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HIGHLIGHTS

Land Snails Consumed

Potential Feed Stocks

The Functional Properties

Potential Antimicrobial Agents

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Phytochemical Investigations on *Elaeagnus Umbellata*

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Abstract - In the course of phytochemical investigations on methanol extract of *Elaeagnus umbellata* lead to the isolation of new isoflavone and phenol compound as a source from this plant. The isolation of plant also gave the terpenoid compound. The name of isolated compounds are; 3-(hydroxymethyl)-4-methoxyphenol (1) and 5, 7-dihydroxy-3(2-hydroxyphenyl-4*H*-chromen-4-one (1) and Stigmasterol (3). This is the first report of the isolation of compounds (1) and (2). The Characterization of the compounds was made on the basis of spectral studies.

Keywords : phytochemical, isoflavone, phenol and spectral studies.

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Phytochemical Investigations on *Elaeagnus umbellata*

Syeda Farina Asghar [α] & Habib-ur-Rehman [σ]

Abstract - In the course of phytochemical investigations on methanol extract of *Elaeagnus umbellata* lead to the isolation of new isoflavone and phenol compound as a source from this plant. The isolation of plant also gave the terpenoid compound. The name of isolated compounds are; 3-(hydroxymethyl)-4-methoxyphenol (1) and 5, 7-dihydroxy-3(2-hydroxyphenyl-4*H*-chromen-4-one (1) and Stigmasterol (3). This is the first report of the isolation of compounds (1) and (2). The Characterization of the compounds was made on the basis of spectral studies.

Keywords : phytochemical, isoflavone, phenol and spectral studies.

I. Introduction

Elaeagnus umbellata is a key plant for the font of indole 3-carbinol alkaloid (Tolkachev & etal.[1]). 'Cervical cancer' is reduced by indole 3-carbinol alkaloid (it is an anti-estrogenic) (Yuan & etal.[2]). For the plant growth and development chitinases play vital role and *Elaeagnus umbellata* is rich in endochitinases in their root nodules (Yaw Joo & etal.[3]).The plant have effective antibacterial activity (Sabir & etal [4]).Vitamins A, C and E, flavonoids, isoflavonoids, essential fatty acids, acids, lycopene, β-carotene, lutein, phytofuene are abundant in the berries of the plant (Chopra, Kohlmeier, Fordham & etals[5-7]).

II. Experimental

Instrumentation : The instruments used during research for collecting data are listed in table A.

a) Plant Collection

The collection of plant material was done at Athmaqam District Neelum Valley Muzaffarabad Azad Kashmir Pakistan. The identification of plant was done by the help of taxonomist at the Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad. The voucher specimen has been kept in the herbarium of the department. The isolation of compounds is given in the scheme A.

i. Compounds isolated from Elaeagnus umbellata

Isolation and Characterization of Compound 1:

The flash column chromatography was used for the purification of fraction U4. The column was eluted with Ethyl acetate/ Chloroform (2.0:8.0) as the solvent system to afford two fractions, U4-1 and U4-2. The fraction U4-1 was rechromatographed on the precoated silica-gel (GF-254) plates with Chloroform/Ethyl acetate (2.0:8.0) as the solvent system. That resulted in the isolation of the pure compound **1** as an amorphous material (20mg, Rf = 0.6).

Spectral Data:

UV (MeOH) λmax (nm): 202, 223, and 276.

IR (CHCl$_3$) υ_{max}, (cm^{-1}): 3405 (O-H), 2925 (aromatic C-H) and 1031 (C-O).

^{1}H-NMR (CDCl$_3$, 300MHz) δ: 3H (s) δ 3.83 (4-OCH$_3$), 2H (s) δ 4.61 (3-CH$_2$OH), 1H (s) δ 6.99 (2-H),1H (d) δ 6.75 (*J*= 8.2 Hz) (5-H), 1H (d) δ 6.93 (*J*= 8.2 Hz) (6-H), 1H (t) δ 5.35 (*J*= 4.0 Hz) (1-OH), 1H (t) δ 3.65 (*J*= 4.0 Hz) (3-OH).

^{13}C-NMR (CDCl$_3$, 100MHz) δ: 1-C (δ 154.7), 2-C (δ 116.8), 3-C (δ 135.7), 4-C (δ 149.3), 5-C (δ 112.7), 6-C (δ 115.8), 3-CH$_2$OH (δ 60.5), 4-OCH$_3$(δ 56.1).

HRMS m/z: 154.020 (C$_8$H$_{10}$O$_3$), 153, 137 and 123.

Isolation and Characterization of Compound 2:

The fraction U4-2 was rechromatographed on the precoated silica-gel (GF-254) plates with Chloroform/Ethyl acetate (3.0:7.0) as the solvent system. That resulted in the isolation of the pure compound **2** as amorphous material (20mg, Rf = 0.9).

Spectral Data:

UV (MeOH) λ_{max} (nm): 203, 215 and 264.

IR (CHCl$_3$) υ_{max} (cm^{-1}): 3430 (O-H), 2953 (aromatic C-H), 1659 (α, β-unsaturated C=O) and 1064 (C=O).

^{1}H-NMR (CDCl$_3$, 400MHz) δ: 1H (s) δ 7.8 (2-H), 1H (s) δ 6.0 (6-H),1H (s) δ 6.2 (8-H), 1H (d) δ 6.7 (*J* = 8.4 Hz) (3'-H), 1H (d) δ 7.0 (*J* = 8.2 Hz) (6'-H), 1H (t) δ 7.1 (*J* = 10.0 Hz) (4'-H), 1H (t) δ 6.9 (*J* = 10.0 Hz) (5'-H), 3H (br) δ 12.5 (5-OH), (7-OH) and (2'-OH) respectively.

^{13}C-NMR (CDCl3, 100MHz) δ: 2-C (δ 153.2), 3-C (δ 121.7), 4-C (δ 180.7), 5-C (δ 161.8), 6-C (δ 98.3), 7-C (δ 166.4), 8-C (δ 94.0), 9-C (δ 160.0), 10-C (δ 105.5), 1'-C (120.2), 2'-C (δ 156.6), 3'-C (δ 117.6), 4'-C(129.3), 5'-C

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(121.2) and 6'-C (130.1).

HRMS, m/z: 270.060 ($C_{15}H_{10}O_5$), 269, 253, 176 and 76.

Isolation and Characterization of Compound 3:

The flash column chromatography was used for the purification of fraction U5. The column was eluted with Ethyl acetate that afforded two fractions, U5-1 and U5-2. The fraction U5-2 was rechromatographed on the precoated silica-gel (GF-254) plates with Chloroform/ Ethyl acetate (1.0:9.0) as the solvent system. That resulted in the isolation of the pure compound **3** as amorphous material (20mg, Rf = 0.7).

Spectral Data:

UV (MeOH) λ_{max} (nm): 203, 215 and 264.

IR ($CHCl_3$) υ_{max} (cm^{-1}): 3484 (O-H), 2868 (C-H), 1659 and (C=C).

^{1}H-NMR ($CDCl_3$, 400MHz) δ: 3H (s) δ 0.69 (18-CH_3), 3H (s) δ 0.99 (19-CH_3), 3H (t) δ 0.78(*J*=7.5Hz) (29-CH_3),3H (d) δ 1.00 (*J*= 6.5 Hz) (21-CH_3), 3H (d) δ 0.82 (*J*= 6.0 Hz) (26-CH_3), 3H (d) δ 0.77 (*J*= 6.0 Hz) (27-CH_3), 1H (bs) δ 5.23 (6-H).

^{13}C-NMR (CDCl3, 100MHz) δ: 1-C (δ37.2), 2-C (δ 31.6), 3-C (δ δ71.8), 4-C (δ 42.2), 5-C (δ140.8), 6-C (δ 121.7), 7-C (δ 31.9), 8-C (δ 31.9), 9-C (δ 51.2), 10-C (36.5), 11-C (21.1), 12-C (δ 39.8), 13-C (δ 42.3), 14-C(56.9), 15-C (24.3) 16-C (28.4) 17-C (δ 56.1), 18-C (δ 11.2), 19-C (δ 21.4), 20-C (δ 40.2),21-C (δ 21.3), 22-C (δ 138.4), 23-C (δ 129.2) 24-C (δ 51.2), 25-C (δ 31.7), 26-C (δ 21.2), 27-C (δ 19.0), 28-C (δ 25.4) and 29-C (δ 12.2).

HRMS, m/z: 412.3861 ($C_{29}H_{48}O$), 413, 396, 283 and 60.

III. Results and Discussions

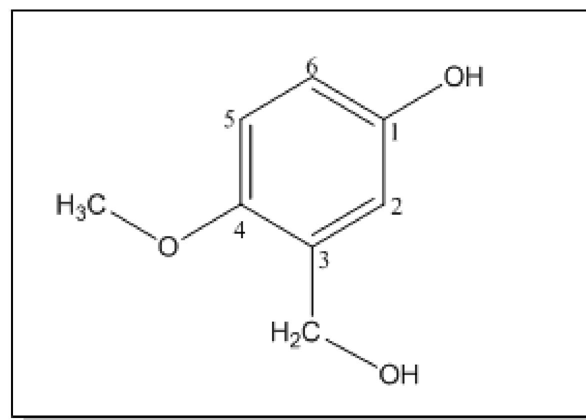
a) 3-(hydroxymethyl)-4-methoxyphenol (1)

The UV spectrum (MeOH) of compound **(1)** showed the λ_{max} absorptions at, 203 nm 223 nm and 276 nm, suggesting the phenol type compound. The IR spectrum ($CHCl_3$) of compound **(1)** showed intense υ_{max} absorptions at 3405 cm^{-1}, 2925 cm^{-1}, and 1031 cm^{-1}, indicating the presence of O-H, aromatic C-H and C-O functions in the molecule.

The ^{1}H-NMR ($CDCl_3$, 400MHz) spectrum of compound **(1)** showed the presence of 10 proton resonances in the molecule. The spectrum showed 3H singlet at δ 3.83, assigned to the methoxy protons (4-OCH_3) of the compound. Another singlet of 2H centred at δ 4.61 given to the methylene protons (3-CH_2OH). 1H singlet appeared at δ 6.99 given to 2-H of benzene proton. Two doublets of 1H each appeared at δ 6.75 (*J*= 8.2 Hz) and δ 6.93 (*J*= 8.2 Hz) were assigned to the 5-H and 6-H benzene protons respectively. Two triplets of 1H each appeared at δ 5.35 (*J*= 4.0 Hz) and δ 3.65 (*J*= 4.0 Hz) were assigned to hydroxyl protons of 1-OH and 3-OH position. The ^{1}H-NMR chemical shift assignments are presented in Table-1.

The ^{13}C-NMR ($CDCl_3$, 100 MHz) spectrum of the compound **(1)** showed the presence of 8 carbon atoms in the molecule. The ^{13}C-NMR chemical shift assignments made by DEPT pulse sequences are presented in Table-2. The downfield signal at δ 154.7 showed the multiplicity of one carbon assigned to the 1-C attached to the hydroxyl group. The signals at δ 116.8, δ 135.7, δ 149.3, δ 112.7 and δ 115.8 were assigned to the 2-C, 3-C, 4-C, 5-C and 6-C carbon atoms of benzene ring respectively. The signal at δ 56.1 was given to the 4-OCH_3. The signal appeared at δ 60.5 assigned to the methylene carbon attached to hydroxyl group 3-CH_2OH.

The mass spectrum of the compound **(1)** showed the molecular ion peak at m/z 154.020, corresponding to the molecular formula $C_8H_{10}O_3$ suggesting the presence of four degrees of unsaturation in the molecule. The prominent peaks were found to occur at m/z 154, 153, 137and 123. The peak appeared at m/z 153 showed the loss of hydrogen atom from the molecule. The peak at m/z 137 showed the loss of hydroxyl group from the molecular ion. The peak at m/z 123 showed the loss of methoxy group from the molecular ion. On the basis of spectral data it is confirmed that the compound **(1)** is two substituted phenol namely; 3-(hydroxymethyl)-4-methoxyphenol.



(1)

b) 5, 7-dihydroxy-3(2-hydroxyphenyl-4H-chromen-4-one(2)

The UV spectrum (MeOH) of the compound **3** showed the λ_{max} absorptions at 203 nm, 215 nm and 264 nm, representing the isoflavone type structure of the molecule. The IR spectrum ($CHCl_3$) showed intense absorptions at 3430 cm^{-1}, 2953 cm^{-1}, 1659 cm^{-1} and 1064 cm^{-1}, indicating the presence of O-H, aromatic C-H, α, β-unsaturated C=O and C-O functionalities in the molecule.

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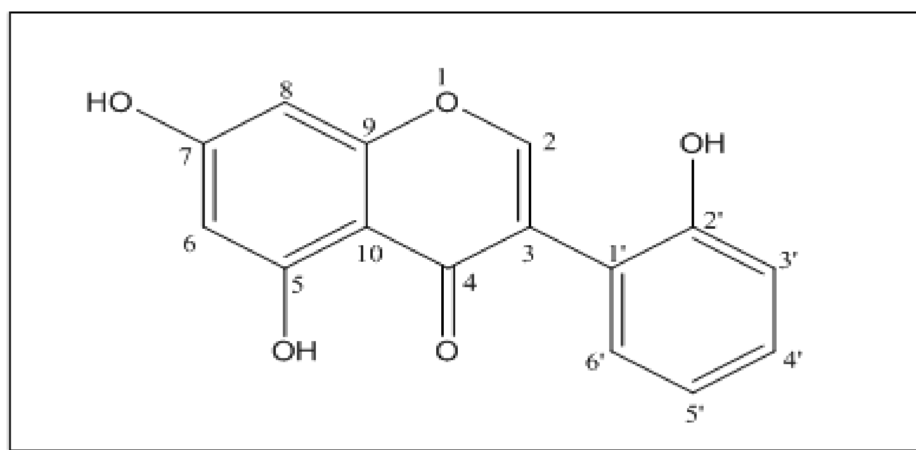
The ^{1}H-NMR ($CDCl_3$, 300MHz) spectrum of the compound **2** showed 10 proton resonances in the molecule. Three singlets of 1H each appeared at δ 7.8, δ 6.0 and δ 6.2 were assigned to the 2-H, 6-H and 8-H respectively. The protons of 3'-H and 6'-H appeared as 1H doublets each at δ 6.7 (*J*= 8.4) and δ 7.0 (*J*= 8.2 Hz), respectively. Two triplets of 1H each viewed at δ 7.1 (*J*= 10.0) and δ 6.9 (*J*= 10.0) given to the 4'-H and 5'-H protons respectively. The broad singlet of 3H at δ 12.5 showed the presence of hydroxyl protons in the compound and the peak assigned to the 5-OH, 7-OH and 2'-OH protons respectively. The ^{1}H-NMR chemical shifts of compound **2** are presented in Table-3.

The ^{13}C-NMR ($CDCl_3$, 100 MHz) spectrum of the compound **(3)** showed the presence of 15 carbon atoms in the molecule. The ^{13}C-NMR (DEPT) chemical shift assignments presented in Table-4. The most downfield signal at δ 180.7 showed the multiplicity of one carbon and assigned to the 4-C carbonyl carbon. The other carbon signals at δ 153.2, δ 121.7, 161.8, δ 98.3, δ 166.4 δ 94.0, δ 160.0, δ105.5, δ 120.2, δ 156.6, δ 117.6, δ 129.3, δ 121.2 and δ 130.1 were given to the 2-C, 3-C, 5-C, 6-C, 7-C, 8-C, 9-C, 10-C, 1'-C , 2'-C, 3'-C, 4'-C, 5'-Cand 6'-C.

The mass spectrum of the compound **2** showed the molecular ion peak at m/z 270.060, corresponding to the molecular formula $C_{15}H_{10}O_5$, showed the 11 degrees of unsaturation in the molecule, Other prominent peaks were found to occur at m/z 269, 253, 176 and 77. The peak at m/z 269 indicated the loss of hydrogen atom while the peak at m/z 253 showed the loss of hydroxyl group from the molecular ion. The peak at m/z 176 suggested the loss of phenol from the molecule



(2)

c) Stigmasterol (3)

The UV spectrum (MeOH) showed the λ_{max} absorptions at 195nm, 204 nm and 387 nm, representing a steroid skeleton. The IR spectrum ($CHCl_3$) showed intense υ_{max} absorptions at 3374 cm^{-1}, 2936, 1462 $cm^{-1}$and 1058 cm^{-1}, indicating the presence of O-H, C-H, C=C and C-O functions in the molecule.

The ^{1}H-NMR ($CDCl_3$, 400MHz) spectrum of **3** showed the presence of 48 proton resonances in the molecule. The spectrum showed two 3H singlets each at δ 0.69 and δ 0.99 given to the 18-CH_3 and 19-CH_3, respectively. A 3H triplet at δ 0.78 (*J*=7.5Hz) was assigned to the 29-CH_3. Three doublets of 3H each at δ 1.00 (*J*= 6.5 Hz), δ 0.82 (*J*=6.0 Hz) and δ 0.77 (*J*=6.0 Hz) assigned to the 21-CH_3, 26-CH_3 and 27-CH_3, respectively. A broad singlet of 1H appeared at δ 5.23 was assigned to the 6-H olefinic proton. The ^{1}H-NMR chemical shift assignments are presented in Table-5.

The ^{13}C-NMR ($CDCl_3$, 400 MHz) spectrum of the compound **3** showed the presence of 29 carbon atoms in the molecule. The ^{13}C-NMR chemical shift assignments determined by DEPT pulse sequence are presented in Table-6. The signals at δ 37.2 δ 31.6, δ 71.8, δ 42.2 , δ 140.8, δ 121.7 and were assigned to the 1-C, 2-C, 4-C, 3-C, 5-C and 6-C respectively. The signal of two carbons multiplicity at δ 31.9 given to the 7-C and 8-C. The signals at δ 51.2, δ 36.5, δ 21.1, δ 39.8, δ 42.3, 56.9, δ 24.3, δ 28.4, δ 56.1, δ 11.2, δ 21.4, δ 40.2 and δ 21.3 and were assigned to the 9-C. and 10-C, 11-C, 12-C, 13-C, 14-C, 15-C, 16-C, 17-C, 18-C, 19-C, 20-C and 21-C respectively. The signals at δ 138.4 and δ 129.2 assigned to the 22-C and 23-C unsaturated carbons respectively. The signals at δ 51.2, δ 31.7, δ 21.2, δ 19.0, δ 25.4 and δ 12.2 were assigned to the 24-C, 25-C, 26-C, 27-C, 28-C and 29-C respectively.

The mass spectrum of the compound **3** showed the molecular ion peak at m/z 412.3861, corresponding to the molecular formula $C_{29}H_{48}O$, indicating five degrees of unsaturation in the molecule. Other prominent peaks were found to occur at m/z, 413, 396, 283 and 60. The peak at m/z 413 indicated the loss of hydrogen atom while the peak at m/z 396 showed the loss of water molecule from the molecular ion (Pateh *et al*[8])

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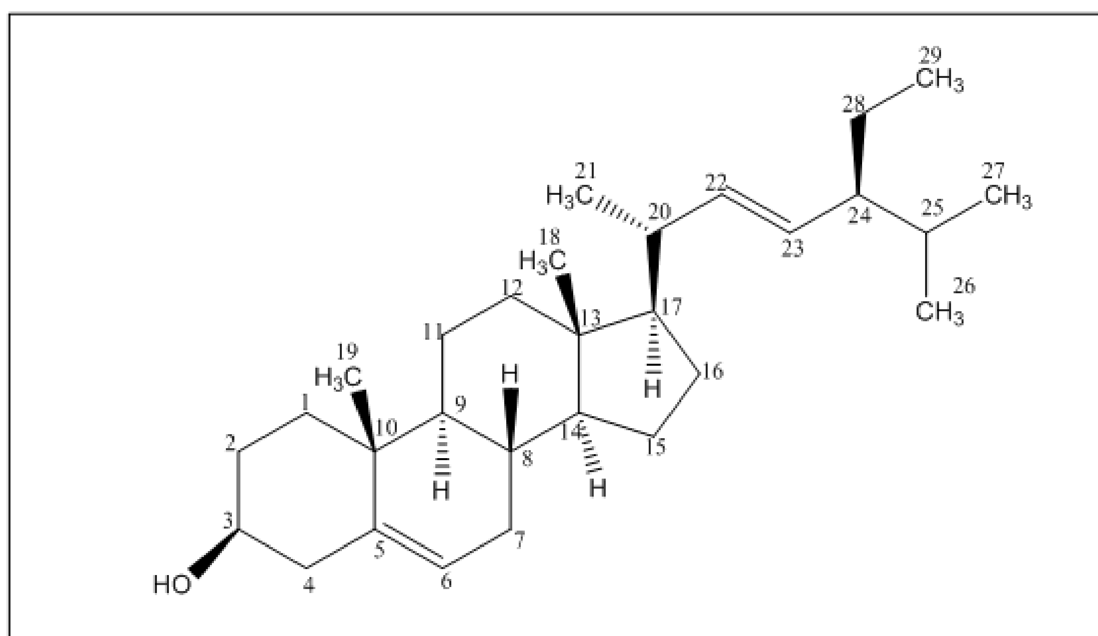

(3)

Table A : list of instruments used during research.

S.#	Data	Instruments
1.	UV spectra	Shimadzu UV-240
2.	IR spectra	JASCO A-302 spectrophotometer
3.	High resolution mass spectra	MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system
4.	^{1}H- NMR spectra	400 MHz on a Bruker AM-300 NMR spectrometer
5.	^{13}C-NMR spectra	100 MHz on a Bruker AM-300 NMR spectrometer
6.	TLC	silica gel plates (GF-254, 0.2 mm, E.Merck)

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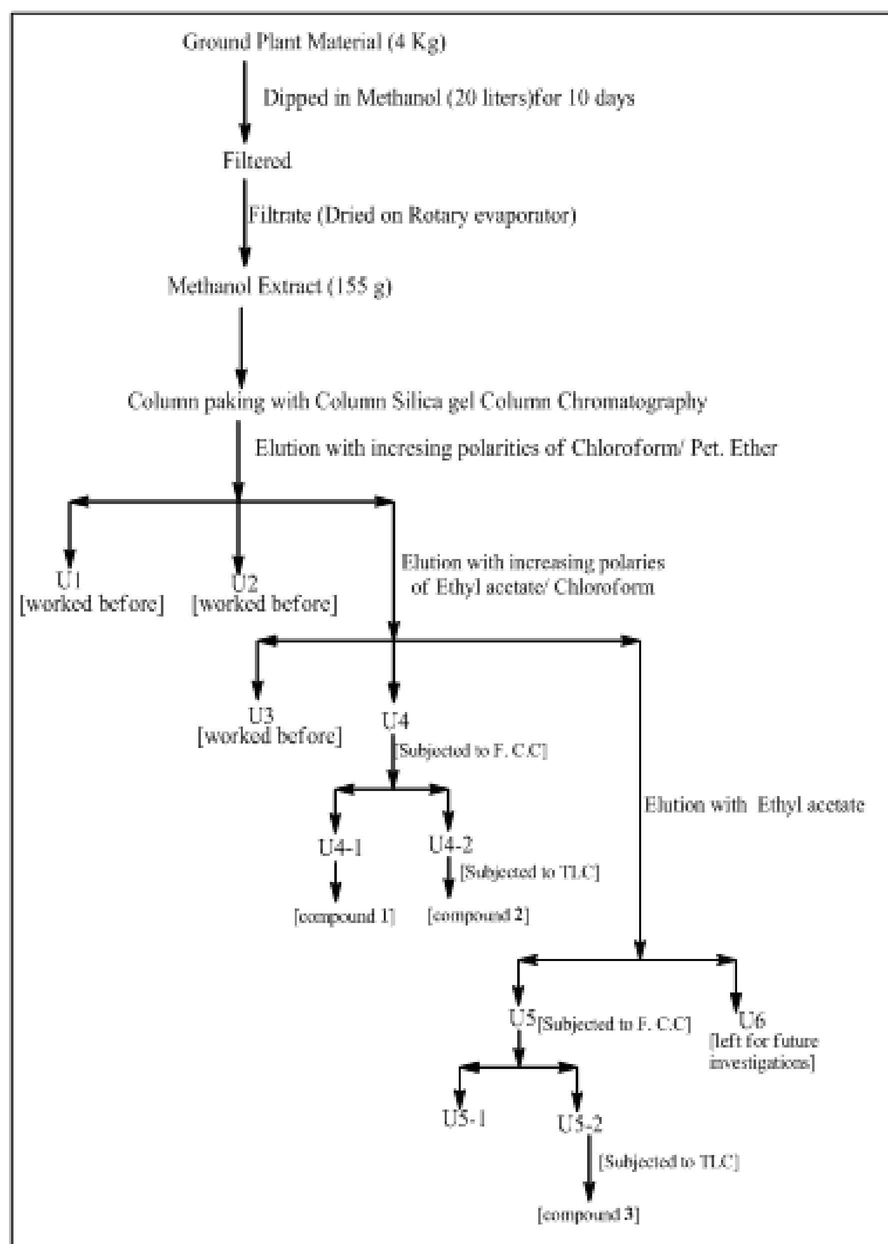

Scheme A : Isolation of compounds.

Table-1 : ^{1}H-NMR ($CDCl_3$, 400MHz) Chemical Shift Assignments of Compound **1**.

Proton No.	Chemical Shift (δ) (ppm)	Integration	Multiplicity	Coupling Constant (*J*) (Hz)
4-OCH_3	3.83	3H	s	-
3-CH_2OH	4.61	2H	s	-
2-H	6.99	1H	s	-
5-H	6.75	1H	d	8.2
6-H	6.93	1H	d	8.2
1-OH	5.35	1H	t	4.0
3-OH	3.65	1H	t	4.0

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Table-2 : ^{13}C-NMR ($CDCl_3$, 400 MHz) Chemical Shift Assignments of Compound **1**.

Carbon No.	Chemical Shift (δ) (ppm)	Carbon No.	Chemical Shift (δ) (ppm)
1-C	154.7	5-C	112.7
2-C	116.8	6-C	115.8
3-C	135.7	3-CH_2OH	60.5
4-C	149.3	4-OCH_3	56.1

Table-3 : ^{1}H-NMR ($CDCl_3$, 400MHz) Chemical Shift Assignments of Compound **2**.

Proton No.	Chemical Shift (δ) (ppm)	Integration	Multiplicity	Coupling Constant (*J*) (Hz)
2-H	7.8	1H	s	-
6-H	6.0	1H	s	-
8-H	6.2	1H	s	-
3'-H	6.7	1H	d	8.4
6'-H	7.0	1H	d	8.2
4'-H	7.1	1H	t	10.0
5'-H	6.9	1H	t	10.0
5,7,2'-OH	12.5	3H	bs	-

Table-4 : ^{13}C-NMR ($CDCl_3$, 100 MHz) Chemical Shift Assignments of Compound **2**.

Carbon No.	Chemical Shift (δ) (ppm)	Carbon No.	Chemical Shift (δ) (ppm)
2-C	153.2	10-C	105.5
3-C	121.7	1'-C	120.2
4-C	180.7	2'-C	156.6
5-C	161.8	3'-C	117.6
6-C	98.3	4'-C	129.3
7-C	166.4	5'-C	121.2
8-C	94.0	6'-C	130.1
9-C	160.0	-	-

Table-5 : ^{1}H-NMR ($CDCl_3$, 400MHz) Chemical Shift Assignments of Compound **3**.

Proton No.	Chemical Shift (δ) (ppm)	Integration	Multiplicity	Coupling Constant (*J*) (Hz)
18-CH_3	0.69	3H	s	-
19-CH_3	0.99	3H	s	-
29-CH_3	0.78	3H	t	7.5
21-CH_3	1.00	3H	d	6.5
26-CH_3	0.82	3H	d	6.0
27-CH_3	0.77	3H	d	6.0
6-H	5.23	1H	bs	-

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Table-6 : ^{13}C-NMR ($CDCl_3$, 100 MHz) Chemical Shift Assignments of Compound **3**.

Carbon No.	Chemical Shift (δ) (ppm)	Carbon No.	Chemical Shift (δ) (ppm)
1-C	37.2	16-C	28.4
2-C	31.6	17-C	56.1
3-C	71.8	18-C	11.2
4-C	42.2	19-C	21.4
5-C	140.8	20-C	40.2
6-C	121.7	21-C	21.3
7-C	31.9	22-C	138.4
8-C	31.9	23-C	129.2
9-C	51.2	24-C	51.2
10-C	36.5	25-C	31.7
11-C	21.1	26-C	21.2
12-C	39.8	27-C	19.0
13-C	42.3	28-C	25.4
14-C	56.9	29-C	12.2
15-C	24.3		

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Lipid Composition of Three Different Types of Land Snails Consumed in Nigeria

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Abstract - The levels of fatty acids, phospholipids and zoosterols were determined in the edible parts of three land snails consumed in Nigeria [Archachatina marginata, Archatina (archatina) archatina and Limicolaria sp.)] on dry weight basis. Results showed crude fat varied from 2.22-2.38 g/100 g; SFA varied from 37.5- 49.8 % of total fatty acids, total unsaturated fatty acids varied from 50.2-62.5 %, PUFA ranged from 25.5-38.7 %. In the phospholipids, phophatidylcholine was highest in all the samples with a range of 1.55-2.88 mg/100 g. Only cholesterol had results > 0.00 mg/100 g in the sterols with a value range of 37.1 – 45.1 mg/100 g. Eicosadienoic acid (C20: 2 cis -11, 14) was the highest PUFA in all the samples with range values of 8.36-16.7 mg/100 g.

Keywords : lipid profiles, three land snails, consumed in nigeria.

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Lipid Composition of Three Different Types of Land Snails Consumed in Nigeria

E.I. Adeyeye

Abstract - The levels of fatty acids, phospholipids and zoosterols were determined in the edible parts of three land snails consumed in Nigeria [Archachatina marginata, Archatina (archatina) archatina and Limicolaria sp.)] on dry weight basis. Results showed crude fat varied from 2.22-2.38 g/100 g; SFA varied from 37.5- 49.8 % of total fatty acids, total unsaturated fatty acids varied from 50.2-62.5 %, PUFA ranged from 25.5-38.7 %. In the phospholipids, phophatidylcholine was highest in all the samples with a range of 1.55-2.88 mg/100 g. Only cholesterol had results > 0.00 mg/100 g in the sterols with a value range of 37.1 – 45.1 mg/100 g. Eicosadienoic acid (C20: 2 cis -11, 14) was the highest PUFA in all the samples with range values of 8.36-16.7 mg/100 g.

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I. Introduction

The conventional sources of meat protein for the Nigerian populace come mainly from livestock in form of poultry, beef, mutton and pork. These traditional sources are being faced by certain constraints such as the persistent and severe sahelian drought, diseases, high cost of feed, primitive animal husbandry techniques and the low productivity of local animal breeds. The rapid growth of human population (Oyenuga, 1968) together with the rising standard of living has also placed great pressure on the existing sources of animal protein.

The constraints earlier enumerated limit radical increase in the domestic livestock production in Nigeria; hence other non-conventional sources of protein are being investigated. Land snails are non-conventional wildlife protein source. Human consumption of the land snails has been practised since the very earliest times. The main users are at present the populations of West Africa and West Europe and their markets are supplied, mainly, with wild snails. In Nigeria, the edible land snails are fast becoming culinary delicacies (referred to as "Congo meat") and demand has been so great that snail farming is gaining importance (Odaibo, 1997). The snail represents food of high nutritive value with a shell mainly composed of calcium carbonate, and flesh consisting of water (at least 70 %) and protein (about 60-70 % on dry basis). The giant snails are rich in lysine and generally low in cholesterol.

Snails now constitute an important source of animal protein for many coastal communities in Nigeria and Ghana. Marine and terrestrial gastropods collected for food include the following species (Yoloye, 1984):

1. The abalome – *Maloitis tuberculata*.
2. The large Ghana cowrie- *Cypraea stecocoratia.*
3. The rock limpet-*Patella safiana*.
4. The lagoon whelk-*Semifuscus morio*.
5. The lined mangrove periwinkle- *Littorina angulifera*.
6. *Thais califera* and *Thais haemastoma* (the dog whelk).
7. The large volute-*Cymbium cymbium*.
8. The Nigeria garden snail (Ipere) *Limicolaria* sp.
9. The giant African land snail-*Archachatina marginata.*
10. The mangrove mud periwinkle-*Tympanotonus fuscutus.*
11. *Archatina* sp. (Ilako).

In West Africa, particularly in Nigeria, various species of *Archatina* and *Archachatina* are eaten to a great extent. In some cases they actually form the largest single item of animal protein in the diet of the common people in rural areas (Odaibo, 1997).

Few publications are available on the nutritional qualities of Nigeria land snails. Published works include Odaibo (1997) on snail and snail farming; Cooper and Knowler (1991) on snails and snail farming (an introduction); Adeyeye (1996) on waste yield, proximate and mineral composition of three different types of land snails found in Nigeria; Adeyeye (1998) on the mineral composition of the haemolymph of three different types of land snails consumed in Nigeria; and Adeyeye and Afolabi (2004) on the amino acid composition of three different types of land snails consumed in Nigeria. The study in this paper is therefore, an attempt to assess the lipid concentration (crude fat, fatty acids, phospholipids and zoosterols) from land snails consumed in the Southwest zone of Nigeria. These are the Nigeria garden snail (*Ipere*) *Limicolaria* sp.; (*Ilako*) *Archatina (archatina) archatina* (Linne) and the giant African land snail, *Archachatina (Calachatina) marginata* (Swaison).

II. Resources and Techniques

a) Materials

Snail samples were collected from the farm at Odo Ayedun-Ekiti, Ekiti State, Nigeria. The samples were

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collected in the month of June, 2011. Samples were then identified.

Samples were washed with distilled water and then wrapped separately in aluminium foil and frozen at -4°C for 5 days before samples were prepared for analysis.

The shells were carefully removed to recover the edible parts. The edible parts were cut into small pieces and dried at 95-105 °C until dried and ground into fine powder.

b) Determination of ether extract

An aliquot (0.25 g) of each part was weighed in an extraction thimble and 200 ml of petroleum ether (40 -60 °C boiling range) was added. The covered porous thimble containing the sample was extracted for 5 h using a Soxhlet extractor. The extraction flask was removed from the heating mantle when it was almost free of petroleum ether, oven dried at 50 °C for 1 h, cooled in a desiccator and the weight of dried oil was determined (AOAC, 2005). Determinations were in duplicate.

c) Preparation of fatty acid methyl esters and analysis

A 50 mg aliquot of the dried oil was saponified for 5 min at 95 °C with 3.4 ml of 0.5 MKOH in dry methanol. The mixture was neutralized by 0.7 MHCl and 3 ml of 14 % boron triflouride in methanol was added. The mixture was heated for 5 min at 90 °C to achieve complete methylation. The fatty acid methyl esters were thrice extracted from the mixture with redistilled *n*-hexane and concentrated to 1 ml for analysis. The fatty acid methyl esters were analysed using an HP 5890 gas chromatograph (GMI, Inc., Minnesota, USA) fitted with a flame ionization detector and using ChemStation software. Nitrogen was used as the carrier gas with a flow rate of 20-60 ml/min. The oven programme was: initial temperature at 60 °C, ramping at 10 °C/min for 20 min, held for 4 min, with a second ramping at 15 °C/min for 4 min and held for 10 min. The injection temperature was 250 °C and the detector temperature was 320 °C. A capillary column 30 m x 0.25 mm was packed with a polar compound (HP INNOWAX) onto a diameter of 0.25 μm was used to separate the esters. A split injection was used with a split ratio of 20:1. The peaks were identified by their relative retention time compared with known standards (AOAC, 2005). Determinations were in duplicate.

d) Sterols analysis

Aliquots of the dried oil were added to screw-capped test tubes. The samples were saponified at 95 °C for 30 min, using 3 ml of 10 % KOH in ethanol, to which 0.20 ml of benzene was added to ensure miscibility. Deionised water (3 ml) was added and 2 ml of hexane was used in extracting the non-saponifiable materials. Three extractions, each with 2 ml of hexane, were carried out for 1 h, 30 min and 30 min respectively, to achieve complete extraction of the sterols. Hexane was concentrated to 1 ml for gas chromatographic analysis (AOAC, 2005). Determinations were in duplicate.

e) Phospholipids analysis

Using a modified method of Raheja *et al.* (1973), 0.01 g of the dried oil was added to test tubes. Any remaining solvent was removed by passing a stream of nitrogen gas over the oil. Then 0.40 ml of chloroform was added, followed by addition of 0.10 ml of the chromogenic solution. The tube was heated to 100 °C in a water bath for 1 min 20 sec, cooled to room temperature, 5 ml of hexane was added and the tube was shaken gently several times. After separation of the solvent and aqueous layers, the hexane layer was recovered and concentrated to 1.0 ml for analysis. Analysis was performed using the gas chromatograph with a capillary column 30 m x 0.25 mm packed with a polar compound (HP 5) onto a diameter 0.25 μm. The oven programme was: initially at 50 °C, ramping at 10 °C/min for 20 min, held for 4 min, a second ramping at 15 °C/min for 4 min and held for 5 min. The injection temperature was 250 °C, and the detector temperature was 320 °C. As previously described, a split injection type was used having a split ratio of 20:1. Peaks were identified by comparison with the known standards. Determinations were in duplicate.

f) Quality assurance

Standard chromatograms were prepared for sterols, phospholipids and fatty acid methyl esters which were then compared with respective analytical results; calibration curves were prepared for all the standard mixtures and correlation coefficient was determined for each fatty acid (34), sterol (7) and phospholipid (5). Correlation coefficient > 0.95 was considered acceptable

g) Statistical analysis

Statistical analysis (Oloyo, 2001) was carried out to determine the mean, standard deviation, coefficient of variation in per cent. Also calculated were the chi-square (X^2) values. The X^2 was subjected to the table (critical) value at $\alpha = 0.05$ to see if significant differences existed in the values of fatty acids, sterols and phospholipids between the snail samples.

III. Results

a) Fatty acids

In Table 1, the crude fat varied between 2.22 g/100 g to 2.38 g/100 g. The values were close with the coefficient of variation per cent (CV %) being low at 3.67. The total energy coming from the crude fat was also low at 82.1-88.1 kJ/100 g.

Table 2 contains the saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) values. The following acids under this section were not detected:

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C2:0, C3:0, C4:0 (in *Archachatina marginata)* and *Archatina archatina,* C6:0, C8:0, C12:0 in *A. marginata* and *Limicalaria* sp. whereas the following fatty acids (FAs) recorded 0.00 % of the total fatty acids: C5:0, C20:0, C24:0, C22:*1 cis*-13 and C24:*1 cis-15.* Low levels of C4:0, C10:0, C12:0, C14:0, C22:0, C14:*1 cis-9*, C16:*1 cis*-9 and C20:*1 cis-11* with each of their value being less than 1.0 % of total fatty acids. It is however interesting to observe that most of their CV % values were mostly 37.2-37.7 with only one (C14:*1 cis-9*) having a CV % of 26.7. Among the SFA, C18:0 was the most concentrated in all the samples and had a range of 23.4-28.7 % total FA with CV % of 10.2. This was closely followed by C16:0 with values of 13.6-19.4 % and CV % of 17.5. For the two FAs the trend of concentration was *Limicolaria* sp > *A. marginata* > *Archatina archatina*. Total SFA range was 49.8 % (*Limicolaria* sp) > 43.0 % (*A.marginata*) > 37.5 % (*Archatina archatina*). Among C18:*1 cis* MUFA, C18:1 *cis*-6 was the most concentrated with values of 2.43-3.41 % and CV % of 17.8 whereas C18: 1*cis*-9 followed with values of 2.41-6.11 % but higher CV % of 57.1. The best source of C18:1 *cis*-9 was in *Limicolaria* sp. (6.11 %). Total MUFA (*cis*) range was 6.11-9.15 % and CV % of 22.4. All the C18:1 *trans* levels were mostly higher than the C18:*1 cis* but with lower levels of CV %. C18:*1 trans*-6 had a range of 6.35-8.44 % and CV % of 15.4; in C18:1 *trans*-9, range was 5.08-6.07 % and CV % of 9.30; and C18:1 *trans*-11 being 3.17-3.94 % and CV % of 11.4. Total C18:1 *trans* was 15.6-17.4 % with CV % of 6.72. The total MUFA values had *a* distribution of 24.8 % (*Limicolaria* sp.) > 24.0 % (*A. marginata*) > 23.8 % (*Archatina archatina*) and low CV % of 2.19.

In Table 3, PUFA *n*-6 and *n*-3 FA composition of the three snail samples were depicted. The following *n-6* PUFA had 0.00 % total fatty acids observed for them: C20:3 *cis*-8, 11, 14, C20:4 *cis*-5, 8, 11, 14 and C22:2 *cis*-13, 16; for the *n*-3 PUFA, the following were in similar category: C20:3 *cis*-11, 14, 17 and C22:6 *cis*-4, 7, 10, 13, 16, 19. Three prominent *n*-6 FAs were observed in the three samples. The first and mostly concentrated in all the samples was C20: 2 *cis*-11, 14 with value range of 8.36-16.7 % and CV % of 33.2. The next highest *n-6* FA was C18:2 *cis*-9, 12 with a range of 7.30-9.51 % and CV % of 13.2. C18: 3 *cis*-6, 9, 12 was low in value with a range of 1.07-1.27 % but least CV % (8.67) among the three. Total *n-6* PUFA (*cis*) was 27.4 % (*Archatina archatina*)> 22.6 % (*A. marginata*) > 16.7 % (*Limicolaria* sp.) with CV % of 24.1. The only *n*-6 but important PUFA *trans* was C18:2 *cis*-9, *trans*-11 (rumenic acid) with comparable levels as C18:2 *cis-9, 12*, rumenic acid had a range of 7.37-9.79 % with CV % of 14.1. Total *n-6* PUFA was 37.2 % (*Archatina archatina*) > 31.4 % (*A. marginata*) > 24.1 % (*Limicolaria* sp.) with CV % of 21.2. The only *n*-3 PUFA having values greater than 0.00 % was C18:3 *cis* -9, 12, 15 with values of 1.37-1.65 % and CV % of 9.28. The values of C18:3 *cis-9*, 12, 15 were much lower than the values of C18: 2 *cis* -9, 12, both are essential fatty acids. The total *n*-6 + *n*-3 PUFA values were 38.7 % (*Archatina archatinna*) > 33.1 (*A. marginata*) > 25.5 % *(Limicolaria* sp.) with CV % of 20.4. Thus the total MUFA +PUFA range of 50.3 -62.5 % showed that the three snails were mostly composed of unsaturated fatty acids in their lipids composition.

The summary of the statistical results from Tables 2 and 3 is shown in Table 4. The X^2 results showed that no significant differences existed among the samples between their SFA, MUFA, DUFA and TUFA values. However, such results within a particular snail sample were highly and positively significantly different at $\alpha_{=0.05}$ since the result values of 30.9-45.1 were all higher (individually) than the critical table value of 7.82.

b) Phospholipids

Phospholipids level (mg/100 g) of the samples are in Table 5. The overall values were generally low at 1.55-2.88 mg/100 g (dry weight) and CV % of 31.3. Only lecithin had values greater than 1.00 mg/100 g in two samples and the percentage concentration ranged from 57.5-61.8 % and CV % of 29.1. Cephalin ranged between 0.226 mg/100 g and 0.450 mg/100 g and it was the second highest phospholipid. Table 5 results were subjected to X^2 analysis as shown in Table 6. At the row and vertical levels, no significant difference was observed at $\alpha_{=0.05.}$

c) Sterols

Table 7 depicted the sterols level (mg/100 g) of the samples. Only cholesterol was having values greater than 0.00 % in the samples. Even the cholesterol levels were low at 37.1-45.1 mg/100 g and close at 9.86 % coefficient of variation. Their X^2 value was also much less (0.805) than the critical value of 5.99 at $\alpha_{=0.05}$ thereby making the results not significantly different among the samples.

IV. Discussion

a) Fatty acids

The crude fat levels of 2.22-2.38 g/100 g in Table 1 were found to be much lower than other animal protein sources found in literature. Some literature crude fat levels were: 67 % (beef fat), 72 % (lamb fat), 71 % (pork fat), duck meat and skin (43 %), calf liver (7 %), chicken, meat and skin (18 %) (Bender, 1992). However the snails crude fat levels compared favourably with what obtains in the skin of *Oreochromis niloticus* fish having a value of 2.25 g/100 g (dry weight) (Adeyeye, 2011) but better than the muscle of *O. niloticus* having a value of 0.228 g/100 g; and also greater than the crude fat levels of the body of Tongue sole fish with: 0.360 g/100 g (skin) and 0.027 g/100 g (muscle) (Adeyeye *et al.*, 2011). The calculated energy from the crude fat gave values of 82.1-88.1 kJ/100 g. For somebody that requires 2500 daily calories and 15 % coming from fat oil consumption, this translates to 41.6 g of fat per day.

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From the present report, a person for optimum weight loss, may reduce the overall fat/oil consumption by eating snail meat. The crude fat levels in the snails showed that each would almost supply equal levels of crude fat and energy as shown by their low CV %.

Among the short-chain fatty acids in the samples is the C4:0. It constituted just 1.09 % total fatty acid in the *Limicolaria* sp. It is mostly found in butterfat from cows. This fatty acid has antimicrobial properties-that is; it protects us from viruses, yeasts and pathogenic bacteria in the gut. They do not need to be acted on by the bile salts but are directly absorbed for quick energy. For this reason, they are less likely to cause weight gain than olive oil or commercial vegetable oils (Portillo *et al*., 1998). Short- chain fatty acids also contribute to the health of the immune system (Kabara, 1978). Medium – chain fatty acids have eight to twelve carbon atoms and are common in butterfat and the tropical oils. In the present samples C10:0 and C12:0 were present in minor quantities in the samples. Like the short-chain fatty acids, these fats have antimicrobial properties; are absorbed directly for quick energy; and contribute to the health of the immune system.

Long-chain fatty acids have from 14 to 18 carbon atoms and can be either saturated, monounsaturated or polyunsaturated. Myristic acid (14:0) is a ubiquitous component of lipids in most living organisms, but usually at levels of 1-2 % only. In the present samples C14:0 ranged from 0.116-0.256. However, it is more abundant in cow's milk fat, some fish oils and in those seed oils enriched in medium-chain fatty acids (e.g. coconut and palm kernel). In *O. niloticus* fish C14:0 formed 6.59 % FA in the skin and 4.19 % in the muscle (Adeyeye, 2011) whereas it was 1.12 % (skin) and 1.05 % (muscle) of Tongue sole fish (Adeyeye *et al*., 2011). This fatty acid is found very specifically in certain proteolipids, where it is linked via an amide bond to an N-terminal glycine residue, and is essential to the function of the protein components. Palmitic acid (16:0) is usually considered the most abundant SFA in nature, and it is found in appreciable amounts in the lipids of animals, plants and lower organisms. It comprices 20-30 % of the lipids in most animal tissues, and it is present in amounts that vary from 10 to 40 % in seed oils. The present results are at variance with these earlier observations. Here it comprised of 13.6 – 19.4 % and it is second to stearic acid. Stearic acid (18:0) is the second most abundant SFA in nature, and again it is found in the lipids of most living organisms. In these samples (18:0) occupied the highest position (23.4-28.7 %) in the SFA group. In lipids of some commercial importance, it occurs in the highest concentrations in ruminant fats (milk fat and tallow) or in vegetables oils such as cocoa butter, and in industrially hydrogenated fats. It can comprise 80 % of the total fatty acids in gangliosides. The other SFA present in minor level was behenic acid (C22:0), a member of the very-long-chain fatty acid. The total SFA of 37.5-49.8 % could easily compare favourably with literature values; they are: 43 % (beef fat), 50 % (lamb fat), 37 % (pork fat), 33 % (chicken, meat and skin), 27 % (duck, meat and skin), 30 % (calf liver) (Bender, 1992).

Oleic acid [9*c*-18:1 or 18:1(*n*-9)] is by far the most abundant monoenoic fatty acid in plant and animal tissue, both in structural lipids and in depot fats. It comprised 6.11 % of *Limicolaria* sp. FA being the highest of the *cis*-MUFA. Olive oil contains up to 78 % of oleic acid, and it is believed to have especially valuable nutritional properties as part of the Mediterranean diet. It has a number of important biological properties, both in the free and esterified form. Oleic acid is the biosynthetic precursor of a family of fatty acid with the (*n*-9) terminal structure and with chain-lengths of 20-24 or more. Petroselinic acid (6*c*-18:1) occurs up to a level of 50 % or more in seed oils of the Umbelliferae family, including carrot, parsley and coriander. In the present report, petroselinic acid occupied the highest position in the *cis*-18:1 FA in both *A. marginata* (3.41 %) and *Archatina archatina* (3.33 %) but second highest position in *Limicolaria* sp. (3.06 %). These values are close having a CV % of 17.8.

Trans-18:1 of reasonable levels were *trans* petroselinic acid (C18:1 *trans*-6) (6.35-8.44 %), elaidic acid (C18:*1 trans-9*) (5.08-6.07 %), vaccenic acid (C18:1 *trans*-11) (3.17-3.94 %). Tissues of ruminant animals, such as cows, sheep and goats, can contain a number of different 18:1 isomers like: C18:1 *trans-9* (5.0 %) and C18:1 *cis*-9 (85 %), C18:1 *trans*-11 (47 %) and C18:1 *cis*-11 (47 %) (Hay and Morrison, 1973) with the *cis*-isomers, 9- and 11-18:1 slightly predominate as might be expected. 11*t*-18:1 makes up 50 % of *trans*-monoenes in ruminant animal tissues (which can comprise 10-15 % of the total monoenes or 3-4 % of the total fatty acids). In the present report C18:1 *trans*-11 had a range of 3.17-3.94 % of the total fatty acids and 20.3-22.3 % of the *trans*-monoenes or 12.8-16.6 % of the total monoenes. *cis*-vaccenic acid [11*c*-18:1 or 18:1 (n-7) is a common monoenoic fatty acid of bacterial lipids, and it is usually present but as a minor component of plant and animal tissues. It is occasionally a more abundant constituent of plants, for example those containing appreciable amounts of its biosynthetic precursor, 9-16:1 (e.g. the fruit of sea buckthorn). Note that vaccenic acid per se is the *trans* isomer. 11-*cis*-Eicosenoic acid [11-20:1 or 20:1 (*n*-9), gondoic] is a common if minor constitutent of animal tissues and fish oils, often accompanied by the 13-isomer. It is also found in rapeseed oil and seed oils of related species. It occupied a level of 0.069-0.152 % in the present snail samples.

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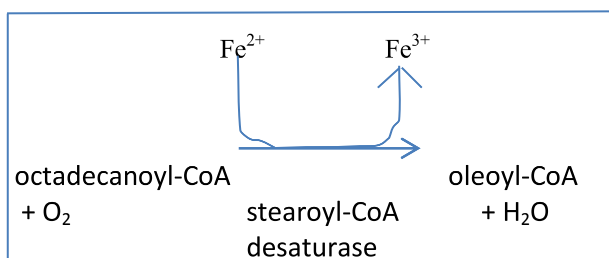
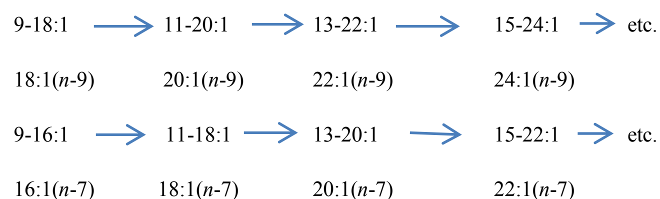
In nearly all higher organisms, including many bacteria, yeasts, algae, plants and animals, double bonds are introduced into fatty acids by an aerobic mechanism that utilizes preformed fatty acids as the substrate. Molecular oxygen and a reduced pyridine nucleotide (NADH or NADPH) are required cofactors. Thus in animals and yeasts, the coenzyme A ester of octadecanoic (stearic) acid is converted directly to oleoyl-CoA by a concerted removal of hydrogen atoms from carbons 9 and 10 (D-stereochemistry in each instance). The stearoyl-CoA desaturase system is in the endoplasmic reticulum membrane with the active centre exposed to the cytosol, and consists of three proteins, cytochrome b5 reductase, cytochrome b5, and the desaturase, which contains two atoms of iron at the active site.



Membrane – bound enzymes are notoriously difficult to purify, but the evidence suggests that the yeast Δ9 desaturase consists of two membrane spanning regions with the bulk of the protein protruding into the cytosol. The enzyme has much in common with hydroxylases and contains eight essential histidine residues that coordinate with the di-iron centre at the active site. The cytochrome b5 component is fused to the desaturase and is believed to facilitate electron transfer from NADH reductase to the catalytic di-iron core.

Palmitoleate is synthesised from palmitate by a similar mechanism. Subsequently, oleate can be chain elongated by two carbon atoms to give longer-chain fatty acids of the (*n*-9) family, while palmitoleate is the precursor of the (*n*-7) family of fatty acids. In mammalian systems the elongases are known to be distinct enzymes that differ from those involved in the production of longer-chain polyunsaturated fatty acids. *Alpha*-and *beta*-oxidation can also occur to give shorter chain components of the two families.



Petroselinic acid (6-18:1) in seed oils of the Unbelliferae is synthesised by an enzyme that removes hydrogens from position 4 of palmitate, before the resulting 4-16:1 is elongated by two carbon atoms.

The relative proportion of SFA/MUFA is an important aspect of phospholipid compositions and changes to this ratio have been claimed to have effects on such disease states as cardiovascular disease, obesity, diabetes, neuropathological conditions and cancer. For example, they have been shown to have cyto-protective actions in pancreatic β-cells. *cis*-Monoenoic acids have desirable physical properties for membrane lipids in that they are liquid at body temperature, yet are relatively resistant to oxidation. They are now recognised by nutritionists as being beneficial in the human diet.

Current nutritional thinking appears to be that dietary *trans*-monoenoic fatty acids, both from ruminant fats and from industrial hydrogenation processes, should be considered as potentially harmful and in the same light as saturated fatty acids.

In Table 3, the five important long-chain and very –long-chain fatty acids were C18:2 *cis*-9, 12, C18: *3 cis-6, 9, 12*, C 18:*2 cis-9, trans-11*, C18:*3 cis-9, 12, 15* (all in the group of long-chain FAs) and C20:*2 cis-11, 14* (under very-long-chain FAs). The two essential fatty acids are C18:*2 cis-9, 12* and C18:*3 cis-9, 12, 15* with respective values of 7.30-9.51 % and 1.37-1.65 %. Another important long-chain fatty acid is gamma-linolenic acid (GLA). It formed a level of 1.07-1.27 % in the snails. It is found in evening primrose, borage and black currant oils. The body makes GLA out of omega-6 linoleic acid and uses it in the production of substances called prostaglandins, localized tissue hormones that regulate many processes at the cellular level. Eicosadienoic acid [C20:2 *cis-11*, 14 or 20:2 (*n*-6) all-*cis*-11, 14-eicosadienoic acid] or homo-gamma-linoleic acid is an uncommon naturally occurring PUFA. It is not enriched in any particular tissue, it is rare in all lipid classes. Dietary sources include herring and menhaden oils, cattle liver, swine brain lipid, shark oil (Yagaloff *et al*., 1995). Homo-γ-LA had levels of 8.36-16.7 % in the total fatty acids of the snails, being the highest concentrated among the total PUFA FAs or 32.8-43.2 % of the PUFA. The FA inhibits the binding of [^{3}H]-ITB$_4$ to pig neutrophil membrane with a Ki of 3μm.

The levels of C18:2 *cis* -9, *trans*-11 ranged from 7.37-9.77 % as seen in Table 3. These levels were more than their corresponding LA (7.30-9.51 %, also Table 3). In Table 2 vaccenic acid levels ranged from 3.17-3.94 %. Conjugated linoleic acids make up a group of polyunsaturated FAs found in meat and milk from ruminant animals and exist as a general mixture of conjugated isomers of LA. Of the many isomers

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identified, the *cis*-9, *trans* -11 CLA isomer (also referred to as rumenic acid or RA) accounts for up to 80-90 % of the total CLA in ruminant products (Nuernberg *et al*., 2002). Naturally occurring CLAs originate from two sources: bacterial isomerization and/or biohydrogenation of trans-fatty acids in the adipose tissue and mammary glands (Griinari *et al*., 2000). Microbial biohydrogenation of LA and aLA by an anaerobic rumen bacterium *Butyrivibrio fibrisolvens* is highly depend on rumen pH (Pariza *et al*., 2000). Grain consumption decreases rumen pH, reducing *B. fibrisolvens* activity, conversely grass-based diets provide for a more favourable rumen environment for subsequent bacterial systhesis (Bessa *et al*., 2000). Rumen pH may help to explain the apparent differences in CLA content between grain and grass-finished meat products. *De novo* synthesis of CLA from 11*t*-*C*18:1 TVA has been documented in rodents, dairy cows and humans. Studies suggest a linear increase in CLA systhesis as the TVA content of the diet increased in human subjects (Turpeinen *et al*., 2002). The rate of conversion of TVA to CLA has been estimated to range from 5 to 12 % in rodents to 19 to 30 % in humans (Turpeinen *et al*., 2002). True dietary intake of CLA should therefore consider native 9*c*11*t*-C18:2 (actual CLA) as well as the 11*t* - C18:1 (potential CLA) content of foods (Adlof *et al*., 2000).

Over the past two decades numerous studies have shown significant health benefits attributable to the actions of CLA, as demonstrated by experimental animal models, including actions to reduce carcinogenesis, atherosclerosis, and onset of diabetes (Kritchevsky *et al*., 2000). Conjugated LA has also been reported to modulate body composition by reducing the accumulation of adipose tissue in a variety of species including mice, rats, pigs, and now humans (Smedman and Vessby, 2001). Optimal dietary intake remains to be established for CLA. It has been hypothesized that 95 mg CLA/day is enough to show positive effects in the reduction of breast cancer in women utilizing epidemiological data linking increased milk consumption with reduced breast cancer (Knekt *et al*., 1996). Ha *et al*. (1989) published a much more conservative estimate stating that 3 g/day CLA is required to promote human health benefits. Ritzenthaler *et al*. (2001) estimated CLA intakes of 620 mg/day for men and 441 mg/day for women are necessary for cancer prevention. Obviously, all these values represent rough estimates and are mainly based on extrapolated animal data. What is clear is that we as a population do not consume enough CLA in our diets to have a significant impact on cancer prevention or suppression. Reports indicate that Americans consume between 150-200 mg/day, Germans consume slightly more between 300-400 mg/day (Ritzenthaler *et al*., 2001), and the Australians seen to be closer to the optimum concentration at 500-1000 mg/day according to Parodi (1994).

The relative values of PUFA in all the samples made them important in diet. The eicosanoids help regulate blood clot formation, blood pressure, blood lipid (including cholesterol) concentration, the immune response, the inflammation response to injury and infection and many other body functions (Whitney *et al*., 1994). A deficiency of *n-6* fatty acids in the diet leads to skin lesions. A deficiency of *n-3* fatty acids leads to subtle neurological and visual problems. Deficiencies in PUFA produce growth retardation, reproductive failure, skin abnormalities and kidney and liver disorders. However, people are rarely deficient in those fatty acids (Tapiero *et al*., 2002). The relative amounts of PUFA and SFA in oils is important in nutrition and health. The ratio of PUFA/SFA (P/S ratio) is therefore important in determining the detrimental effects of dietary fats. The higher the P/S ratio the more nutritionally useful is the oil. This is because the severity of atherosclerosis is closely associated with the proportion of the total energy supplied by saturated fats and polyunsaturated fats (Honatra, 1974). The present PUFA/SFA varied between 0.512-1.03 which were averagely normal. The *n*-6 and *n*-3 FAs have critical roles in the membrane structure (Kinsella, 1990) and as precursors of eicosanoids, which are potent and highly reactive compounds. Since they compete for the same enzymes and have different biological roles, the balance between the *n*-6 and *n*-3 FAs in the diet can be of considerable importance (WHO/FAO, 1994). The ratio of *n-6* to *n-3* or specifically LA to aLA in the diet should be between 5:1 and 10:1 (WHO/FAO, 1994) or 4-10 g of *n-6* FAs to 1.0g of *n-3* FAs (Canadian Government Publishing Center, 1990). As LA is almost always present in foods, it tends to be relatively more abundant in animal tissues. This is supported in the present report as follows: C18:2 (*n*-6) ranged as 7.30-9.51 % whereas C18:3(*n*-3) ranged as 1.37-1.65 %. In turn, these FAs are the biosynthetic precursors in animal systems of C20 and C22 PUFAs, with 3-6 double bonds, via sequential desaturation and chain-elongation steps (desaturases in animal tissues can only insert a double bond on the carboxyl side of an existing double bond) (Berg *et al*., 2007). Whilst it would be easy for the body to synthesize arachidonic acid [20:4(*n*-6)] from [18:2(*n*-6)], it may be difficult to synthesize the *n*-3 PUFA series especially eicosapentaenoic acid [20:5(*n*-3) or EPA] because of the low level of C18:3(*n*-3) and so the diet must be enhanced in this PUFA. However, the 2*n*-6/3*n*-3 fell within the above ratio as 5.3:1, 5.3:1 and 6.2:1.

Literature results for MUFA were: beef fat (48 %), lamb fat (39 %), pork fat (41 %), chicken, meat and skin (42 %), duck, meat and skin (54 %) and calf, liver (54 %); their corresponding PUFA were: beef fat (4 %), lamb fat (5 %), pork fat (15 %), chicken, meat and skin

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(19 %), duck, meat and skin (12 %), calf, liver (26 %) (Bender, 1992). All the snail MUFA levels were lower than the literature values shown above as the MUFA snail levels were 23.8-24.8 %. On the other hand all the PUFA levels in the snails were higher than the literature PUFA levels shown above since snails had levels of 25.5-38.7 %. The C18:2 levels from the literature were (in %): rabbit, lean (13.5), brain, sheep (0.4), liver: ox (7.4), sheep (5.0), pig (14.7), calf (15.0) (Paul and Southgate, 1978) which were highly comparable with the snail results at 7.30-9.51 %. From literature for C18:3, we had (in %): rabbit, lean (0.7), brain, sheep (-), liver: ox (2.5), sheep (3.8), pig (0.5), calf (1.4) (Paul and Southgate, 1978); these results were highly comparable to the snail C18:3 results of 1.37-1.65%.

The statistical analysis of the results in Tables 2 and 3 as summarised in Table 4 showed that all the results in the row columns were not significantly different at $\alpha = 0.05$. On the other hand all the vertical column results were significantly different among themselves at $\alpha = 0.05$. It should be noted particularly for cases where there are more than two categories or groups that X^2 cannot indicate or specify where the significant difference lies, a situation similar to that found in ANOVA. However, post hoc tests that provide solution to the problem when encountered in ANOVA cannot be applied to Chi Square test. In case of X^2 – test, the category that contributes the highest proportion is declared as one that differs significantly from others (Oloyo, 2001). This meant that the SFA was significantly different from other members in each vertical column since it contributed the highest proportion.

b) Phospholipids

Table 5 shows the level of various phospholipids in the samples. Phospholipids are not essential nutrients: they are just another lipid and, as such, contribute 9 kcalories per gram of energy. Minor contributors to the phospholipids level were phosphatidylethanolamine (PE), phosphatidylserine (Ptd-L-Ser or PS), lysophosphatidylcholine and phosphatidylinositol (PI), each of them contributed less than 1.0 mg/100 g in each of the snail samples. The total phospholipids level ranged from 1.55-2.88 mg/100 g showing the snails to be low in phospholipid content. The only phospholipid with values closer to 1.0 mg/100 g was lecithin (phosphatidylcholine) with values range of 0.958-1.72 mg/100 g. Lecithin is usually the most abundant phospholipid in animals and plants, often amounting to almost 50 % of the total, and as such it is the key building block of membrane bilayers. This observation is true for lecithin values in these results with percentage values ranging from 57.5-61.8 %. Phosphatigylcholines (PC) are a class of phospholipids that incorporate choline as a headgroup. They are a major component of biological membranes and can be easily obtained from a variety of readily available sources such as egg yolk or soy beans from which they are mechanically extracted or chemically extracted using hexane. They are also a member of the lecithin group of yellow-brownish fatty substances occurring in animal and plant tissues. Phosphatidylcholines are such a major component of lecithin that in some contexts the terms are sometimes used as synonyms. However, lecithin extract consists of a mixture of phosphatidylcholine and other compounds. It is also used along with sodium taurocholate for stimulating fed- and fasted-state biorelevant media in dissolution studies of highly-lipophilic drugs. Phosphatidylcholine is more commonly found in the exoplasmic or outer leaflet of a cell membrane. It is thought to be transported between membranes within the cell by phosphatidylcholine transfer protein (PCTP) (Wirtz, 1991). Phosphatidylcholine also plays a role in membrane-mediated cell signalling and PCTP activation of other enzymes (Kanno *et al.*, 2007). At birth and throughout infancy, phosphatidylcholine concentrations are high (as high as 90 % of the cell membrane), but it is slowly depleted throughout the course of life, and may drop to as low as 10 % of the cellular membrane in the elderly. As is such, some researchers in the fields of health and nutrition have begun to recommend daily supplementation of phosphatidylcholine as a way of slowing down senescence (Mei-Chu, 2001) and improving brain functioning and memory capacity (Chung *et al.,* 1995). In addition to the increased caloric burden of a diet rich in fats like phosphatidylcholine, a recent report has linked the microbial catabolites of phosphatidylcholine with increased atherosclerosis through the production of choline, trimethylamine oxide and betaine (Wang *et al.*, 2011). The present snail samples were all low in both total fat and phosphatidylcholine. In Table 6, the X^2 values at both the row column and vertical column were not significantly different at $\alpha_{= 0.05.}$

c) Sterols

The sterol results in Table 7 showed the values to be low at 37.1-45.1 mg/100 g and close in the samples with CV % of 9.86. Only cholesterol was greater than 0.00 mg/100 g in the samples. Cholesterol is a high-molecular-weight alcohol that is manufactured in the liver and in most human cells. Like saturated fats, the cholesterol we make and consume plays many vital roles. Along with saturated fat, cholesterol in the cell membrane gives our cells necessary stiffness and stability. This is why serum cholesterol levels may go down temporarily when we replace saturated fats with polyunsaturated oils in the diet (Jones, 1997). Cholesterol acts as a precursor to vital corticosteroids, hormones that help us deal with stress and protect the body against heart disease and cancer; and to the sex hormones like androgen, testosterone, estrogen and progesterone. Cholesterol is a precursor to vitamin D, a

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very important fat-soluble vitamin needed for healthy bones and nervous system, proper growth, mineral metabolism, muscle tone, insulin production, reproduction and immune system function. The bile salts are made from cholesterol. Bile is vital for digestion and assimilation of fats in the diet. Recent research shows that cholesterol acts as an antioxidant (Cranton and Frackelton, 1984). This is the likely explanation for the fact that cholesterol levels go up with age. As an antioxidant, cholesterol protects us against free radical damage that leads to heart disease and cancer. Cholesterol is needed for proper function of serotonin receptors in the brain (Engelberg, 1992). Serotonin is in the body's natural "feel-good" chemical, low cholesterol levels have been linked to aggressive and violent behaviour, depression and suicidal tendencies. Mother's milk is especially rich in cholesterol and contains a special enzyme that helps the baby utilise the nutrient. Babies and children need cholesterol-rich foods throughout their growing years to ensure proper development of the brain and nervous system. Dietary cholesterol plays an important role in maintaining the health of the intestinal wall (Alfin-Slater and Aftergood, 1980). This is why low - cholesterol vegetarian diets can lead to leaky gut syndrome and other intestinal disorders.

Cholesterol levels in literature from many animal protein sources were much higher than the snail results. Values in mg/100 g were: fish (50-60), egg yolk (1260), meat and poultry (60-120), brain (2000-3000), liver (300-350) (Bender, 1992); others were rabbit, lean (71), brain, sheep (2200), liver: ox (270), sheep (430), pig (260) and calf (370) (Paul and Southgate, 1978). However the snail cholesterol levels were higher than in the fish (mg/100 g): 6.86 (skin) and 0.303 (muscle) of Tongue sole fish (Adeyeye *et al*., 2011); 31.6 (skin) and 4.35 (muscle) of *Oreochromis niloticus* (Adeyeye, 2011). Most authorities, but not all, recommend a reduction in dietary cholesterol to around 300 mg or less per day (Bender, 1992); all the snail results were much lower than 300 mg.

d) Quality assurance

The correlation determined for all the standards: fatty acids, phospholipids and sterols, all had values ranging as follows: 0.99833-0.99997 (fatty acids), 0.99909-0.99999 (phospholipids) and 0.99920-0.99994 (sterols); all the correlation values were greater than 0.95 which is the critical correlation for acceptance of these types of analytical results, thus attesting to the quality assurance of the determinations.

V. Conclusion

The findings of this study showed that the samples demonstrated the lipid concentration of ruminants with slight unequal distribution of all parameters determined. The samples were low in total fats, low concentration of cholesterol and phospholipids. All the samples had unsaturated acids as the predominant fatty acids with good percentage levels of C18:*1 cis-6*, C18:1 *cis*-9, C18:1 *trans*-6, C18:1 *trans*-11, C18:2 *cis* 9, 12, C20: 2 *cis*-11, 14 and *C*18:2 *cis*-9, *trans* 11. Significant differences occurred in the fatty acid levels. Quality assurances of the determinations were highly satisfactory.

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Table 1 : Crude fat and total energy levels of three different types of land snails in Nigeria

Parameter	*A marginata*	*Archatina archatina*	*Limicolaria* sp	Mean	SD	CV%
Crude fat (g/100 g)	2.38	2.35	2.22	2.32	0.09	3.67
Total energy (kJ/100 g)*	88.1	87.0	82.1	85.7	3.19	3.73

**Crude fat x 37 kJ; SD = standard deviation; CV % = coefficient of variation.*

Table 2 : Saturated and monounsaturated fatty acid composition of three different types of land snails in Nigeria (% total fatty acid)

Fatty acid	*A marginata*	*Archatina archatina*	*Limicolaria* sp	Mean	SD	CV%
Acetic acid (C2:0)	Nd	Nd	Nd	-	-	-
Propionic acid (C3:0)	Nd	Nd	Nd	-	-	-
Botanic acid (C4:0)	Nd	Nd	1.09	-	-	-
Pentanoic acid (C5:0)	0.00	0.00	0.00	0.00	0.00	0.00
Hexanoic acid (6:0)	Nd	Nd	Nd	-	-	-
Octanoic acid (C8:0)	Nd	Nd	Nd	-	-	-
Decanoic acid (C10:0)	0.143	0.069	0.152	0.122	0.046	37.7
Lauric acid (C12:0)	Nd	0.179	Nd	-	-	-
Myristic acid (14:0)	0.240	0.116	0.256	0.204	0.077	37.6
Palmitic acid (C16:0)	16.6	13.6	19.4	16.5	2.90	17.5
Stearic acid (C18:0)	25.8	23.4	28.7	26.0	2.65	10.2

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Arachidic acid (C20:0)	0.00	0.00	0.00	0.00	0.00	0.00
Behenic acid (C22:0)	0.215	0.104	0.229	0.183	0.068	37.5
Lignoceric acid (C24:0)	0.00	0.00	0.00	0.00	0.00	0.00
Total SFA	**43.0**	**37.5**	**49.8**	**43.4**	**6.16**	**14.2**
Myristoleic acid(C14:1*cis*-9)	0.445	0.256	0.363	0.355	0.095	26.7
Palmitoleic acid (C16:1*cis*-9)	0.086	0.042	0.092	0.073	0.027	37.2
Petroselinic acid(C18:1*cis*-6)	3.41	3.33	2.43	3.06	0.544	17.8
Oleic acid (C18:1 *cis*-9)	2.53	2.41	6.11	3.68	2.10	57.1
Gondoic acid (C20:1 *cis*-11)	0.143	0.069	0.152	0.121	0.046	37.5
Erucic acid (C22:1 *cis*-13)	0.00	0.00	0.00	0.00	0.00	0.00
Nervonic acid (C24:1*cis*-15)	0.00	0.00	0.00	0.00	0.00	0.00
MUFA (*cis*)	**6.61**	**6.11**	**9.15**	**7.29**	**1.63**	**22.4**
trans-Petroselinic (C18:1*trans*-6)	8.44	8.37	6.35	7.72	1.19	15.4
Elaidic acid (C18:1 *trans*-9)	5.08	5.35	6.07	5.50	0.512	9.30
Vaccenic acid (C18:1*trans*-11)	3.83	3.94	3.17	3.65	0.416	11.4
MUFA (*trans*)	**17.4**	**17.7**	**15.6**	**16.9**	**1.14**	**6.72**
MUFA (totals)	**24.0**	**23.8**	**24.8**	**24.2**	**0.529**	**2.19**

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; Nd = not deleted; - = not determined.

Table 3 : PUFA *n*-6 and *n*-3 fatty acid composition of three different types of land snails in Nigeria (% total fatty acid)

Fatty acid	*A marginata*	*Archatina archatina*	*Limicolaria* sp	Mean	SD	CV%
Linoleic acid (C18:2 *cis*-9, 12)	8.75	9.51	7.30	8.52	1.12	13.2
Gamma-linolenic acid (C18:3 *cis*-6,9,12)	1.27	1.21	1.07	1.18	0.103	8.67

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Eicosadienoic acid						
(C20:2 *cis*-11,14)	12.6	16.7	8.36	12.6	4.17	33.2
Dihomo-γ-linolenic acid						
(C20:3 *cis*-8, 11, 14)	0.00	0.00	0.00	0.00	0.00	0.00
Arachidonic acid (AA)						
(C20:4 *cis* 5, 8, 11, 14)	0.00	0.00	0.00	0.00	0.00	0.00
Docosadienoic acid						
(C22:2*cis*-13,16)	0.00	0.00	0.00	0.00	0.00	0.00
***n*-6 PUFA (*cis*)**	**22.6**	**27.4**	**16.7**	**22.2**	**5.36**	**24.1**
Rumenic acid						
(C18:2 *cis*-9, *trans*-11)	8.84	9.79	7.37	8.67	1.22	14.1
***n*-6 PUFA (totals)**	**31.4**	**37.2**	**24.1**	**30.9**	**6.56**	**21.2**
Alpha-linolenic acid (ALA)						
(C18:3 *cis*-9, 12, 15)	1.65	1.54	1.37	1.52	0.141	9.28
Eicosatrienoic acid (ETE)						
(C20:3 *cis-11, 14, 17*)	0.00	0.00	0.00	0.00	0.00	0.00
Cervonic acid (DHA)						
(C22:6 *cis*-4,7,10,13,16,19)	0.00	0.00	0.00	0.00	0.00	0.00
***n*-6 + *n*-3 (PUFA)**	**33.1**	**38.7**	**25.5**	**32.4**	**6.63**	**20.4**
Totals (SFA+MUFA+PUFA)	100	100	100	100	0.00	0.00
Totals (MUFA +PUFA)	**57.1**	**62.5**	**50.3**	**56.6**	**6.11**	**10.8**
PUFA/SFA	0.770	1.03	0.512	0.771	0.259	33.6
MUFA/SFA	0.558	0.635	0.498	0.564	0.069	12.2
2*n*-6/3*n*-3	5.30	6.18	5.33	5.60	0.500	8.92
Ratio	1:1	1:1	1:1	-	-	-

PUFA = unsaturated fatty acid.

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Table 4 : Statistical analysis of the results from Table 2 and 3

Fatty acid	*A. marginata*	*Archatina archatina*	*Limicolaria* sp	X^2	Remark
SFA	43.0	37.5	49.8	1.77	NS
MUFA	24.0	23.8	24.8	0.021	NS
DUFA	30.2	36.0	23.0	2.85	NS
TUFA	2.92	2.75	2.44	0.045	NS
X^2	33.5	30.9	45.1	-	-
Remark	*	*	*	-	-

*X^2 = chi-square; NS = not significant at $\alpha_{=0.05}$ and critical value of 5.99; * = significant at $\alpha_{=0.05}$ and critical value of 7.82; DUFA = diunsaturated fatty acid; TUFA = triunsaturated fatty acid.*

Table 5 : Phospholipids level (mg/100 g) of three different types of land snails in Nigeria

Phospholipid	*A marginata*	*Archatina archatina*	*Limicolaria* sp	Mean	SD	CV%
Cephalin (PE)	0.450 (15.6)	0.383 (13.4)	0.226 (14.6)	0.353	0.115	32.6
Lecithin	1.72 (59.7)	1.64 (57.5)	0.958 (61.8)	1.44	0.419	29.1
Ptd-L-Ser (PS)	0.244 (8.47)	0.35 (12.3)	0.186 (12.0)	0.260	0.084	32.2
Lysophosphati-dylcholine	0.209 (7.26)	0.214 (7.51)	0.088 (5.68)	0.170	0.071	41.9
PtdIns (PI)	0.263 (9.13)	0.261 (9.16)	0.096 (6.19)	0.207	0.096	46.4
Totals	2.88	2.85	1.55	2.43	0.759	31.3

PE = phosphatidylethanolamine; Lecithin = phophatidylcholine; PS = phosphatidylserine; PI = phosphatidylinositol; values in parentheses are in percentages. 14

Table 6 : Statistical analysis of the results from Table 5

Phospholipid	*A. marginata*	*Archatina archatina*	*Limicolaria* sp	X^2	Remark
PE	0.45	0.383	0.226	0.075	NS
Lecithin	1.72	1.64	0.958	0.243	NS
PS	0.244	0.351	0.186	0.054	NS
Lysophospha-tidylcholine	0.209	0.214	0.088	0.059	NS
PI	0.263	0.261	0.096	0.088	NS
X^2	2.88	2.56	1.73	-	-
Remark	NS	NS	NS	-	-

NS = not significant at $\alpha_{=0.05}$ (critical value = 5.99) on the row and critical value 9.49 at the column.

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Table 7 : Sterol level (mg/100 g) of three different types of land snails in Nigeria

Sterol	*A marginata*	*Archatina archatina*	*Limicolaria* sp	Mean	SD	CV%
Cholesterol$^+$	42.7	45.1	37.1	41.6	4.11	9.86

$^+X^2$ value was 0.805 and not significant at $\alpha_{= 0.05}$ and critical value of 5.99.

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Abstract - Fossil fuel resources are decreasing daily while biodiesel fuels are attracting increasing attention worldwide as blending components or direct replacements for diesel fuel in vehicle engines. This study investigated the physicochemical properties of oils extracted from Jatropha, neem, moringa and castor seeds for their suitability in biodiesel production. This is with a view to compare which of the oils has better functional properties for a quality biodiesel production. Our results has shown that all the oil from the plants seed study, have good physiochemical properties and are very good precursor for biodiesel synthesis.

Keywords : biodiesel, feedstock, biofuel, renewable.

GJSFR-B Classification : FOR Code: 090405

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Comparative Studies on the Functional Properties of Neem, Jatropha, Castor, and Moringa Seeds Oil as Potential Feed Stocks for Biodiesel Production in Nigeria

S.G. Zaku [α], S. A. Emmanual [σ], A.H. Isa [ρ] & A. Kabir [ω]

Abstract - Fossil fuel resources are decreasing daily while biodiesel fuels are attracting increasing attention worldwide as blending components or direct replacements for diesel fuel in vehicle engines. This study investigated the physicochemical properties of oils extracted from Jatropha, neem, moringa and castor seeds for their suitability in biodiesel production. This is with a view to compare which of the oils has better functional properties for a quality biodiesel production. Our results has shown that all the oil from the plants seed study, have good physiochemical properties and are very good precursor for biodiesel synthesis.

Keywords : biodiesel, feedstock, biofuel, renewable.

I. Introduction

The major percentages of energy used in the world today are being generated from fossil fuel sources. These fossil fuels are non-renewable resources that take millions of years to form and their reserves are being depleted faster than they are being regenerated. They are the major contributors and sources of green house gases, air pollution and global warming. Some of the emissions generated from these fossil fuels are CO, CO_2, NO_X, SO_X, unburnt or partially burnt hydrocarbon and particulate(Ndana et al.,2011). This rate of depletion and environmental issue is seriously calling for an alternative.

Biodiesel, a form of Biofuel is an answer to this call. It is a fuel derived from renewable biological sources that can be added to petroleum diesel as a blend or used on its own in diesel engines. The first diesel engines by Rudolph Diesel in the 1890s were designed to run on refined vegetable oils. Biodiesel fuel is now attracting increasing attention worldwide as a blending component or a direct replacement for diesel fuel in vehicle engines (Demirba, 2009). Biodiesel blends of up to B_{20} can be used in nearly all diesel equipment and are compatible with most storage and distribution equipment. These low-level blends generally do not require any engine modifications. Based on these criteria, Jatropha curcas , neem, moringa oleifera and castor oils have been found to be useful renewable sources for biodiesel production.

Jatropha curcas is drought-resistant oil bearing multi-purpose shrub/small tree, belonging to the family of *Euphor-biaceae* (Wang et al., 2011). It originates from Central America and is widely grown in Mexico, China, and north-east Thailand, India, Nepal, Brazil, Ghana, Mali, Zimbabwe, Nigeria, Ma-lawi, Zambia and some other countries (Baroi et al., 2009). The plants grow quickly forming a thick bushy fence in a short period of time of 6 – 9 months, and growing to heights of 4m with thick branches in 2–3 years. It grows in arid and semi arid climates and in a wide range of rainfall regimes, from 200 to 1500 mm per annum [5]. It can survive in poor stony soils, and has a life span of 50 years (Baroi et al., 2009). *Jatropha curcas* can produce significant amounts of oil in their respective seeds. The oil content of the seeds varies from 30 to 60% depending on the variety, place and the method of oil extraction (Baroi et al., 2009).

Neem (*Azadirochta indica A. Juss*) is a native Indian tree well known for its medicinal features. Most of the parts such as leaves, bark, flower, fruit, seed and root have applications in the field of medicine (Muthu et al., 2010). It is an evergreen tree related to mahogany, growing in almost every state of India, South East Asian countries and West Africa (Muthu et al., 2010). It grows in drier areas and in all kinds of soil. It contains several thousands of chemicals which are terpenoids in nature. A mature neem tree produces 30 to 50 kg fruit every year and has a productive life span of 150 to 200 years (Ragit et al., 2011). It has the ability to survive on drought and poor soils at a very hot temperature of 44°C and a low temperature of up to 4°C, and its high oil content of 39.7 to 60% (Ragit et al., 2011).

Moringa *oleifera* Lam belongs to an onogeneric family of shrubs and tree, Moringaceae and is considered to have its origin in Agra and Oudh, in the northwest region of India, south of the Himalayan Mountains. There is evidence that the cultivation of this tree in India dates back many thousands of years. The Indians knew that the seeds contain edible oil and they

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used them for medicinal purposes. It is probable that the common people also knew of its value as a fodder or vegetable. This tree can be found growing naturally at elevations of up to 1,000 m above sea level. It can grow well on hillsides but is more frequently found growing on pastureland or in river basins. It is a fast growing tree and has been found to grow to 6 – 7m in one year in areas receiving less than 400 mm mean annual rainfall (Odee, 1998). In English it is commonly known as Horseradish tree, Drumstick tree, Never Die tree, West Indian Ben tree, and Radish tree (Ramachandran *et al.*, 1980). The seed contain between 30 - 50% oil.

Castor oil plant (Ricinus communis L.) Is a species of flowering plant in the spurge family, Euphorbiaceae. It belongs to a monotypic genus, Ricinus, and subtribe, Ricininae. It seed is the castor bean; it is indigenous to the Southeastern Mediterranean basin, Eastern Africa, and India, but widespread throughout tropical regions (Muthu et al., 2010). Castor seed is the source of castor oil, which has a wide variety of uses. The seed contain between 40 - 60% oil that is rich in triglycerides, mainly ricinolein. The seed contain ricin, a toxin, which is also present in lower concentrations throughout the plant.

To date, reports on the use of these oils, in particular neem, castor and moringa oil in Nigeria, for the production of biodiesel are not available while that of Jatropha curcas is limited (Belewu et al., 2010). The fatty acid compositions of the oils (Table I) and their physicochemical properties (Table II) have been investigated. The results of this study would form a basis for the development of a database for biodiesel production from these feedstocks, especially in countries where they are in abundance. Hence, this paper evaluates the physiochemical properties of oils extracted from the selected plants seeds.

II. Materials and Methods

Seeds sample Source;

The studied plant seeds were collected from different places within the Northern part of Nigeria. Neem seeds (Azadirachta indica) and Castor seed (Ricinus communis) were collected dry from Yelwa, Plateau state. Moringa seed (Moringa oleifera) from Sheda Science and Technology Complex, Abuja while Jatropha (Jatropha curcas) seeds were obtained from National Research Institute for Chemical Technology Zaria.

Sample preparation and oil extraction

The seeds collected were cleaned by removing foreign materials such as ticks, stains, leaves, other seeds, sand and dirt. After cleaning, the seeds were dried in the oven at 500C for 72 hours until constant masses were obtained. The dried seeds were then mechanically dehauled to remove the seed coat. Removal of the seed coat is imperative because the seed coat contains little or no oil and more importantly inclusion would make extraction less efficient. The dehauled seeds were further dried at 500C for another 48hours and ground to powder using mortar and pestle. The oil was extracted separately from each type of seeds using Soxhlet extractor with n-hexane as a solvent. The percentage oil yield and free fatty acid level were determined. The physicochemical analysis of oils was carried out according to AOAC (1990), AOCS (1997) and Standard methods were used for the determination of oils properties.

III. Results

Table I : percentage oil yield and free fatty acid level of the samples.

Samples	Percentage yield (%)	Acid value (mg KOH/g)	FFA (mg KOH/g)
Jatropha seed oil	46.4	8.43	4.22
Castor seed oil	47.2	12.48	6.24
Neem seed oil	45.3	17.40	8.70
Moringa seed oil	40.2	4.96	2.48

Note : Free fatty acids (FFA) value is half of the acid value

Table II : Physicochemical properties of oils obtained from four different plants seed.

Parameters	Jatropha Seed oil	Castor seed oil	Neem seed oil	Moringa seed oil
Moisture Content (%)	2.39	3.48	2.53	0.043
Ash content (%)	12.5	15	11.10	7.5

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Saponification Value (mgKOH/g)	191.8	164.1	186.4	188.1
Iodine value (meq/g)	62.12	45.26	58.20	66.2
Peroxide value (meq/g)	40	10	78.40	3.50
S. G. at 15/4^{0}C	0.9156	0.9178	0.9327	0.9080
Viscosity (Mpa)	88.15	81.95	88.40	49.96

IV. Discussion

The oil yields of all the seeds (Jatropha curcas 46.4%, Neem 45.3%, Castor 47.2% and Moringa seed 40.2%) are shown in Table I. These results fall within the range of the percentage oil content (30 - 60%) reported by Azam et al., (2005), and (Sneha et.al (2009). The results indicate that all the sample seed contains appreciable quantity of oil enough to be extracted for commercial scale production of biodiesel. The obtained values for the free fatty acid level of the oils are presented on Table I. The acid value is a measure of the amount of carboxylic acid groups present per gramme of the oil and the higher value significantly affect efficiency of transesterification and consequently result in low yield, (Conackci et al., 2001). The result on Table 1 shows that all the oils contain high acid value except that of moringa seed; as such the oil cannot be directly transesterified. Transesterification can only be achieved when the acid value is 2% or 1% FFA. There is therefore the need to carry out acid esterification of the oil as to reduce high acid value to 2% or less prior to alkaline transesterification, and this could probably lead to optimal biodiesel yield. The quality of oils expressed in terms of the physicochemical properties such as moisture content, ash content, saponification value, iodine value, peroxide value, specific gravity and viscosity are shown in Table II. The moisture content of the samples shown in table 2 are Jatropha 2.39%, Castor seed 3.48% , Moringa seed 0.043 %and Neem seed 2.53%. These values are low especially that of Moringa signifying that the seeds can dry well and can be stored for a long time. The ash contents are fairly low indicating that mineral contents are low. The values for specific gravity of oils samples shown in Table II were found to be within the range of 0.717- 0.921as reported by Danguguwa (1983) except neem seed oil that is insignificantly above the range. The saponification values were low when compared with the value of 190 - 194 reported by Eteshola (1990); this signifies that these oils are of good quality for use as feedstock for biodiesel production.

V. Conclusions

In this article, a comparative study on the functional properties of oils extracted from jatropha, neem,castor and moringa seeds for their suitability in biodiesel production, shown that all the oils can be used as raw materials to obtain biodiesel fuel of high quality and could be suitable alternative to fossil diesel.

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Evaluation of 6, 8-Dichloro-2-methyl-4H-Chromen-4-one Derivatives as Antileishmanial Agents

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Evaluation of 6, 8-Dichloro-2-methyl-4H-Chromen-4-one Derivatives as Antileishmanial Agents

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Abstract - The present work aims at study of antileishmanial activity of some 6, 8-dichloro-2-methyl-4H-chromen-4-one derivatives. The synthesized chromenes have been screened for antileishmanial activity on *L. major* parasite. The results were promising and showed that all compounds under investigation have some degree of activity against leishmania *L. major* parasite and compound 5 showed potential activity with IC_{50} value 0.58± 0.09 μg/ml, comparatively with the standard drug Amphotericin B.

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I. Introduction

The chromone system (benzo-γ-pyrone) is present in many compounds widely found in plants and particularly in flavones and isoflavones. It also forms the important components of pharmcophores of large number of molecules of medicinal significance [1]. Moreover, chromone-fused heterocyclic derivatives have attracted a great deal of interest due to their wide applications in the field of pharmaceuticals [2]. Some flavonoids have been reported to possess anticancer, anti HIV, anti-inflammatory and several other activities [3-5]. It was also reported that chromones have different biological activities and could be utilized as cytotoxic (anticancer) [6-12], antihepasmodic, estrogenic [13], antimicrobial [14-16], antifungal [17], antibacterial [18-20]. Due to their abundance in plants and their low mammalian toxicity, chromone derivatives are present in large amounts in the diets of human [21, 22].

Leishmaniasis is arising as a severe public health problem. It is epidemic in 88 countries and 350 million are at risk to be infected world wide. Balochistan and Sindh provinces of Pakistan are vulnerable to cutaneous leishmaniasis. The appearance of new cases of leishmaniais is around 2 million annually. Currently, there are no effective drugs available for leishmaniasis. The available drugs to treat the disease are frequently ineffective. Thus, there is a growing interest to investigate inexpensive, low side effect and more potent compounds against leishmaniasis.

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Herein, and in continuation of our previous work [23-31], the authors aimed at utilization of the reactivity of 6, 8-dichloro-2-methyl-4H-chromen-4-one (1) towards carbon electrophiles and nucleophiles to get chromene derivatives and evaluated them as antileishmanial agents.

II. Experimental

a) Instrumentation

All melting points were measured on a Gallenkamp electric melting point apparatus and are uncorrected. The infrared spectra were recorded using potassium bromide disks on a Pye Unicam SP-3-300 infrared spectrophotometer. ^{1}H NMR and ^{13}C NMR experiments were run at 300 MHz on a Varian Mercury VX-300 NMR spectrometer using tetramethylsilane (TMS) as internal standard in deuterated chloroform or dimethyl sulphoxide. Chemical shifts are quoted as δ. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometers at 70 eV. All the spectral measurements as well as the elemental analyses were carried out at the Micro analytical Center of Cairo University. All the newly synthesized compounds gave satisfactory elemental analyses.

b) Synthesis

6,8-Dichloro-2-styryl-4H-chromen-4-one(2a),2-(4-fluoro styryl)-6,8-dichloro-4H-chromen-4-one (2b) and 2-(4-methoxystyryl)-6,8-dichloro-4H-chromen-4-one (2c)

To a solution of 2-methylchromone derivative (**1**) (10 mmol, 2.29 g) in dry ethanol (20 mL), the appropriate aldehyde such as benzaldehyde, 4-flourobenzaldehyde and 4- methoxybenzaldehyde (10 mmol) was added. The reaction mixture was stirred at room temperature for 2h in the presence of sodium ethoxide (prepared by reaction 0.33 g sodium metal with 10 mL dry ethanol). The solid product that formed was collected by suction, dried and then recrystallised from benzene (Scheme 1). *6, 8-Dichloro-2-styryl-4H-chromen-4-one* (**2a**): Pale brown crystals. Yield: 96%. M.p.: 163-166 oC. FT-IR (KBr, cm-1): 1658 -(C=O)(chromone), 1631 -(C=C). 1H NMR (300 MHz, CDCl3, δ, ppm): 8.08-7.40 (m, 7H, Ar-H), 6.83 (d, 1H, -C*H*=CH-), 6.78 (d, 1H, -CH=C*H*-), 6.35 (s, 1H, pyran ring). MS (EI, *m/z*, %): 316 (M+., 25.1). Anal. calcd. for

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$C_{17}H_{10}Cl_2O_2$: C, 64.38; H, 3.18; Cl,22.36; Found: C, 64.24; H, 2.98, Cl, 22.24%.

2-(4-fluorostyryl)-6,8-dichloro-4H-chromen-4-one (**2b**): Pale brown crystals. Yield: 63%. M.p.: 186-188 °C. FT-IR (KBr, cm-1): 1649 -(C=O) (chromone).

1H NMR (300 MHz, DMSO-*d*6, δ, ppm): 8.16-7.60 (m, 6H, Ar-*H*), 7.33 (d, 1H, -C*H*=CH-), 7.24 (d, 1H, -CH=C*H*-), 6.54 (s, 1H, pyran ring). 13C NMR (75 MHz, DMSO-*d*6, δ, ppm): 175.0 (C-3), 161.6 (C-1 & C-6'), 149.8 (C-9), 135.9 (C-7), 133.4 (C-2'), 131.2 (C-6), 130.1 (C-3'), 130.0 (C-5), 129.4 (C-4'), 125.4 (C-8), 123.0 (C-4), 119.9 (C-1'), 116.0 (C-5'), 110.0 (C-2) (Scheme 1). MS (EI,*m/z*, %): 334 (M+., 27.4). Anal. calcd. for $C_{17}H_9Cl_2FO_2$: C, 60.92; H, 2.71; Cl, 21.16. Found: C, 60.84; H, 2.59; Cl, 21.00%.

2-(4-methoxystyryl)-6,8-dichloro-4H-chromen-4-one (**2c**): Green crystals. Yield: 38%. M.p.: 195-198 oC. FT-IR (KBr, cm-1):1652 -(C=O) (chromone), 1643 -(C=C). 1H NMR (300 MHz, DMSO-*d*6, δ, ppm): 8.07-7.35 (m, 6H, Ar-*H*), 7.05 (d, 1H, -C*H*=CH-), 6.98 (d, 1H, -CH=C*H*-), 6.44 (s, 1H, pyran ring), 3.79 (S, 3H, -OC*H3*). MS (EI, *m/z*, %): 348 (M+2 , 27.8). Anal. calcd. for $C_{18}H_{12}Cl_2O_3$: C, 62.27; H, 3.48; Cl, 20.42. Found: C, 62.14; H, 3.38; Cl, 20.20%.

7,9-Dichloro-4-phenyl-1H-furo[3,4-a]xanthene-1,3,11-trione (3a)

A mixture of 2-styryl derivative **2a** (2 mmol) and maleic anhydride (20 mmol) in molar ratio 1:10 was fused on sand bath at fused temperature for 3 h and left to cool. The solid that formed was triturated with warm ethanol, filtered and recrystallized from ethanol to afford xanthone derivative **3a** as brown crystals (Scheme 1). Yield: 58%. M.p.: >300 oC. FT-IR (KBr, cm-1): br. centered at 1722 -(C=O) anhydride, 1631 -(C=O) (chromone). 1H NMR (300 MHz, DMSO-*d*6, δ, ppm): 8.18- 7.26 (m, 7H, Ar-*H*), 6.58 (s, 1H, C5-*H*). MS (EI, *m/z*, %): 315 (MC4O3,+ H , 17.8). Anal. calcd. For $C_{21}H_8Cl_2O_5$: C, 61.34; H, 1.96; Cl, 17.24. Found: C, 61.22; H, 1.78; Cl, 17.17%.

Reaction of 2-styrylchromone derivatives (2b) with N-Phenylmaleimide; formation of adducts (3b)

2 mmol of 2-Styryl derivatives (**2b**) was fused with *N*-phenylmaleimide (4 mmol) in molar ratio 1:2 on sand bath at fused temperature for 3 h; left to cool. The solid that formed was triturated with warm ethanol; filtered and recrystallised from the proper solvent to afford the expected adducts (**3b**) (Scheme 1).

7,9-Dichloro-4-(4-fluorophenyl)-2-phenylchromeno[3,2-e] isoindole-1,3,11(2H)-trione (**3b**): Re crystallized from acetic acid to afford the adduct as brown crystals. Yield: 80%. M.p.: 197- 200 oC. FT-IR (KBr, cm-1): 1776, 1707 -(C=O) imide. 1H NMR (300 MHz, CDCl3, δ, ppm): 8.10-7.10 (m, 11H, Ar-*H*), 6.36 (s, 1H, C5-*H*). MS (EI, *m/z*, %): 503 (M+., 50). Anal. calcd. For C27H12Cl2FNO4: C, 64.31; H, 2.40; Cl, 14.06; N, 2.78. Found: C, 64.22; H, 2.35; Cl, 13.97; N, 2.67%.

Ethyl3-(6,8-dichloro-4-oxo-4H-chromen-2-yl)-2-oxo-propanoate (4)

To a mixture of chromone derivative **1** (5 mmol, 1.14 g) and diethyl oxalate (25 mmol, 3.6 g) in dry diethyl ether (50 mL), sodium metal (0.5 g) was added at once. The reaction mixture was stirred for 0.5 h and left overnight at room temperature. Acidification with cold dilute acetic acid, the crude solid product that deposited was collected by suction, dried and then recrystallized from toluene to give pyruvic ester derivative **4** as orange crystals (Scheme 1). Yield: 65%. M.p.: 218-220 oC. FT-IR (KBr, cm-1): 1730 -(C=O) ketoester, 1653 -(C=O) chromone. 1H NMR (300 MHz, DMSO-*d*6, δ, ppm): 8.12-7.84 (2s, 2H, Ar-*H*), 6.96 (s, 1H, -C*H*=C-OH), 6.08 (s, 1H, pyran ring), 4.30 (q, 2H, -C*H*2CH3, *J* = 7.2 Hz), 3.75 (s, 1H, OH, exchangeable), 1.31 (t, 3H, -CH2C*H*3, *J* = 7.2 Hz). 13C NMR (75 MHz, DMSO-*d*6, δ,ppm): 184.0 (C-3), 177.6 (C-2'), 162.6 (C-1), 161.1 (C-3'), 149.9 (C-9), 133.4 (C-7), 129.4 (C-6), 125.1 (C-5), 123.5 (C-8), 122.8 (C-4), 110.3 (C-1'), 109.3 (C-2), 62.4 (C-4'), 14.0 (C-5'). MS (EI, *m/z*, %): 328 (M+., 47.9). Anal. calcd. for $C_{14}H_{10}Cl_2O_5$: C, 51.09; H, 3.06; Cl, 21.54. Found: C, 51.00; H, 2.98; Cl, 21.32%.

2-(6,8-Dichloro-2-methyl-4H-chromene-4-ylidene)malononitrile (5)

A mixture of chromone derivative **1** (5 mmol, 1.14 g) and malononitrile (5 mmol, 0.33 g) in freshly distilled acetic anhydride (12.5 mL) was heated under reflux for 3 h., left to cool. Excess acetic anhydride was distilled off and the crude product was filtered and washed with water, dried and then recrystallized from ethanol to give malononitrile derivative (**5**) as brown crystals (Scheme 2). Yield: 59%. M.p.: 121-123 oC. FT-IR (KBr, cm-1): 2212 -(C≡N), 1652 -(C≡C). 1H NMR (300 MHz, DMSO, δ, ppm): 8.14 (s, 1H, Ar-*H*), 7.89 (s, 1H, Ar-*H*), 6.37 (s, 1H, C3-*H*), 2.32 (s, 3H, C*H*3). Anal. calcd. For $C_{13}H_6Cl_2N_2O$: C 56.35; H, 2.18; Cl, 25.59; N, 10.11. Found: C, 56.19; H, 2.10; Cl, 25.45; N, 10.03%.

2-Amino-3-(6,8-dichloro-2-methyl-4H-chromen-4-ylidene) prop-1-ene-1,1,3-tricarbonitrile (6)

A mixture of chromone derivative **1** (5 mmol, 1.14 g) and malononitrile (10 mmol, 0.66 g) in ethanol (20 mL) in presence of few drops of piperidine was heated under reflux for 4 h. The crude solid product that deposited was collected by suction, dried and then recrystallized from ethanol to give compound **6** as yellow crystals (Scheme 2). Yield: 59%. M.p.: 198-200 °C. FT-IR (KBr, cm-1): 3411, 3322 -(NH2), 2212 -(C≡N). 1H NMR (300 MHz, DMSO, δ, ppm): 8.25 (s, 2H, N*H*2, exchangeable), 7.65 (s, 1H, Ar-*H*), 7.28 (s, 1H, Ar-*H*), 6.79 (s, 1H, C3-*H*), 2.32 (s, 3H, C*H*3). MS (EI, *m/z*, %): 317 (M-CN+H , 17.7). Anal. calcd. for $C_{16}H_8Cl_2N_4O$: C,

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56.00; H, 2.35; Cl, 20.66; N, 16.33. Found: C, 55.92; H, 2.17; Cl, 20.49; N, 16.29%.

c) Antileishmanial Assay

Each compound (1 mg) was dissolved in DMSO (1 mL) and Amphotercin B (1 mg) was also dissolved in DMSO (1 mL) as positive control. Parasites at log phase were centrifuged at 3,000 rpm for 3 minutes. Parasites were diluted in fresh culture medium to a final density of 2 × 106 cells/mL. In 96-well plates, 180 μL of medium was added in different wells. Twenty μL of the compound was added in medium and serially diluted. Parasite culture (100 μL) was added in all wells. Three rows were left for negative and positive controls. In the negative controls, DMSO was serially diluted in medium while the positive control contained varying concentrations of the standard antileishmanial compound Amphotercin B. The plates were incubated for 72 hours at 24 °C. The culture was examined microscopically on an improved neubaur counting chamber and IC50 values of compounds possessing antileishmanial activity were calculated. All assays were run in duplicate. IC50 of samples was determined by using the Prism software [27].

III. Results and Discussions

a) Synthesis

6, 8-Dichloro-2-methyl-4*H*-chromen-4-one (**1**) was prepared *via* acid-catalyzed cyclodehydration of the β-diketone; 1- (3,5-dichloro-2-hydroxyphenyl)butane-1, 3-dione [32]. 2- Methylchromones are typical substances containing an active methyl group due to the considerable stabilization of the produced carbanion by abstracting a proton from the methyl group as a result of conjugation with the double bond and carbonyl functionality. Thus, 6, 8-dichloro-2-methyl-4*H*chromen-4-one (**1**) condensed under Knovenagel reaction conditions, with different aromatic aldehydes namely, benzaldehyde, 4-flurorobenzaldehyde and 4-methoxybenzaldehyde to afford the corresponding 2-styrylchromones (**2a-c**) [7, 24, 25, 32-35].

2-Styryl chromones (**2a-c**) are typical dienes which underwent cycloaddition reactions under Diels Alder reaction conditions with maleic anhydride and/or *N*-arylmaleimides as dienophiles, to yield the initial adducts which subsequently underwent dehydrogenation to afford the desired adducts (**3a-b**).

Condensation of 2-methylchromone **1** with diethyl oxalate in the presence of sodium metal gave the corresponding pyruvate esters (**4**) [36], which exists as keto- enol tautomers.

The reaction of 2-methylchromones with malononitrile as an example of compounds containing active methylene groups yields a product, which depends upon the reaction conditions. Thus, when 2-methylchromone **1** was allowed to react with malononitrile (1:1) in boiling acetic anhydride, the corresponding condensation product **5** was obtained [25]. The product **5** is formed *via* carbon nucleophile attack of the active methylene on the electronically deficient carbonyl carbon of chromone nucleus.

On the other hand, when 2-methylchromone, **1** was allowed to react with excess malononitrile in refluxing ethanol containing few drops of piperidine, the product was identified to be the tricarbonitrile (**6**) which is formed from the attack of a second malononitrile molecule on the initially formed condensation intermediate of type (**5**). The attack occurs at one cyano group but not on both probably due to steric hindrance.

b) Antileishmanial activity

Antileishmanial activity of nine 4H-chromen-4-one derivatives was evaluated in order to utilize as antileishmanial agents. Compounds (**5** and **6**) showed significant activity with IC_{50} values 0.58±0.09 and 0.59±0.05 μg/ml respectively. These IC_{50} values are comparable with IC_{50} of the standard drug Amphotericin B. Compounds **2a, 2b, 2c 3b** and **4** showed good activity with IC_{50} values between 0.61±0.02 μg/ml to 0.69±0.07 μg/ml. on other hand compounds **1** and **3a** showed moderate activity with IC_{50} values 0.72±0.04μg/ml, 0.78±0.02 μg/ml.

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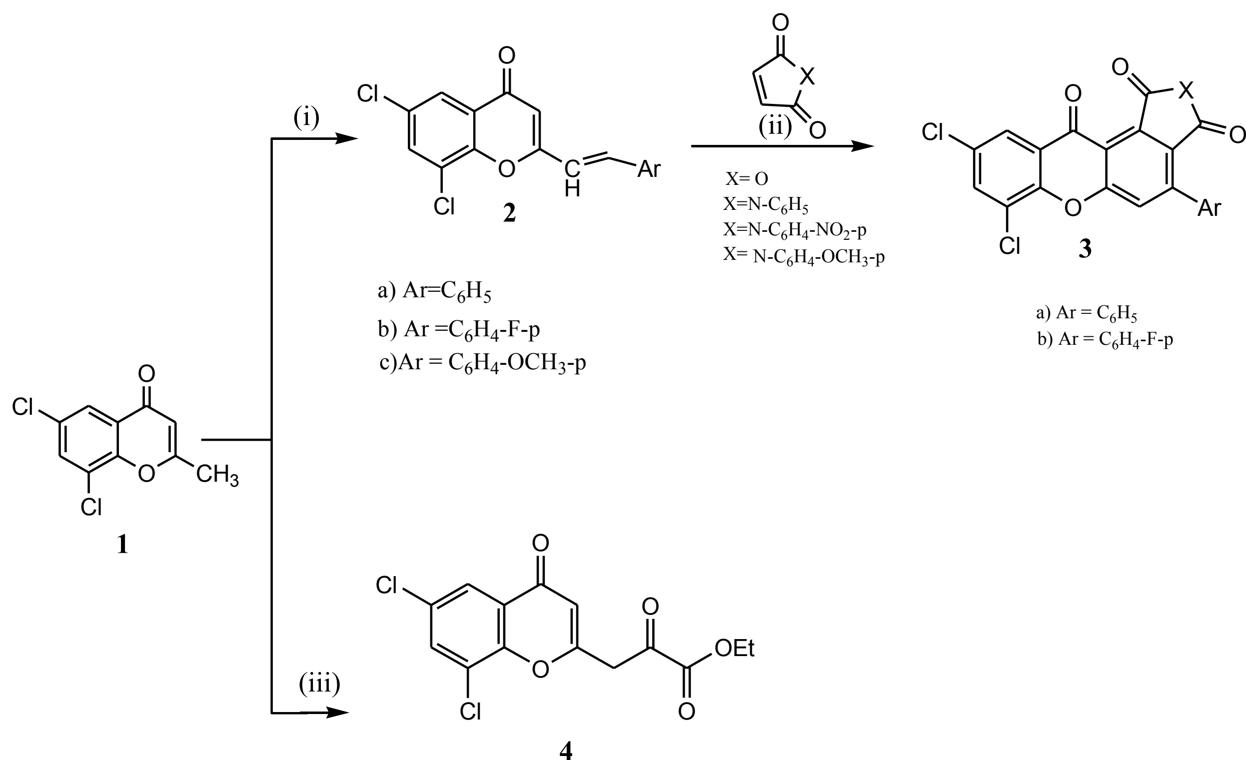
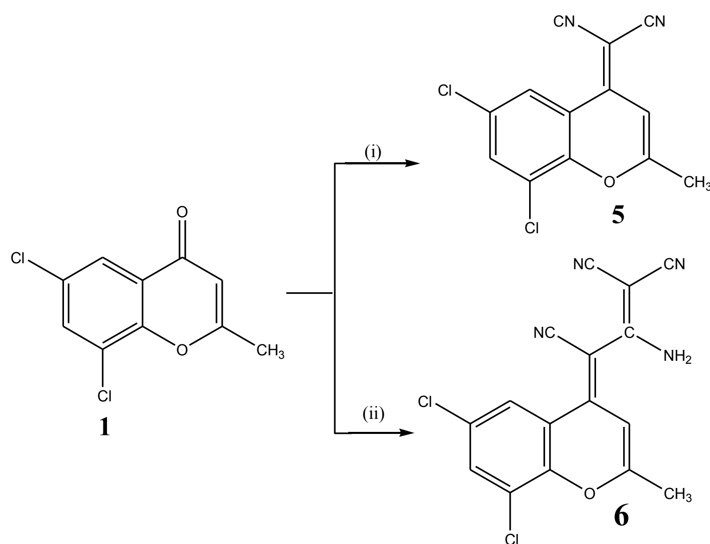


(i) Ar-CHO, EtONa, EtOH, stirring; (ii) fusion; (iii) Diethyloxalate, dry diethyl ether, sodium metal stirring, rt;

Scheme 1



(i) alononitile, Ac_2O, reflux; (ii) Malononitile, piperidine, EtOH, reflux

Scheme 2

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Table 1 : %Inhibition ofcompounds **1-6** against *L. major*

Compound	*L.major* $IC_{50\ value}$
1	0.78±0.02
2a	0.68±0.08
2b	0.69±0.07
2c	0.61±0.02
3a	0.72±0.04
3b	0.66±0.07
4	0.62±0.01
5	0.58±0.09
6	0.59±0.05
DMSO	0.99±0.00
Standard IC_{50}	0.56±0.01

[a] *percentage inhibition activity: 100 = (non-significant; 0.95−0.80 = low; 0.79−0.70 = Moderate;0.69−0.60 = Good; below 0.59-0.56 = Significant activity).*

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Synthesis and Biological Evaluation of Some New 1-Pheyl, 3-ethoxycarbonyl, 5-hydroxy Indole Derivatives as a Potential Antimicrobial Agents

By Basavaraj M. Kalshetty , Ramesh S. Gani & M.B.Kalashetti
Basaveshwar Science College Bagalkot and Bharathiar University

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Keywords : biological evaluation, Indole derivatives potential and microbial agents.

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Synthesis and Biological Evaluation of Some New 1-Pheyl,3-ethoxycarbonyl,5-hydroxy Indole Derivatives as a Potential Antimicrobial Agents

Basavaraj M. Kalshetty [α], Ramesh S. Gani [σ] & M.B.Kalashetti [ρ]

Abstract - The derivatives of Indole show biological activities including herbicidal. The newly synthesized compounds 1-phenylethyl,2-methyl,3-ethoxy carbonyl,5-methoxycarbonyl ,2-methoxy Indole (Compound 2) were prepared by treating 1-phenyl,3-ethoxycarbonyl,5-hydroxy,2-methyl Indole (Compound 1) successively with methyl bromo- acetate and refluxing with K2CO3 / KI in the presence of dry acetone. Newly synthesized compound 2 refluxed with hydrazine hydrate in alcoholic media forming 1-Phenylethyl,3-ethoxy carbonyl,2-methyl Indole,5-yl oxy acetic acid hydrizide (Compound 3). This drug which on separately reacting with carbon disulphide, phenyl iso-thiocynide, acetylacetone, triethylorthoformate gave condensed bridge head heterocyclic's such as 1-Phenyl ethyl,2-methyl,3-ethoxy cabonyl,5(5'-mercapto,1'-3'-4'-oxadiazol,2'-yl)-methoxy Indole (Compound 4), 1-Phenylethyl,3-ethoxycarbonyl,2-methyl,5-yl (methoxy carbothisemi cabazide) (Compound 5), 1-Phenyl,2-methyl,3-ethoxycarbonyl,5(2,5, dimethyl Pyrrole,1-yl) amino carbonyl methoxy Indole (Compound 8) and 1-Phenyl,3-ethoxycarbonyl,2-methyl,5-(1',3',4'-oxadiazole,2'-yl-methoxy Indole (Compound 9) respectively. Compound 6 and Compound 7 were also synthesized heterocycles of the Indole derivatives. The structures of the compounds were established with the help of the elemental analysis and spectral date (IR, NMR and Mass). Compounds were screened for their antimicrobial potential.

Keywords : biological evaluation, Indole derivatives, potential and microbial agents.

I. Introduction

Newly synthesized derivatives of Triazole, Coumarin and Indole show diverse types of Biological activities such as analgesic & anti-inflmmatory[1],anti-tumaor[2], anti-mycobecterial[3], anti-cancer[4], anti-convulsant[5], diuretic[6], anti-microbial[7] and anti-diabatic[8]. The literature survey reveals that the heterocyclic compounds of Indole may enhance the biological activity [14]. Keeping in view of these reports, in the present investigation, it was planned to synthesis various bisheterocycles interesting in Indole moiety is linked to Oxidiazole, Pyrrole and Triazole. The 1,3,4-oxidiazoles have been shown to possess muscle relaxant, tranquilizing and anti-tubercular[15]. In the light of biological activities shown by Oxadiazole [16] the continuation of our work on Chemo-selectivity of Indole dicarboxylates towards hydrazine hydrates [17] and bridged heterocycles as various Schemes.

1-Phenyethyl ,3-ethoxy carbonyl,2-methyl Indole,5-yl oxy acetic acid hydrizide (Compound 3) have been found to show anti-bacterial[9], anti-microbial[10,11], anti-flammatory[12] and anti-convulsant[13] activities. Hence the derivatives of compound in Scheme 1, Scheme 2, Scheme 3 and derivatives of compound 2 in Scheme 4 have been found to possess varying Pharmacological activities.

Hence, we started to link oxadiazoles, Pyrrole, Triazoles to C-5 position of biological active Indole moiety leading to the synthesis of hitherto unknown title compounds with view to study their pharmacological profile. In the present investigation the required starting material 1-Phenylethyl,3-ethoxycarbonyl,5-hydroxy,2-methyl Indole (Compound 1) was prepared by adopting the Nenitzescue method 18 where as by reacting Ethyl,3-Phenylethyl aminocrotanate (Compound B) with p-benzoquinone (Compound A) as reported in scheme 01. Thus formed compound 1 was allowed to react with methyl bromo acetate in the presence of anhydrous KOH, produced Indole dicarboxylates (Compound 2). This compound 2 was refluxed with hydrazine hydrate in the presence of ethanol, produced Indole monocarbohydrazine (Compound 3) was observed that the C-3 ethoxy carbonyl group in the ester of compound 2 did not react with hydrazine hydrate under the above reaction conditions and dicarbohydrazide was not produced, the observed resistant of C-3 ethoxycarbonyl of the diester (Compound 2) to wards nucleophilic attack of hydrazine hydrate may be attributed to the canonical form of the diester (Scheme 04). Where in C-3 ethoxycarbonyl group has less double bond character.

The monocarbohydrazide (Compound 3) was further reacted separately with alcoholic KOH and disulphide (Scheme 01), with alcoholic PhNCS (Scheme 02(, with Acetylacetone in the presence of glacial acetic acid (Scheme 03) and Triethylorthoformate in boiling alcohol (Scheme 03) to afford respectively. 1-Phenyl ethyl,2-methyl,3-ethoxycabonyl,5(5'-mercapto,1'-3'-4'-ox adiazol,2'-yl)-methoxy Indole **(Compound 4)** (Scheme 01), 1-Phenylethyl,3-ethoxycarbonyl,2-methyl,5-yl (meth oxy carbothisemi cabazide) **(Compound 5)** (Scheme

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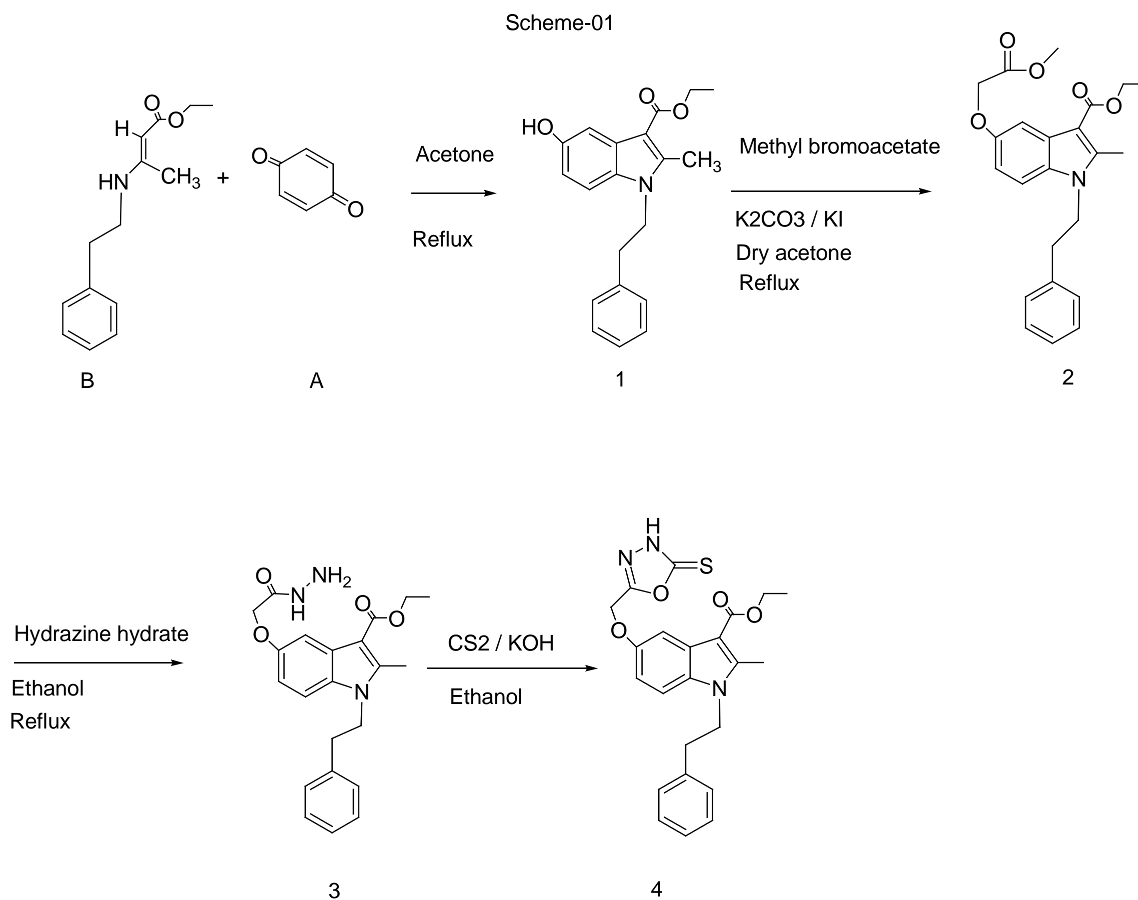
02), 1-Phenyl,2-methyl,3-ethoxycarbonyl,5 (2,5, dimethyl Pyrrole,1-yl) amino carbonyl methoxy Indole **(Compound 8)** and 1-Phenyl,3-ethoxycarbonyl,2-methyl,5-(1',3',4'-oxadiazole,2'-yl-methoxy Indole **(Compound 9)** 0f Scheme 03.

In the compound 5 the thiosemicarbazide was oxidiatevely cyclised to the desired 1-Phenyl,3-ethoxy carbonyl,2-methyl,5(5'-analino,1',3',4',-Oxadiazole,2'-yl, 1-methylethoxy Indole **(Compound 6)** Scheme 02. This compound 5 specifically reacting with Iodine and KI in 4% sodium hydroxide solution forming 1-Phenylethyl,3-ethoxycarbonyl,2-methyl,5(4'-phenyl,5'-mercapto,1',2',4' -Triazole,3-yl) methoxy Indole **(Compound 7).** The structures of these newly synthesized compounds were confirmed by their spectral and analytical data tested for their anti- bacterial activities by Cup Plate method. Analgesic activity by aceti acid induced writhing response, hot plate reaction time and tail immersion method and anti- inflammatory activity by Carrageenan induced Paw edema method.



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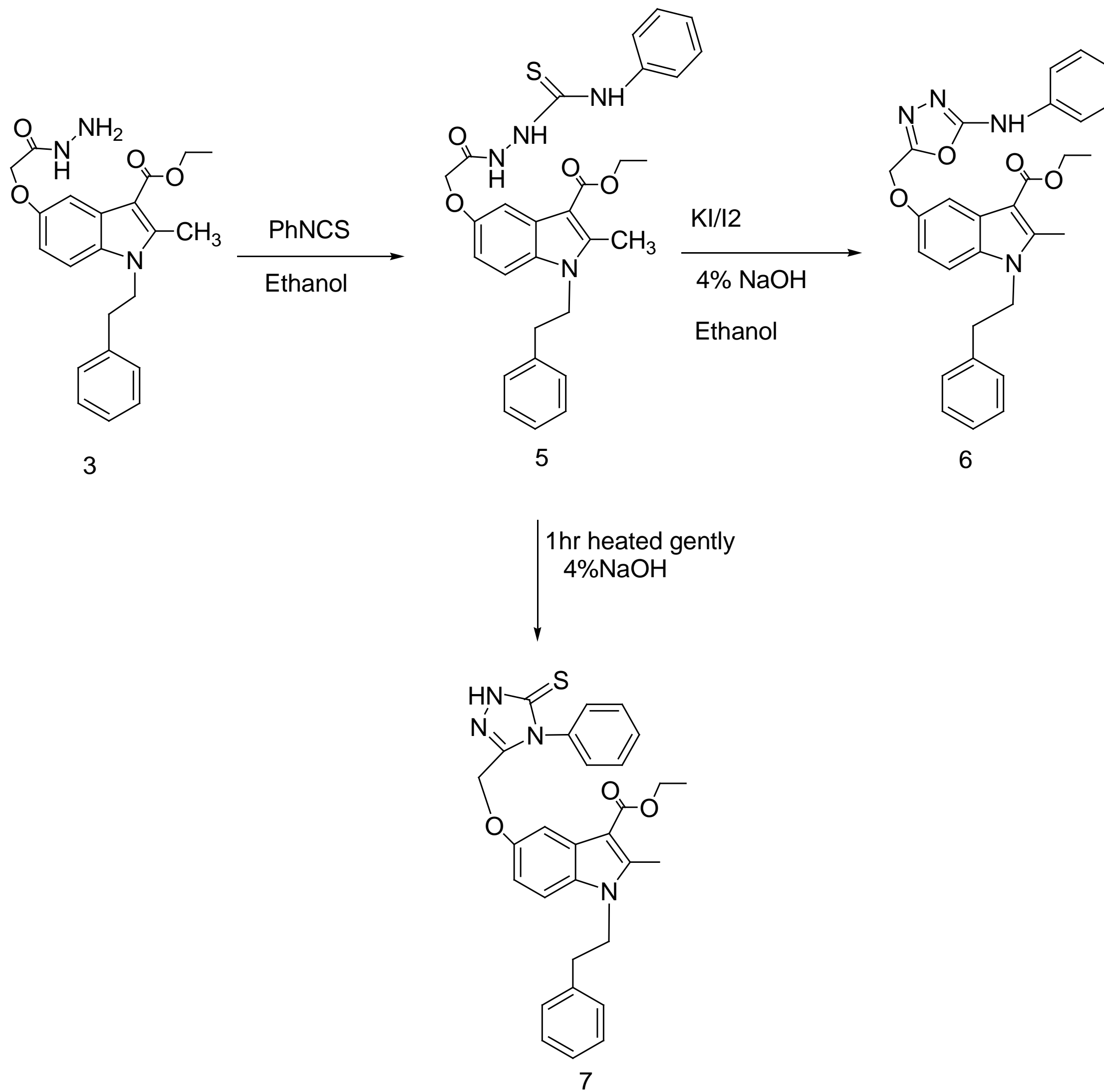

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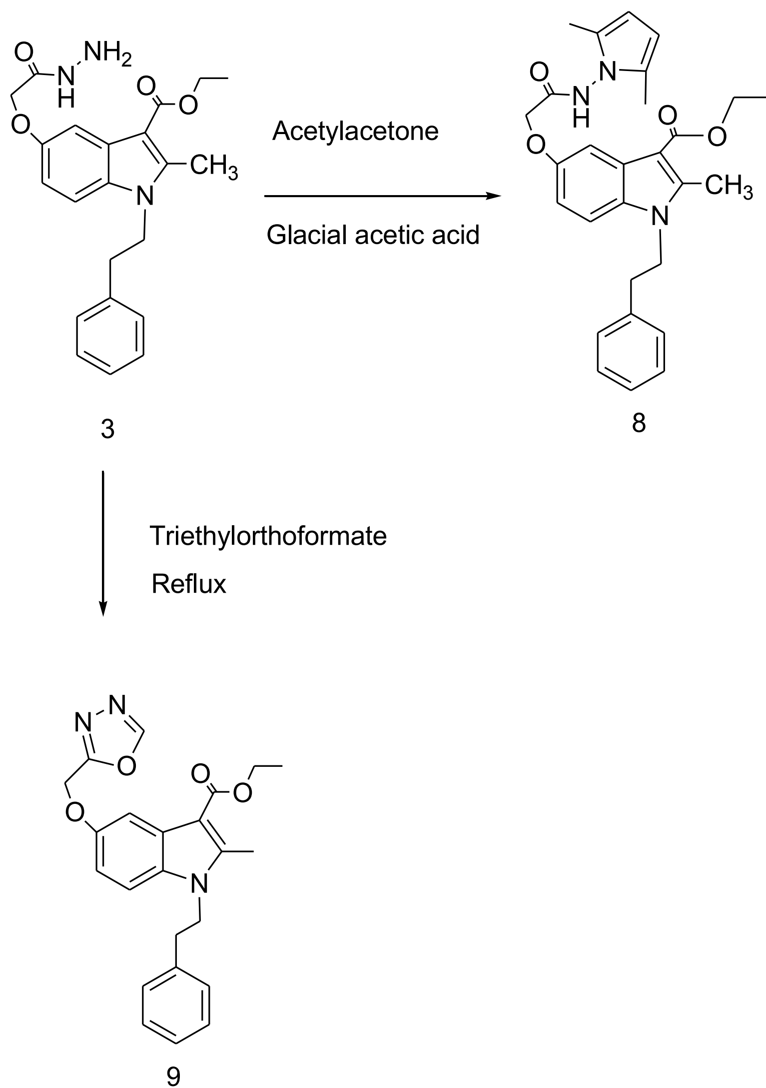

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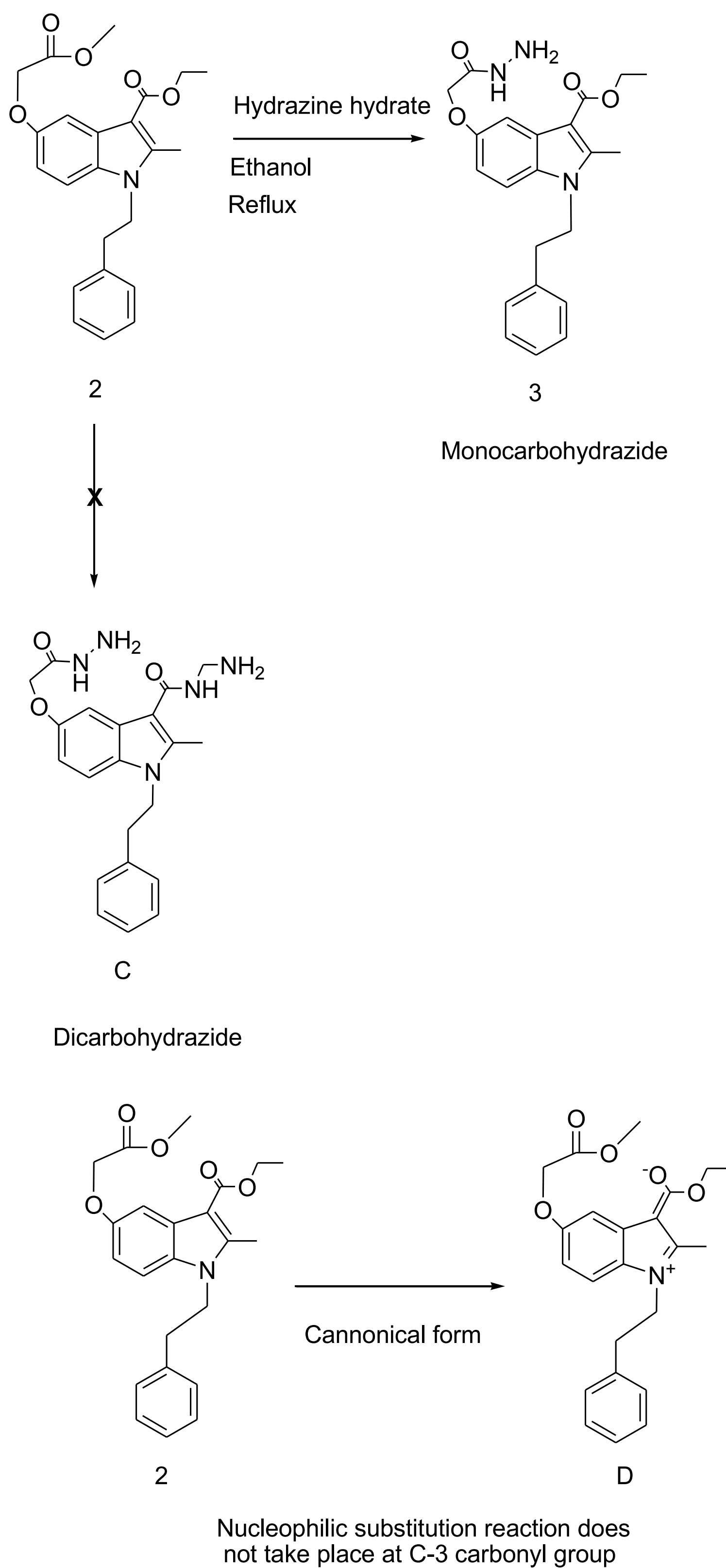

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II. Results and Discussion

All the synthesized compounds were screened for analgesic and anti-inflammatory activity in rats and mice, Wister rats (230-250g) ND Swise mice (25-30g) were used. The animals were kept in 26°C±2°C with the relative humidity of 44-56% continuously for 12 hours in light / dark cycle. They were fedded with standard diet and water. An approval for the experimental protocol was obtained and the procedure was carried at H.S.K College of Pharmacy, Bagalkot, Karnataka State, India. The rats had been fasted for 18-24 hours, were used for the experiments. The test compounds were suspended in 0.5% Sodium carboxymethyl cellulose (Na-CMC) and administered at the doses of 3 and 10 mg/kg of the body weight (bw), diclofence and pentazocine were administered as reference standard srugs for anti-inflammatory and analgesic respectively; at a dose of 10mg / kg body weight. The control group received 0.5% Na-CMC in distilled water,

The mass spectrum of newly synthesized compounds was good agreement with their molecular ion peaks. The characterization data of new compounds were given in **Table 1**. The synthesized compounds were evaluated in vitro for anti-bacterial activity against Escherichia Coli and Sacillus Cirraflagellous by Cup Plate method. The results were summarized in **Table 2**.

a) Anti-bacterial and Anti-fungal activity

The compounds 1 – 9 were screened for their in vitro anti-fungal potential activity by Cup Plate method[20] against Aspergillus fumigates (A F), human pathogenic yeast [Candida alb cans (OA)], Griseofulvin used as a standard. the compounds were tested, the anti-fungal activity results indicated that the some of the Indole derivatives possessed a broad spectrum of activity against the reference drugs, the compound which have no anti-fungal activity are not included in **Table 2**.

The compounds were tested at 1 mg / ml concentration in DMSO by Tube Dilution Technque[21]. The drug dilution were made serially, the test was performed at 28-29oC and Minimum Inhibitory Concentration (MIC) in mg / ml was recorded by Visual observations after 24-60 hours incubation. The suitable controls and standard drugs were set under identical conditions. Hence, the synthesized compounds have shown varying degree of anti-fungal activity against Candida albicans (human pathogenic yeast) although they have shown their major potential against Aspergillus fumigates (AF). However, the anti-fungal activity of the compounds 1 to 9 was screened and was found broad spectrum of activity against the reference drugs.

i. *Anti-inflammatory activity*

The anti-inflammatory activity of the test compounds was evaluated as described by winter et al [22] and Diwan et al [23] .One hour after administration of the test compounds. Rates in all groups were challenged with carrageenan (1% prepared in 0.4% NaCl) in the sub planter region of the right hind paw. The paw volume was measured at different intervals of time (0.5, 1, 2, 3, and 5h) using a digital plethysmometer (UGO Basil.Italy) and a zero hour reading, before administration of the carrageenan was taken. The percentage inhibition of the paw volume for each test group was calculated using following equation;

Percentage of inhibition (%) = [1-volume in ml]

(test compound) / Volume in ml (control)]x 100

The results in Table 3 and **Table 4** showed that some of the synthesized compounds have significant anti-inflammatory activity among these compounds. The compounds 1, 3, 6 showed significant anti-inflammatory activities at third and fifth hour, where as these were found to be non-significant at the 30 min. similarly, the other compounds showed more or less significant activity at the third and fifth hour were non-significant at the first hour.

ii. *Analgesic Activity*

The Eddy and Leimback hot plate test [24] was carried out in mice for evaluating analgesic activity. Albino mice of either sex were divided in to 12 groups, containing six animals each. Animals were administered with control (0.4% NaCl), test compoundes (3 and 10mgkg^{-1}) and pentazocine (10mgkg^{-1}) as an aqueous suspension of 1% sodium carboxy methyl cellulose .One hour after administration of compoundes , mice were kept on hot plate pre heated to 50^{0}C for 15 seconds . The time taken to lick the hind paw was recorded at 60, 120, and 180min.increasein the reaction time (time interval taken by the animal to lick paw) was considered as proportional to analgesic activity as shown in **Table 5.**

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Table 1 : Characterization of synthesized New compounds

Compound	Substituent	m.p. (^{0}C)	Yield(%)	Nature (solvent)	Molecular formula	Elemental analysis for Calcd) % C	H	N
Compound 01	1-Phenylethyl	177-178	71	Brown granules (Ethanol)	$C_{20}H_{21}NO_3$	74.2 74.12	6.55 6.51	4.33 4.31
Compound 02	1-phenylethyl		66	Pale yellow (Ethanol)	$C_{23}H_{25}NO_5$	69.86 69.78	6.37 6.31	3.54 3.52
Compound 03	1-phenylethyl		61	Colorless needle (Ethanol)	$C_{22}H_{25}N_3O_4$	66.82 66.71	6.37 6.30	10.63 10.59
Compound 04	1-phenylethyl		69	yellow granules (Ethanol)	$C_{23}H_{23}N_3O_4S$	63.14 63.10	5.30 5.29	9.60 9.57
Compound 05	1-phenylethyl		82	Yellow flakes (Ethanol)	$C_{29}H_{30}N_4$O4S	65.64 65.61	5.70 5.66	10.56 10.51
Compound 06	1-phenylethyl		69	Pale yellow (Ethanol)	C29H28N_4O_4	70.15 70.09	5.68 5.61	11.28 11.17
Compound 07	1-phenylethyl		68	Pale yellow (Ethanol)	$C_{29}H_{28}N_4O_3S$	67.95 67.91	5.51 5.49	10.93 10.88
Compound 08	1-phenylethyl		71	Pale yellow (Ethanol)	$C_{28}H_{31}N_3O_4$	71.01 71.0	6.60 6.56	8.87 8.76
Compound 09	1-phenylethyl		68	Pale yellow (Ethanol)	$C_{23}H_{23}N_3O_4$	68.13 68.09	5.72 5.69	10.36 10.29

Table 2 : Antibacterial activity of compounds 3-11 were against B, cirroflagellosus and Escherichia coli. Antifungal activity of compounds 3-11 were against Candida albicans and Aspergillus.

Compound/Code	Concentration ; 1mg/ml Zone of inhibition in mm after 48hr		Compound/Code	Concentration ; 1mg/ml Zone of inhibition in mm after 48hr	
	E.col	B.cirroflagellosus		Candida albicans	Aspergillus Niger
Compound 01	++	+	Compound 01	++	++
Compound 02	-	++	Compound 02	+	++
Compound 03	+	+	Compound 03	+	+
Compound 04	+++	+++	Compound 04	++	+++
Compound 05	++	++	Compound 05	++	+++
Compound 06	+	+	Compound 06	+	+
Compound 07	+++	++	Compound 07	+++	+++
Compound 08	++	++	Compound 08	++	+++
Compound 09	+++	+++	Compound 09	++	+++
Norfloxicin	+++	+++	Griseofulvin	+++	+++

Symbols: Zone diameter of growth inhibition (-) inactive; (<12mm); (+) = weakly active (12-16mm); (++) moderately active (16-21mm); (+++)=highly active (22-28mm)

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Table 3 : In vivo anti-inflammatory activity of 1-Phenylethyl-2-methyl-3-ethoxycarbonyl-5-hydroxyindole derivatives

Treatment	½ hr		1hr		3hr		5hr	
	Paw-volume (ml)	%EI	Paw-volume (ml)	%EI	Paw-volume (ml)	%EI	Paw-volume (ml)	%EI
Normal	0.6625±0.01315	--	0.6225±0.06415	--	0.6675±0.01109	--	0.6675±0.01652	--
Control	1.2523±0.45213 c	--	1.2950±0.64300 c	--	1.3520±0.07325 c	--	1.1983±0.02314 c	--
Diclofenac (10mg/kg)	0.2263±0.0149***		0.2763±0.0239***		0.2838±0.0171***		0.2988±0.028***	
Compound-02 (3mg/kg)	0.2475±0.02414	80.23	0.3876±0.01724*	70.06	0.3975±0.01248	70.59	0.2925±0.00212	75.59
Compound-02 (10mg/kg)	0.2600±0.01475	79.23	0.3427±0.01318*	73.53	0.3575±0.01215*	73.55	0.3050±0.0102**	74.54
Compound-01 (3mg/kg)	0.2825±0.01047*	77.44	0.3612±0.011**	72.10	0.4075±0.02179	69.85	0.3755±0.01287	68.66
Compound-01 (10mg/kg)	0.2560±0.02911	79.55	0.3310±0.0130*	74.44	0.4108±0.02202	69.61	0.3955±0.02200	66.99

*All the values are expressed as Mean±SEM, Analysis of Variance (ANOVA) followed by Dunnett's test. *P<0.05; **P<0.01 and ***P<0.001*
as comparison of test groups to control group; P<0.05; [b]P<0.01 and [c]P<0.001 as comparison of normal group to control group.

Table 4 : In vivo anti-inflammatory activity of 1-Phenylethyl-2-methyl-3-ethoxycarbonyl-5-hydroxyindole derivatives

Treatment	½ hr		1hr		3hr		5hr	
	Paw-volume (ml)	%EI	Paw-volume (ml)	%EI	Paw-volume (ml)	%EI	Paw-volume (ml)	%EI
Normal	0.6625±0.01315	--	0.6225±0.06415	--	0.6675±0.01109	--	0.6675±0.01652	--
Control	1.2523±0.45213 c	--	1.2950±0.64300 c	--	1.3520±0.07325 c	--	1.1983±0.02314 c	--
Diclofenac (10mg/kg)	0.2263±0.0149***		0.2763±0.0239***		0.2838±0.0171***		0.2988±0.0281***	
Compound-08 (3mg/kg)	0.4231±0.821*	66.21	0.3327±0.08315	74.30	0.3984±0.03352	70.53	0.3764±0.0321	68.58
Compound-08 (10mg/kg)	0.3981±0.0283	68.21	0.3527±0.04251	72.76	0000.4192±0.6035	68.99	0.3847±0.0172	67.89
Cmpound-04 (3mg/kg)	0.2561±0.0372	79.55	0.2738±0.01154*	78.85	0.3193±0.0362	76.38	0.29837±0.0172	75.10
Compound-08 (10mg/kg)	0.2451±0.0372	80.42	0.2392±0.03416*	81.52	0.3291±0.0364*	75.65	0.3150±0.3261**	73.71
Compound-04 Tracho-2 (3mg/kg)	0.3261±0.0364	73.96	0.35647±0.03212*	72.47	0.42918±0.0253	68.25	0.3982±0.0162	66.76
Compound-04 Tracho-2 (10mg/kg)	0.3012±0.0374*	75.94	0.4103±0.02718**	68.31	0.4343±0.0241	67.87	0.3873±0.02342	67.67

*All the values are expressed as Mean±SEM, Analysis of Variance (ANOVA) followed by Dunnett's test. *P<0.05; **P<0.01 and ***P<0.001*
as comparison of test groups to control group; [a]P<0.05; [b]P<0.01 and [c]P<0.001 as comparison of normal group to control group.

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Table 5 : In vivo analgesic activity of 1-Phenylethyl-2-methyl-3-ethoxycarbonyl-5-hydroxyindole derivatives

Compound	Reaction Time (X ± SE) in seconds (difference in reaction time compared to basal value)			
	Basal	60 min	120 min	180 min
Control	4.60 ± 0.11	10.01 ± 1.20	9.550 ± 0.62	10.35 ± 1.24
Pentazocine (5mg/kg)	4.93 ± 0.23	11.21 ± 1.23 (6.28 ± 0.30	14.32 ±1.23*** (9.39± 0.20)	15.00 ±0.00*** (10.07± 1.53)
Compound-02 (3mg/kg)	4.29 ± 0.21	12.43 ± 0.62 (8.14± 1.05)	11.30 ± 0.48** (± 0.31) 7.01	12.22 ± 1.43 (7.91± 0.84)
Compound-02 (10mg/kg)	5.67 ± 0.41	14.12 ± 0.88 (8.45± 0.01)	13.60 ± 0.20*** (7.93± 0.19)	12.95 ± 0.22* (7.28± 0.77)
Compound-01 (3mg/kg)	6.30 ± 0.17	10.23 ± 0.62 (± 0.25) 3.93	11.30 ± 0.38** (5.00± 0.41)	12.37 ± 0.23 (6.07± 0.14)
Compound-01 (10mg/kg)	5.61 ± 0.29	11.22 ± 0.35 (5.61± 0.34)	12.24 ± 0.13** (6.63± 0.36)	13.15 ± 1.20* (7.54± 0.19)
Compound-08 (3mg/kg)	6.25 ± 0.54	09.21 ± 021 (2.96± 0.38)	11.35 ± 0.13** (5.1± 1.08)	12.12 ± 2.73* (5.87± 0.21)
Compound-08 (10mg/kg)	7.65 ± 0.35	10.01 ± 021* (2.36± 0.25)	13.31 ± 0.10*** (5.66± 0.27)	14.02 ± 1.03** (6.37± 0.24)
Compound-04 (3mg/kg)	6.17± 0.66	10.21 ± 0.21 (4.04± 0.25)	12.10 ± 0.18** (5.93± 0.41)	13.45 ± 0.33 (7.28± 0.14)
Compound-04 (10mg/kg)	6.63 ± 0.16	10.22 ± 0.21 (3.59± 0.34)	11.33 ± 0.31** (4.7± 0.36)	14.21 ± 2.13* (7.58± 0.19)
Compound-04 Tracho-2 (3mg/kg)	4.35 ± 0.98	09.28 ± 1.45 (4.93± 0.10)	12.65 ± 1.15 (8.3± 0.31)	14.32 ±1.43* (9.97± 1.60)
Compound-04 Tracho-2 (10mg/kg)	7.55 ± 0.87	10.61 ± 0.32* (3.06± 0.23)	14.78 ±0.33*** (7.23± 0.51)	15.00 ±0.00*** (7.45± 0.23)

*Results expressed in mean ± SEM (n=6) Significance level *p<0.5, **p<0.01, *** p<0.001;*

III. Experimental

Melting points were determined in open capillary tubes and are uncorrected. IR spectra(cm-) recorded on Perkins-Elmer 881; 1HNMR spectra in $CdCl_3$ or TMS ON BRUKERS 400MHz NMR spectrometer (chemical shift in delta ppm); and mass spectra on a Auto spec E1 mass spectrometer. Elemental analysis was carried out on Heraeus CHN rapid analyzer.

a) Synthesis of Heterocyclic's

1. Ethyl,3-phenylethylaminocrotanate (Compound B):

Ethylacetoacetate was added drop wise to mixture of phenyl ethylamine and concentrated hydrochloric acid (2drops) with stirring at such a rate so that temperature remained at 40-45°C.The addition required one hour and stirring was continued for additional 2 hr at 40-45°C. The mixture was set aside overnight at room temperature, then extracted with ether. Ethereal solution was dried over anhydrous sodium sulfate and ether was evaporated to get β aminocrotanate as violet oil.(80-90% yield).IR (KBR): 1651(ester C=O) 3289cm (NH).

2. 1-Phenylethyl ,3-ethoxycarbonyl,5-hydroxy,2-methyl Indole (Compound 1) :

To a cooled solution of p-benzoquinone 1(.0.1mol)) in dry acetone (40ml) was added Ethyl-3-phenylethylaminocrotanate(Compound B) 0.1M with shaking. The reaction mixture was allowed to stand at room temperature for one hour and then it was heated for 1.5 hour on steam bath. Excess of solvent was removed under reduced pressure and the residue was recrystalized from suitable solvent (75-80% yield) ;Molecular formula $C_{20}H_{21}NO_3$;

IR (KBr): 1657(C3 ester C=0) and 3252cm- (C5-OH):

^{1}HNMR ($CdCl_3$)/TMS)δ 1.44(t 3 H,J=7.01 Hz of C_3–ester CH_3),2.43 (s, 3H, C_2-CH_3), 3.0(t 2H, J= 7.32Hz, of Ph-CH_2), 4.29(t, 2H,J=7.32Hz, N-CH_2), 4.44(q,2H, J=7.32Hz, , C_3-OCH_2) 5.36 (s, 1H, C_5-OH),6.82(dd, J_{1-3}=8.44,Hz, J_{2-4}=8.83Hz, Ar C_6-H), 7.28(d, J=2.7Hz, Ar C_7-H), 7.65(d, J=2.44Hz, Ar C4-H), 7.03(d, J=7.62, Ar 6'-H), 7.16&7.14(d,J=8.54Hz, Ar 4'& 8'-H), 7.25&7.23 (dd, J1-3=6.7Hz, J2-4=6.40Hz Ar 5' and 7' H);

^{13}CNMR(200MHz, $CDCl_3$) ;δ=11. 14. 35. 38. 40. 44. 58. 101. 105. 110. 111. 126. 126. 127. 128. 129. 138. 144. 152. 165.

MS (m/z relative intensity); 346(M^++23), (10), 324(M^+ +1), (100), 278(10),

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3. 1-phenylethyl,2-methyl,3-ethoxycarbonyl,5-methoxycarbonyl, 2-methoxy Indole (Compound 2) :

To a solution of cooled solution of 5-hydroxyindole (0.03mole,) in dry acetone (500ml) were added methyl bromo acetate (0.06mole),anhydrous potassium carbonate(8gm) and potassium iodide (0.1gm). The reaction mixture was heated at reflux for 57hr. It was filtered and solvent was removed under reduced pressure. The residue was collected and crystallized from suitable solvent. Residue was purified by colum by using 10% ethyl acetate in hexane.mp 74-77°C, yield 63%. Molecular formula $\mathbf{C_{23}H_{25}NO_5}$ IR (KBr); 1686(C_3- ester- C=0) 1731(C_5 –ester C=O) and absent (C_5-OH group was observed);

^{1}HNMR, delta 1..42 (t, 3H,J=7.32Hz, C_3-ester CH_3), 2.44(s,3H,C_2-CH_3),3.03(t, 2H J=7.32, Phenyl CH_2), 3.82(s, 3H, C-5-OCH3), 4.26(q, 2H,J= 7.32Hz, and C3-OCH_2),4.72(s,2H,C-5-OCH2),4.39,(t,J=7.32Hz,1NCH_2),-6.86(dd, J_{1-3}=8.85Hz, J_{2-4}=8.85Hz, 1H,Ar-C_6-H), 7.03(d, 1H,J=1.52Hz,Ar-C_7-H),7.63(d,1H,J=2.44HzAr-C_4H,)7.05 (dd,J_{13}=7.62Hz,&J_{24}=7.32Hz1Hof'6'Phenyl),7.19 & 7.21 (d,J=8.85Hz,2H of "4&"8 phenyl),7.27&7.24(dd,J1-3= 6.7Hz, J2-4,7.01Hz,2H of '5&'7 Phenyl), MS (m/z relative intensity); 438(M^++1), (100),392,(30), 324(10),

4. 1-phenylethyl,3-ethoxycarbonyl,2-methylindole,5-yl, oxyaceticacid hydrazide (Compound 3) :

A mixture of 4(0.02mole) in ethanol (200ml) hydrazine hydrate (9ml 99%), and pyridine (1drops) was heated on a boiling water bath for 25hr and was concentrated to half volume and left overnight. The separated solid was filtered, washed with little ethanol and crystallized from suitable solvent mp116-117°C, Yield, 67%.Molecular formula $\mathbf{C_{22}H_{25}N_3O_4}$, IR (KBr) 1673($C_3$-easter C=O), 1633($C_5$-amode C=O);3273, 3557cm^{-1} (NH/NH_2);

^{1}HNMR ($CDCl_3$/TMS); 1..33(t, 3H,J=7.01Hz, C_3-ester CH_3), 2.41(s,3H,C_2-CH_3),2.98(t,J=7.01, 2H, Phenyl CH_2), 4.23(q, 2H,J= 7.09Hz, C3-OCH_2) ,4.49 (s, 2H,C-5-OCH2),4.36,(t,J=7.01,1N-CH_2),-6.87(dd, J_{1-3}=8.85Hz, J_{2-4}=8.85Hz,1H,Ar-C_6-H), 7.09(d, 1H,J=1.52 Hz,Ar- C_7-H),7.48(d,1H,J=2.1Hz,,Ar-C_4-H,)7.05(dd,J_{13}=7.62Hz, & J_{2-4}=7.32Hz1Hof'6'-Phenyl),7.20&7.22(d, J=7.32Hz,2H of "4&"8 phenyl), 7.26&7.25(dd,J1-3=6.7Hz,J2-4,7.01 Hz, 2H of '5&'7 Phenyl), 9.38, (s 1H, Amide NH, Disappeared on D_2O exchange,) MS (m/z relative intensity); 396(M+1)(18), 350(M-45)(100),

5. 1-phenylethyl,2-methyl,3-ethoxycarbonyl,5(5'mercapto-1',3',4'-oxadiazol-2'-yl)-methoxyindole(Compound 4) :

A mixture of carbohydrazide (Compound 3) 0.0015M in absolute ethanol (20ml) KOH (0.003M) dissolved in water (3ml) and carbon disulfide (0.0045M) was heated under reflux until the evolution of H_2S ceased (20hr). The reaction mixture was cooled to room temperature and poured in to ice-cold water. It was then neutralized with dil. HCl. The precipitated solid was filtered washed with water and dried. The product was recrystalised from ethanol. Yield mp206-208°C, Yield, 78%. Molecular formula $\mathbf{C_{23}H_{23}N_3O_4S}$ IR (KBr) 1655 (C_3-easter C=O),3084cm (NH);

^{1}HNMR ($CDCl_3$/TMS); δ1.34(t, 3H J=6.96.Hz,, C3-ester CH3), 2.43(s,3H, C_2-CH_3),2.98(t,2H, J=6.96 Hz,Phenyl-CH_2), 4.28(q,2H, J=7.32Hz,C3-OCH_2) 4.39 (t, 2H,J=7.32Hz,1N-CH_2),5.25(s,2H,C5-O-CH_2),6.94(dd,J_{1-3} =8.79Hz, J_{2-4}= 8.79, Ar,C_6-H), 7.1(d, 1H,J=1.52Hz,Ar-C_7-H), 7.57(d, 1H,J=2.44Hz,,Ar-C_4-H,),7.05(dd,J_{1-3}=7.62 Hz,&J_{2-4}=7.32Hz1Hof'6'-Phenyl),7.19&7.21(d,J=8.85Hz, 2H of "4&"8 phenyl), 7.27&7.24(dd,J1-3=6.7Hz, J2-4,7.01Hz,2H of '5&'7 Phenyl),14.68(s, amide NH dis-appeared on D_2O exchange),

^{13}CNMR(200MHz,$CDCl_3$);δ=11.14. 35. 38. 39. 40. 40. 44. 58. 60. 102. 105., 111., 121. 126. 128.,131.,138.,145.152.,159.164.178.02,MS (m/z relative intensity); 460(M+Na), (60),392,(M-45), (100),

6. 1-phenylethyl,3-ethoxycarbonyl,2-methyl,5-yl(meth oxycarbothiosemicarbazide) (Compound 5) :

To a solution of carbohydrazide (Compound 3) 0.095M in ethanol (50ml) was added phenylisothiocynate (0,095M) with stirring. The mixture was heated under reflux for 12hr. The yellow solid that separated on cooling to room temp was filtered, and recrystalized from alcohol. Yield, 69%. Molecular formula $\mathbf{C_{29}H_{30}N_4O_4S}$ IR (KBr), 3222, 3310 cm^{-1} secondary amide NH. 1693 cm^{-1} (C-5-ester C=O) and 1673cm^{-1}(C-3-ester C=O),

^{1}HNMR ($CDCl_3$/TMS); δ1.45(t, 3H,,J=7.0 Hz,3h, C-3-ester CH3), 2.48(s,3H, C_2-CH_3), 3.03(t,2H, J=6.96 Hz, Phenyl-CH_2), 4.39(t,2H, J=7.12Hz,1N-CH_2), 4.27(q, 2H, J=6.96Hz,C3-OCH_2), 7.78(d, J=2.19Hz, Ar-C_4-H), 6.96(dd,J_{1-3}=8.79Hz, J_{2-4}=8.79, Ar,C_6-H), 7.29(d, J=2.7 Hz, 1H, Ar-C_7-H), 7.2 to7.4 (m 10H , Aromatic H),

8.39, 9.1, 9.9(s 1H, Amide NH, Disappeared on D_2O exchange,) ; ^{13}CNMR(200MHz, $CDCl_3$);11,14,,25, 53,45,59,76,77,78,81,103,109,114,117,121,124,125,126, 126,127,128,129,129,132,137,145,148,165,171,188,MS(m/z relative intensity); 540(M+1), (100). 495(M-45) (60).

7. 1-phenyl,3-ethoxycarbonyl,2-methyl,5(5'analino-1', 3',4'-oxadiazol-2'-yl-methoxyindole) (Compound 6) :

To a solution of thiosemicarbazide (Compound 5) 0.005M in ethanol (15ml) was added NaOH solution (1 ml, 4%) with cooling and shaking. Then a solution of iodine in KI (aq 5%) was added gradually to it with shaking till the colur of iodine persisted at room temp. The contents were heated at reflux on a water bath for 7 hr. The solvent was removed under reduced pressure and residue was recrysatlized from ethanol. Yield, 76%. IR (KBr)

1674cm^{-1} (C_3-easter C=O), 3222cm^{-1}(NH);

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^{1}HNMR ($CDCl_3$/TMS); δ1.32(t,3H J=7.33.Hz,, C3-ester CH3), 2.41(s,3H, C_2-CH_3) 3.01(t, J=7.32 Hz, Phenyl-CH_2),4.34 (t,J =7.32Hz,1N- CH_2),4.26(q,,2H,C3-OCH_2), 6.6 to7.5 (m 13H , Aromatic H), 10.6,(s, 1H of phenyl NH , disappeared on D_2O exchange,) MS (m/z relative intensity); 503(M+NH3), (100),458,(M-45).(15).

8. 1-phenylethyl,3-ethoxycarbonyl,2-methyl,5(4'-phenyl,5'-mercapto-1',2',4'-trizole-3'-yl) methoxyindole (Compound 7) :

To the suspension of thiosemicarbazide (Compound 5) 0.0015 M in a sodium hydroxide solution (4%) (10ml) was heated gently under reflux for 1hr. The reaction mixture after cooling room temperature was poured in to crushed ice (20gm) and acidified carefully with dilute acetic acid. The precipitation thus obtained was filtered, washed with water, dried and recrystallised from suitable solvent. Yield, 78% Molecular formula $C_{29}H_{28}N_4O_4$;1HNMR ($CDCl_3$ / TMS); δ1.32(t, 3H J=6.2.Hz,C3-ester CH3), 2.48(s,3H, C_2-CH_3) 3.04(t, J= 7.32Hz, Phenyl-CH_2),4.32(t, J=7.32Hz,1N-CH_2) 4.39(q, J=7.0Hz,C3-OCH_2), 6.6 to7.7 (m 8H , Aromatic H),MS (m/z relative intensity); 483(M+1) (100),438(M-45),(15).

9. 1-phenyl,2-methyl,3-ethoxycarbonyl 5(2,5 dimethyl pyrrole,1-yl) amino carbonyl 1-methoxy Indole (Compound 8) :

To a solution of (Compound 3) 0.0015M in absolute ethanol (10ml) were added acetyl acetone 0.0015M and glacial acetic acid (1ml). The reaction mixture was heated on a boiling water-bath for 3 hr. the reaction mixture was concentrated to half of its original volume and poured into ice-cold water (20ml) .The reaction the separated solid was collected by filtration. Washed with water .dried and recrystalised from ethanol. ,mp 147-148^{0}C Yield, 63%. Molecular formula $C_{28}H_{31}N_3O_4$;IR (KBr) 1619cm (C_3-easter C=O), 1692(C_5-amode C=O); 3298cm(NH);

^{1}HNMR ($CDCl_3$/TMS); δ1.42(t,3H J=7.32.Hz,,C_3-ester CH_3), 2.08(s,6H, pyrrole 2 CH_3) 2.49(s,3H, C_2-CH_3) 3.05(t, 2H,J=6.96 Hz, Phenyl-CH_2),4.32(t, 2H, J=6.96 Hz,1N-CH_2) 4.36(q, 2H,J=6.96Hz,C3-OCH_2), 5.80(s, 2H, Ar pyrrole- H), ,6.90(dd,J_{1-3}=8.79Hz, J_{2-4}=8.79, Ar,C_6-H), 7.24 (d, J=1.83Hz, 1H, Ar-C_7-H), 7.72(d, J=2.56,Hz, Ar-C_4-H),7.01(dd,J_{1-3}=7.62Hz,1H of 6'-Phenyl) 7.16&7.14 (d,J=8.85Hz, 2H of 4'&8' Phenyl group)7.26&7.23 (dd, of 5'&7'Phenyl; group), 8.98,(s,1H of Amide NH disappeared on D_2O exchange), ^{13}CNMR(200MHz, $CDCl_3$) ;δ=11. 11. 14. 25. 35. 45. 59. 76. 77, 63, 103. 104. 105. 110. 111. 126. 127. 127. 128. 128.131.137. 145, 146. 148, 152. 165. 167. MS (m/z relative intensity); 574(M+1), (100),

10. 1-phenyl,3-ethoxycarbonyl,2-methyl,5(1', 3', 4'-oxadi azol,2'-yl,methoxy Indole (Compound 9).

Triethyl orthoformate was added to indole carbohydrazide (Compound 3) 0.002M and heated at reflux for 10-12hr. The excess of triethyl orthoformate was removed under reduced pressure and the residue was triturated with pet ether the resulting solid was filtered and recrystalised from ethanol, Yield, 73%. Molecular formula $C_{23}H_{23}N_3O_4$ IR (KBr) 1657cm^{-1} (C_3-easter C=O),

^{1}HNMR ($CDCl_3$/TMS); δ1.28(t,3H J=6.72.Hz,, C3-ester CH3), 2.48(s,3H, C_2-CH_3),3.94 (t,2H, J=7.33Hz, Phenyl-CH_2),4.35(t,, 2H,J=7.32Hz,1N-CH_2),4.24 (q,,2H, J= 7.33Hz,C3-OCH_2), 6.6 to7.5 (m 13H, Aromatic H), MS (m/z relative intensity); 506(M+1), (100),

IV. Conclusions

1-phenylethyl-2-methyl-3-ethoxycarbonyl-5(5'-mercapto-1',3',4'-oxadiazol-2'-yl)-methoxyindole (**Compound 4)** and 1-phenyl-2-methyl-3-ethoxy carbonyl5(2,5 dimethyl pyrrole -1-yl) amino carbonyl methoxyindole (**Compound 8)** ,1-phenyl-3-ethoxy carbonyl-2-methyl-5(1',3',4'-oxadiazol-2'-yl-methoxyin dole (**Compound 9)** prepared as a part of our ongoing Structure Activity Relationship study showed good analgesic activity, These compounds also exhibited systematic as well as a topical anti-inflammatory, antifungal and antibacterial activity. The research and development of new 5-hydroxy Indole derivatives linked with Oxadiazole, Triazole and Pyrrole in conjugation with metal complex will provide the focus of future research in the development of new Indole effective drugs.

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3. Submission of Manuscripts,

4. Manuscript’s Category,

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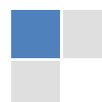

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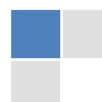

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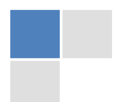

- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

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

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing 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 approach 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. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than 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 maintain the initial two items to no more than one ruling each.

- Reason of the study - 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 quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness 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 to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled 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 with every section. If you make the four points listed above, you will need a least of four paragraphs.
- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
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This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement 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 for the least amount of information that would permit another capable scientist to spare 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 object, mention the specific item describing a way but draw the basic

principle while stating the situation. The purpose is to text 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:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not 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 - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in 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 a 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. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
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- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise 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 in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.

- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- 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 part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. 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 implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly 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 with prospect, and let it drop at that.

- Make a decision if each premise is supported, 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 the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One 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 available information
- Submit to work done by specific persons (including you) in past tense.
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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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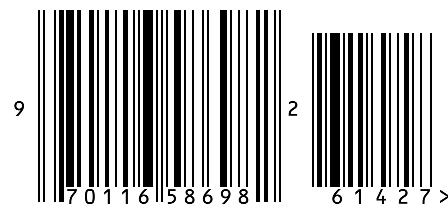

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