coumaroyl-CoA and p-hydroxyphenyllactic acid is followed by RA synthesis (54). Chlorogenic acid is synthesized by the condensation of *p*-coumaroyl-CoA with quinic acid (55). These metabolites which were found in the *Nepeta pilinux* extracts, support the survival of plants against harsh environmental conditions, therefore they have been seen by humans as a remedy for various diseases for many years. It has been reported that RA, chlorogenic acid and its metabolite caffeic acid have a neuroprotective effect due to their antioxidant capacity (56, 57).

c) *Total Antioxidant Characterization of the Methanol Extracts*

Total phenolic content (TPC) and radical scavenging activities (RSA) against DPPH and ABTS radical were evaluated to characterize the antioxidant capacity of the extracts and results were presented in Table 3. TPC of the NP-LF and NP-S extract from *Nepeta pilinux* was 50.81 and 13.37 mg GAE/g respectively. Consistent with the TPC of NP-LF which was found around fourfold of NP-S, RSA of NP-LF were higher (433.18 and 82.29 µg / mL for DPPH and ABTS respectively) than NP-S as expected (Table 3). The antioxidant capacity of this species was found to be quite consistent comparing with other species in the literature reports. Antioxidant activity of methanolic extracts from flower and leaf parts of *Nepeta rtanjensis* were reported by Bošnjak-Neumüller et al. (2017). They have found that the leaf extract had higher antioxidant capacity with TPC of 62.73 mg of GAE/g and IC_{50} value of RSA against DPPH as 112.59 µg/mL than those of flower extract. The antioxidant activities of ethanol, methanol, acetone, and water extracts from *Nepeta cadmea* were presented by Kaska et al. (2018). They have found that the water extract had the highest RSA (IC_{50} value of DPPH as 25.54 µg/mL and ABTS, 14.51 µg/mL) in these four extracts. Highest TPC with 79.84 mg GAE/g, was found in the methanol extract while the highest total flavonoids with 77.09 mgQE/g was in the acetone extract. It was reported by Teber and Bursal (2020) that ethanol and water extracts of *Nepeta nuda* subsp. *albiflora* had strong antioxidant effects with IC_{50} values of DPPH as 54.4 and 113.0 µg/mL, respectively. The TPC of flowers, leaves and roots methanol extracts of *Nepeta humulis* were found as 123.18, 66.20 and 54.77 mg GAE/g extract, respectively. Flower extract which had the highest TPC displayed best RCA with IC_{50} of 1290 and 350 $\mu g.mL^{-1}$ against DPPH and ABTS respectively (58).

Table 3: Total phenolic content, radical scavenging activity and enzyme inhibition of the extracts

Samples and standards	TPC[a]		RSA[b]		Enzyme inhibition	
	mgGAE/g	mgQE/g	DPPH SC_{50}	ABTS SC_{50}	urease IC_{50}	XO IC_{50}
NP-LF	50.81±1.50	35.40±1.06	433.18±12.74	82.29±0.98	62.47±0.10	48.48±0.10
NP-S	13.37±0.34	9.03±0.24	523.49±5.82	381.58±5.15	230.59±0.23	222.67±0.13
Gallic Acid			1.52±0.06	3.29±0.09		
Quercetin			5.87±0.13	8.52±0.16		
Acetohydroxamic acid					24.56±0.29	
Allopurinol						0.54±0.04

GAE, Gallic acid equivalent; QE, quercetin equivalent; SC_{50}, value of the concentration of extract required to scavenge 50% of DPPH and ABTS radicals (µg extract per mL methanol); IC_{50}, value of the concentration of extract required to inhibite 50% of *Jack bean* urease and bovine milk xanthine oxidase enzymes (µg extract per mL methanol). [a]Total phenolic contents are expressed in mg GAE/g extract and mg QE/g extract. [b]Radical Scavenging Activity

d) *Urease and Xanthine oxidase inhibitions of the methanol extracts*

The urease enzyme inhibition of the NP-LF with IC_{50} value of 62.47 $\mu g.mL^{-1}$ was only three times lower than the inhibition of acetohydroxamic acid (24.56 $\mu g.mL^{-1}$) which is standard medicine *(Table 3)*. NP-S had low inhibition against to this enzyme with IC_{50} value of 230.59 $\mu g.mL^{-1}$. The xanthine oxidase inhibition of the NP-LF and NP-S extracts, 48.48 and 222.67 $\mu g.mL^{-1}$ respectively, were quite lower comparing to the inhibition of allopurinol (0.54 $\mu g.mL^{-1}$) which is standard medicine reducing the production of uric acid in the body caused by certain cancer medications and kidney stones. Akdeniz et al. (2020) were screened urease inhibition effect of the essential oils and ethanolic extracts of *Nepeta heliotropifolia and Nepeta congesta* subsp. *cryptantha* comparing with the standard thiourea. They reported that none of them exhibited urease inhibitory activity (47). In another study, ethyl acetate sub fraction of *Nepeta praetervisa* showed significant urease inhibitory activity (68%) (59). The structure-activity relationship revealed that the planar flavones and flavonols with a 7-hydroxyl group such as chrysin, luteolin, kaempferol, quercetin, myricetin, and isorhamnetin inhibited xanthine oxidase activity at low concentrations, while the nonplanar flavonoids, isoflavones and anthocyanidins were less inhibitory (35). Although the inhibitory effects of the extracts cannot compete with acetohydroxamic acid and allopurinol, the fact that they are a natural herbal inhibitor source shows that these extracts are more suitable for use.

IV. Conclusion

This was the first investigation on the chemical composition and bioactivities of the *Nepeta pilinux.* The polar and apolar extracts of aerial parts had high amount of phenolic and essential oil compounds and demonstrated the potential antioxidant capacities. The polar extract with 3.1% rosmarinic acid had urease and

xanthine oxidase inhibition as well. This comprehensive evaluation of *Nepeta pilinux* revealed that this endemic plant could be the source of valuable therapeutic compounds. Besides, this report would be the incentive for further works on this plant's metabolites.

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Disclosure Statement

No potential conflict of interest was reported by the authors

References Références Referencias

1. K.H.C. Baser, N. Kirimer. M. Kurkcuoglu and B. Demirci, *Essential oils of Nepeta species growing in Turkey*, Chemistry of Natural Compounds, *36*(4), 356-359 (2000).
2. A. Kaya and T. Dirmenci, *Nutlet surface micromorphology of the genus Nepeta L. (Lamiaceae) in Turkey*, Turkish Journal of Botany, 32(2), 103-112 (2008).
3. I.C.Hedge I. C. and J. M. Lamond, *Flora of Turkey and the East Aegean Islands*, Vol. 7, ed. P. H. Davis, p. 264-288*, University Press Edinburgh*, Edinburgh (1982).
4. A. Guner, N. Ozhatay, T. Ekim and K.H.C. Baser, *Flora of Turkey and the East Aegean Islands*, Vol. *11*. Second Supplement, Edinburgh (2000).
5. F. Celep and T. Dirmenci, *Systematic and biogeographic overview of Lamiaceae in Turkey*, Natural Volatiles and Essential Oils, *4*(4), 14-27 (2017).
6. C. Formisano, D. Rigano and F. Senatore, *Chemical constituents and biological activities of Nepeta species*, Chemistry & Biodiversity, 8(10), 1783-1818 (2011).
7. B.R. Lichman, G.T. Godden, J.P. Hamilton, L. Palmer, M.O. Kamileen, D. Zhao, and L.K. Henry, *The evolutionary origins of the cat attractant nepetalactone in catnip,* Science Advances, 6(20), eaba0721 (2020).
8. U.R. Bernier, K.D. Furman, D.L. Kline, S.A. Allan and D.R. Barnard, *Comparison of contact and spatial repellency of catnip oil and N, N-diethyl-3-methylbenzamide (DEET) against mosquitoes*, Journal of Medical Entomology, *42*(3), 306-311 (2005).
9. G. Schultz, E. Simbro, J. Belden, J. Zhu and J. Coats, *Catnip, Nepeta cataria (Lamiales: Lamiaceae)-A closer look: Seasonal occurrence of nepetalactone isomers and comparative repellency of three terpenoids to insects*, Environmental Entomology, 33(6), 1562-1569 (2004).
10. W. Reichert, J. Ejercito, T. Guda, X. Dong, Q. Wu, A. Ray and J.E. Simon, *Repellency Assessment of Nepeta cataria essential oils and ısolated nepetalactones on Aedes aegypti.* Scientific Reports, *9*(1), 1524 (2019). https://doi.org/10.1038/s41598-018-36814-1.
11. D. Rigano, N.A. Arnold, F. Conforti, F. Menichini, C. Formisano, F. Piozzi and F. Senatore, *Characterisation of the essential oil of Nepeta glomerata Montbret et Aucher ex Bentham from Lebanon and its biological activities*, Natural Product Research, 25(6), 614-626 (2011).
12. K.H.C. Baser and T. Ozek, *Composition of the essential oil of Nepeta caesarea Boiss. from Turkey*, Journal of Essential Oil Research, 6(6), 645-646 (1994).
13. K.H.C. Baser, T. Ozek and G. Tumen, *Composition of the essential oil of Nepeta viscida Boiss. from Turkey*, Journal of Essential Oil Research, 7(5), 569-570 (1995).
14. G. Kokdil, S. Kurucu and G. Topcu, *Composition of the essential oil of Nepeta nuda L. ssp. albiflora (Boiss.) Gams*, Flavour and Fragrance Journal, *11*(3), 167-169 (1996).
15. G. Kokdil, S. Kurucu and G. Topcu, *Chemical constituents of the essential oils of Nepeta italica L. and Nepeta sulfuriflora PH Davis*, Flavour and Fragrance Journal, *12*(1), 33-35 (1997).
16. G. Kokdil, M. Tanker, S. Kurucu and G. Topçu, *Essential oil analysis of Nepeta cilicia Boiss*, Flavour and Fragrance Journal, *12*(2), 99-101 (1997).
17. K.H.C. Baser, B. Demircakmak, A. Altintas and H. Duman, *Composition of the essential oils of Nepeta cadmea Boiss*, Journal of Essential Oil Research, *10*(3), 327-328 (1998).
18. G. Tumen, K.H.C. Baser, M. Kurkçuoglu, B. Demirci and B. Yildiz, *Composition of the essential oil of Nepeta trachonitica post from Turkey*, Journal of Essential Oil Research, *11*(1), 21-22. (1999).
19. M. Dabiri and F. Sefidkon, *Chemical composition of the essential oil of Nepeta racemosa Lam. from Iran*, Flavour and Fragrance Journal, *18*(2), 157-158 (2003).
20. O. Kilic, S. Hayta and E. Bagci, *Chemical composition of essential oil of Nepeta nuda L. subsp. nuda (Lamiaceae) from Turkey*, Asian Journal of Chemistry, 23(6), 2788-2790 (2011).
21. A. Adiguzel, H. Ozer, M. Sokmen, M. Gulluce, A. Sokmen, H. Kilic and O. Baris, *Antimicrobial and antioxidant activity of the essential oil and methanol extract of Nepeta cataria,* Polish Journal of Microbiology, 58(1), 69-76 (2009).
22. A. Gormez, S. Bozari, D. Yanmis, M. Gulluce, G. Agar and F. Sahin, *Antibacterial activity and chemical composition of essential oil obtained from Nepeta nuda against phytopathogenic bacteria*,

Journal of Essential Oil Research, *25*(2), 149-153 (2013).
23. N. Hasimi, S. Kizil and V. Tolan, *Essential oil components, microelement contents and antioxidant effects of Nepeta italica L. and Achillea filipendulina Lam*., Journal of Essential Oil Bearing Plants, *18*(3), 678-686 (2015).
24. F. Bozok, M. Cenet, G. Sezer and Z. Ulukanli, *Essential oil and bioherbicidal potential of the aerial parts of Nepeta nuda subsp. albiflora (Lamiaceae),* Journal of Essential Oil Bearing Plants, 20(1), 148-154 (2017).
25. C. Sarikurkcu, O. Ceylan, S. Targan and S. Ć. Zeljković, *Chemical composition and biological activities of the essential oils of two endemic Nepeta species*, Industrial Crops and Products, *125,* 5-8 (2018).
26. A.H. Gilani, A.J. Shah, A. Zubair, S. Khalid, J. Kiani, A. Ahmed and V.U. Ahmad, *Chemical composition and mechanisms underlying the spasmolytic and bronchodilatory properties of the essential oil of Nepeta cataria L*., Journal of Ethnopharmacology, 3(121), 405-411 (2009).
27. Kaska A, Deniz N, Çiçek M, Mammadov R. Evaluation of Antioxidant Properties, Phenolic Compounds, Anthelmintic, and Cytotoxic Activities of Various Extracts Isolated from Nepeta cadmea: An Endemic Plant for Turkey. J Food Sci. 2018 Jun;83(6):1552-1559.
28. Teber İ., and Bursal E., 2020. Phenolic compounds and antioxidant activity of *Nepeta nuda* subsp. *albiflora*, International Letters of Natural Sciences, Vol. 79, pp.1-8.
29. Goldansaz, S. M., Festa, C., Pagano, E., De Marino, S., Finamore, C., Parisi, O. A., Borrelli, F., Sonboli, A., & D'Auria, M. V. (2019). Phytochemical and Biological Studies of *Nepeta asterotricha* Rech. f. (Lamiaceae): Isolation of Nepetamoside. *Molecules (Basel, Switzerland)*, *24*(9), 1684. https://doi.org/10.3390/molecules24091684
30. Bošnjak-Neumüller J, Radaković M, Djelić N, Vuković-Gačić B, Stevanović ZD, Kolarević S, Mišić D, Stanković M, Knežević -Vukčević J, Spremo-Potparević B, Stanimirović Z. *Nepeta rtanjensis* (Lamiaceae), a plant endemic to the Balkans: Phenolic composition, antioxidant activity, and in vitro antigenotoxic effects in triiodothyronine-induced DNA damage in human lymphocytes. Pak J Pharm Sci. 2017 Mar;30(2(Suppl.)):625-634.
31. Balasubramanian A, Ponnuraj K. Crystal structure of the first plant urease from jack bean: 83 years of journey from its first crystal to molecular structure. J Mol Biol. 2010 Jul 16;400(3):274-83. doi: 10.1016/j.jmb.2010.05.009. Epub 2010 May 13. PMID: 20471401.
32. Shi D.H. , You Z.L. , Xu C. , Zhang Q. , Zhu H.L., (2007). Synthesis, crystal structure and urease inhibitory activities of Schiff base metal complexes Inorg. Chem. Commun., 10, 404-406.
33. Ohta T., Shibata H., Kawamori T., Iimuro M., Sugimura T., Wakabayashi K., (2001). Marked Reduction of Helicobacter pylori-Induced Gastritis by Urease Inhibitors, Acetohydroxamic Acid and Flurofamide, in Mongolian Gerbils, Biochemical and Biophysical Research Communications, 285, 3, 728-733.
34. Hayashi T.,. Sawa K, Kawasaki M., Arisawa M., Shimizu M., Morita N., (1988). Inhibition of cow's milk xanthine oxidase by flavonoids, J. Nat. Prod. 51, 2, 345-348. https://doi.org/10.1021/np50056a030.
35. Nagao A. Seki, M. Kobayashi, H. (1999) Inhibition of Xanthine Oxidase by Flavonoids, Bioscience, Biotechnology, and Biochemistry, 63:10, 1787-1790, DOI: 10.1271/bbb.63.1787.
36. Lin, S.; Zhang, G.; Liao, Y.; Pan, J.; Gong, D. Dietary Flavonoids as Xanthine Oxidase Inhibitors: Structure–Affinity and Structure–Activity Relationships. J. Agric. Food Chem. 2015, 63, 7784–7794.
37. Van Hoorn, D.E.C.; Nijveldt, R.J.; Van Leeuwen, P.A.M.; Hofman, Z.; M'Rabet, L.; De Bont, D.B.A.;Van Norren, K. Accurate prediction of xanthine oxidase inhibition based on the structure of flavonoids. *Eur. J. Pharmacol.* 2002, *451*, 111–118.
38. F. Abak, Şanlıurfa ili Lamiaceae (Ballıbabagiller) Familyasının Florası, Bazı Taksonların Fitokimyasal ve Etnobotanik Özellikleri , PhD Thesis, Recep Tayyip Erdogan University, Department of Biology) (2018).
39. F.W. McLafferty and D.B. Stauffer, *The Wiley/NBS Registry of Mass Spectral Data*, J.Wiley and Sons: New York (1989).
40. R.P. Adams, *Identification of Essential Oil Components by Gas chromatography/Mass Spectrometry, Ed. 4.1*. Allured Publishing Corporation, Illinois (2017).
41. D.H. Hochmuth, *MassFinder-4*, Hochmuth Scientific Consulting, Hamburg, Germany (2008).
42. J.M.P.M. Curvers, J. Rijks, C.A.M.G. Cramers, K. Knauss and P. Larson, *Temperature programmed retention indices: Calculation from isothermal data. Part 1: Theory*, Journal of High Resolution Chromatography, 8(9), 607-610 (1985).
43. Singleton, V.L. (1985). Citation Classic - Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Cc/Agr Biol Environ, 18-18.
44. Brand-Williams W., Cuvelier M.E., Berset C. (1995). Use of a free radical method to evaluate antioxidant activity, LWT - Food Science and Technology, 28, (1), 25-30.
45. Arnao, M.B., Cano, A., and Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem 73, 239-244.

46. Weatherburn M.W., Phenol-hypochlorite reaction for determination of ammonia, Anal. Chem. 39 (1967) 971–974.
47. Akdeniz M., Ertas A., Yemer I., Firat M. and Kolak U., Phytochemical and biological investigations on two Nepetaspecies: Nepeta heliotropifolia and N. congesta subsp. Cryptantha, J Food Biochem. 2020; 44:e13124. DOI: 10.1111/jfbc.13124.
48. K.H.C. Baser, G. Ozek, T. Ozek, A. Duran and H. Duman, *Composition of the essential oils of Rhabdosciadium oligocarpum (Post ex Boiss.) Hedge et Lamond and Rhabdosciadium microcalycinum Hand.-Mazz.,* Flavour and Fragrance Journal, *21*(4), 650-655 (2006). DOI: 10.1002/ffj.1639.
49. A. Ali, N. Tabanca, B. Demirci, E.K. Blythe, K.H.C. Baser and I.A. Khan, *Chemical composition and biological activity of essential oils from four Nepeta species and hybrids against Aedes aegypti (L.) (Diptera: Culicidae)*, Records of Natural Products, *10*(2), 137-147 (2016).
50. E. Sezik, E. Kocakulak, K.H.C. Baser and T. Ozek, *Composition of the essential oils of Juniperus oxycedrus subsp. macrocarpa from Turkey*, Chemistry of Natural Compounds, *41*(3), 352-354 (2005).
51. V.I. Babushok, P.J. Linstrom and I.G. Zenkevich, *Retention indices for frequently reported compounds of plant essential oils*, Journal of Physical and Chemical Reference Data, *40*(4), 043101 (2011).
52. Zeynep Tunalıer, N. Kırımer and K.H.C. Başer, A Potential New Source of Cedarwood Oil: Juniperusfoetidissima Willd., J. Essent. Oil Res., 16, 233-235 (2004).
53. Margraf M., 2015. Comparison of Compounds in Bourbon Vanilla Extract and Vanilla Flavour. LC GC Europe 28(7):415-417.
54. M. Petersen, Rosmarinic acid: new aspects, Phytochem Rev (2013) 12:207–227. DOI 10.1007/ s11101-013-9282-8.
55. G.E. Bartley, R.J. Avena-Bustillos, W.-X. Du, M. Hidalgo, B. Cain and A. P. Breksa III, Transcriptional regulation of chlorogenic acid biosynthesis in carrot root slices exposed to UV-B light, Plant Gene 7, 1-7 (2016).
56. F. Taram, A.N. Winter and D.A. Linseman, Neuroprotection comparison of chlorogenic acid and its metabolites against mechanistically distinct cell death-inducing agents in cultured cerebellar granule neurons, Brain Research, 1648, 69-80 (2016).
57. F. Taram, E. Ignowski, N. Duval and D. A. Linseman, Neuroprotection Comparison of Rosmarinic Acid and Carnosic Acid in Primary Cultures of Cerebellar Granule Neurons, Molecules, 23, 2956 (2018). doi:10.3390/molecules23112956.
58. Gökbulut A., Yılmaz G., 2020. Nepeta humulis Bentham: First evaluation of phenolic profile and radical scavenging potential. J. Res. Pharm; 24 (6), 901-907.
59. Fareed, G., Afza, N., Mali, A., Fareed, N., Lateef, M., Iqbal, L., & Mughal, U.R (2013). Phytochemical screening, total phenolic contents and biological evaluation of aerial parts of nepeta praetervisa. Journal of the Chemical Society of Pakistan, 35(5), 1366-1370.

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Recurrent Relationships as an Important Class of Mathematical Equations in Chemistry

By Igor G. Zenkevich

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Abstract- The unique potential and different areas of applications of recurrent (synonymous: recursive) relationships in chemistry and chromatography are considered. Recurrent relations can be used in two forms: as functions of integer arguments, $y(x + 1) = ay(x) + b$, and as functions of equidistant argument values, $A(x + \Delta x) = aA(x) + b$, $\Delta x = \text{const}$. The first form applies to all physicochemical properties of homologs in organic chemistry, because the number of carbon (and other) atoms in a molecule can be integer only. The second one applies to chemical variables depending on temperature, pressure, concentrations, etc., when the chemists should provide equal "steps" of their variations.

Recurrent relations combine the properties of arithmetic and geometric progressions, which accounts for their unique approximation abilities. This was illustrated by approximating the number of isomers of alkanes, the boiling points of homologs (nonlinear dependencies), the melting points of homologs (alternation effects), the temperature dependence of the solubility of inorganic salts in water, and by revealing the anomalies of gas chromatographic retention indices and retention times in reversed-phase high performance liquid chromatography.

Keywords: recurrent relations, chemistry, chromatographic retention, chemical variables, number of isomers, physicochemical properties, linear approximation.

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Recurrent Relationships as an Important Class of Mathematical Equations in Chemistry

Igor G. Zenkevich

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Recurrent relations combine the properties of arithmetic and geometric progressions, which accounts for their unique approximation abilities. This was illustrated by approximating the number of isomers of alkanes, the boiling points of homologs (nonlinear dependencies), the melting points of homologs (alternation effects), the temperature dependence of the solubility of inorganic salts in water, and by revealing the anomalies of gas chromatographic retention indices and retention times in reversed-phase high performance liquid chromatography. In the latter case, specific anomalies in the recurrent approximation of retention times allow detecting the reversible hydration of analytes.

The restricted text cannot represent all the examples of the applications of recurrent relations in chemistry and chromatography. However, even this short review allows us to conclude that the potential of recurrences should not be underestimated.

The author confirms that the objective difficulties of the perception of recurrences seem to be the main reason for their rare use in chemistry. In such a situation, any examples of demonstrating their capabilities are significant. Consequently, the author is very grateful to the Editorial Board of GJSFR for the kind offer to submit a manuscript on this topic.

Keywords: *recurrent relations, chemistry, chromatographic retention, chemical variables, number of isomers, physicochemical properties, linear approximation.*

I. Introduction

Mathematics is an essential tool for data processing in all natural sciences, including chemistry [1–4]. However, in all of them, there are several stereotypes of presenting different mathematical equations. Most of them are the following: in equations of the form $y = f(x, \ldots)$ the argument(s) (x, …) are typically on the right side, while the function is on the left. The number of possible examples is so large that it is difficult to estimate it. For instance, the well known Antoine equation which has many chemical applications relates the absolute temperature (T, argument), and the vapor pressure of pure liquids (P, function): $\log P = a/T + b$ (the coefficients a and b should be precalculated). If necessary, the argument and function can be swapped and this relationship can be used to calculate the temperature from the pressure. Besides that numerous analogues of Antoine equation are used in different areas. For instance, the dependence of retention times *vs*. temperature of gas chromatographic column is described with Antoine-like equation $\log(t_R') = a/T + b$.

Numerous other forms of representing dependences (e.g., parametric) are known for mathematicians, but in chemistry they are perceived as rather unusual.

Recurrent equations are precisely such unusual forms of mathematical relationships. The main feature of them is the absence of arguments in their writing: recurrent equations relate the current value of a function to its previous value(s). The rare use of these relations is confirmed by the fact that many contemporary manuals have no information about them. Recurrent (recursive) progressions are mentioned in [2, P. 174] only as a particular case for comparing with other progressions (arithmetic, geometric, etc.). Such an attitude towards recurrences cannot be accepted, since the properties of recurrences make them indispensable mathematical object in chemistry. The main reason of that is, probably, just unusual mathematical form of recurrences, namely the absence of arguments.

The purpose of this article is to illustrate the application of recurrences with examples from various areas of chemistry and chromatography.

Generally, the most straightforward (first-order) linear recurrences can be represented in two ways. *The first kind of recurrence* combines the values of functions of discrete integer arguments ($n + 1$ and n) in relation (1):

$$y(n + 1) = ay(n) + b \qquad (1)$$

The coefficients a and b should be precalculated by the least-squares method using data for a preselected training set.

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At first glance, the scope of application of such relations with integer arguments seems to be somewhat limited. However, it is not; in organic chemistry many properties of organic compounds are considered as the functions of their positions in the corresponding homologous series, or, in other words, of the number of carbon (or other elements) atoms in the molecule. Meanwhile, *the number of atoms of any element in a molecule can take integer values only*. It is the principal reason for the applying of recurrent dependences to various properties of organic compounds. One can only be surprised that this was not been done before 2006, but it may only be explained by the unfamiliarity of these relations.

The second form of recurrent dependences applies to any functions for which it is possible to provide the equidistant argument values, namely, in the form ($x + \Delta x$), where Δx = const (relation 2). In other words, the experimenter must select appropriate argument values from their possible multitude:

$$y(x + \Delta x) = ay(x) + b, \quad \Delta x = \text{const} \qquad (2)$$

Such an expansion of the domain of definition allows applications of recurrences to functions of continuous variables such as temperature (T), pressure (P), concentrations (C), etc., i.e., not only to properties of homologs in organic chemistry, but also to characteristics of chemical systems in physical, analytical, inorganic chemistry, etc.

The general feature of recurrences is the absence of argument values in both parts (left and right) of equalities. Every point on the plots of recurrences is specified by two values of functions: current and previous, or current and subsequent ones. The second feature of equation (2) is the appearance of the additional (artificial) variable Δx: the shape of the graph begins to depend on this value.

As a result, both forms of recurrences are somewhat difficult to visualize, which explains in some way their rare use in scientific practice. To illustrate this, let us consider as an example the recurrent representation of any well-known function $y(x)$. Let us select 36 points within the domain of its definition with the increment $\Delta x = 10$ and build a plot corresponding to the equation $y(x + 10) = ay(x) + b$, shown in Fig. 1.

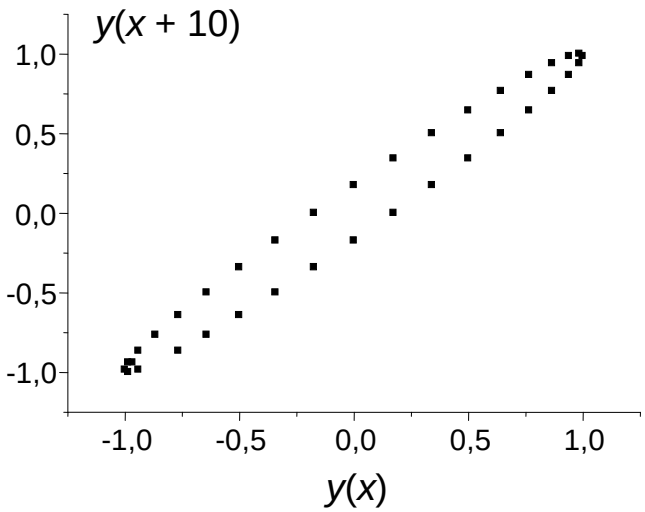


Fig. 1: Recurrent representation of a well-known mathematical function in the form $y(x + 10) = ay(x) + b$. It is not so easy to recognize it

We obtain some kind of closed ellipsoidal curve, which has no analogues among the plots of commonly used functions. If we change the value Δx and select $\Delta x = 1$ (or less) instead of $\Delta x = 10$, the ellipsoidal curve will turn into a practically straight segment located between points (–1, –1) and (+1,+1). So as not to continue the intrigue, let us note that this function is $y = \sin x$, but it is rather difficult to "recognize" it from the recurrent graph without special preparation.

First-order recurrent relations like (1) or (2) look like simple mathematical expressions only at a first glance. These equations have the non-recurrent algebraic solution that is an unusual type of polynomial of variable degree, because the values of the argument are the power:

$$y(x) = y(0)a^x + b(a^x - 1) / (a - 1) \qquad (3)$$

This solution can be easily found using MAPLE software. The value of the auxiliary parameter $y(0)$ should be predetermined, for example, using the value $y(1)$, because at $x = 1$ $y(1) = y(0)a + b$, and $y(0) = [y(1) - b]/a$.

This solution is the row, because $(a^x - 1) / (a - 1) = a^{x-1} + a^{x-2} + \ldots + 1$. The variable degree of this polynomial is a crucial explanation of the usefulness of

applying recurrences to approximation of chemical variables. This fact deserves special comments.

Example 1: Let us try to approximate the nonlinear dependence of normal boiling points of 1-chloroalkanes $C_nH_{2n+1}Cl$ on the number of carbon atoms in a molecule: 46.2°C (n = 3), 78.4°C (n = 4), 107.8°C (n = 5), 135.6°C (n = 6), 160.6°C (n = 7), 183.8° C (n = 8), 205.4°C (n = 9), and so on. The commonly used way to do this is choosing the fixed-degree polynomial (e.g., second or third). At the same time, the polynomial of variable degree (3) will have the degree x = 3 for propyl chloride (n = 3), x = 4 for butyl chloride (n = 4), x = 5 for pentyl chloride (n = 5), etc.

In addition, a noticeable consequence of the application of this approach can be derived from this solution. If $a \equiv 1$ and $b \neq 0$, equation (3) transforms into the relation for a simple arithmetic progression, $y(x) = k + bx$. At the same time, if $0 < a \neq 1$ and $b \equiv 0$, this equation transforms into the expression for geometric progression, $y(x) = ka^x$. Hence, in the general case (at arbitrary values of coefficients a and b), the recurrent equation (1) and/or (2) combines the mathematical properties of both kinds of progressions in variable proportions. It explains us the excellent approximating "power" of recurrences for numerous chemical variables. Thus, we must conclude that recurrences are not the particular case of other progressions [2] but are their generalization.

The second noticeable consequence from equation (3) is the behavior of function $y(x)$ or $A(n_C)$ with a hypothetical increase in the number of carbon atoms in a molecule, $x \rightarrow \infty$, or $n_C \rightarrow \infty$. If the value of coefficient a obeys the inequality $a < 1$, the limit of polynomial (3) exists, and it is equal to:

$$\lim A(n_C)\big|_{nC\rightarrow\infty} = b / (1 - a) \qquad (4)$$

If $a > 1$, the initial numerical sequence has no limit and tends to infinity.

The author cannot cite all his papers devoted to the properties and chemical applications of recurrences due to the necessity to restrict the self-citation. Only six of them published within the period from 2009 to 2024 are mentioned as references [5–10].

To conclude the Introduction, it should be noted that, probably, the most famous and widely mentioned examples of recurrences are so-called Fibonacci numbers, F_n, known since ancient times [11–13]:

$$F_n = F_{n-2} + F_{n-1}\ , \quad (F_0 = F_1 = 1) \qquad (5)$$

A special journal named *The Fibonacci Quarterly* has been published since 1963 (see ref. [11]).

II. Experimental

This semi-review article does not imply specific experimental conditions. The necessary data are available from the references cited. In general, recurrent relationships can be applied to any non-linear set of equidistant variables not only in chemistry or chromatography. For example, the temperatures of the cooling kettle, T(°C) = $f(t$, min) are: 72(0), 62(5), 55(10), 50(15), 46(20), etc. All these data correspond to the linear recurrent dependence $T(t + 5\ \text{min}) = aT(t) + b$ with the following parameters: a = 0.72 ± 0.01. b = 10.0 ± 0.8, R = 0.9996, S_0 = 0.2.

Another important example is the approximation of the carbon dioxide content in the atmosphere of Earth (data from site http://climate.gov),(CO_2 concentration (ppm)) = f(year): 324(1970), 337(1980), 353(1990), 369(2000), 390(2010), and 412(2020). Parameters of the recurrence C(year + 10) = aC(year) + b are: a = 1.14 ± 0.01, b = -33 ± 8, R = 0.9994, S_0 = 1.2. After that we can easily (because the dependence is linear) estimate the concentration of CO_2 in the atmosphere at 2030: it should be 1.14 × 412 - 33 = 437 ppm.

The values of different physicochemical properties of organic compounds (boiling points, melting points, etc.) were taken from all available sources of reference information, e.g., [14], as well as from Internet. The necessary stage of processing the initial information was the rejection of outliers, followed by averaging the data. It is required because recurrences are very "sensitive" even to minor errors in data sets.

The simplicity of recurrent calculations requires special comments. The use of, e.g., Origin software implies the following stages. First, the initial data on the function of integer (equation 1) or equidistant (equation 2) arguments are entered into column A[X]. The next step is copying all data from this column excluding the value from the first line into column B[Y] with shifting one line up. Just such shifting is the sense of recurrences. After that, we can operate with the obtained two-dimensional data array in the usual way, i.e., plot this dependence and calculate all the parameters of the linear regression $y(x+1) = ay(x) + b$. For example, let us make the recurrent approximation of the squares of natural numbers from 3 to 10. Hence, the first column (Origin or Excel software) contains the values 9, 16, 25, 36, 49, 64, 81, and 100 (eight values). The second column (shifted) in the same lines contains the values 16, 25, 36, 49, 64, 81, and 100. In the result we obtain seven pairs of numbers for calculating the parameters of linear regression by least squares method: a = 1.16 ± 0.01, b = 4.5 ± 0.5, R = 0.9998, S_0 = 0.7. The plot of this dependence looks like "ideal" straight line. Such calculations are very simple, and the application of no special functions is required.

III. Results and Discussion

a) Chemical Applications

i. Number of Isomers

In this section, it seems advisable to avoid considering the simplest artificial examples and start directly with real chemical problems. The first is the dependence of the number of structural isomers (N) of homologs on the number of carbon atoms in their molecules (n_C). The number of such isomers for n-alkanes C_5–C_{18} is listed in Table 1; it is the rapidly increasing function [15]. It is noteworthy that estimates of the number of isomeric alkanes with n_C = 18 and above, obtained by different methods, appeared to be slightly different, e.g., 60524 *vs*. 60523 for $C_{18}H_{38}$. However, such small differences for many practical purposes are negligible.

Table 1: Number of structural isomers of alkanes C_nH_{2n+2}

Number of Carbon Atoms, N_c	Number of Structural Isomers, N	Log n
5	3	0.477
6	5	0.699
7	9	0.954
8	18	1.255
9	35	1.544
10	75	1.875
11	159	2.201
12	305	2.550
13	802	2.904
14	1858	3.269
15	4347	3.638
16	10359	4.015
17	24894	4.396
18	60524 (60523)*	4.782

The plot of this function $N = f(n_C)$ for alkanes with $5 \le n_C \le 17$ is presented in Fig. 2(a). It seems rather challenging to precalculate the number of isomeric alkanes with a higher number of carbon atoms because we do not know the type of this function (it should be evaluated or assumed preliminarily). However, if we transform this dependence to the linear form, the problem's solution enormously simplifies, because we can evaluate other values using the simplest linear extrapolation. Just recurrent approximations allow us to implement this approach.

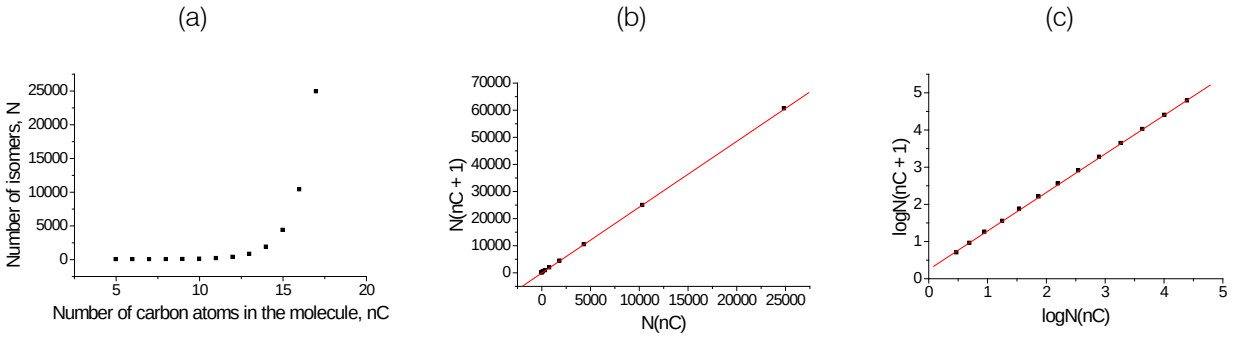


Fig. 2: (a) Plot of the number of structural isomers of alkanes C_nH_{2n+2} (N) vs. the number of carbon atoms in the molecule (n_C); (b) plot of the recurrent dependence $N(n_C + 1) = aN(n_C) + b$; parameters of the linear regression: a = 2.429 ± 0.004, b = –62 ± 30, R = 0.99999, S_0 = 98; (c) plot of the recurrent dependence of logarithms, $\log N(n_C + 1) = a\log N(n_C) + b$; parameters of the linear regression: a = 1.036 ± 0.005, b = 0.248 ± 0.012, R = 0.9998, S_0 = 0.02

Approximating the data set in the form $N(n_C + 1)$ *vs*. $N(n_C)$ gives the plot presented in Fig. 2(b). It is a linear dependence with the correlation coefficient R = 0.99999. Other parameters of the linear regression are indicated in the footnote to Fig. 2. If the correlation coefficients exceed 0.999, the plots of such dependencies are visually perceived as absolutely straight lines. It is an essential moment in the

characterization of recurrent dependencies. For numerous chemical variables, these dependencies are linear, but if not, the reasons of deviations from linearity should be investigated. If we will use this result for evaluating the number of isomers of alkane $C_{18}H_{38}$ using the number of isomers for the previous alkane $C_{17}H_{36}$, we should make the following simple calculations:

$$24894 \times 2.429 - 62 \approx 60406$$

For the example considered the evaluation of uncertainty appeared to be approximately ±98. The correct answer is 60524 or 60523 isomers (Table 1). Hence, the relative error is approximately 0.19%. Such accuracy seems to be enough for many practical purposes.

This example illustrates another feature of recurrent approximation. If it is linear for variable x, it remains linear for any monotonic functions of this variable, e.g., x^n, $\log(x)$, $\exp(x)$, etc. Hence, we can approximate not the number of isomeric alkanes (N) but the $\log N$ values. Fig. 2(c) illustrates the linear recurrent dependence for logarithms of isomers' number.

The number of isomers of homologs of other series [16, 17] can be approximated using recurrent relations without any restrictions.

ii. *Approximation of Boiling Points of Homologs*

To illustrate the potential of recurrent relations in form (1) (functions of integer argument), let us consider the approximation of values of any physicochemical properties of homologs. The simplest example is normal (at the atmospheric pressure) boiling points (T_b) of *n*-alkanes C_nH_{2n+2} within the set of compounds C_2–C_{14} (if necessary, this range can be expanded); the reference data are readily available [14]. The plot in Fig. 3(a) illustrates the initial nonlinear dependence $T_b(n_C)$; the linear recurrent approximation $T_b(n_C + 1) = aT_b(n_C) + b$ is illustrated in Fig. 3(b). The parameters of the linear regression are listed in the footnote to this figure.

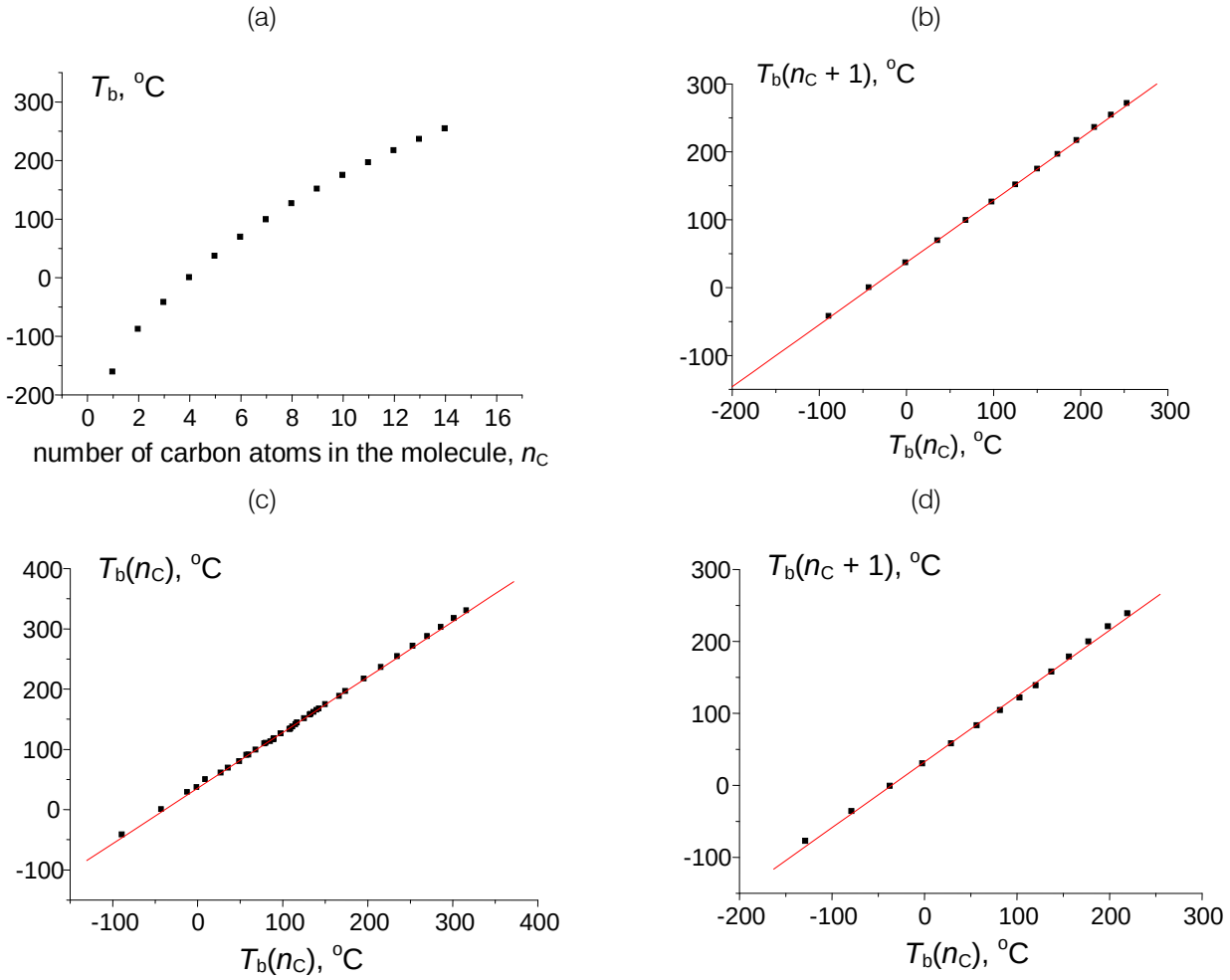


Fig. 3: (a) Dependence of the normal boiling points of n-alkanes C_1–C_{14} (°C) on the number of carbon atoms in the molecule; (b) recurrent approximation of the normal boiling points (equation 1); parameters of linear regression: a = 0.916 ± 0.003, b = 36.9 ± 0.5, R = 0.99993, S_0 = 1.2; (c) recurrent approximation of the normal boiling points for all alkanes (including branched isomers); parameters of the linear regression: a = 0.922 ± 0.003, b = 35.6 ± 0.5, R = 0.9997, S_0 = 2.0; (d) recurrent approximation of the normal boiling points for perfluoro-1-alkanes C_nF_{2n+2}; parameters of the linear regression: a = 0.91 ± 0.01, b = 32.5 ± 1.4, R = 0.9991, S_0 = 4.2

The next exciting feature of recurrences does not follow from their mathematical properties but has a critical chemical sense. Namely, if the recurrent linearization is valid for normal alkanes (with non-branched carbon skeleton), the equation with practically the same coefficients will be valid for all isomeric structures of the same class, i.e., for alkanes with branched carbon skeletons. It is illustrated by Fig. 3(c) (39 points for *iso*-alkanes were added). Comparing the coefficients of recurrences (b) and (c) confirm their identity: 0.916 and 0.922 (values of coefficient *a*), as well as 36.9 and 35.6 (values of coefficients *b*). Moreover, combining the data for 189 homologs of 10 different homologous series confirms the existence of a single linear relationship for all of them with the following parameters: $a = 0.930 \pm 0.002$, $b = 33.5 \pm 0.3$, $R = 0.9995$, $S_0 = 2.4$ [7]. It allows us to conclude that coefficients *a* and *b* or recurrent regressions are close to each other for all homologous series with the same homologous differences, at first CH_2.

The following example illustrates the applicability of recurrences not to alkanes but compounds of other chemical nature. The plot of the recurrent approximation of boiling points of perfluoro-*n*-alkanes C_1–C_{14} is presented in Fig. 3(d).. The correlation coefficient of this dependence exceeds 0.999.

The linearity of recurrent relations makes it possible to evaluate the boiling points of any next homologs using the data for previous ones.

Example 2: Let us evaluate the normal boiling point of 1-chlorodecane using T_b value for 1-chlorononane (205.4°C, see Example no. 1). The values of recurrent coefficients for the series of chloroalkanes are $a = 0.923 \pm 0.002$, $b = 35.7 \pm 0.3$, $R = 0.99999$, and $S_0 = 0.3$. The next step of calculations is simple: $205.4 \times 0.923 + 35.7 = 225.3$°C (the reference value is 225.8°C).

Example 3: Let us evaluate the normal boiling point of *n*-butylisocyanate using T_b value for *n*-propylisocyanate (88°C). As the data for the series of alkyl isocyanates are insufficient to calculate the coefficients of equation (1), let us use the "universal" values of these coefficients for any homologous series (see above). The result is $88 \times 0.930 + 33.5 = 115.6$°C (the reference value is 115°C).

Recurrent relations provide linear approximations of not only normal boiling points but also the values of other physicochemical properties of organic compounds within homologous series, $A(n_C)$. These include, for example, relative density (d_4^{20}), refractive index (n_D^{20}), dynamic viscosity (μ), surface tension (σ), ionization potential (*I*), acidity constant (pK_a), dielectric permittivity (ε), water solubility (*w*, p*S*), and many others. The fundamental requirement to the dependences $A(n_C)$ is the same: these functions should be monotonic (the signs of their first derivatives should be constant). The example of nonmonotonic dependencies is, for instance, the temperature dependence of the solubility of organic compounds in water.

Due to the problem of the monotony of changes in the values of physicochemical properties of organic compounds, it is interesting to consider another form of recurrent relations, namely, second-order recurrences:

$$y(n+2) = ay(n) + b \tag{5}$$

iii. *Melting Points of Homologs*

Similarly to the previous ones, the coefficients *a* and *b* of equation (5) should be precalculated by the least-squares method. The algebraic solution of equation (5) can be found in the same way as the solution of equation (1) using MAPLE software. Surprisingly, it turned out to be much more complex than the solution of (1):

$$y(x) = \frac{(y(0)a - y(1))\sqrt{a})(-\sqrt{a})\uparrow x - (y(1)\sqrt{a} - ay(0))(\sqrt{a})\uparrow x}{2a} - \frac{b}{a-1} - \frac{b(\sqrt{a}-a)(-\sqrt{a})\uparrow x + b(\sqrt{a}+a)(\sqrt{a})\uparrow x}{2a(a-1)} \tag{6}$$

Relation (6) obviously cannot be recommended for any calculations due to its complexity, but it allows us to make important conclusions. First, this solution contains negative terms to the *x* power, namely (-sqr(*a*)) ↑x. It means that these terms have different signs depending on whether the *x*-values are even or odd. Therefore, the presence of terms with alternating signs in the equation makes it applicable for approximation of variables with pronounced alternation effects. The most important of these variables are the melting points of homologs with even and odd numbers of carbon atoms in the molecules. The alternation of the melting point is well known for *n*-alkanes, *n*-alkenes, 1-alkanols, carboxylic acids, 1-alkylamines, numerous 1,ω-difunctionalized alkanes, etc. Fig. 4 illustrates different kinds of the recurrent data approximation for 1-alkylamines. Plot (a) represents the set of initial data, "distorted" with alternation effects. The first-order linear recurrent approximation (equation 1) converts this plot to two nonparallel straight lines (b). Finally, the application of equation (5) to this data set gives a single straight line with the correlation coefficient $R = 0.9996$ (no less than the *R*-values in the other cases mentioned above), but with irregular arrangement of points (c). The parameters of linear regression (c) are listed in the footnote.

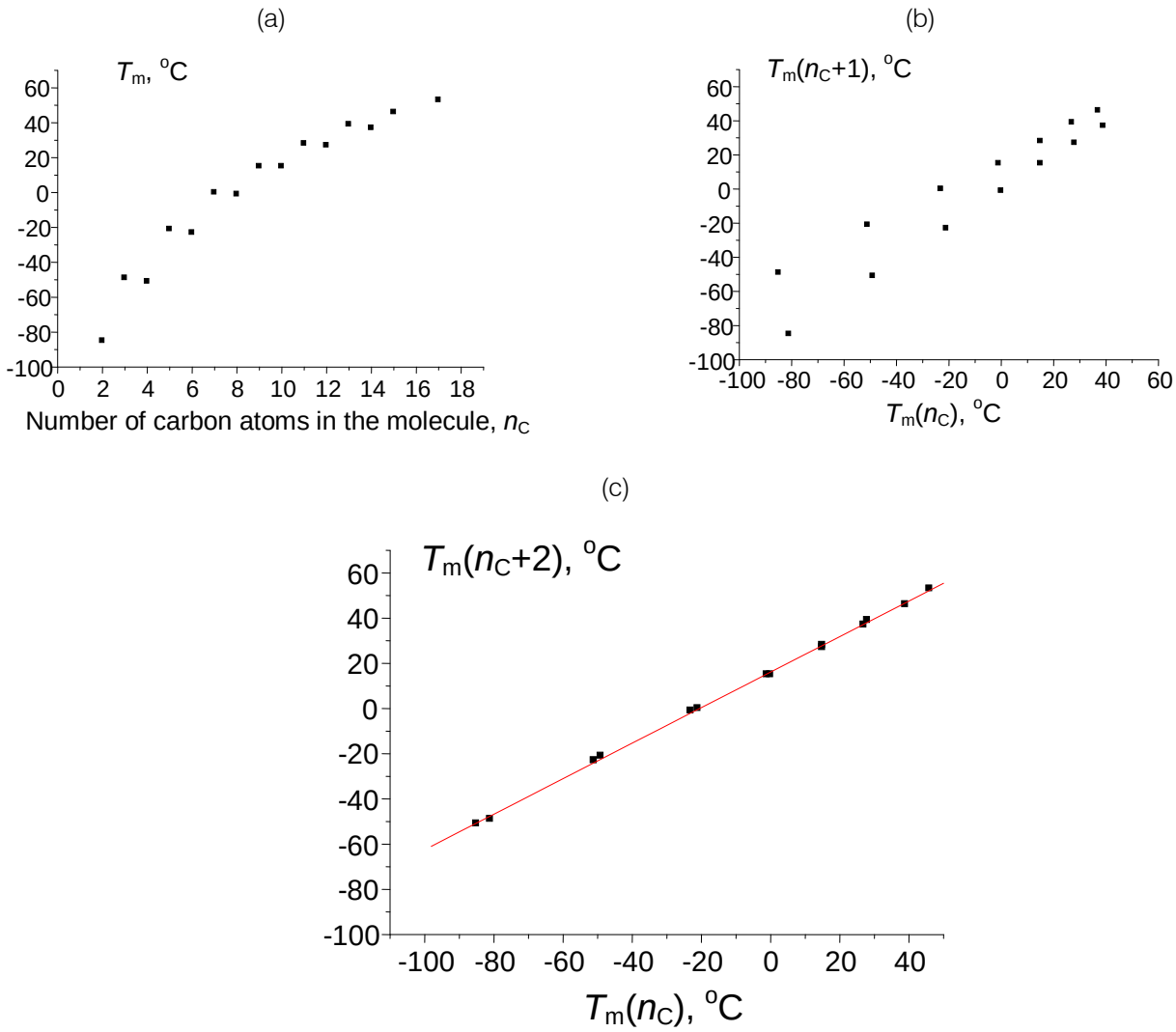


Fig. 4: (a) Dependence of the melting points of n-alkylamines C_2–C_{18} (°C) on the number of carbon atoms in the molecule; the alternation effect is easily noticeable; (b) recurrent approximation of the melting points (equation 1); all points belong to two non-parallel lines; (c) recurrent approximation of the melting points for n-alkyl amines (equation 4); parameters of the linear regression: a = 0.786 ± 0.006, b = 16.1 ± 0.2, R = 0.9996, S_0 = 0.9

Secondly, despite the complexity of equation (6), it is easy to conclude that the limiting value of $y(x)$ at $x \to \infty$ is the same as that of the polynomial (3), namely, $b/(1 - a)$. Hence, we can evaluate the melting point for hypothetical 1-alkylamine with $n_C = \infty$, i.e.,16.1×(1 – 0.786) ≈ 75°C. The highest-molecular-mass 1-alkylamine for which the melting point has been determined is 1-octadecylamine with T_m 53°C [14].

Another example with alternating melting points of homologous *n*-alkane carboxylic acids is considered in [5].

iv. *Temperature Dependence of Solubility of Inorganic Salts in Water*

The next possible application of recurrences is approximating the temperature dependence of the solubility of inorganic salts in water, $w(T)$, g/100 g of water. It is known that numerous inorganic and some organic compounds form hydrates in aqueous solutions. Using the recurrences allows us to detect important features of the data sets on solubility, namely, the formation of hydrates. If the dissolved compound exists in the single chemical form (either hydrated or non-hydrated) at different temperatures of the solution, the recurrent approximation of $w(T)$ dependence has no anomalies; i.e., it is linear. This case can be illustrated with plots for the solubility of copper sulfate within the temperature range $0 \le w(T) \le 100$°C presented in Fig. 5. Despite the nonlinearity of the initial dependence $w(T)$ (a), its recurrent approximation has the correlation coefficient $R = 0.9998$ (b).

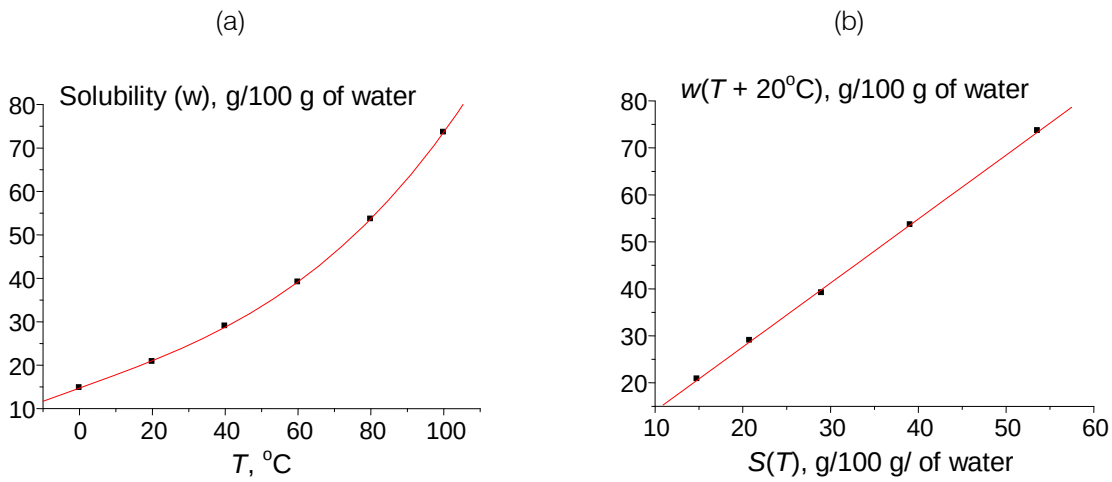


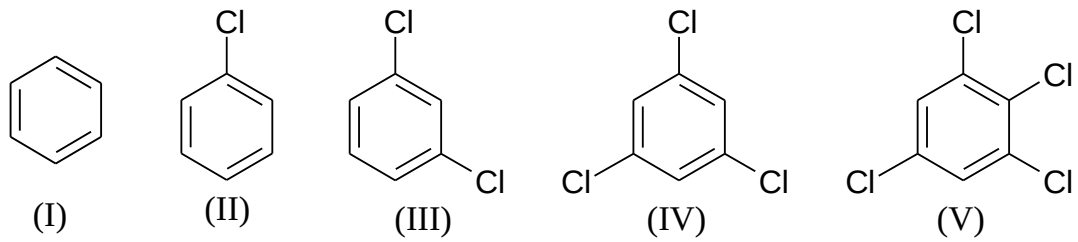
Fig. 5: (a) Nonlinear temperature dependence of the solubility of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$); (b) linear recurrent approximation of this dependence, $w(T + 20°C) = aw(T) + b$; parameters of the linear regression: $a = 1.36 \pm 0.02$, $b = 0.4 \pm 0.6$, $R = 0.9998$, $S_0 = 0.5$

Another example is the solubility of potassium acetate (Fig. 6). Considering the initial $w(T)$ dependence (a) does not allow us to decide whether the leftmost point is weird or belongs to a general nonlinear dependence. The red line in plot 6(a) results from approximation $w(T)$ by a fourth-degree polynomial. However, if we present the same data in the recurrent form, we obtain the linear dependence for all the points ($R = 0.9998$), excluding the leftmost one, which is an obvious outlier. It follows from this fact that, at low temperatures (e.g., 0°C), this salt exists in another form, less soluble than we can expect from the dependence $w(T)$ at other temperatures.

b) Applications of Recurrences in Chromatography

i. *Revealing the anomalies of gas chromatographic retention indices of chlorinated benzenes*

Gas chromatographic retention indices (RI) are important chromatographic characteristics of analytes and are informative for their GC-MS identification. Let us consider the sequence of GC retention indices (RI) for benzene and its chlorinated derivatives with chlorine atoms in unhindered positions (the series of so-called congeners). It includes data for only four compounds, namely, unsubstituted benzene (I), chlorobenzene (II), 1,3-dichlorobenzene (III), and 1,3,5-trichlorobenzene (IV) (all chlorine atoms are in *meta*-positions relative to each other). It should be noted that all RI values were taken from an independent source of reference information, namely, the NIST RI Database [18]:



RI values on standard non-polar polydimethylsiloxane stationary phases [18]:

654 ± 7 839 ± 7 989 ± 10 1117 ± 14 1322 ± 11

Adding the next chlorine atom (1,2,3,5-tetrachlorobenzene, V) will cause it to be in an *ortho*-position to two other chlorine atoms, which gives rise to steric tension in the molecule. However, it is difficult to identify this effect directly from the dependence $RI = f(n_{Cl})$ (Fig. 7(a)). At first glance, only the second point from the right on this plot seems to be located slightly below the regression line, which is not understandable. At the same time, plotting the recurrent dependence $RI(n_{Cl} + 1) = aRI(n_{Cl}) + b$ (Fig. 7(b)) immediately reveals the anomaly just for the rightmost point corresponding to tetrachlorobenzene (V). The steric hindrance in this tetrachlorobenzene increases its retention index above the value extrapolated from data for other (non-

hindered) congeners. Similar effects are also observed for retention indices of bromo- and methyl-substituted benzenes.

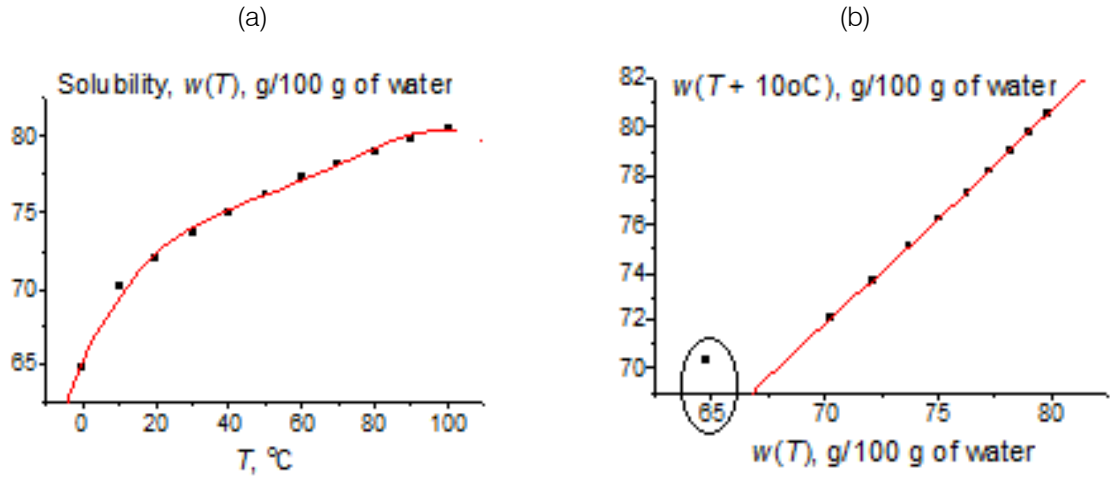


Fig. 6: (a) Nonlinear temperature dependence of the solubility of potassium acetate (CH_3CO_2K) in water; (b) recurrent approximation of this dependence, $w(T + 10°C) = aw(T) + b$; parameters of the linear regression without the leftmost point: a = 0.882 ± 0.005, b = 10.1 ± 0.4, R = 0.9998, $S_0 = 0.5$.

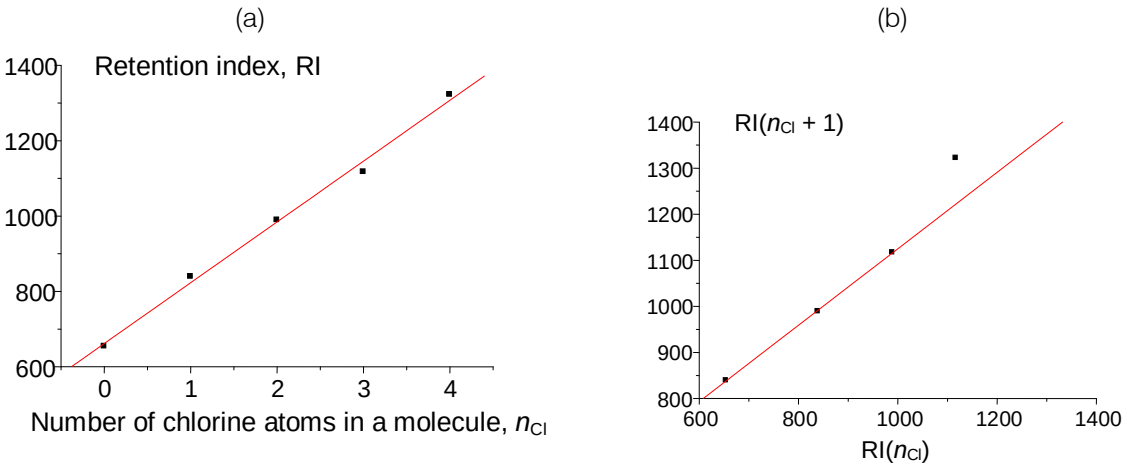


Fig. 7: (a) Dependence of the retention indices of benzene and its four chloroderivatives (see text) on the number of chlorine atoms (standard nonpolar polydimethylsiloxane stationary phase); (b) recurrent approximation of this dependence, $RI(n_{Cl} + 1) = aRI(n_{Cl}) + b$; parameters of the linear regression without the rightmost point: a = 0.83 ± 0.01, b = 296 ± 10, R = 0.9998, $S_0 = 2.9$

The last example illustrates the next important feature of recurrences. In many cases, the deviations of recurrent approximations from the linearity seem to be no less informative than the linearity of these dependencies. The explanation of this fact appears to be relatively simple: Recurrent relations (1) or (2) allow linear approximations of numerous monotonic dependencies, but preferably if they can be described with a single equation. If these dependencies are distorted by additional effects, the high "linearization ability" of recurrences appeared to be insufficient for their linearization. This feature allowed us to detect the steric effects of the fourth chlorine atom in RI values of chlorinated benzenes.

ii. *Revealing the anomalies of retention parameters in reversed-phase high-performance liquid chromatography*

Considering the results of the last example, let us discuss the unusual applications of recurrent relations in reversed-phase high-performance liquid chromatography (RP HPLC). It is the contemporary analytical method based on the different distribution of smallest amounts of analyzed compounds (analytes) between moving liquid phase (eluent) and stationary

phase fixed in a chromatographic column (modified silica gels).

The dependences of retention times of analytes on the content of organic solvent in an eluent, $t_R(C)$, should be considered first. Establishing the regularities of these dependencies seems to be the general approach in characterization both analytes and sorbents in chromatographic columns [19-22].

First, let us illustrate the general statement mentioned in the previous subsection: If the dependence $t_R(C)$ has no anomalies, its recurrent approximation is linear with a high R-value. The absence of anomalies means the single law of variations of retention times *vs*. concentration of organic modifier in an eluent throughout the concentration range under consideration. It can be illustrated with data for acetophenone (methanol-water eluents). Chromatographic analyses were performed using a Shimadzu LC-20 Prominence liquid chromatograph equipped with a diode-array detector and Phenomenex C18 columns 250 mm long and 4.6 mm i. d. with a sorbent particle size of 5 μm. Water–methanol mobile phases were used in several isocratic modes with 5 or 10% concentration steps of methanol at an eluent flow rate of 1.0 mL min^{-1} and column temperature of 30°C. The samples were injected using an SIL-20A/AC autosampler; the sample volume was 20 μL. To prepare eluents, we used deionized water (resistivity 18.2 MΩ cm) prepared using a Milli-Q device (Millipore, USA), and methanol (analytical grade, Kriokhrom, St. Petersburg). The pH values of the eluent were 6.2–6.3. The number of replicate injections of each sample was 2–3. The interinjection variations of the retention times in all the cases did not exceed 0.01–0.02 min.

The plot in Fig. 8(a) presents the initial nonlinear (close to exponential or hyperbolic) dependence of retention times of acetophenone on the methanol content in the eluent. The recurrent approximation of these data (Fig. 8(b)) is linear with $R = 0.99999$. Such R-value means that 99.999% of this function is a linear component, and only 0.001% is its distortion.

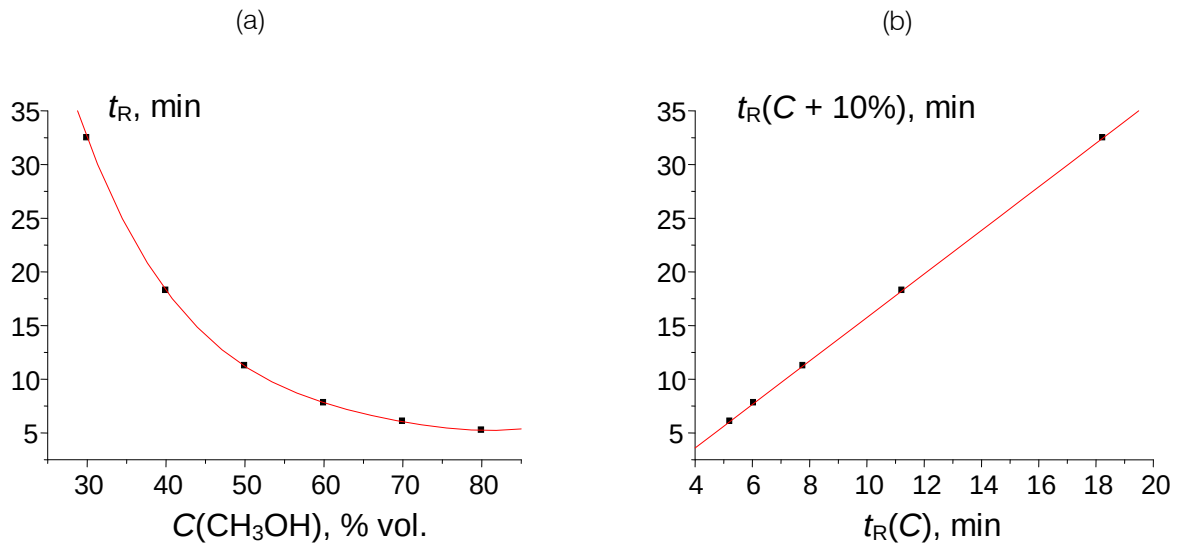


Fig. 8: (a) Plot of the acetophenone retention time (min) *vs*. methanol concentration in the eluent, $t_R(C)$: 32.464(30), 18.244(40), 11.230(50), 7.774(60), 6.052(70), and 5.226(80); (b) recurrent approximation of these data: $t_R(C + 10\%) = at_R(C) + b$. Parameters of the linear regression: $a = 0.4932 \pm 0.0005$, $b = 2.231 \pm 0.008$, $R = 0.99999$, $S_0 = 0.01$ (no outliers)

Let us combine two theses. *First*, some organic compounds form hydrates and some of these hydrates are pretty stable. Several examples of such compounds are, for example:

Compound	CAS no. of anhydrous form	CAS no. of hydrate
Glycine	56-40-6	130769-54-9
Oxalic acid	144-62-7	856335-90-5 (mono) 6153-56-6 (di)
Toluene	108-88-3	112270-21-0
Phenol	108-52-2	217182-78-0 144796-97-4
Nicotinamide	98-92-0	917925-73-6
Caffeine	58-08-2	5743-12-4
Methane (!)	74-82-8	14476-19-8

CAS numbers obviously are different both for anhydrous and hydrated forms of the compounds mentioned. Such a number for a hydrate does not confirm its stability or isolation; it is the confirmation of interest in this hydrated form. It is interesting that this list even includes the simplest hydrocarbon, methane. *Second*, the separation of analytes in RP HPLC takes place in water-containing eluents, when the probability of hydrate formation is high.

To identify the area in which anomalies should be expected, let us look again at Fig. 6. So far as the formation of hydrate of potassium acetate is most probable at low temperature of the aqueous solution, one anomalous leftmost point corresponds to the minimal values of solubility, $w(T)$. In HPLC eluents, the hydrate formation is most probable at the maximal water content of an eluent. Hence, the weird point in the $t_R(C)$ plot should be located on the right. The plot in Fig. 9 represents the recurrent approximation of the set of retention times $t_R(C)$ for 2-methoxybenzaldehyde oxime at retention times within the range of approx. 5–40 min. Four points belong to the single straight line with the correlation coefficient $R = 1.000$, but the rightmost point deviates downward from the regression line [10]. The formation of hydrates was confirmed experimentally for some oximes of aromatic carbonyl compounds [23, 24].

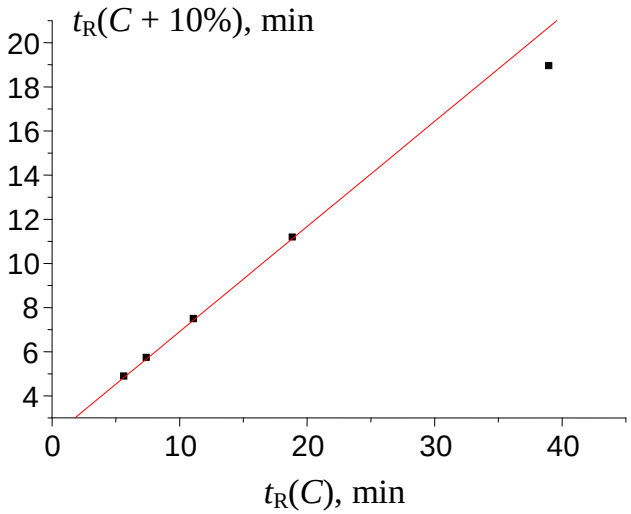


Fig. 9: The plot illustrating the anomaly (the rightmost point below the regression line) in the recurrent approximation of the retention times for 2-methoxybenzaldehyde oxime. Parameters of the linear regression (without outlier): **a** = 0.4763 ± 0.0006, b = 2.144 ± 0.007, R = 1.000, S_0 = 0.006.

It is worth noting that deviations of the rightmost points in the plots of recurrent approximations of retention times in RP HPLC were detected for the first time for complex organic compounds, namely, synthetic antitumor sulfonamide drugs containing the polar functional group –SO_2-NRR′ [9]. Most sulfonamides form stable hydrates, which can be isolated in a solid form [25–28].

Thus, the recurrent approximation of retention times in RP HPLC allows us to detect the reversible formation of hydrates of analytes. The possibility is all the more important considering that detecting hydrates using other methods is much more difficult.

IV. Conclusion

This manuscript provides a brief exploration of the applications of recurrent relations in chemistry and chromatography, acknowledging that it cannot encompass all possible examples. Nonetheless, the review highlights the significant potential of recurrent relations in simplifying calculations through the linearization of diverse chemical dependencies.

The linearization achieved by recurrent relations not only streamlines mathematical manipulations but also reveals valuable insights from deviations in chemical dependencies. For instance, in reversed-phase high performance liquid chromatography (RP HPLC), non-linear variations in retention times can indicate hydration phenomena during analyte separation.

Formally, two alternative conclusions emerge from our discussions. Firstly, a broad hypothesis posits that many monotonic chemical variables follow first-order recurrent dependencies — a potential general law in chemistry. However, a more modest yet preferable conclusion emphasizes the exceptional extrapolation capabilities of recurrent relations, which justify their widespread adoption in approximating a wide array of chemical variables.

In conclusion, to make a reader smile, one humorous application of recurrences should be

mentioned. There is a very nonlinear dependence of the time of drop falling of liquid (including wine) from an empty bottle, $t(n)$, on the serial number of the drop (n). No idea concerning the analytical form of this function can be proposed (or, at least, it is rather difficult). However, the recurrent representation of this dependence, $t(n+1) = at(n) + b$, demonstrates the excellent linearity [5].

References Références Referencias

1. Gutman I., Polanskii O.E. *Mathematical Concepts in Organic Chemistry.* Springer-Verlag, Berlin, 1986. 212 p.
2. Cockett M., Doggett G. *Maths for Chemists.* 2nd Edn. RSC Publ, Cambrige, 2012. 417 p.
3. Cunningham A., Whelan R. *Math for Chemists.* University of Birmingham, University of Leeds, 2014. 119 p.
4. Oriakhi C.O. *Chemistry in Quantitative Language.* 2nd Edn. Oxford Univ. Press, 2021. 528 p.
5. Zenkevich I.G. Approximation of any physicochemical constants of homologues with the use of recurrent functions. *J. Math. Chem.* 2009. Vol. 46. P. 913-933.
6. Zenkevich I.G. Application of recurrent relationships in chromatography. *J. Chemometr.* 2009. V. 23. P. 179-187. doi: 10.1002/cem.1214.
7. Zenkevich I.G. Application of recurrent relations in chemistry. *J. Chemometr.* 2010. V. 24. P. 158-167. doi: 10.1002/cem.1297.
8. Zenkevich I.G. Recurrent Relationships in Separation Science. In "Chemometrics in Chromatography" Eds. L. Komsta, Y.V. Heyden, J. Sherma. New York, Taylor & Francis, 2017, Ch. 24. P. 449-468.
9. Zenkevich I.G., Derouiche A., Nikitina D.A. Detection of organic hydrates in reversed phase high performance liquid chromatography using recurrent approximation of their retention times. *J. Liquid Chromatogr. Related Technol.* 2021. V. 44. № 11-12. P. 588-598. doi: 10.1080/10826076.2021.1998905.
10. Zenkevich I.G., Derouiche A. Features and advantages of the recurrent approximation of retention times in reversed-phase high performance liquid chromatography. *Separations.* 2024. V.11. № 69. doi: 10.3390/separations11030069.
11. Basin S.L. The Fibonacci sequence as it appears in nature. *The Fibonacci Quarterly.* 1963. V. 1. № 1. P. 53-56.
12. Singh P. The so-called Fibonacci numbers in ancient and medieval India. *Historia Mathematica.* 1985. V. 12. № 3. P. 228-244. doi: 10.1016/0315-0860(85)90021-7.
13. Brasch T., Bystrom J., Lystad L.P. Optimal control and the Fibonacci sequence. *J. Optimization Theory Applns.* 2012. V. 154. № 3. P. 857-878. doi: 10.1007/s10957-012-0061.
14. Lide D.R. Ed. *Handbook of Chemistry and Physics.* 84th Edn. CRC Press, Boca Raton, FL. 2003-2004.
15. Henze H.P., Blair C.M. The number of the isomeric hydrocarbons of the methane series. *J. Am. Chem. Soc.* 1931. V. 53. P. 3077-3085.
16. Henze H.P., Blair C.M. The number of structurally isomeric alcohols of the methanol series. *J. Am. Chem. Soc.* 1931. V. 53. P. 3042-3046.
17. Perry D. The number of structural isomers of certain homologs of methane and methanol. *J. Am. Chem. Soc.* V. 54. P. 2918-2920.
18. The NIST Mass Spectral Library (NIST/EPA/NIH EI MS Library, 2017 Release). Software/Data Version; NIST Standard Reference Database, Number 69, August 2017. National Institute of Standards and Technology, Gaithersburg, MD 20899: http://webbook.nist.gov (Accessed: June 2024).
19. Soczewinski E. Quantitative retention-eluent composition relationships in liquid chromatography. *J. Liquid Chromatogr*. 1980. V. 12. P. 1781-1806.
20. Snyder L.R., Kirkland J.J., Dolan J.W. Introduction to modern liquid chromatography. 3rd Edn. New York: J. Wiley & Sons, 2009.
21. Saifutdinov B.R., Davankov V.A., Il'in M.M. Thermodynamics of the sorption of 1,3,4-oxadiazole and 1,2,4,5-tetrazine derivatives from solutions on hypercrosslinked polystyrene. *J. Phys. Chem. A.* 2014. V. 88. № 3. P. 358-364. doi: 10.1134/S0036024414030224.
22. Saifutdinov B.R., Buryak A.K. The influence of water-acetonitrile solvent composition on thermodynamic characterization of adsorption of aromatic heterocycles on octadecyl-bonded silica gels. *Colloid J.* 2019. V. 87. № 6. P. 754-761. doi: 10.1134/S1061933X1 9060176.
23. Jerslev B., Larsen S. Hydrogen bonding and stereochemistry of ring-hydroxylated aromatic aldehyde oximes, Crystal structure of three 4-OH-substituted benzaldehyde oximes. *Acta Chem. Scand*. 1991. V. 45, P. 285-291.
24. Jerslev B., Larsen S. Crystallization and crystal structure at 122 K of anhydrous (E)-4-hydroxybenzaldehyde oxime. *Acta Chem. Scand*. 1992. V. 46. P. 1195-1199.
25. Guo M.-L. 4-Acetamido-N-(4-methylpiperazinyl) benzenesulfonamide monohydrate. *Crystallogr. Commun.* 2004. V. 60. № 4. P. 574-575. doi: 10.1107/S1600536804005446.
26. Suchetan S.F., Foro S., Gowda B.T., Prakash M.S. 4-Chloro-N-(3-methylbenzoyl)benzenesulfonamide monohydrate. *Acta Crystallogr. Sect. E Struct. Rep. Online.* 2012. V. E68. № 1. P. o46. doi: 10.1107/S1600536811051932.
27. Kompella A., Kasa S., Balina V.S., Kusumba S., Adibhatla B.R.K., Muddasani P.R. Large scale

process to prepare highly pure Bosentan monohydrate. *Science J. Chem.* 2014. V. 2. № 6-1. P. 9-15. doi: 10.11648/j.sjc.s.2014020601.12.

28. Jatezak M., Sidoryk K., Kossykowska M., Luniewski W., Zagrodzka J., Lipiec-Abramska E. Development and validation of a UHPLC UV method for the in-process control of bosentan monohydrate synthesis. *Chromatographia*. 2016. V. 79. P. 1131-1141. doi: 10.1007/s10337-016-3124y.

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Medicinal Mushroom Biotechnology

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Abstract- Since most of medicinal mushrooms are rare in nature production of fungal fruiting bodies using artificial cultivation in a form of farming has been intensively established during the last 40 years. Solid state cultivation of various medicinal mushroom mycelia in various types of bioreactors, suitable for veterinary use, appears slightly in last few decades. Developing submerged technologies, using stirred tank and air lift bioreactors, are the most promising technologies for fast and large cultivation of medicinal pharmaceutically active products for human need. This potential initiates the development of new drugs and some of the most attractive over the counter human and veterinary remedies. This article is an overview of the engineering achievements in comprehensive medicinal mushroom mycelia cultivation.

Keywords: *medicinal mushrooms, cultivation techniques, bioreactors.*

GJSFR-B Classification: *LCC: QK617, RS431.M8*

Medicinal Mushroom Biotechnology

Marin Berovic [α], Bojana Boh [σ] & Andrej Gregori [ρ]

Abstract- Since most of medicinal mushrooms are rare in nature production of fungal fruiting bodies using artificial cultivation in a form of farming has been intensively established during the last 40 years. Solid state cultivation of various medicinal mushroom mycelia in various types of bioreactors, suitable for veterinary use, appears slightly in last few decades. Developing submerged technologies, using stirred tank and air lift bioreactors, are the most promising technologies for fast and large cultivation of medicinal pharmaceutically active products for human need. This potential initiates the development of new drugs and some of the most attractive over the counter human and veterinary remedies. This article is an overview of the engineering achievements in comprehensive medicinal mushroom mycelia cultivation.

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I. Introduction

A total of more than 200 medicinal functions are thought to be produced by medicinal mushrooms (MM) and fungi including antitumor, immunomodulating, antioxidant, radical scavenging, cardiovascular, cholesterol-lowering, antiviral, antibacterial, anti-parasitic, antifungal, detoxification, hepatoprotective, anti-diabetic, anti-obesity, neuroprotective, neuroregenerative, and other effects. Also, substances derived from MM can be used as painkillers or analgetics [4, 5, 6]. The best implementation of MM drugs and dietary supplements has been in preventing immune disorders and maintaining a good quality of life, especially in immunodeficient and immuno-depressed patients, patients under chemotherapy or radiotherapy, patients with different types of cancers, chronic blood-borne viral infections of Hepatitis B, C, and D, different types of anemia, the human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), herpes simplex virus (HSV), Epstein Bar virus, Influenza viruses A and B, H5N1 [7], COVID-19 [8-10], West Nile virus, chronic fatigue syndrome, patients with chronic gastritis and gastric ulcers caused by *Helicobacter pylori*, and people suffering from dementia (especially Alzheimer's disease) [1, 2, 3, 5].

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To combat these threats, humankind is focusing more and more attention on mushrooms and mushroom products. Mushrooms themselves are consumed regularly as part of the human diet and are treated as healthy or functional foods. On the other hand, the term mushroom nutriceuticals or dietary supplements has been applied to products derived from medicinal mushrooms that are taken to enhance general health and fitness but are not a regular food but a dietary food supplements [1].

II. Main Pharmaceutically Active Compounds

The main components of these supplements are polysaccharides, triterpenes and immunomodulatory proteins. Polysaccharide components, in particular, have been widely investigated as a source of anti-tumor and immunostimulating agents. They are widely distributed in mushrooms, with over 660 species from 183 genera reported to contain pharmacologically active polysaccharides. About 77% of all medicinal mushroom products are derived from the fruiting bodies, which have been either commercially farmed or collected from the wild, 21% from culture mycelium and 2% from culture broths. Precisely how these products work is still a matter of conjecture, but numerous laboratory animal tests as well as human clinical trials have shown them to be effective. In some cases, attention has focused on a single bioactive mushroom component and its effectiveness in treating specific disease conditions, much like a pharmaceutical. In the case of nutriceuticals/dietary supplements, emphasis has been placed on a combination of components that collectively impact on an individual's overall health and quality of life [2].

Many such products are currently available, and their market value worldwide increased from 1.2 billion in 1991 to 3.6 billion USD in 1994. The combined market value of medicinal mushrooms, mushroom extracts and derived products in 1999 was estimated to be 6.0 billion USD. That year, the United States nutraceutical market alone was valued at 35 million USD. Since then, demand has increased between 20% and 40% annually depending on the species, with *Ganoderma*-based dietary supplements alone valued at 1.6 billion USD. The MM industry has grown from small-scale (cottage-based) operations aimed at supplementing household incomes, to medium and mega-sized industrial

ventures. This review examines the past, present and future of MM development, and includes a pyramid model addressing key issues [3].

They are of different chemical composition, such as polysaccharides, glycopeptide-protein complexes, proteoglycans, proteins and triterpenoids, with most scientific attention focussed to the group of non-cellulosic β-glucans with β-(1-3) linkages in the main chain of the glucan, and additional β-(1-6) branch points, that are characteristic for the antitumor and immuno-stimulating action. Mushroom polysaccharides do not attack cancer cells directly, but produce their antitumor effects by activating different immune responses in the host. Their mechanisms of action involve them being recognized by several immune cells receptors as non-self molecules, so the immune system is stimulated by their presence. Structurally different β-glucans have different affinities toward receptors and thus generate different host responses [12].

Immunomodulating and antitumor activities of these metabolites are related to immune cells such as hematpoietic stem cells, lymphocytes, macrophages, T cells, dendritic cells, and natural killer cells, involved in the innate and adaptive immunity, resulting in the production of biologic response modifiers [13]. Clinical evidence for antitumor and other medicinal activities come primarily from some commercialised purified polysaccharides, such as lentinan from Shiitake - *Lentinula edodes,* krestin from *Coriolus versicolor*, grifolan from *Grifola frondosa*, and schizophyllan from *Schizophyllum commune* [12,14], but polysaccharide preparations of some other medicinal mushrooms also show promising results.

III. Wood Degrading *Basidiomycetes* with Pharmacological Effects

Almost unknown in Western Scientific Research only three decades ago, some of the wood degrading *Basidiomycetes* became intensely and systematically studied due to their promising pharmacological effects (Figures 1 A-E). Among them, *Ganoderma lucidum* – Ling zhi or Reishi, *Grifola frondosa* – Maitake, *Hericium erinaceus* – Lion mane and *Cordyceps militaris* have been known from the traditional Asian medicine, and *Trametes versicolor* Turkey tale, (previous *Coriolus versicolor*) are the subject of this review.

Figure 1: *Ganoderma lucidum* (A), *Trametes versicolor* (B) (previous *Coriolus versicolor*), *Grifola frondosa* (C), *Hericium erinaceus* (D) and *Cordyceps militaris* (E)

Technological possibilities for commercial cultivation gave rise to the number of patents, which are protecting inventions related to new methods and technologies for cultivation of fruit bodies and/or mycelium biomass, methods of active compounds isolation, and development of new commercial formulations and products. *Ganoderma lucidum* has been identified as a medicinal mushroom with the largest number of patented inventions [11].

a) Ganoderma Lucidum

Ganoderma (W. Curt.: Fr.) Lloyd is a white rot wood *Basidiomycete* that degrades lignin and possess hard fruiting body. *G. lucidum* is from *Ganoderma sp.* the most often reported as a source of various medicinal compounds. In Asian traditional medicine, the fruiting body of *G. lucidum,* named Ling-zhi in Chinese and Reishi in Japanese language, has been used for treatment of several diseases for thousands of years, as

reported in Shen Nong's Materia Medica (Leung et.al., 2002; Kim and Kim, 2002; Lin, 2009).

Modern uses of *Ganoderma* include treatment of coronary heart diseases, arteriosclerosis, hepatitis, arthritis, nephritis, bronchitis, asthma, hypertension, cancer and gastric ulcer [15,16] Publications also report on *Ganoderma* antiallergenic constituents [17], immunomodulatory action [18-20], antitumor activity [21-23] cardiovascular effects [24], liver protection and detoxification, and effects on nervous system [22,25]. New reports emphasize its potential in treatment of viral, especially HIV infections [26, 27].

Pharmaceutically active compounds from *Ganoderma lucidum* include triterpenoids, polysaccharides (1,6-β-D-glucans and 1,3-β-D-glucans), proteins, proteoglycans, steroids, alkaloids, nucleotides, lactones and fatty acids, amino acids, nucleotides, alkaloids, steroids, lactones and enzymes. Over 100 triterpenoids were found in *Ganoderma* spp., such as ganoderic (highly oxygenated C_{30} lanostane-type triterpenoids), lucidenic, ganodermic, ganoderenic and ganolucidic acids, lucidones, ganoderals and ganoderols [11].

A large and diverse spectrum of chemical compounds with a pharmacological activity has been isolated from the mycelium, fruiting bodies and sclerotia of *Ganoderma* mushrooms: triterpenoids, polysaccharides, proteins, amino acids, nucleotides, alkaloids, steroids, lactones, fatty acids and enzymes [28, 29]. There is abundant evidence that polysaccharides isolated from *G. lucidum* are immunomodulatory effective [4, 30-34,]. Studies have shown that the most active immunomodulatory polysaccharides are (1→3)-β-D and (1→6)-β-D glucans, that can be precipitated by ethanol. Their prevailing structure is β-1,3 D-glucopyronan with 1–15 units of β-1,6 monoglucosyl side chains. Their 1,3-linked backbone, relatively small side chains, and an organized helical structure are beneficial for the immunostimulation. Although they are chemically heterogeneous, these polysaccharides are usually termed as *β*-glucans [30-32, 36, 37].

Bioactive polysaccharides have been isolated from different sources of *G. lucidum*: basidiocarps, spores and from the mycelial biomass cultivated in liquid culture. Few have been isolated from the culture medium [37]. Though different models of the fungal cell wall differ somewhat, scientists agree that β-glucan is not located on the surface of the wall but is more or less immersed in the wall material. Generally, the cell wall of most fungi contains five main components: (1→3)-β-D glucan, (1→6)-β-D glucan, chitin, and glycoproteins. β-glucan forms 9–46 % of cell wall mass [36, 37]. A large number of studies have shown that polysaccharides of *G. lucidum*, especially β-D glucan, can modulate the functions of many components of the immune system such as the antigen-presenting cells, T and B lymphocytes, NK cells, neutrophil granulocytes, dendritic cells and, on cytokine production [30, 32, 38, 39].

b) Grifola Frondosa

Grifola frondosa also known as Maitake is white rot lignin degrading *Basidiomycete* with excellent nutritional and medicinal properties. *Grifola frondosa* active compounds primarilly belong to the group of polysaccharides (especially 1,6-β-D-glucans and 1,3-β-D-glucans), glycoproteins, and proteins. This products have been used for treatment of a series of diseases, including hepatitis, arthritis, nephritis, bronchitis, coronary heart diseases, asthma, arteriosclerosis, hypertension, cancer and gastric ulcer. Newer investigations report on *Grifola frondosa* antiallergenic constituents, immunomodulatory action and treatment of HIV infections, antitumor and cardiovascular effects, liver protection and detoxification and effects on nervous system [40].

G. frondosa has gained in popularity among consumers, not only because as a gastronomic delight of its taste and flavor, but also because of its reported medicinal value. Its active compounds primarily belong to the group of polysaccharides (especially 1,6-β-D-glucans and 1,3-β-D-glucans), glycoproteins, and proteins. Medicinal effects of *G. frondosa* are numerous, including anti-cancer activity [41, 42], immune system stimulation [43,44], effects on angiogenesis [45], reduction of benign prostatic hyperplasia [46], antibacterial [47] , and antiviral effects [48], effects on lipid metabolism and hypertension [41], antidiabetic activity [49], vitality and performance enhancement [50], antioxidant effects, and beneficial cosmetic effects on skin [51]. According to Shen (2001), more than 20 anti-tumor polysaccharides have been isolated and purified from *G. frondosa* [52].

c) Hericium Erinaceus

In *H. erinaceus* various pharmaceutically active substances were found. Phytosterols (β-sitosterol and ergosterol), lower the content of low density lipoproteins (LDL) and triglycerides that operate anticarcinogenic as well they reduce the metabolism of fats [53]. In *H. erinaceus* fruitbody numerous constituents such as are polysaccharides, proteins, lectins, phenols, hericenones, erinacines and terpenoids were identified. They strengthen the immune system, relieve gastritis and gastrointestinal infections, reflux and upset stomach due to stress [54].

H. erinaceus water-soluble polysaccharides increased activity of macrophages and other immune cells in the fight against cancer cells, but also demonstrated the reduction of formation of metastases. The most outstanding activity of the extract of *H. erinaceus* is that it strengthens the immune system and

activate the synthesis of nerve growth factor [55]. Due to the increased proliferation of T and B-lymphocytes it strengthens the immune system and strengthens the body's natural defences Thus, *H. erinaceus* expresses very positive effects on prolongation of quality of life of the cancer patients [53].

Among the compounds isolated from fruiting bodies and cultured mycelia of *H. erinaceum*, most interesting are the low-molecular-weight compounds belonging to a group of cyathin diterpenoids (erinacines A–K, P, and Q). Several of them, i.e. erinacines A–H, are known to have a potent stimulating effect on nerve growth factor (NGF) synthesis *in vitro* [56-61].

H. erinaceus polysaccharides (HEP) derived from fruiting bodies and mycelium severed as effective therapeutic agents in liver damage-associated diseases. A study demonstrated that the serum levels of aspartate aminotransferase and glutamic pyruvic transaminase activities in carbon tetrachloride-induced hepatic injury were decreased by administration with extracellular and intracellular HEP (200, 400, and 800 mg/kg/day) from mycelium, but the blood lipid levels in the serum of mice were enhanced [62]. Moreover, Kim *et al*. found that 10 mg/kg/day HEP markedly alleviated *Salmonella typhimurium*-induced liver damage and reduced infected mice mortality [63]. Zhang *et al*. revealed that endo-HEP potent hepatoprotective effect *in vivo*, which may be due to its powerful antioxidant capacity. Taken together, the HEP could be exploited as a supplement in the prevention of hepatic diseases [64].

d) Trametes Versicolor

Trametes versicolor, previous *Coriolus versicolor,* also known as Turkey tail mushroom is one of the most attractive medical fungi. It is known for its use and success as a remedy in Asian traditional medicine [65-67]. *T. versicolor* pharmaceutical activities include immunomodulation, antibody production, activation of apoptosis etc.

The two most prominent products of *T. versicolor* are polysaccharide Krestin (PSK) and polysaccharide peptide (PSP) both potentially highly active pharmaceutical substances in complementary cancer treatments. PSP has a variety of physiological effects, such as immunological enhancement, antitumor, liver protecting, oxidation resistance, and reducing blood fat. PSP has been clinically used in treating cancers, hepatitis, hyperlipidemia, chronic bronchitis, and other diseases. The clinical data also demonstrate that PSP has diverse functions such as improving the quality of patients' life, enhancing learning and memory, and antiulcer effects [70-73].

Current studies support PSP as an immunotherapeutic. PSP activates and enhances the function and recognition ability of immune cells, strengthens the phagocytosis of macrophages, increases the expressions of cytokines and chemokines such as tumor necrosis factor-α (TNF-α), interleukins (IL-1β and IL-6), histamine, and prostaglandin E, stimulates the filtration of both dendritic cells and T-cells into tumors, and ameliorates the adverse events associated with chemotherapy. In recent years, immunotherapy has been widely used in cancer treatment. However, to use PSP as an immunotherapeutic at world stage, further chemical, biochemical and pharmacological studies of PSP are needed [66].

In vitro and *in vivo* studies have shown that the mixture of PSP and PSK has a synergistic action that highly affects immune cell proliferation and highly expresses antitumor activities [65, 67, 68, 74-76].

e) Cordyceps Militaris

The usage of natural/herbal medicines over the synthetic ones has seen an upward trend in the recent past. *Cordyceps* being an ancient medicinal mushroom used as a crude drug for the welfare of mankind in old civilization is now a matter of great concern because of its unexplored potentials obtained by various culture techniques and being an excellent source of bioactive metabolites with more than 21 clinically approved benefits on human health [77-78].

The studies by many researchers in the past on *Cordyceps* have demonstrated that it has anti-bacterial, anti-fungal, larvicidal, anti-inflammatory, anti-diabetic, anti-oxidant, anti-tumor, pro-sexual, apoptotic, immunomodulatory, anti-HIV, remarkable clinical health effects including action on hepatic, renal, cardiovascular, respiratory, nervous, besides having anti-cancer, anti-oxidant, anti-inflammatory and anti-microbial activities [79-82].

Cordycepin has received much attention due to its broad-spectrum biological activity. It is known to interfere with various biochemical and molecular processes including purine biosynthesis [83, 84], DNA/RNA synthesis [85] and mTOR (mammalian target of rapamycin) signaling transduction [86]. *Cordyceps* has been included as one of the growing numbers of fungal traditional Chinese medicine (FTCM) used as cures for modern diseases with many products available commercially.

Great potential of pharmaceutical active compounds and *Cordyceps militaris* extract contains many biological bioactive materials, such as the terpenoids cordycepin and cordycepic acid, polysaccharides, sterols and other compounds [87]. *Cordyceps militaris* main active component is terpenoid cordycepin that inhibit the development of cancer cells including antitumor, anti-metastatic, insecticidal, anti-proliferative, anti-bacterial properties, anti-fungal, larvicidal, anti-inflammatory, anti-diabetic, anti-oxidant, pro-sexual, apoptotic, immunomodulatory, anti-HIV, remarkable clinical health effects including action on hepatic, renal, cardiovascular, respiratory, nervous,

besides having anti-cancer, anti-oxidant, anti-inflammatory and anti-microbial activities, anti-leukemia and antimalarial activities [77-82].

The second main active components are polysaccharides, which research have shown to be effective in regulating blood sugar and also have anti-metastatic and antitumor properties [87-89]. The most outstanding active substance of *C. militaris* is Cordycepin. The structure of Cordycepin is very much similar with cellular nucleoside, adenosine. Cordycepin, i.e., 3′-deoxyadenosine, is the main active constituent which is most widely studied for its medicinal value having a broad spectrum biological activity and acts like a nucleoside analogue [90].

Cordycepin alone has been widely explored for its anti-cancer/anti-oxidant activities, thus, holding a strong pharmacological and therapeutic potential to cure many dreadful diseases in future. Further investigations need to be focused on to study the mechanistic insight into the mysterious potential of this medicinal mushroom on human health and promoting its cultivation strategies for commercialization and ethno-pharmacological use of this wonderful herb [91, 92].

IV. Cultivation Technologies

Since medicinal mushrooms are scarce in nature, cultivation of fruit bodies on artificial media has been introduced. Traditional cultivation of fruit bodies on wood logs has been known for centuries. With time, cultivation methods have diversified, modified and developed (Figure 2). [11].

Besides on wood logs, fruit bodies are being produced on sawdust substrates in trays or beds, and in sterilised plastic bags or in bottles. In addition, production of fungal mycelia has been developed in bioreactors, utilizing submerged cultivation in liquid media, or solid state cultivation on various secondary wastes substrates from wood and agricultural industry [93].

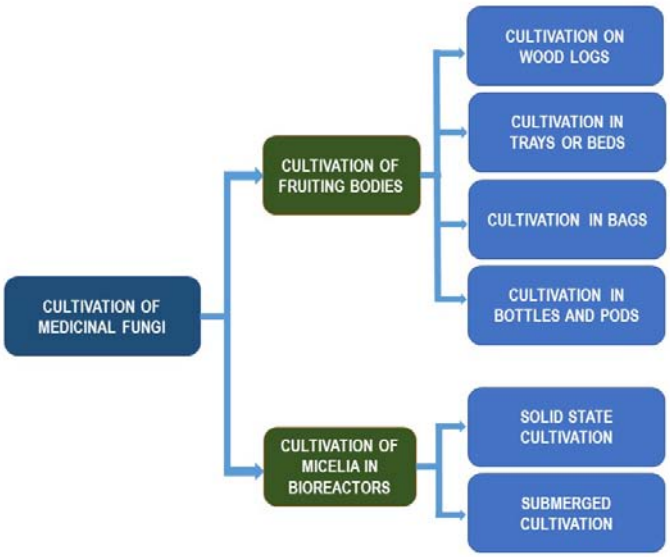

Figure 2: Technological possibilities of cultivating medicinal fungi fruit bodies or fungal biomass on a commercial scale adapted from [11]

a) Farming Fruit Bodies Cultivation

In the wild, wood degrading mushrooms grow primarily on hardwood of trees. However, under artificial cultivation conditions, they thrive on various other substrates containing lignin and cellulose, and therefore have a high potential for recycling different types of organic waste materials from wood and agriculture industry. As lignocellulose containing wastes are produced worldwide in large quantities, and in many instances they pose a threat to the environment. Cultivation of edible and medicinal wood degrading fungi on lignocellulosic substrates offers almost unlimited possibilities and economically viable potentials for large scale commercial cultivation on a World scale [94].

In farming fruit bodies cultivation substrate cultivation methods are divided mostly into bottle cultivation and bag cultivation, sometimes also called synthetic logs. Bag cultivation has more advantages, such as the use of more substrate, a strong body and convenient manipulation, so it is more widely used. Both production processes include the following main steps: raw material preparation, mixing, bagging (bottling) and sterilization, inoculation, spawning, embedding in soil (or transfer to mushroom house), fruiting body development, management and harvesting [94].

Figure 3: Farming production of *Ganoderma lucidum* fruit boddies (Photo A.Gregori)

For a production of *G. lucidum* fruit bodies supplemented sawdust is performed in heat-resistant polypropylene bottles or bags. Sawdust can be supplemented with rice bran (10%) and $CaCO_3$ (3%), moistened with water and filled (700 g) into plastic bags. A plastic collar is then fitted onto each bag and stopped with a cotton plug. After 5h of heat treatment (95-100°C) and cooling, substrate is inoculated with grain or sawdust spawn, and incubated for 3 to 4 weeks (or until the spawn fully colonizes the substrate) [95].

b) Solid State Cultivation

Solid state cultivation (SSC) is a three-phase heterogeneous process taking place in various bioreactors, comprising solid, liquid and gaseous phases, which offers potential benefits for the microbial cultivation for bioprocesses and products development.

Microbial growth on solid state substrate particles is very close to the growth of fungi in the natural environment. Main source of water, carbohydrates, phosphorous, nitrogen and sulphur are intrapartically bounded, therefore the microbial culture applied have to posses the abilities to access the water and essential element sources out the solid matrix Concerning that on the tips of young growing hiphae fungal polysaccharides are produced. Polysaccharide gel has actually two functions. Primary it serve as a gel media where from lignocellulitic enzymes are secreted in the solid wooden matrix and secondary as a sticky material used for anchoraging of hyphae and for moving on the solid surface. Produced fungal polysaccharides have also a whole palette of their pharmaceutical activities that are used in traditional Eastern medicine already for centuries [96].

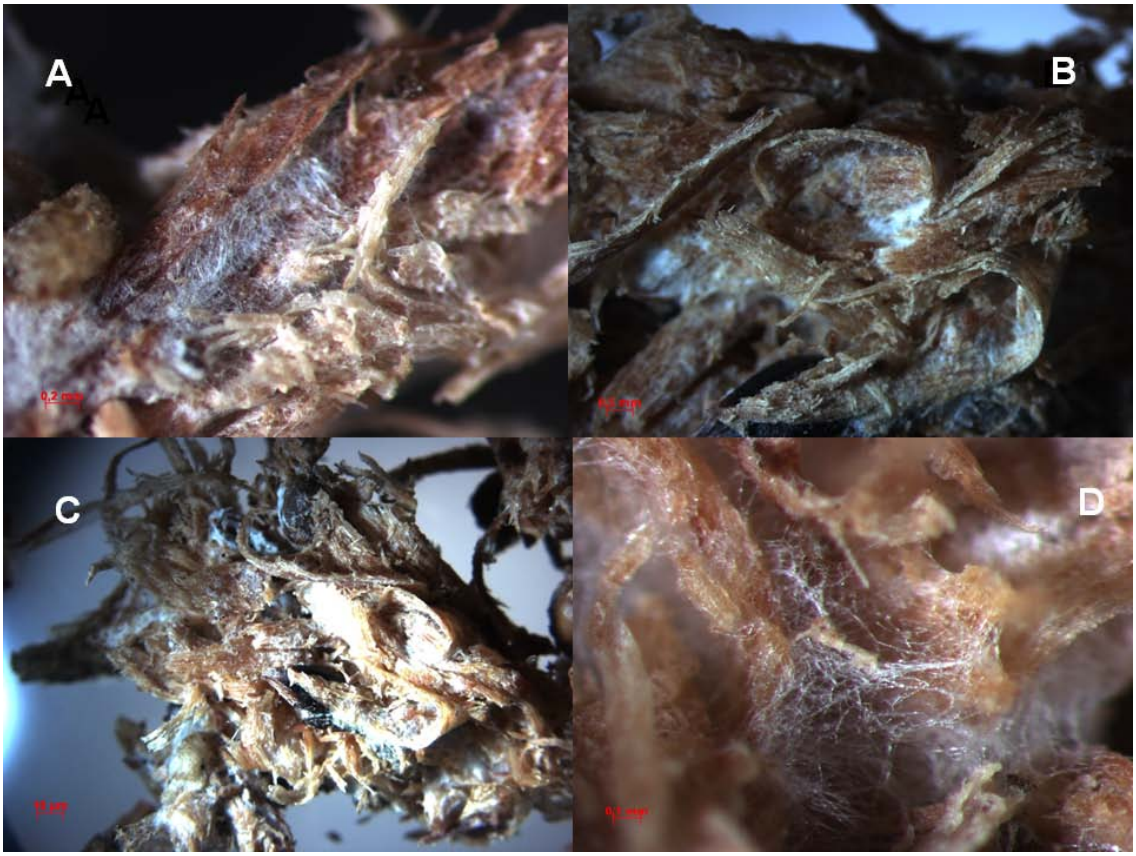

Figure 4: Growth of *Hericium erinaceus* on solid substrate 124 h (D), 145 h (A), 234h (B) and 280 h (C), (Photo M. Vittori)

SSC in bioreactors involves the growth and metabolism of microorganisms in fully control environment. Mycrobial growth in takes place in aerated beds of moist solid materials in which the interparticle spaces contain a continuous gas phase and little or no free water. The upper limit of moisture content for solid state cultivations is determined by the absorbancy of the solid, which varies between substrates, although for most substrates a free water becomes apparent before 80% moisture level is reached. Although fungal mycelia growth in SSC is very close to the growth in nature habitats fungal fruit boddies are not produced in this technology [93]. An example of SSC mycelia growth in presented in Figure 4.

Over the last two decades, SSC has gained significant attention for the development of industrial bioprocesses, particularly due to lower energy requirement associated with higher product yields and less wastewater production with lesser risk of bacterial contamination.

An important advantage of solid state cultivation over other techniques is that a concentrated product can be obtained from a cheap substrate, such as wood and agricultural secondary residue with little pretreatment or enrichment. For this reason, solid state cultivation seems to be most appropriate for the production of pharmaceutically active animal feed supplements, for which the whole fermented substrate can be used as the product [97]. Results of solid state medicinal mushroom cultivation on various substrates are presented in Table 1.

Table 1: Solid state cultivation of various medicinal mushroom in 15 L horizontal stirred tank bioreactor (HSTR). Own results

Fungus	Substrate	Biomass (mg/g)	Intracellular IPS (mg/g)
*Ganoderma lucidum**	Beech saw-dust	68	7.45
Grifola frondosa	Beech saw-dust	54	4.70
Trametes versicolor	Corn straw	83.5	5.95
Hericium erinaceus	Husked and paddy millet	350	3.07
Cordyceps militaris	Husked paddy millet+ rice	236	10.42

**European strain MZKI G93*

An important advantage of solid state cultivation over other techniques is that a concentrated product can be obtained from a cheap substrate, such as an agricultural residue with little pretreatment or enrichment. On the other hand, the use of an undefined medium, such as sawdust, might make the product purification more difficult. For this reason, solid state cultivation seems to be most appropriate for the production of pharmaceutically active animal feed supplements, for which the whole fermented substrate can be used as the product [98].

In recent years, substantial credibility in employing solid state cultivation (SSC) technique has been witnessed owing to its numerous advantages over submerged bioprocessing (SC). In spite of enormous advantages, true potential of SSC technology has not yet been fully realized at industrial scale [96].

c) Submeged Cultivation

Submerged cultivation of mushrooms represent the best and the fastest technology for a large scale production of medicinal mushroom mycelia and their products for a human use. In recent years, its submerged cultivation has received great interest in Asian countries as a promising alternative for efficient production of medicinal mushroom mycelia and its valuable metabolites [99].

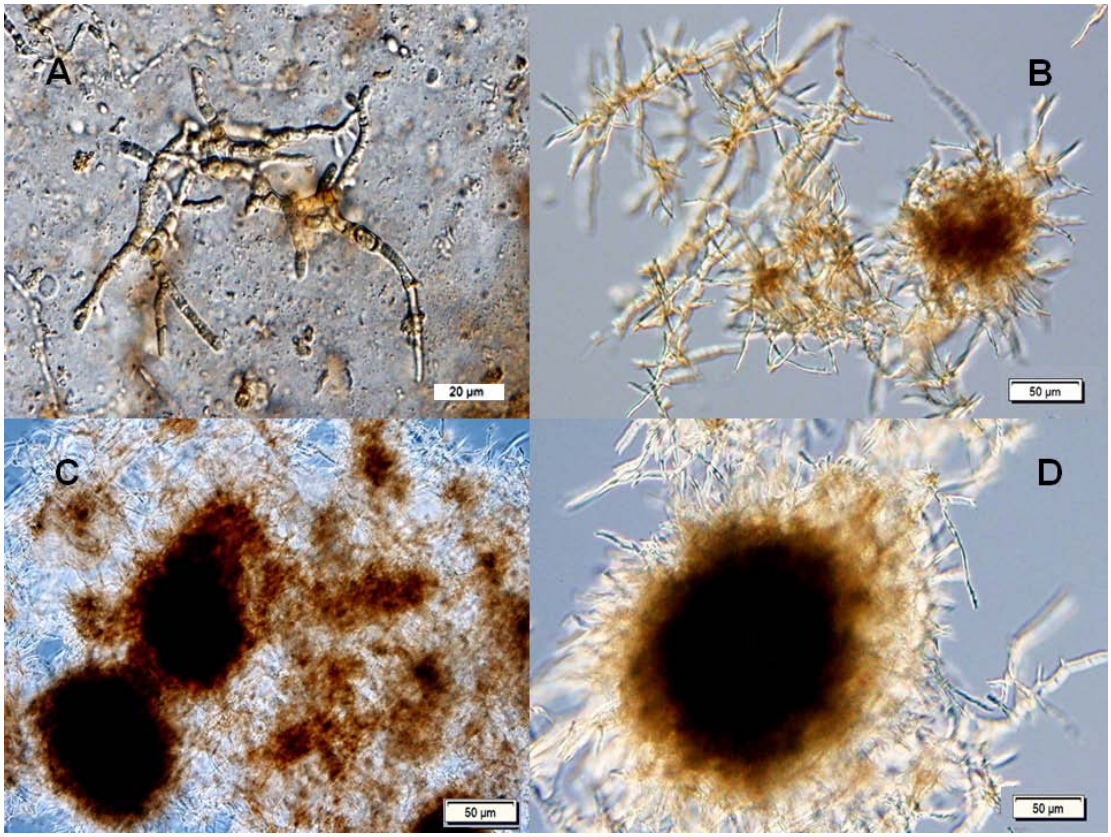

Figure 5: Growth of *Hericium erinaceus* in submerged cultivation. (A) fungal mycelia (14 h)(magnification 1000x); (B) mycelia clumps (218 h) (400x), (C) mycelia pellets (262 h) (100x), (D) mycelia pellets (346 h) (100x) (Photo M.Vittori)

Mycelial growth and the results of submerged medicinal mushroom cultivation of five species on various substrates are presented in Figure 5 and Table 2.

The problems in submerged cultivation of fungal biomass increase with increase the broth viscosity during cultivation because of changes in the morphology, fungal biomass and extracellular polysaccharide (EPS) production. Therefore one of the most important factors of large-scale submerged cultures in bioreactors is related to the heath and mass transfer liquid phase oxygen supply. It is necessary to characterize the variations that occur during the submerged cultivation in bioreactors and their effects on growth and product formation [100,101].

Table 2: Results of submerged cultivation of five species of medicinal mushrooms in bioreactors

Species	Substrate	Bioreactor	Products	
Grifola frondosa	Optimized medium for mycelial biomass: 45.2 gL^{-1} glucose, 2.97 gL^{-1} KH_2PO_4, 6.58 gL^{-1} peptone Optimized medium for extracellular polysaccharides 58.6 gL^{-1} glucose, 4.06 gL^{-1} KH_2PO_4 3.79 gL^{-1}peptone	15 L STR bioreactor innoculum 10% (v/v), T 25 °C, initial pH 5.5, aeration rate 8.0 vvm, agitation speed 80 rpm	Biomass 22.50 gL^{-1} Extracellular polysaccarhides 1.32 gL^{-1}	[103]
Ganoderma lucidum	Potato dextrose 101.2 gL^{-1} glucose 2 % olive oil	10 L STR bioreactor innoculum 10% (v/v) T 30 °C, initial pH 5.8, aeration rate 1.0 vvm, agitation speed 300 rpm	Biomass 15.9 gL^{-1} Extracellular polysaccarhides 9.6 gL^{-1} Intracellular polysaccarhides 6.3 gL^{-1}	[104]
Hericium erinaceus	30 gL^{-1} corn flour 10 gL^{-1} glucose 3.0 gL^{-1} yeast extract 1.0 gL^{-1} KH_2PO_4, 0.5 gL^{-1} $CaCO_3$ 15 mL of corn steep liquor	15 L STR bioreactor innoculum 10% (v/v), T 28 °C initial pH 5.7 aeration rate 0.8 vvm, agitation speed 80 rpm	Biomass 20.5 gL^{-1} Extracellular polysaccarhides 4.25 gL^{-1}	[105]
Trametes versicolor	35 gL^{-1} glucose 0.5 gL^{-1} yeast extract, 5.0 gL^{-1} pepton 1.0 gL^{-1} KH_2PO_4 0.5 gL^{-1} $MgSO_4 \times 7 H_2O$ 0,05 gL^{-1} tiamin	10 L STR inoculum 10% (v/v) , T 28 °C initial pH 5.7 aeration rate 1.0 vvm, agitation speed 400 rpm	Biomass 18,5 gL^{-1} Extracellular polysaccharide 3.8 gL^{-1}	[106]
Cordyceps militaris	80 gL^{-1} glucose, 10 gL^{-1} yeast extract, 0.5 gL^{-1} $MgSO_4 \cdot 7H_2O$ 0.5 gL^{-1} KH_2PO_4	5 L STR bioreactor T 24 °C, pH 5.8, agitation 200 rpm, aeration 1.5 vvm,	Biomass 40.60 gL^{-1} Extracellular polysaccharide 6.74 gL^{-1}	[107]

d) Submerged Cultivation of the other Species in Bioreactors

Besides the cultures present in this chapter, there are also some other medicinal mushrooms that were submerge cultivated in mostly in lab scale bioreactors.

Inonotus levis and *Agaricus nevoi* cultivation was proceed in a 10 L stirred-tank bioreactor using substrate based on glucose and corn steep liquor at pH 5.5. Agitation speed gradually increased from 50 to 300 rpm and T 28 °C. *I.levis* developed very rapidly and after 5 days of cultivation the culture reached the stationary phase of growth with a high level of mycelia biomass of 16 g/L at level of EPS concentration 4.2 g/L. *A. nevoi* was distinguished by a much lower growth rate and entered the stationary growth phase on day 10 with mushroom biomass 12 g/L and EPS of 3.9 g/L [102].

Submerged cultivation of *Agaricus brasiliensis* was studied in 1 L stirred tank reactor. Sucrose was found to be most effective for EPS production. Yeast extract was the best for EPS among the inorganic and organic nitrogen sources tested. The factorial experiment demonstrated that a temperature of 30 °C and a pH of 6.1 were the best for the EPS production. Glucose 10 g/L, yeast extract 3 g/L , K_2HPO_4 0.6 g/L and $MgSO_4$ 0.3 g/L [108].

Armillaria mellea was cultivated on glucose 40 g/L and yeast extract based substrate in 5 L stirred tank reactor at 22°C; the two-stage aeration rate strategy (1.2→0.6 vvm); 150 rpm, controlled pH 4.0, 6.65 g/L fungal biomass and 233.2 m g/L of extracellular polysaccharides with antioxidant properties were obtained [109].

Pleurotus pulmonarius was studied in submerged cultivation in a 2 L stirred-tank reactor. Substrate was composed by 20 g/L of brown sugar, 4 g/L rice bran, 4 g/L malt extract, and 4 g/L of yeast extract with initial pH of 5.5 Incubated at 28◦C with agitation speed of 250 rpm and oxygen partial pressure of 30–40%. Maximum *P. pulmonarius* dry biomass production of 11.8 g/L was achieved after 3 days of cultivation [110].

Pleurotus saca was submerged cultivated on substrate consist of beer worth substrate (batch mode in 10-L stirred tank reactor. Agitation speed was 500 rpm and aeration 5 $Lmin^{-1}$ and pH 6.2, up to 48.5 g/L of dry biomass was obtained [111].

Pleurotus ostreatus was cultivated in a 20 L stirred tank bioreactor in a submerged process with enhanced glucan and dietary fibres content, using 57 g/L xylose and 37 g/L corn steep liquor. High yields 39.2 g/L of dry biomass was obtained [112,113].

Ganoderma lingzhi were studied in 5 L stirred bioreactor. The optimum conditions were an initial pH of 5.9, 20.0% DO and T 29° C. These conditions resulted in a triterpene acids (TA) of 0.31 g/L. Furthermore this optimized conditions were then successfully scaled up to a production scale of 200 L, and maximum TA production and productivity of 0.29 g/L and 0.05 g/L day^{-1} were achieved. [114].

e) *Differences between Solid State and Submerged Cultivation*

Main difference and benefits of solid state and submerged medicinal mushroom cultivation are presented in Table 3.

Table 3: Comparison of solid-state and submerged cultivation [96]

Solid state cultivation	Submerged cultivation
Some products can only be produced well under low moisture conditions. For other products, if the producing organisms require free water, solid state cultivation cannot be used.	A wide range of products can be produced, from a wide range of micoorganisms and fungi. Many products are produced best under submerged cultivation.
The medium is relatively simple (eg. grain) and unrefined. It may contain all nutrients necessary for growth, or only require wetting with a mineral solution. Pretreatment can be as simple as cooking or grinding. However, the substrate composition and characteristics can be variable.	The medium often contains more highly processed ingredients and is therefore more expensive. Unprocessed ingredients may need processing to extract and solubilize the nutrients. With defined media good reproducibility is possible
The low water availability helps to select and protect against growth of contaminants.	The water activity is usually very high and many contaminants can grow well.
Media are concentrated and smaller bioreactors can be used, leading to higher volumetric productivities, even when growth rates and yields are lower.	Media are dilute and therefore occupy larger volumes, leading to lower volumetric productivities.
High substrate concentrations can enable high product concentrations.	High substrate concentrations can cause rheological problems. Substrate feeding systems may be required.
Aeration requires less power since pressures are lower. Gas transfer is easier since the particles have a large surface area.	High air pressures can be required. Gas transfer from the gas to liquid phase is slow and can be limiting.
Mixing within particles is not possible, and growth can be limited by the diffusion of nutrients.	Vigorous mixing can be used, and diffusion of nutrients is usually not limiting.
Ability to remove metabolic heat is restricted, leading to overheating problems.	High water content and more dilute nature makes temperature control easier.
Process control can be difficult due to difficulties in making on-line measurements, and in measuring biomass. The addition of substances during the process is difficult.	Many on-line sensors are available and more are being developed. Additions of substances can be made to control the process.
Downstream processing may be simpler since products are more concentrated. However, extracts can be contaminated with substrate components.	Downstream processing requires removal of large volumes of water, and is more expensive. However, with defined media, product purification may be easier.
Liquid waste is not produced.	Usually large volumes of liquid wastes are produced.
Growth kinetics and transport phenomena have received little attention and are poorly characterized.	Much kinetic and transport information is available in literature, which can guide reactor design and operation.
Research results and information from the solid-state cultivation can be scaled-up, or even transferred and applied in liquid-state cultivation.	In scaling up fungal submerged cultivation processes, various technical problems need to be solved, such as an increased broth viscosity and oxygen supply.
Solid-state cultivation of fungal mycelia is less labor intensive.	Submerged cultivation is more demanding and labor intensive.

V. Down Stream Processing

Disruption of medicinal mushrooms by mechanical, chemical or enzymatic methods is greatly required for the efficient extraction of active compounds from them. In addition, ultrafine powder of medicinal mushroom by mechanical method can be used for functional food or dietary supplement. Other products used for pharmaceuticals are produced at the stages of extraction, fractionation and purification by varying techniques including microwave assisted extraction, membrane separation, adsorptive separation and chromatograpy. Recrystallization, lyophilisation, drying and formulation are used as final product treatments [115].

Current disruption methods can be classified into mechanical, chemical and enzymatic methods in terms of their principles and characteristics. Mechanical methods are often preferred due to short residence time and lower operating costs [116]. The most common mechanical means for disruption are bead mill and homogenizer [117]. Another most frequently used sample disruption method is air jet milling which uses high velocity jets of gas to impart energy to particles for size reduction. The main features of air jet mills include [118].

Extraction is the first step to separate the desired products from the raw materials. Nearly 80–85 % of all medicinal mushroom products are extracted from their fruiting bodies while only 15 % are derived from mycelium culture [119].

Solvent is one of the most important parameters for a successful extraction. Selectivity, solubility, cost and safety should be considered in selection of solvents. Alcohols (ethanol and methanol) are universal solvents for the extraction of natural products although their low selectivity.

In recent years, advanced and greener extraction methods such as supercritical fuid extraction, pressurized liquid extraction, ultrasound assisted extraction, microwave assisted extraction, pulsed electric field extraction and enzyme assisted extraction have also been applied for extraction of natural products, and they offer some advantages such as lower organic solvent consumption, shorter extraction time and higher selectivity. In particular, supercritical fluid extraction gains increasing attention due to its higher efficiency and greener characteristics.

A brief summary of the various extraction methods used for medicinal mushroom products is shown in Table 4 [120].

Table 4: A brief summary of various extraction method used for medicinal mushroom product adapted from [120]

Applied Method	Solvent	Volume of consumed solvent	Temperature	Time	Pressure	Polarity of extracted products
Maceration	Various solvent	Large	Room temperature	Atmospheric	Long	Dependent on the solvent
Percolation	Various solvent	Large	Room temperature, sometimes under heat	Atmospheric	Long	Dependent on the solvent
Decoration	Water	None	Under heat	Atmospheric	Moderate	Polar compounds
Reflux extraction	Various solvent	Moderate	Under heat	Atmospheric	Moderate	Dependent on the solvent
Soxhlet extraction	Organic solvent	Moderate	Under heat	Atmospheric	Long	Dependent on the solvent
Pressurized liquid extraction	Organic solvent	Small	Under heat	High	Short	Dependent on the solvent
Supercritical fluid extraction	Supercritical fluid (usually CO_2)	None or small	Near room temperature	High	Short	None polar or moderate polar
Ultrasound assisted extraction	Various solvent	Moderate	Room temperature or under heat	Atmospheric	Short	Compounds dependent on the solvent
Microwave assisted extraction	Various solvent	Moderate	Room temperature or under heat	Atmospheric	Short	Dependent on the solvent
Pulsed electric field extraction	Various solvent	Moderate	Room temperature or under heat	Atmospheric	Short	Dependent on the solvent

Enzyme assisted extraction	Various solvent	Moderate	Room temperature or under heat	Atmospheric	Moderate	Dependent on the solvent
Distillation	Water	Moderate	Under heat	Atmospheric	Long	Essential oil

The obtained fraction containing the desired products via fractionation steps described above may consist of several compounds with highly similar chemical and physical properties. Sometimes these compounds are analoges or even isomers as already stated, presenting a huge challenge for the purification. Column chromatography packed with separation media with small particle size is the most prevailing technique to implement the task, because it can offer very high number of theoretical plates and thus high resolution. However, in industrial scale, the particle size of the packed material is much larger because no pump can generate the pressure in manufacturing scale as that in a HPLC system [120].

VI. Conclusion

Various pharmaceutically active substances from medicinal mushrooms represent effective potential in human life. Great demand for medicinal fungi biomass production could be fulfilled using various cultivatiom techniques. Medicinal fungi biomass in a present time is mostly covered by farming. Farming cultivation represents cheap but long time consuming technology. Using cultivation on a wooden locks a few year coughing time is need. Cultivation on sawdust substrates in trays or beds and in sterilised plastic bags or in bottles represents an advance and much faster production of fungal fruit boddies than conventional farming.

Solid state a few week time cultivation is a comprehensive, well controlled technology that enables much faster medium scale technology for medicinal mushroom mycelia production. In this technology various secondary waste from wood and agriculture industry are successfully used. No fungal fruit boddies are produced. Final product delignolized, wooden material is overgrown by medicinal fungi biomass in two to four weeks, enreach with proteins and various pharmaceutically products need to be dryed and pulverized and in such form it could be directly used in a veterinary need. Solid state cultivation of medicinal mushroom biomass is cheap in non time consuming technology perfectlly suitable in veterinary use.

Submerged cultivation of medicinal fungi biomass represents fast and comprehensive technology method. Fungal biomass is in its final state from 10 to 28 days. The main benefit of cultivation of medicinal mushroom biomass in bioreactors is in using of higher sterility, comprehensive technology and bioprocess control, for large scale production of various pharmaceutically active compounds as are fungal polysaccharides, terpenoides or proteinoglucans in much shorter cultivation time. Reports on pharmacological activity of extracts, partly purified preparations and isolated compounds from biomass of *G.lucidum, G.frondosa, T.versicolor, H.erinaceus* and *C.militaris* and the other reported species in laboratory and pilot scale, are very convincing. Production of medicinal fungi polysaccharides enhanced by fed-batch or two-stage cultivation strategy was found very useful for improving the production. Some of present results of lab scale research of various medicinal fungi are already transferred to pilot and lower industrial scale and they represents suitable starting platform for development of medicinal fungi biomass in large scale pharmaceutical industrial production. Comparing the economy of the same product production solid state processing is 30 % less than those of the one produced in submerged cultivation.

Isolation of medicinal fungi pharmaceutically compounds in all three technologies is is based on precipitation with hot water and ethanol. Crude extracts often show equal or stronger pharmacological activity as purified compounds, which suggests potential synergistic effects of several naturally occurring compounds. In the future, more precise capture (or enrichment) and separation techniques such as affinity separation, and more integrated bioprocesses for medicinal mushroom products should be developed which would enable a higher product yield and better process performance.

As pointed out submerged cultivation of medicinal mushrooms has significant large scale industrial potential, but its success on a commercial scale depends on existing field-cultivation technology as well as pharmaceutical market economy. Production of various medicinal mushrooms products, terpenoids, polysaccharides and proteinoglucans represents great business in Asiatic space where it is traditional. Great demand of this active ingredients definitively need to include new and fast large scale industrial production technologies as are solid state and submerged cultivations. In opposite, Western Pharmaceutical Industry has no tradition in natural isolates from herbal and fungal sources. Unfortunately, it is too convinient and much basesed on classical pharmaco-chemical biosynthesis including its all side effects.

However, from the viewpoint of Western Science, pharmaceutical legislation and regulations might be one of the main obstacles hindering the introduction of medicinal mushrooms products as registered pharmaceuticals. In any case, further

research is needed to fully understand all mechanisms of pharmaceutical effects of medicinal mushrooms products and to identify their potential side effects.

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References Références Referencias

1. Wasser, S.P. Medicinal mushroom science: history, current status, future trends, and unsolved problems. *Int J Med Mushrooms,* 2010, *12*, 1-16.
2. Chang, S.T.; Wasser, S.P. The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. *Int J Med Mushrooms,* 2012, *14*, 95–134.
3. Chang, S. T.; Wasser, S.P. Current and future research trends in agricultural and biomedical applications of medicinal mushrooms and mushroom products (Review). *Int J Med Mushrooms,* 2018, *20,* 11-21.
4. Boh, B. *Ganoderma lucidum:*a potential for biotechnological production of anti-cancer and immunomodulatory drugs. *Recent Pat Anticancer Drug Discov*, 2013, *8*,255-287.
5. Zmitrovich, I.V.; Belova, N.V.; Balandaykin, M.E.; Bondartseva, M.A.; Wasser, S.P. Cancer without pharmacological illusions and a niche for mycotherapy (Review). *Int J Med Mushrooms* 2019, *21,*105–109.
6. Griffiths, S. The fossilized fungus that "kick started" life on Earth: 440-million-year-old mushroom is oldest organism to have lived on dry land. *Daily Mail*. Australia, 2016. https://www.dailymail.co.uk/sciencetech/article-3472781/The-fossilised-fungus-kickstarted-life-Earth-440-million-year-old-mushroom-oldest-organism-lived-dry-land.html
7. Chang, S.T.; Buswell, J.A. *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (*Aphyllophoromycetideae*) – A mushrooming medicinal mushroom. *Int J Med Mush*, 1999, *1*,139-146.
8. Baldauf, S.L.; Roger, A.J.; Wenk-Siefert, I., Doolittle, W.F. A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science*, 2000, *290,*972–977.
9. Katz, L.A.; Grant, J.R.; Parfrey, L.W.; Burleigh, J.G. Turning the crown upside down: gene tree parsimony roots the eukaryotic tree of life. *Systematic Biology*, Oxford Academic, 2012, *61,* 653–660.
10. Chang, S.T.; Buswell, J.A. Medicinal Mushrooms – Past, Present and Future, *Advances in Biochemial Engineering and Biotechnology*. In Berovic M., Zhong J.J. Eds. *Medicinal Mushrooms Bioengineering and Biotechnology*, (In Press) 2023.
11. Boh Podgornik, B.; Berovic, M. *Ganoderma lucidum* Production of Pharmaceuticals. In: Biochemistry Research Trends, Liong, M.T. Ed. *Bioprocess Sciences and Technology*. Hauppauge: Nova Science Publishers, New York, USA, 2011. pp. 233-268.
12. Chen, J. Z.; Seviour, R. Medicinal importance of fungal ß-(1-3), (1-6)-glucans. *Mycological Research*, 2007,*111*, 635-652.
13. Moradali, M. F.; Mostafavi, H.; Ghods, S.; Hedjaroude, G.A. Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi), *International Immunopharmacology*, 2007,*7*, 701-724.
14. Wasser, S.P. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, 2002, *60*, 258-274.
15. Jong, S.C.; Birmingham, J.M. Medicinal benefits of the Mushroom *Ganoderma*. *Adv. Appl. Microbiol.* 1992, *37*, 101-134.
16. Perumal, K. Indigenous technology on organic cultivation of Reishi (*Ganoderma lucidum*) in India. *Proceedings of the 5th International medicinal mushroom conference,* Nantong, China, September 2009, *5-8*, pp.592-595.
17. Tasaka, K.; Akagi, M.; Miyoshi, K.; Mio, M.; Makino, T. Anti-allergic constituents in the culture medium of *Ganoderma lucidum*. 1. Inhibitory effect of oleic acid on histamine release. *Agents Action* 1988, *23*, 153-156.
18. Kino, K.; Sone, T.; Watanabe, J.; Yamashita, A.; Tsuboi, H.; Miyajima, H.; Tsunoo, H. Immunomodulator, LZ-8, prevents antibody-production in mice, *Int. J. Immunopharmacol.* 1991, *13*, 1109-1115.
19. Ji, Z.; Tang, Q.; Zhang, J.; Yang, Y.; Liu, Y.; Tang, C.; Pan, Y. Immunomodulation of bone marrow macrophages by GLIS, a proteoglycan fraction from *Ganoderma lucidum*. *Proceedings of the 5th International medicinal mushroom conference,* Nantong, China, September 5-8, 2009, pp.596-603.
20. Ye, L.; Zheng, X.; Zhang, J.; Tang, Q.; Yang, Y.; Wang, X.; Li, J.; Liu, Y.; Pan, Y. Biochemical characterization of a proteoglycan complex from an edible mushroom *Ganoderma lucidum* fruiting bodies and its immunoregulatory activity. *Food Res. Int.* 2011, *44*, 367-372.
21. Yuen, J.W.M.; Gohel, M.D.I.; Ng C.F. The differential immunological activities of *Ganoderma lucidum* on human pre-cancerous uroepithelial cells. *J. Ethnopharmacol.* 2011,*135*, 711-718.
22. Zhang, J.; Tang, Q.; Zhou, C.; Jia W.; Da Silva, L.; Nguyen, D.; Reutter, W.; Fan, H. Mice and inhibition

of tumour growth by GLIS, a bioactive proteoglycan from *Ganoderma lucidum*. *Proceedings of the 5th International medicinal mushroom conference*, Nantong, China, 5-8.September, 2009, pp. 547-562.
23. Jiang, Z.; Jin, T.; Gao, F.; Liu, J.; Zhong, J.; Zhao, H. Effects of Ganoderic acid Me on inhibiting multidrug resistance and inducing apoptosis in multidrug resistant colon cancer cells. *Proc. Biochem.* 2011,*46*, 1307-1314.
24. Jakopovich, I. Medicinal mushroom preparations against lung cancer. *Abstracts of the 5th International medicinal mushroom conference*, Nantong, 5-8. September, 2009, 71-73.
25. Lee, S.Y.; Rhee, H.M. Cardiovascular effects of mycelium extract of *Ganoderma lucidum* - inhibition of sympathetic outflow as a mechanism of its hypotensive action. *Chem. Pharm. Bull.* 1990, *38*, 1359-1364.
26. El-Mekkawy, S.; Meselhy, M.R.; Nakamura,N.; Tezuka,Y.; Hattori,M.; Kakiuchi, N.; Shimotohno, K.; Kawahata, T.; Otake, T. Anti-HIV-1 and anti-HIV-1 protease substances from *Ganoderma lucidum*. *Phytochemistry* 1998, *49*, 1651-1657.
27. Pan, H.; Wang, Y.; Xie, Y.; Li, X.; Li, S.; Zhi, Z.; Chong, L.; Peng, T. The Anti-Simian immunodeficiency virus effect of compounds extracted from three kinds of medicinal mushrooms. *Proceedings of the 5th International medicinal mushroom conference*, Nantong, China, 5-8. September, 2009, pp. 686-690.
28. Wasser, S.P.; Weiss, A.L. Medicinal mushrooms - *G. lucidum*, Reishi mushroom. *Pedeifus Publishing House*, Haifa, Israel, 1997.
29. Lin Z.B.; Yang B.X. *Ganoderma* and Health: Biology, Chemistry and Industry, 2019, 107-134.
30. Lin, Z.B. Cellular and molecular mechanisms of immuno-modulation by *Ganoderma lucidum*. *J. Pharmacol. Sci.* 2005, *99*,144-153.
31. Russell, R.; Paterson, M. *Ganoderma lucidum* - A therapeutic fungal biofactory. *Phytochemistry*. 2006,*67*,1985-2001.
32. Boh, B. ; Berovic, M. ; Zhang, J. ; Zhi-Bin, L. *Ganoderma lucidum* and its pharmaceutically active compounds. In: El-Gewely MR, editor. *Biotechnology Annual Review*. 13, Elsevier, Amsterdam, 2007, pp. 265-301.
33. Lai, C.Y.; Hung, J.T.; Lin, H.H.; Yu, A.L.; Chen, S.H.; Tsai, Y. C. Immunomodulatory and adjuvant activities of a polysaccharide extract of *Ganoderma lucidum* in vivo and in vitro. *Vaccine.* 2010, *28*,4945-4954.
34. Zhang, J.; Tang, Q. ; Zhou, C.; Jia, W.; De Silva, L.; Nguyen, L.D. GLIS, a bioactive proteoglycan fraction from *Ganoderma lucidum*, displays anti-tumor activity by increasing both humoral and cellular immune response. *Life Sci.* 2010, 87,628-637.
35. Novak, M.; Vetvicka, V. β-glucans, history, and present: immunomodulatory aspects and mechanisms of action. *J. Immunotoxicol.* 2007,*5*,47-57.
36. Chan, G.C.; Chan, W.C., Sze, D.M. The effect of β-glucan on human immune and cancer cells. *J. Hematol. Oncol.* 2009, *2*,1-11.
37. Zhe, J.; Tang, Q.; Zhang, J.;Yang, Y.; Jia, W., Pan, Y.. Immunomodulation of RAW264.7 macrophages by GLIS, a proteopolysaccharide from *Ganoderma lucidum* . *J Ethnopharmacol.* 2007, *112*,445-450.
38. Chen, H.S.; Tsai, Y.F.; Lin, S.; Lin, C.C.; Khoo, K.H.; Lin, C.H. Studies on the immuno-modulating and anti-tumor activities of *Ganoderma lucidum* (Reishi) polysaccharides. *Bioorg. Med. Chem.* 2004, *12*, 595-601.
39. Berovic, M.; Habijanic, J.; Zore, I.; Wraber, B.; Hodzar, D.; Boh, B. Submerged cultivation of *Ganoderma lucidum* biomass and immunostimulatory effects of fungal polysaccharides. *J. Biotechnol.* 2003,*103,*77-86.
40. Hirose, K.; Muto, S.; Niimura, K.; Ohara, M.; Oguchi, Y.; Matsunaga, K.; Kadochi, J.; Sugita, N.; Furushu, T.; Yoshikumi C.; Takahashi, M. Antiviral agent. *J.P. Patent.* 63316734, 1988.
41. Frank, J.A.; Xiao, R.; Yu, S.; Ferguson, M.; Hennings, L.J.; Simpson, P.M.; Ronis, M.J.; Fang, N.; Badger, T.M.; Simmen, F.A. Effect of Shiitake mushroom dose on colon tumorigenesis in azoxymethane-treated male Sprague-Dawley rats. *Nutr. Res.* 2006, *26,*138– 145
42. Nanba, H.; Kubo, K. Effect of Maitake D-fraction on cancer prevention. *Ann. N.Y. Acad. Sci.* 1997, *833,* 204-207.
43. Harada, N.; Kodama, N.; Nanba, H. Relationship between dendritic cells and the D-fraction-induced Th-1 dominant response in BALB/c tumor-bearing mice. *Cancer Lett.* 2003,*192,* 181–187.
44. Kodama, N.; Murata, Y.; Asakawa, A.; Inui, A.; Hayashi, M.; Sakai, N.; Nanba, H. Maitake D-fraction enhances antitumor effects and reduces immunosuppression by mitomycin-C in tumor-bearing mice. *Nutrition*, 2005, *21*, 624-629
45. Lin, H.; She, Y.H.; Cassileth, B.R.; Sirotnak, F.; Cunningham Rundles, S. Maitake beta-glucan MD-fraction enhances bone marrow colony formation and reduces doxorubicin toxicity in vitro. I*nt. Immunopharmacol.* 2004, 4, 91–99.
46. Barry, R.J.; Govindan, G.; Miller, A. *Grifola* extracts and methods of use thereof. *Patent application* PCT WO 03/011217 A2, 2003.
47. Kodama, N.; Yamada, M.; Nanba, H. Addition of Maitake D-fraction reduces the effective dosage of vancomycin for the treatment of Listeria-infected mice. *Jpn. J. Pharmacol.* 2001,*87*, 327-332
48. Mlinarič A, Kač, M.; Pohleven, F. Screening of selected wood-damaging fungi for the HIV-1 reverse

transcriptase inhibitors. *Acta Pharm*. 2005, 55, 69–79.
49. Manohar, V.; Talpur, N.A.; Echard, B.W.; Lieberman, S.; Preuss, H.G. Effects of a water-soluble extract of Maitake mushroom on circulating glucose/insulin concentrations in KK mice. *Diabetes Obe. Metab.* 2002, *4*, 43-48.
50. Jung, K.; Kim, I.H.; Han, D. Effect of medicinal plant extracts on forced swimming capacity in mice. *J. Ethnopharmacol*. 2004, *93,*75-81.
51. Bae, J.T.; Sim, G.S.; Lee, D.H.; Lee, B.C.; Pyo, H.B.; Choe, T.B.; Yun, J.W. Production of exopolysaccharide from mycelial culture of *Grifola frondosa* and its inhibitory effect on matrix metalloproteinase -1 expression in UV-irradiated human dermal fibroblasts. *FEMS Microbiol Lett.* 2005, *251,* 347-354.
52. Shen, Q. Molecular phylogenetic analysis of *Grifola frondosa* (Maitake) and related species and the influence of selected nutrient supplements on mushroom yield, Ph.D. Thesis, The Pennsylvania State University, The Graduate School, 2001.
53. Mizuno, T. 1995, Bioactive biomolecules of mushrooms: food function and medicinal effect of mushroom fungi, *Food Rev. Int.* 1995, *11 (1)*, 7-21.
54. Mizuno, T. Bioactive Substances in *Hericium erinaceus* (Bull.:Fr.) Pers. (Yamabushitake), and its Medicinal Utilization. *Int J Med Mushrooms*, 1999,1, *2,* 105-120.
55. Kolotushkina, E.V., Moldavan, M.G.; Voronin, K.Y.; Skibo, G.G. The influence of *Hericium erinaseus* extract on myelination process in vitro; *Fiziologichniy Zhurnal,* 2003, *49(1),* 38-46.
56. Kawagishi, H.; Shimada, A.; Shirai, R.; Okamoto, K.; Ojima, F.; Sakamoto, H.; Furukawa, S. Erinacines A, B and C, strong stimulators of nerve growth factor (NGF)-synthesis, from the mycelia of *Hericium erinaceum*. *Tetrahedron Lett.* 1994, *35(10)*, 1569–1572.
57. Kawagishi, H.; Shimada, A.; Shizuki K.; Mori H. Erinacine D, a stimulator of NGF-synthesis, from the mycelia of *Hericium erinaceum*. *Heterocycl. Commun.* 1996a, *2,* 51–54.
58. Kawagishi H.; Shimada, A.; Hosokawa S.; Mori H. Erinacines E, F, and G, stimulators of nerve growth factor (NGF)-synthesis, from the mycelia of *Hericium erinaceum*. *Tetrahedron Lett.* 1996b, *37,* 7399–7402.
59. Kawagishi, H.; Masui, A.; Tukuyama, S.; Nakamura, T. Erinacines J and K from the mycelia of *Hericium erinaceum*. *Tetrahedron Lett.* 2006, *62,* 8463–8466.
60. Kenmoku, H.; Sassa, T.; Kato, N. Isolation of Erinacine P, a new parental metabolite of cyathane-xylosides, from *Hericium erinaceum* and its biomimetic conversion into erinacines A and B. *Tetrahedron Lett.* 2000, *41*, 4389–4393.
61. Kenmoku, H.; Shimai, T.; Toyomasu, T.; Kato, N.; Sassa, T. Erinacine Q, a new erinacine from *Hericium erinaceum* and its biosynthetic route to erinacine C in the Basidiomycete.*Biosci. Biotechnol. Biochem.* 2002, *66*, 571–575.
62. Cui, F.Y.; Gao, X.; Zhang, J.J.; Liu, M.; Zhang, C.; Xu, N.; Zhao, H.J.; Lin, L.; Zhou, M.; Jia, L. Protective effects of extracellular and intracellular polysaccharides on Hepatotoxicity by *Hericium erinaceus* SG-02. *Current Microbiol.* 2016, *73*, 379-385.
63. Kim, S.P.; Moon, E.; Nam, S.H.; Friedman, M. *Hericium erinaceus* mushroom extracts protect infected mice against Salmonella Typhimurium-Induced liver damage and mortality by stimulation of innate immune cells. *J.Agric.Food Chem.* 2012, *60,* 5590-5596.
64. Zhang, Z.F.; Li, G.Y.; Pan, H.J.; Pandey, A.; He, W.Q.; Fan, L.F. Antioxidant and hepatoprotective potential of endo-polysaccharides from *Hericium erinaceus* grown on tofu whey. *Int. J. Biol. Macromol.* 2012, *51,* 1140-1146.
65. Chu, K.K.; Ho, S.S.; Chow, A.H. *Trametes versicolor*: a medicinal mushroom with promising immunotherapeutic values, *J. Clin. Pharm.* 2002, *42*, 976-984.
66. Dou, H.; Chang, Y.; Zhang, Z.F. *Coriolus versicolor* polysaccharopeptide as an immunotherapeutic in China. *Progress Molecular Biology Translational Science,* 2019*, 163*, 361-381.
67. Cheng, K.F.; Leung, P.C. General review of polysaccharopeptides (PSP) from *C. versicolor*: Pharmacological and clinical studies. *Cancer Therapy*, 2008, *6,* 117-130.
68. Ng, T.B. A Review of Research on the Protein-Bound Polysaccharide (Polysaccharopeptide, PSP) from the Mushroom *Trametes versicolor* (Basidiomycetes: Polyporaceae), *Gen. Pharmacol.* 1998, *30 (1),* 1-4.
69. Hattori, T.S.; Komatsu, N.; Shichijo, S.; Itoh, K. Protein-bound polysaccharide K induced apoptosis of the human Burkitt lymphoma cell line, Namalwa. *Biomed. Pharmacotherapy,* 2004, *58 (4),* 226-230.
70. Ho, C.Y.; Lau, C.B.S.; Kim, C.F. Differnetial effect of *Trametes versicolor* (Yunzhi) extract on cytokine production by murine lymphocytes in vitro, *Intern. Immunopharm.* 2004, *4*, 1549-1557.
71. Ho, C.Y.; Kim, C.F.; Leung, K.N. *Trametes versicolor* (Yunzhi) extract attenuates growth of human leukemia xenografts and induces apoptosis through the mitochondrial pathway. *Oncol. Reports,* 2006, *16*, 609-616.
72. Harhaji, L.; Mijatović, S.; Maksimović -Ivanić, D. Anti-tumor effect of *Trametes versicolor* methanol extract against mouse B16 melanoma cells: In vitro and in vivo study. *Food Chem. Toxicol.* 2008, *46*, 1825-1833.
73. Sevastere, B.; Prodan, I.; Manalachioae, R. Antitumor Effect of *Trametes versicolor* Queil

Alcoholic Extract, *Bulletin UASVM, Vet. Med.* 2010, *67(1),* 263-269.
74. Kidd, P.M. The use of mushroom glucans and proteoglycans in cancer treatment. *Altern. Med. Rev.* 2000,*5(1),* 4-27.
75. Cui, J.; Chisti, Y. Polysaccharopeptides of *Trametes versicolor*: physiological activity, uses, and production, *Biotechnol. Adv.* 2003, *21*,109-122.
76. Price, L.A.; Wenner, C.A.; Sloper D.T. Role for toll-like receptor 4 in TNF-alpha secretion by murine macrophages in response to polysaccharide Krestin, a *Trametes versicolor* mushroom extract, *Fitoterapia,* 2010, *81,* 914-919.
77. Wang, Z.M.; Peng, X.; Lee, K.L.D.; Tang, J.C.O.; Cheung, P.C.K.; Wu, J.Y. Structural characterisation and immunomodulatory property of an acidic polysaccharide from mycelial culture of *Cordyceps sinensis* fungus Cs-HK1. *Food Chem.* 2011, *125*, 637–643.
78. Zhou, X.; Luo, L.; Dressel, W.; Shadier, G.; Krumbiegel, D.; Schmidtke, P.; Zepp, F.; Meyer, C.U. Cordycepin is an immunoregulatory active ingredient of *Cordyceps sinensis*. *Am J Chin Med.* 2008, *36*,967–980.
79. Lee, B.; Park, J.; Park, J.; Shin, H.J.; Kwon, S.; Yeom, M.; Sur, B.; Kim, S.; Kim, M.; Lee, H.; Yoon, S.H.; Hahm, D.H. *Cordyceps militaris* improves neurite outgrowth in neuro2A cells and reverses memory impairment in rats. *Food Sci Biotechnol.* 2011, *20*, 1599–1608.
80. Lee, J.H.; Hong, S.M.; Yun, J.Y.; Myoung, H.; Kim, M.J. Anti-cancer effects of cordycepin on oral squamous cell carcinoma proliferation and apoptosis in vitro. *J Cancer Ther.* 2011,*2*,224–234.
81. Patel, S.; Goyal, A. Recent developments in mushrooms as anti-cancer therapeutics: a review. *3 Biotech.* 2012, *2*,1–15.
82. Yue, K.; Ye, M.; Zhou, Z.; Sun, W.; Lin, X. The genus *Cordyceps*: a chemical and pharmacological review. *J. Pharm. Pharmacol.* 2013, *65 (4)*, 474–493.
83. Overgaard, K.H. The inhibition of 5-phosphoribosyl-1- pyrophosphate formation by Cordycepin triphosphate in extracts of Ehrlich ascites tumor cells. *Biochim Biophys Acta.* 1964, *80,* 504–507.
84. Rottman, F.; Guarino, A.J. The inhibition of phosphoribosyl-pyrophosphate amidotransferase activity by cordycepin mono phosphate. *Biochim Biophys Acta.* 1964,*89*,465–472.
85. Holbein, S.; Wengi, A.; Decourty, L., Freimoser, F.M.; Jacquier, A.; Dichtlrna, I.B. Cordycepin interferes with 3′ end formation in yeast independently of its potential to terminate RNA chain elongation. *RNA.* 2009,*15*,837–849.
86. Wong, Y.Y.; Moon, A.; Duffin, R.; Barthet-Barateig, A.; Meijer, H.A.; Clemens, M.J.; de Moor, C.H. Cordycepin inhibits protein synthesis and cell adhesion through effects on signal transduction. *J Biol Chem.* 2010, *285*, 2610–2621.
87. Chen, X.; Wu, G.; Huang, Z. Structural analysis and antioxidant activities of polysaccharides from cultured *Cordyceps militaris. Int. J. Biol. Macromol.* 2013, *58,* 18-22.
88. Guan, J.; Zhao, J.; Feng, K.; Hu, D.J.; Li, S.P. Comparison and characterization of polysaccharides from natural and cultured *Cordyceps* using saccharide mapping, *Anal. Bioanal. Chem*. *2011*, *399,* 3465-3474.
89. Russell, R.; Paterson, M. *Cordyceps* - A traditional Chinese medicine and another fungal therapeutic biofactory? *Phytochemistry.* 2008, *69*, 1469-1495.
90. Cunningham, K.G.; Manson, W.; Spring, F.S.; Hutchinson, S.A. Cordycepin, a metabolic product isolated from cultures of *Cordyceps militaris* (L.) Link. *Nature.*1950;166:949–954.
91. Das, S.K.; Masuda, M.; Hatashita, M.; Sakurai, A.; Sakakibara, M. Effect of inoculation on production of anti-cancer drug-cordycepin in surface liquid culture using *Cordyceps militaris* mutant: a minor factor may greatly affect the result. *Indian J. Biotechnol.* 2010;9:427–430.
92. Das, S.K.; Masuda, M.; Sakurai, A.; Sakakibara, M. Medicinal uses of the mushroom *Cordyceps militaris*: current state and prospects. *Fitoterapia.* 2010;81:961–968.
93. Boh Podgornik, B.; Berovic, M. Cultivation of *Ganoderma lucidum* and *Grifola frondosa* and production of their pharmaceutical active compounds. In: Petre M., Berovic, M. (Eds.). *Mushroom biotechnology and bioengineering*. Bucharest: CD Press Publishing House, 2012. pp. 21-84.
94. Nikšić, M.; Boh Podgornik, B.; Berovic, M. Farming of Medicinal Mushrooms., *Advances in Biochemial Engineering and Biotechnology.* In Berovic M., Zhong J.J. Eds. Medicinal Mushrooms Biochemical Engineering and Biotechnology, 2023; DOI 10.1007/10 2021 201.
95. Royse, D.J. (1996) Specialty mushrooms. In: J. Janick Ed., Progress in new crops. *ASHS Press*, Arlington, VA. 1996, 464-475.
96. Mitchell, D.A.; Berovic, M. Solid state cultivation. In: Bioprocess Engineering Course, Ed. Berovic M, *University of Ljubljana Press*, Ljubljana 1998, pp. 128-167.
97. Gregori, A., Kretschmer, N.; Wagner, S.; Boechzelt, H.; Klinar, D.; Bauer, R.; Pohleven F, Influence of Olive Oil Press Cakes on Shiitake Culinary-Medicinal Mushroom, *Lentinus edodes* (Berk.) Singer (Higher Basidiomycetes) Fruiting Bodies Production and Effect of their Crude Polysaccharides on CCRF-CEM Cell Proliferation, *Int. J.Med. Mush.* 2012, *14(4),* 419–424.

98. Arora, S.; Rani, R.; Ghosh, S. Bioreactors in solid state cultivation technology: Design, applications and engineering aspects. *J Biotechnol.* 2018, *26,* 916–34.
99. Hikino, H.; Konno, C.; Mirin, Y.; Hayashi, T. Isolation and hypoglycemic activity of ganoderans A and B, glycans of *Ganoderma lucidum* fruit bodies. *Planata Med.* 1985,(4),339–40.
100. Berovic, M.; Boh Podgornik, B. Engineering aspects in production of various medicinal mushrooms biomass in submerged bioreactors. *Int. J. Med. Mush.* 2019, *21(8),* 735-753.
101. Berovic, M.; Popovć, M. Submerged cultivation of *Ganoderma lucidum* biomass in stirred tank reactor. *Chem Biochem Eng Quart.* 2018, *32(4),*465-472.
102. Elisashvili, V. Submerged Cultivation of Medicinal Mushrooms: Bioprocesses and Products (Review), *Int.J.Med. Mush.* 2012; *14(3),* 211–239.
103. Cui, F.J.; Li, Y.; Xu, Z.H.; Xu, H.Y.; Sun, K.; Tao, W.Y. Optimization of the medium composition for production of mycelial biomass and exo-polymer by *Grifola frondosa* GF9801 using response surface methodology. *Biores.Technol.* 2006,*10*, 209-1216.
104. Zore, I.; Berovic, M.; Boh Podgornik, B.; Hodzar, D,; Pohleven, F. Procedure for preparation of inoculum for growing of fungus *Ganoderma lucidum* by submersion cultivation, *Slovenian patent* SI 9700014, 1998.
105. Huang, D.; Cui, F.J.; Li, Y.; Zhang, Z.; Zhao, J.; Han, X.M.; Xiao, X.; Qian, J.; Wu, Q.; Guan, G. Nutritional Requirements for the Mycelial Biomass and Exopolymer Production by *Hericium erinaceus* CZ-2. *Food Technol. Biotechnol.* 2007, *45(4),* 389–395.
106. Smole, J. Submerged cultivacition of *Trametes versicolor* in stirred tank bioreactor. B.Sc.Thesis, University of Ljubljana, Slovenia, 2015.
107. Kwon, J.S.; Lee, J.S.; Shin, W.C.; Lee, K.E.; Hong, E.K. Optimization of culture conditions and medium components for the production of mycelial biomass and exo-polysaccharides with *Cordyceps militaris* in liquid culture biotechnology. *Bioproc. Engineering.* 2009,*14,*756-762.
108. Fana, L.; Soccol, A.T.; Pandey, A.; Soccol, R.C. Effect of nutritional and environmental conditions on the production of exo-polysaccharide of *Agaricus brasiliensis* by submerged cultivation and its antitumor activity, *LWT Food Sci. Technol.* 2007, *40,* 30–35.
109. Lung, M.Y.; Hsieh, C.W. Antioxidant property and production of extracellular polysaccharide from *Armillaria mellea* in submerged cultures: effect of culture aeration rate. *Eng. Sci.* 2011, *11*, 482–90.
110. Noorlidah, A.; Rozila, I.; Norjuliz, M.K.J.; Annuar, M.S.M. Production of liquid spawn of an edible grey oyster mushroom, *Pleurotus pulmonarius* (Fr.) Quél by submerged cultivation and sporophore yield on rubber wood sawdust, *Scientia Horticulturae.* 2013, *161*, 65–69.
111. Berovic, M.; Kneževic, M. *Pleurotus saca* submerged cultivation. *Biotechnol. Lett.* 1995, *17(8)*, 839-844.
112. Papaspyridi, L.M.; Katapodis, P., Gonou-Zagouc, Z.; Kapsanaki-Gotsic, E.; Christakopoulos, P. Optimization of biomass production with enhanced glucan and dietary fibres content by *Pleurotus ostreatus* ATHUM 4438 under submerged culture, *Biochemical Engineering Journal* 2010, *50*, 131–138.
113. Papaspyridi, L.M.; Aligiannis, N.; Christakopoulos, P.; Skaltsounis, A.L.; Fokialakis, N. Production of bioactive metabolites with pharmaceutical and nutraceutical interest by submerged cultivation of *Pleurotus ostreatus* in a batch stirred tank bioreactor, *Procedia Food Science*, 2011,*1*,1746 – 1752.
114. Wang, X.L.; Ding, Z.Y.; Zhao, Y.; Liu, G.Q.; Zhou, G.Y. Efficient Accumulation and *In-Vitro* Antitumor Activities of Triterpene Acids from Fed-Batch Cultivation of Ling-Zhi or Reishi Medical Mushroom *Ganoderma lucidum* (*Agaricomycetes*). *In.J.Med.Mushr.* 2017, *19(5),* 419–431.
115. Shi, H.; Zhang, M.; Devahastin, S. New development of efficient processing techniques on typical medicinal fungi: A Review. *Food Reviews Int.* 2020, *36*, 39-57.
116. Hatti-Kaul, R.; Mattiasson, B. Isolation and purifaction of proteins.*Marcel Dekker*, NewYork, 2003.
117. Middelberg, A.P.J. Process-scale disruption of microorganisms. *Biotech Adv*. 1995, *13*, 491-504.
118. Xiao, Y.Z. ; Qiang, A.; Lian, W.Y.; Yiang F.Y.; Sun, J.C.; Guo, S.G. Application of superfine pulverization technology in biomaterial industry. *J Taiwan Institute Chem E*. 2009,*40*,337-343.
119. Lindequist, U.; Niedermeyer, T.H.J.; Jülich, W.D. The pharmacological potential of mushrooms. *Evid Based Complement Alternat Med.* 2005;*2*:285–299.
120. Luo, H.Y.; Li, Y.B. Down Stream Processing of Medicinal Mushrooms Products, *Advances in Biochemial Engineering and Biotechnology*. In Berovic M., Zhong J.J. Eds. Medicinal Mushrooms Biochemical Engineering and Blotechnology, 2023; DOI 10.1007/10 2021 187

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The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Fellows are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Fellow Members.

Benefits

To the institution

Get letter of appreciation

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

Exclusive Network

Get access to a closed network

A FSFRC member gets access to a closed network of Tier 1 researchers and scientists with direct communication channel through our website. Fellows can reach out to other members or researchers directly. They should also be open to reaching out by other.

Career | Credibility | Exclusive | Reputation

Certificate

Receive a print ed copy of a certificate

Fellows receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member's university.

Career | Credibility | Exclusive | Reputation

Designation

Get honored title of membership

Fellows can use the honored title of membership. The “FSFRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., FSFRC or William Walldroff, M.S., FSFRC.

Career | Credibility | Exclusive | Reputation

Recognition on the Platform

Better visibility and citation

All the Fellow members of FSFRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation. All fellows get a dedicated page on the website with their biography.

Career | Credibility | Reputation

Future Work

Get discounts on the future publications

Fellows receive discounts on future publications with Global Journals up to 60%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

Career | Financial

GJ Internal Account

Unlimited forward of Emails

Fellows get secure and fast GJ work emails with unlimited forward of emails that they may use them as their primary email. For example,
john [AT] globaljournals [DOT] org.

Career | Credibility | Reputation

Premium Tools

Access to all the premium tools

To take future researches to the zenith, fellows and associates receive access to all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

Financial

Conferences & Events

Organize seminar/conference

Fellows are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

Career | Credibility | Financial

Early Invitations

Early invitations to all the symposiums, seminars, conferences

All fellows receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.

Exclusive

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Earn 60% of sales proceeds

Fellows can publish articles (limited) without any fees. Also, they can earn up to 60% of sales proceeds from the sale of reference/review books/literature/ publishing of research paper. The FSFRC member can decide its price and we can help in making the right decision.

Exclusive | Financial

Reviewers

Get a remuneration of 15% of author fees

Fellow members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

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Become a member of the Editorial Board

Fellows may join as a member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer. Additionally, Fellows get a chance to nominate other members for Editorial Board.

Career | Credibility | Exclusive | Reputation

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Get access to scientific museums and observatories across the globe

All members get access to 5 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 10 GB free secure cloud access for storing research files.

ASSOCIATE OF SCIENCE FRONTIER RESEARCH COUNCIL

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Organize seminar/conference

Associates are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

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Exclusive

Publishing Articles & Books

Earn 30-40% of sales proceeds

Associates can publish articles (limited) without any fees. Also, they can earn up to 30-40% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.

Exclusive | Financial

Reviewers

Get a remuneration of 15% of author fees

Associate members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

And Much More

Get access to scientific museums and observatories across the globe

All members get access to 2 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 5 GB free secure cloud access for storing research files.

Associate	Fellow	Research Group	Basic
$4800 **lifetime designation**	$6800 **lifetime designation**	$12500.00 **organizational**	APC **per article**
Certificate, LoR and Momento **2** discounted publishing/year **Gradation** of Research **10** research contacts/day **1 GB** Cloud Storage **GJ** Community Access	**Certificate,** LoR and Momento **Unlimited** discounted publishing/year **Gradation** of Research **Unlimited** research contacts/day **5 GB** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**Certificates,** LoRs and Momentos **Unlimited** free publishing/year **Gradation** of Research **Unlimited** research contacts/day **Unlimited** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**GJ** Community Access

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template.zip

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

Authorship Policies

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

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Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

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Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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