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Plant of Cleome Rutidosperma

Kinetic Characterization of a Protease

Discovering Thoughts, Inventing Future

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Cytotoxic Effects, Phytochemical and GC/MS Analyses of *Boscia senegalensis. L* Leave Extracts

By Manal A Ibrahim & Adam Kh Musa
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Abstract- *Boscia senegalensis* leaves which were traditionally used to relief intestinal pain in Sudan were tested for secondary metabolites and cytotoxic effects. The result indicated a moderate presence of alkaloids, highly presence of tannins, weakly presence of flavonoids and steroids and negative results for saponin, phenolic compound and triterpens. Methanolic extract was tested for cytotoxicity against brine shrimp larvae. Remarkable cytotoxicity was revealed with high value equal to 1.975 μg/ml. Also the extract was subjected to separation by column chromatography technique, four fractions were obtained. The fractions tested for cytotoxicity against brine shrimp larvae again. F_1, F_2, F_3 and F_4 represented high values equal to 66.13. 11.07, 1.74 and 99.41 μg/ml respectively. F_3 with high cytotoxicity was chosen for gas chromatography / mass spectrometry analysis. Thirty-five compounds were not recorded in any previous work in available literature were obtained. The high cytotoxicity of this fraction due to presence of octadecenoic and n-hexadecenoic acid which are known to have anticancer activity.

Keywords: *boscia senegalensis, brine shrimp larvae, GC/MS analysis, tramadol, D allose.*

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Cytotoxic Effects, Phytochemical and GC/MS Analyses of *Boscia senegalensis. L* Leave Extracts

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Also presence of tramadol may increase the cytotoxicity, which was found to cure sever and moderate pain. Amazingly *B.seneglansis* leaves were used in folkloric medicine in some parts of Sudan for intestinal pain without any knowledge of their chemical constituents.

Moreover D.allose sugar (3.67) was increase toxicity of this fraction since it had promising antitumor proliferation and apoptotic activity.

Keywords: boscia senegalensis, brine shrimp larvae, GC/MS analysis, tramadol, D allose.

I. Introduction

Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. (Davidson-Hunt, 2000). And Traditional herbal medicine as a major African socio-cultural heritage, obviously in existence for several hundreds of years, was once believed to be primitive and wrongly challenged with animosity, especially by foreign religions. However, today traditional medicine has been brought into focus for meeting the goals of a wider coverage of primary health care delivery, not only in Africa but also to various extents in all countries of the world (Elujoba, et al., 2005).

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In Sudan, people have been tapping their herbal remedies from education for time immemorial. For this purpose, they use a vast variety of plants ranging from the rain forest vegetation in the south, to the desert vegetation of the north, and from the semi-Mediterranean climatic zone of the red sea, to the rich savanna of the west (Elghazali et al., 2003). The Sudan has been home to indigenous civilization, such as Meroe, and road for others, namely pharaonic, Christian and Islamic civilizations. The country has been heavily influenced by fusion of different cultures. The immigrant Arab culture and the neighboring cultures (mainly Egyptian and West African cultures) have strongly influenced Sudanese culture. However, there is a wide range of practices, which fall under the umbrella of traditional medicine (Elkhalifa, 2003),

Boscia seneglansis is a member of the family Capparaceae is locally known as Elcrasan and Elmekheat. Its occurs across area that in recent decades has faced more hunger than any other in the world—the vast swath of Sahel and Sahara savannas stretching from Mauritania, Senegal, and Mali all the way to southeastern Egypt, Sudan, Ethiopia, Somalia, and Kenya (NRC, 2008). and western Sudan. (Arbonnier, 2002).It is usually eaten as a food with oil and salt. Alternatively, seeds are ground to flour which is consumed in the form of kisra, flat thin bread popular in Sudan or Asida, a local form of porridge. The taste of the final product can be improved by blending with millet or sorghum flour (NRC, 2008).

The leaves are used to protect stored food against parasites (Hans, 2000). According to the African folk medicine, an infusion of leaves is used to remove intestinal parasites from camels. leaves mixed with millet flour taken each morning on an empty stomach for anthelminthic; dried leaves or dried bark are taken for schist osmosis. Infusion of the leaves is used as an eyewash. pruritus of the eye due to syphilis and to relief intestinal pain. (Orwa *et al*., 2009).The seeds of *B. senegalensis* are a valuable source of glucocapparin. This component which presents an interesting anti-hyperglycemic effect could be related to the traditional use of the seeds in Chad against type 2 diabetes. However, the cytotoxicity effect pointed out suggests that further investigations extended to would be needed to make the glucocapparin a potential anti-diabetic drug

(Mahamat, *et al*., 2012).One active principle in *Boscia* has been identified as a glucocapparin, a sulfonated glucose which exhibited not only hypoglycemic effect, but also cytotoxicity (Ngomvougat*et al*., 2015). Phenolic compounds of *Boscia seneglansis*, especially flavonoids. Kampferol, quercetin and their derivatives proved to be effective against numerous cancer cell lines. (Carocho*et al.,* 2013).

Now-a-days brine shrimp (*Artemiasalina,* fairy shrimp or sea monkeys) lethality assay is commonly used to check the cytotoxic effect of bioactive chemicals. It is a preliminary toxicity screening of plant extracts. (Ghosh, *et al*., 2015; It is also an internationally accepted test for detecting antitumor potential of the drugs (Hazra and Chatterjee 2008). The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxicity and anti-tumor properties. (Indabawa 2009).since the brine shrimp responds similarly to the corresponding mammalian system (Solis *et al*., 1993).

Cytotoxicity via the brine shrimp test is studied in order to reveal new anticancer compounds (Harborne, 1998). Thus, the aim of this study is toestimate the cytotoxicity of extract and fractions of *Boscia seneglansis* against brine shrimp larvae as a new potential source of natural anti-tumor agent and subjected the bioactive fractions to GC/MS analysis.

II. Materials and Methods

a) Plant materials

The Plant *Boscia senegalensis* was Collected in April 2017 from Gabrat Kadogly in South Kordofan, Sudan, authenticated and identified by Dr. Manal A. Ibrahim, Department of Botany, Faculty of Science and Technology, Omdurman Islamic University.

b) Preparation of Extract

The leaves were dried at room temperature in order to avoid any changes that may alter their chemical composition. Then, they were ground to a coarse powder, 100gm of plant materials were soaked overnight with 350 ml 98% methanol in 500ml conical flask. Then the extracts were filtered, evaporated to dryness under reduced pressure in a rotatory evaporator and weighted.

c) Qualitative phytochemical Analysis

Phytochemical screening for the identification of major groups of chemical constituents using standard procedures (Harborne, 1998). The phytochemical compounds which tested were, tannins, saponins, flavonoids, terpenoids, Steroids, Alkaloids and phenolic compound.

d) Column chromatography

i. *Extraction and fractionation procedures*

The dried leaves (1kg) of the *B.Senegalensis* were soaked for 2 days in 1500 ml methanol. It gave 13 g, with dark green residue and were subjected to silica gel (230 – 400 mesh) column chromatography separation using stepwise gradient elution of n-hexane to chloroform to ethyl acetate and finally washing with water. 100 ml portions were collected, concentrated and combined according to their similarity in spectrometric and TLC separation behaviors; using suitable solvent systems.

ii. *Brine Shrimp Lethality Test*

Brine shrimp lethality bio-assay was carried out to investigate the cytotoxicity of plants extract. *Artemia Salina* (leach) eggs (50mg) were added to a hatching chamber containing sea water (45ml). The hatching chamber was kept under an inflorescent bulb for 48h for the eggs to hatch into shrimp larvae. Test extract and fractions (20 mg) were separately dissolved in 2 ml of methanol, then 5, 50 and 500 μl of each solution were transferred into vials corresponding to 10, 100 1000 μg / ml, respectively. Each dosage was tested in triplicates. The vials 9 for each test) and one control containing 500 μl of the solvent were allowed to evaporate to dryness in 48h at room temperature. Ten larvae of A. Salina leach (taken 48 – 72 h after the initiation of hatching) were added to each vial and the final volume of the solution in each vial was adjusted to 5ml with sea water, immediately after adding the shrimps. One drop of dimethyl sulphoxide (D M SO) was added to the test and control vials before the addition of the shrimps to enhance the solubility of the plant extract. (Meyer *etal*.,1982). LC_{50} values were determined at 95% confidence intervals by analyzing the data on a computer loaded with a Finney program (McLaughlin, 1991). The LC_{50} values of the brine shrimps obtained for the studied plant extracts were recorded. Etoposide, the reference cytotoxic drug, was used as a positive control with LC_{50} (7.46).

iii. *Gas Chromatography / Mass Spectrometer (GC/MS)*

The qualitative and qualitative analysis of the sample was carried out by using GC / MS technique model (GC/MS –QP2010 –Ultra) from japans, Simadzu company, with serial number 0205101565SA and capillary column (Rtx – 5ms – 30m x 0.25mm x 0.25um). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61ml/min, the temperature program was started from 60c^{o} with rate 10c^{o}/min to 300c^{o} as final temperature degree with 3minutes holt time, the injection port temperature was 300c^{o}, the ion source temperature was 200c^{o} and the interface temperature was 250c^{o}. The sample was analyzed by using scan mode in the range of m/z 40 – 500 charges to ratio and the total run time was 27 minutes. Identification of components for sample was

achieved by comparing their retention index and mass fragmentation patents with those available in the library, the national Institute of Standards and Technology (NIST)., results were recorded.

III. Results and Discussion

a) *Phytochemical Studies*

The presence or absence of some secondary plants products were tested by procedures described by Harborne (1973). The results indicated a moderate presence of alkaloids, weakly presence for flavonoids, steriods and triterpenes, highly presence for tannins, and negative presence for phenolic compounds and saponin (Table 1). Capparaceae family, showed moderate to abundant presence of alkaloids, although some novel alkaloids have been isolated from fruits and aerial parts of some Capparaceae (Foster *et al*., 2016). The seeds and leaves of *B. seneglansis* were characterized by the presence of alkaloids, saponins and tannins (Adam *et al*., 2011).

Table (1): Phytochemical screening test for the secondary products of plants

Compound	Alkaloid	Phenolic compound	Saponin	Tannin	Flavonoid	steroid	Triterpene
presence	++	-	-	+++	+	+	+

Keys:

+ + + = High presence.

+ + = Moderate presence.

+ = Weakpresence.

- = Absent.

b) *Brine Shrimp Lethality Test*

The importance of the cytotoxicity from the fact that it is linked with the discovery of anticancer compounds (Moshi *et al*., 2004). A good relationship has been found with the brine shrimp lethality test to detect anti-tumoral compounds in terrestrial plant extracts (Mackeen *et al*., 2000). The significant correlation between the brine shrimp assay and in vitro growth inhibition of human solid tumor cell lines were demonstrated by the national cancer institute (NCI, USA). It is significant because it shows the value of this bioassay as a pre- screening tool for antitumor drug research (Anderson *et al*., 1991). Not only that there is positive correlation between brine shrimp toxicity and 9 KB (human nasopharyngeal carcinoma) cytotoxicity ($p = 0.036$ and kappa = 0.56). The brine shrimp test was being used as a prescreen for a panel of six human solid tumor cell lines at the cell culture laboratory of the Purdue Cancer Center (Mclaughin *et al*., 1998). This is an internationally accepted bioassay for screening of antitumor compounds (Meyer *et al*., 1982).

In this regard, a simple bioassay was used for screening purposes (Hostettmann, 1991). Thus *Artemiasalina* larvae (brine shrimp nauplii) has been used target organism to detect bioactive compounds in plant extract and toxicity to this crustacean has a good correlation with anti-tumor activities in man (Mclaughlin, 1991) since the brine shrimp responds similarly to the corresponding mammalian system (Solis *et al*.,1993).

According to the method described by Meyer (*et al*., 1982), methanol extracts were used to determine its cytotoxicity against brine shrimp larvae (LC_{50}) after 24 hours:

The results of this study are classified as: LC_{50} less than 20 μg / ml and was considered as highly toxic, LC_{50} from 20 to 100 μg / ml as toxic, LC_{50} from 100 to 500 μg /ml as moderately toxic and from 500 to 1000 μg / ml was weakly toxic according to Padmaja et al (2002). However, meyer (1982). considered the LC_{50} values > 1000 μg / ml as non – toxic or safe.

The brine shrimp lethality test revealed the cytotoxicity effects of plants extracts and *B. seneaglenses* fractions which studied in this investigation. The crude extract of plant materials were showed considerable results. The highly effect against brine shrimp larvae was shown by *B. senegalensis* extract euql to1. 97 μg/ml (Table 2)

As for the fractions of the plant materials, The F_3 and F_2 gave the highest result which was equal to 1.74 and 11.07 μg/ml respectively (Table 3).

On the other hand, F_4 and F_1 exhibited toxic result (66. 13 μg/ml, 90. 41μg/ml). It's to be noted that the F_3 found in *B.senegalensis* which was considered as responsible for the high cytotoxicity of this plant since Azaizah *et al*., (2003) stated that medicinal plants with bioactive compounds may act individually, additively or synergistically to improve health. The result clearly indicated that the plant had high cytotoxic effect which was attributed to synergistically effects of the compounds. However, this is disagreement with what was reported by Sakine, *et al* (2012), who examined clucocapparin, which is a compound isolated from plant seeds, against brine shrimp larvae (16. 48 μg/ml).

Table (2): Brine shrimp bioassay results of plant extracts

Plants	Part used	LC_{50} µg / mL
B. Senegalensis	Leaves	1.975

Keys: LC_{50}> 20 µg / ml = highly toxic, 20 -100 µg / ml as toxic, 100 -500 µg /ml moderately toxic, > 1000 µg / ml weakly toxic

Table (3): Brine shrimp bioassay results of plant fractions

NO	Fractions	LC_{50} (µg /ml)
1	F_1	66.130
2	F_2	11.075
3	F_3	1.740
4	F_4	90.417

c) GC/MS Analysis of B. senegalensis leaves fraction

This technique was used for identification of fractions which were selected according to their high cytotoxic effect against brine shrimp larvae (F_3). The results showed different constituents, molecular weights, formula and retention times (Table 4). Thirty-five compounds not recorded in any previous work in the available literature were shown. Out of these compounds, 12 compounds showed high percentage with values ranging from 2.23 to 20.69%.

Table (4): GC/MS Analysis of *B. senegalensis* leaves fraction

NO.	Compounds	R.T	%	Formula	Class type
1	3-Buten-1-amine,N,N-dimethyl-	3.652	1.67	$C_6 H_{13} N$	Amines
2	2-pyrrolidinemethanol,1-methyl-	4.781	1.06	$C_6 H_{13} NO$	Amines
3	N-Methyl-L-prolinol	5.017	4.59	$C_6 H_{13} NO$	FA
4	Arecoline	5.463	0.51	$C_8 H_{13} NO_2$	Amines
5	1-But-2-enylpyrrolidine	5.812	1.28	$C_8 H_{15} N$	Amines
6	4-(4-methyl-piperazin-1-yl)-1,5,dihydro-l	6.099	5.88	$C_8 H_{14} N_4 O$	Aromatic aldehyde
7	Acetic acid,9-methyl-9-aza-bicyclo[3.3.1]n	6.699	0.94	$C_{11} H_{17} NO_2$	Ester
8	Methanamine,N-[3-methyl1-2-butenyliden	6.864	0.84	$C_6 H_{11} N$	Amine
9	4-hydroxy-2-methylpyrrolidine-2-carboxy	7.124	1.74	$C_6 H_{11} NO_3$	Amine
10	2-pyrrolidine methanol, 2-methyl-,(s)	7.284	20.69	$C_6 H_{11} NO$	Amines
11	8-Azabicycol[3.2.1]oct-6-en-3-one,8-methyl	7.525	1.34	$C_8 H_{11} NO$	Amine
12	1,4:3,6-Dianhydro-.alph.-d-glcopyranos	7.665	1.67	$C_6 H_8 O_4$	Mo
13	1,1-Dimethylamino-1-butene	7.847	3.05	$C_6 H_{13} N$	Alkene
14	2-Tertrazoline-5-thione,1-cyclohexyl-4-	7.934	5.37	$C_{12} H_{21} N_5 OS$	Amine
15	L-Homoserinelactone, N, N-dimethyl-	8.175	0.09	$C_6 H_{11} NO_2$	Alkene
16	Tropinone	8.225	0.27	$C_8 H_{13}$	No Alkene
17	2-Methoxy-4-vinylphenol	9.109	2.92	$C_9 H_{10} O_2$	Phenol
18	Tramadol	9.150	1.51	$C_{16} H_{25} NO_2$	Phenol
19	1,2-Ethanediamine,N.N-dimethyl-	9.304	1.78	$C_4 H_{12} N_2$	Amine
20	6-Amino-1-hexanol,N.N-dimethyl-,methyl	9.552	0.27	$C_9 H_{21} NO$	Amine
21	Phenol1,2,6-dimethoxy-	9.640	0.43	$C_8 H_{10} O_3$	Phenol
22	1-Tetradecen	9.959	0.14	$C_{14} H_{28}$	Alkene
23	2-Buten-1-one,1-(2,6,6-trimethyl-1,3	10.029	0.50	$C_{13} H_{18} O$	Alkene
24	1-Methyl-2- pyrrolidine ethanol	10.112	0.31	$C_7 H_{15} NO$	Amine
25	4-Morpholine ethanol	10.581	0.36	$C_6 H_{13} NO_2$	SH
26	1,3-propanediol,2-(hydroxymethyl)-2-nitr	11.246	3.26	$C_4 H_9 NO_5$	Alkane
27	4-(2,4,4-Trimethyl-cyclohexa-1,5dienyl)-b	11.374	0.44	$C_{13} H_{18} O$	Triterpenes
28	D-Allose	11.846	3.67	$C_6 H_{12} O_6$	Carbohydrate
29	3',5'-Dimethoxy acetophenone	12.494	2.23	$C_{10} H_{12} O_3$	Ketones
30	Lidocaine	16.422	1.08	$C_{14} H_{22} N_2 O$	Amine
31	n-hexadecanoic acid	16.865	7.13	$C_{16} H_{33} O_2$	FA
32	Benzenmethanol,2,5dimethoxy-,acetate	17.478	1.83	$C_{11} H_{14} O_4$	Ester

33	Oleic Acid	18.649	7.39	$C_{18}H_{34}O_2$	FA
34	Octadecanoic acid	18.842	4.48	$C_{18}H_{36}O_2$	FA
35	Stigmasta-7,16,25,-trien-3-ol,(3.beta.,5.alp	24.001	9.26	$C_{29}H_{46}O$	Triterpenes

OM = oxygenated monoterpene. *SH = sesquiterpene hydrocarbon*

FA = Fatty acid.

Table (5): Statistics chemical classes of F_3

Compounds	No.Compounds	Concentration %
Amine compounds	13	37.94
Fatty acid	4	23.59
Alkene	5	4.05
Phenol	3	4.86
Triterpenes	2	9.70
Ester	2	2.77
Aromatic aldehyde	1	5.88
Oxygenated monoterpene	1	1.67
Sesquiterpene hydrocarbon	1	0.36
Carbohydrate	1	3.67
Ketone	1	2.23
Alkane	1	3.26
Total	35	99.98

F_3 contains thirty-five compounds (Table11). Out of these compounds: octadecanoic acid (4,48%) and n-hexadecnoic acid (7.13%) which are possible causes for the cytotoxicity of this fraction, since Isidrov*et al*., (2011) reported that hexadecnoic acids are known to have anticancer activities. Another explanation to increase the cytotoxicity of this fraction it might present of triterpenes (9.70%) which was attracted particular interest in the19 and 20 centuries, due to its extensive antitumor activities (Gadzikowska and Grynkaiewiy, 2001). Furthermore, tramadol which is present in the fraction was found to be toxic in rats (samy*et al.,* 2017). However, tramadol is a centrally acting opioid analgesic which is mainly used to cure sever and moderate pain (Nossaman *et al*., 2010). Amazingly *B. seneglansis* leaves were used in folkloric medicine of the native of some parts of Sudan for the remedy of intestinal pains without any knowledge of its constituents.

N-methyl –L-prolinol and pyrrolidine methanol 2-methyl – (S) are a derivative of proline which might considered as the cause of high toxicity of the fraction, since free proline was found to inhibit the growth of tumors induced by N-methyl –N – Nitrosourea in rats as reported by Kalinovsky *et al*., (2004). Addition to the compound pyrroldine methanol have the highest percentage with the value equal to (20.69 %). Further possible explanation for the increased cytotoxicity might be due to presence of oleic acid. This fatty acid promotes apoptosis and necrosis of the junket Cell. The mechanism of cell death induced by this fatty acid seemed to involve mitochondrial depolarization and lipid accumulation. (John- Fernada., 2005). Also a D-allose is a rare sugar with a similar structure to 2-DG produced from D-ribose for which a recent mass production process has been developed. (Menavuvu *et al.,* 2006). D-allosehas been studied in multiple cancer cell line models including ovarian cancer and was demonstrated to have promising anti-tumor proliferation and pro-apoptotic activity (Sui *et al*., 2005).This a clear indication of first time accomplishment of results which were not preceded by any other ones reported in the available literature.

IV. Conclusion

The plant *Boscia senegalensis* which contains bioactive compound as revealed by using brine shrimp larvae was subjected to GC/MS analysis. The identified compounds represented many constituents which have pharmacological uses and anticancer compounds, as well as tramadol which used in sever and moderate pains. Hence, the plant may be used as a new and promising natural source of intestinal tumor remedies.

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Results of Application of Clinpro White Varnish® and Tooth Mousse® during the Enamel Mineral Maturation and its Focal Demineralization

By Marina Mitush Markaryan, Izabella Frunze Vardanyan, Mikayel Ervand Manrikyan & Gayane Ervand Manrikyan
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Abstract- According to some authors, the final maturation of tooth enamel occurs in 1-2 years after eruption, and then for 2-3 years this process continues in just the fissure area. Full-fledged mineralization during this period is carried out due to the absorption of minerals from saliva, especially in respect to fluorine, calcium and phosphorus ions. As a result of the course of prophylactic treatment with Clin Pro White Varnish® and Tooth Mooth®, an activation of remineralization process was observed ended with the stabilization of the process or complete remineralization in the junior (6-7 years old) and the middle school group children (12-13 years old), which was confirmed by the obtained indices of the "DiagnodentKaVo" laser fluorometry test and the colorimetric test. The use of these preparations is a convenient and effective method in these age groups of children during the preventive treatment provision.

Keywords: *remineralization, laser fluorometry, colorimetric test, schoolchildren.*

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Abstract- **According to some authors, the final maturation of tooth enamel occurs in 1-2 years after eruption, and then for 2-3 years this process continues in just the fissure area. Full-fledged mineralization during this period is carried out due to the absorption of minerals from saliva, especially in respect to fluorine, calcium and phosphorus ions. As a result of the course of prophylactic treatment with Clin Pro White Varnish® and Tooth Mooth®, an activation of remineralization process was observed ended with the stabilization of the process or complete remineralization in the junior (6-7 years old) and the middle school group children (12-13 years old), which was confirmed by the obtained indices of the "DiagnodentKaVo" laser fluorometry test and the colorimetric test. The use of these preparations is a convenient and effective method in these age groups of children during the preventive treatment provision.**

Keywords: remineralization, laser fluorometry, colorimetric test, schoolchildren.

I. Introduction

The period of physiological maturation (mineralization) of the enamel of immature teeth in children can last from 2 to 5 years, and throughout the entire period of mineral maturation (especially during the first year after eruption) the child's teeth need careful and effective care[1].

The initial stage of dental caries is characterized by the efflux of calcium, phosphorus and fluoride ions from the enamel without disturbing its structural integrity, i.e. without the carious cavity formation[2,3]. In such a situation the process is reversible, since the enamel of children's teeth has a high ability to restore its structure owing to the penetration of necessary macro- and microelements from the surrounding medium. A widely used method is the remineralizing therapy with calcium, phosphorus and fluoride preparations, which is usually being performed by a specialist in a dental office[4,5,6]. More recently, such preparations as ClinPro White Varnish® and Tooth Mooth® have appeared in the arsenal of modern dentists for the treatment of caries in the chalky spot stage[7].

At the same time, making a precise diagnosis in the absence of visible manifestations of the pathological process is a very difficult problem due to the extremely small size of the focus of carious lesions and the depth of localization [3,8]. According to Costa A.M. et al. (2007), the frequency of detecting caries in the fissure area by examination with a sharp dental probe is making up 56-58%, with panoramic radiography - 67%, by the method of fiberglass transillumination - 30%, by measuring the electrical resistance of tooth hard tissues - 83%, and by the laser fluometry - more than 90% [9].

Thus, the development and scientific substantiation of new methods of diagnostics, prognosis and increasing the resistance of tooth enamel is an urgent problem in dentistry.

The aim of the study was to objectively evaluate the effectiveness of the use of preparations Clinpro White Varnish® and Tooth Mousse®in the initial form of caries in children to improve the quality of treatment.

II. Material and Methods

In a study conducted with the participation of 213 children in two age groups of 6-7 years (98 people) and 12-13 years (115 people), we treated caries in the chalky spot stage using Clinpro White Varnish® and Tooth Mousse®. Children were divided into groups not only by age, but also by the medicinal means used. To identify the risk of a carious spot, a colorimetric test was used, for which the subjected to examination rinsed his mouth with 1% glucose solution, and then with 0.1% methylene red solution, which stained dental plaque yellow. After a lapse of time (up to 5 seconds), the result was evaluated by a color marking on the test strips (pH 7 - green, pH 6.5 - yellow, pH 6 - orange, pH 5.5 - red). Later on namely in these areas that focal demineralization of the enamel can occur. The level of

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caries detection by this test usage is making up 74.8%[10]. Measuring of fluorescence with the "Diagnodent (KaVo)" at various stages of caries development implies high efficiency and reliability in the demineralization foci revealing in order to plan the adequate and easily implemented treatment options. Numerical values of the scale (5) from 0 to 14 correspond to a healthy enamel structure; from 15 to 25 – to caries within the terms of enamel; from 21 to 90 – to caries within the terms of dentin [11,12].

On control examinations after 6 and 12 months, the areas of foci of enamel demineralization of the studied teeth were assessed. Ethical clearance has been guaranteed from ethical committee in YSMU. The obtained data was statistically processed in the SPSS program.

III. Research Results

The study conditions were maximally standardized for both groups, which was of fundamental importance for assessing the effectiveness of the preparations used. In the junior school group, the children did not complain of caries. In the middle school group, 2 children complained of the presence of a chalky spot (in 3.4 ± 0.18% of cases).

In 6-7 years old schoolchildren in the group using Clinpro White Varnish®, 46 incisors (with smooth surfaces - 41.8%) and 64 first molars (58.2%) were affected. In this given group, after 6 months of using the preparation remineralization was observed just in one schoolchild (2.04%) on the first two molars, which made up 1.82%. The laser fluorescence index at the initial examination averaged 19.2 ± 2.5. The intensity of the colorimetric test before remineralizing therapy made up 5.6 ± 0.2 points on the incisors and 5.7 ± 0.3 - on the molars (Table 1). Among the schoolchildren of the middle age group, the initial indices of the colorimetric test corresponded to 5.7 ± 0.4 on the incisors and 6.2 ± 0.4 points - on the molars. After using the remineralizing agent Clinpro White Varnish® in the children with incomplete mineralization, we observed a noticeable significant increase ($p<0.01$) to 6.1 ± 0.3 and 6.2 ± 0.3 on incisors and molars, respectively. The indices of "Diagnodent (KaVo)" corresponded on average to enamel caries (Table 1).

Table 1: Changes in the colorimetry indices and those of DiagnodentKaVo device in the dynamics of Clinpro White Varnish® preventive treatment

Name of the study	Initial indices	Indices after 6 months	Indices after 12 months
primary group school children			
Colorimetry	7.8±0.84	3.5±2.01	2.3±2.3
Diagnodent indices	19.2±2.5	16.1±2.7	14.5±3.3
middle-aged group schoolchildren			
Colorimetry	7.2±1.36	2.8±1.5	1.4±1.2
Diagnodent indices	17.3±2.3	13.8±1.5	12.6±1.5

The study of the activity of initial caries in the clinic showed that in the group of primary schoolchildren prior to the remineralizing therapy with Tooth Mousse® the intensity of staining in the colorimetric test was 5.6 ± 0.3 points on incisors and 5.85 ± 0.5 points - on molars. Colorimetry indices in the group of middle schoolchildren using Tooth Mousse® before the startup of preventive measures corresponded to 5.9 ± 0.4 points both on incisors and molars.

When carrying out laser fluorometry, the values of the "Diagnodent" device during the initial examination varied within the range of 14-18 units in the group of junior schoolchildren, and from 14 to 24 - in the middle schoolchildren. The average values made up 16.2 ± 1.2 and 17.6 ± 1.9, respectively. This corresponds to the manufacturer's data of the "Diagnodent" device for enamel caries. The initial mean values of LFM before treatment in the study groups did not differ significantly ($p>0.05$, Table 2).

Table 2: Dynamics of indices of the demineralization foci during the preventive treatment with Tooth Mousse®.

Terms of observation		Age groups	
		6-7 years	12-13 years
Before treatment	Colorimetry	4.7±1.6	5.1±1.95
	Data of «DiagnodentKaVo»	16.2±1.2	17.6±1.9
6 months	Colorimetry	2.9±1.6	3.58±2.2
	Data of «DiagnodentKaVo»	12.3±1.45	14.8±1.6
12 months	Colorimetry	1.2±1.05	1.89±1.8
	Data of «DiagnodentKaVo»	11.2±1.9	13.4±1.3

IV. Discussion

In two 6-7 years old schoolchildren (4.1%) in the group using Clinpro White Varnish® after 12 months there was a progression of "Diagnodent" (KaVo) indices, which testifies about further development of the carious process despite the preventive treatment. Complete remineralization of the foci of demineralization after 12 months was observed in 12 schoolchildren (24.5%) on 26 teeth (23.6%). The laser fluorescence index after 12 months of usage of the preparation decreased 1.32 times (Table 1). In 22.4% of children of this age group a stabilization of the process was observed both on the smooth surfaces of the incisors and on the tubercles and fissures of the first molars.

The intensity of the colorimetric test after the preventive measures also tended to decrease 1.1 times ($p < 0.01$) (Table 1). Among the schoolchildren of the middle age group, the caries of fissure (fissures of the first two permanent molars) was observed in 1.71% in one child (1.7%). Stabilization of caries in 12 months after the preventive treatment occurred in only 5 people (8.6%). A decrease in the readings of the "Diagnodent" (KaVo) device as a sign of remineralization was diagnosed in 30 people (51.7%), and complete remineralization was observed in 23 schoolchildren (39.7%). At the same time, in the fissure area, complete remineralization of caries occurred in 14 people (24.1%), and on smooth surfaces of teeth - in 9 children (15.5%), while signs of mineralization prevailed on smooth surfaces of teeth in 27.6% schoolchildren of this age group (Table 1).

The initial values of the colorimetric test were above the critical level. As a result of the measures taken, a tendency to alkalization of dental plaque appeared, as evidenced by an increase in the indices of the colorimetric test 1.1 times.

In the group of junior schoolchildren after remineralizing therapy with Tooth Mousse® the susceptibility to the dye became 1.1 times lower on both incisors and molars ($p < 0.01$). Colorimetry indices in the group of middle schoolchildren using Tooth Mousse® after remineralizing therapy were 6.4 ± 0.3 and 6.25 ± 0.3 on incisors and molars, respectively.

The indices of laser fluorometry also had a pronounced significant tendency towards a decrease in indices to the normal range compared to the indices of this method before treatment in 15.8% of cases in the middle age group and 73.5% of cases in the junior schoolchildren group, which is reflected in Table 2.

In 12 months after the remineralization treatment provided there was a pronounced significant reduction in the number of complaints of stains availability in 15 cases (26.3%) in the Tooth Mousse® applying group.

V. Conclusion

The analysis of indices characterizing the resistance of tooth enamel to the action of acids showed that after the use of Clinpro White Varnish® and Tooth Mousse® preparations, the state of the surface layer of the enamel turned more steady to the effects of acids, and the enamel has become more resistant to cariogenic factors. In general, in all the observed children the intensity of the colorimetric test and the parameters of laser fluorometry after the preventive treatment procurement decreased, although the differences between the means applied were not significant ($p > 0.05$), which makes it possible to equally recommend these preparations for the widespread use.

Conflicts of Interest

The authors have declared that no conflict of interest exists.

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Growth Rate of Young African Mahogany Plants under Saline Stress Conducted with Nutritive Solution

By Willian Viana Campos

Abstract- The study of the growth of african mahogany plants in saline environments is extremely important for the adequate productivity of the crop. This study aimed to evaluate the relative and absolute growth rate of young african mahogany plants, *Khaya senegalensis*, under saline stress, conducted with nutrient solution. The plants were grown in pots containing washed sand, in a greenhouse. Salinity was established by adding NaCl, with levels of electrical conductivity: 1.0 • 3.38 • 15.14 • 29.90 • 42.61 • 53.60 dS m^{-1}, in a completely randomized design. The height of the plants was measured with the aid of a graduated ruler, placed parallel to the stem, measuring the height from the neck to the apical bud of the stem. With the height results in mind, the relative and absolute growth rate was evaluated by the relationship between final height, initial height and time period. The results were subjected to analysis of variance, using the F test, for the comparison of means, regression analysis for the quantitative study of the characteristics, using the SISVAR 5.6 statistical program and principal component analysis. In the different treatments with salinity there was no difference in plant height, indicating that the species *Khaya senegalensis* adapted to changes in electrical conductivity, due in part to the use of nutrient solution.

Keywords: *salinity, plant height, principal component analysis, determination coefficient, woody plants.*

GJSFR-C Classification: *FOR Code: 060799*

GROWTHRATEOFYOUNGAFRICANMAHOGANYPLANTSUNDERSALINESTRESSCONDUCTEDWITHNUTRITIVESOLUTION

Strictly as per the compliance and regulations of:

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Keywords: salinity, plant height, principal component analysis, determination coefficient, woody plants.

I. Introduction

Among african mahogany species, *Khaya senegalensis* has greater rusticity and relative advantage for cultivation in areas under adverse conditions, especially in regions with high concentration of salts in the soil solution and low rainfall (RIBEIRO; FILHO; SCOLFORO, 2017) . Salinity is a term related to the excessive accumulation of salts, especially NaCl, in soil or water, which negatively affects the growth and development of many living organisms (PEDROTTI et al., 2015). It has been one of the environmental factors that can limit plant growth and productivity, especially in arid and semi-arid regions, due to the great environmental contrasts (AKÇA et al., 2020).

The harmful effect of salinity on plants can vary, depending on climatic conditions, light intensity, species and soil conditions (TANG et al., 2015). Due to the addition of salts to water, their osmotic potential is reduced, decreasing the availability of water in the roots and, therefore, exposes plants to secondary osmotic stress (MITTAL; KUMARI; SHARMA, 2012).

The salts absorbed by plants do not directly control growth, but influence the turgor, photosynthesis and activity of specific enzymes (ACOSTA-MOTOS et al., 2015). Saline stress is initially perceived by the root system and impairs plant growth by decreasing hydraulic conductivity, both in the short term, inducing osmotic stress caused by less water availability, and in the long term, by ionic toxicity, due to the imbalance of nutrients in the cytosol (TANG et al., 2015).

High concentrations of Na^+ and Cl^- ions in soil or water reduce water potential, which leads to an initial reduction in growth and low productivity (PETROPOULOS, 2012). Plant growth is inhibited by salinity through osmotic stress and by a decrease in cell turgor (SEMIDA et al., 2016).

The reduction in plant growth, due to salinity, is mainly determined by the following factors, which contribute to the decline in photosynthetic activity: i) increase in the osmotic pressure of the medium, which reduces the plant's ability to absorb water (similar to stress) water); ii) ionic excess (for example, Na^+ and Cl^-) to a toxic level for plant cells; iii) ionic imbalance, which affects the nutritional status of the plant and acts on biochemical and metabolic components, related to plant growth (BOARI et al., 2019).

The high salinity alters the ionic relationship in the vacuoles of plants, which, directly or indirectly, result in changes in the nutrient removal processes (SUN et al., 2017). In this condition, the photosynthetic rate is lower, but the respiration rate increases, associated with reduced growth (SANDOVAL-GIL; MARÍN-GUIRAO; RUIZ, 2012). Thus, the increase in salinity alters the absorption of nutrients in different uptake pathways in the plant (SUN et al., 2017). Under saline conditions, the absorption and translocation of Na^+ and Cl^- induced by salinity compete with nutrient elements, such as K^{+1}, N, P and Ca^+, which generally develop a nutritional imbalance resulting in reduced yield (GIRSOVA et al., 1999).

In view of the potential expansion of the crop in regions of saline soils, this study aimed to evaluate the relative and absolute growth rate of young african mahogany plants, *Khaya senegalensis*, under environmental stress, maintained with a nutritive

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solution, allowing for this adequate investigation. culture in saline conditions, showing the adaptability of the species to semiarid regions.

II. Material and Methods

a) Characterization of the experimental área

The experiment was conducted in a greenhouse, at the State University of Southwest Bahia (UESB), Vitória da Conquista *campus* (Figure 01), whose geographical coordinates are 14° 53 '08" south latitude and 40° 48' 02" from west longitude of Greenwich, with an altitude of 881 m. The treatments were administered during the phase of the first four months of plant growth (June 2018), time counted at the time of planting.

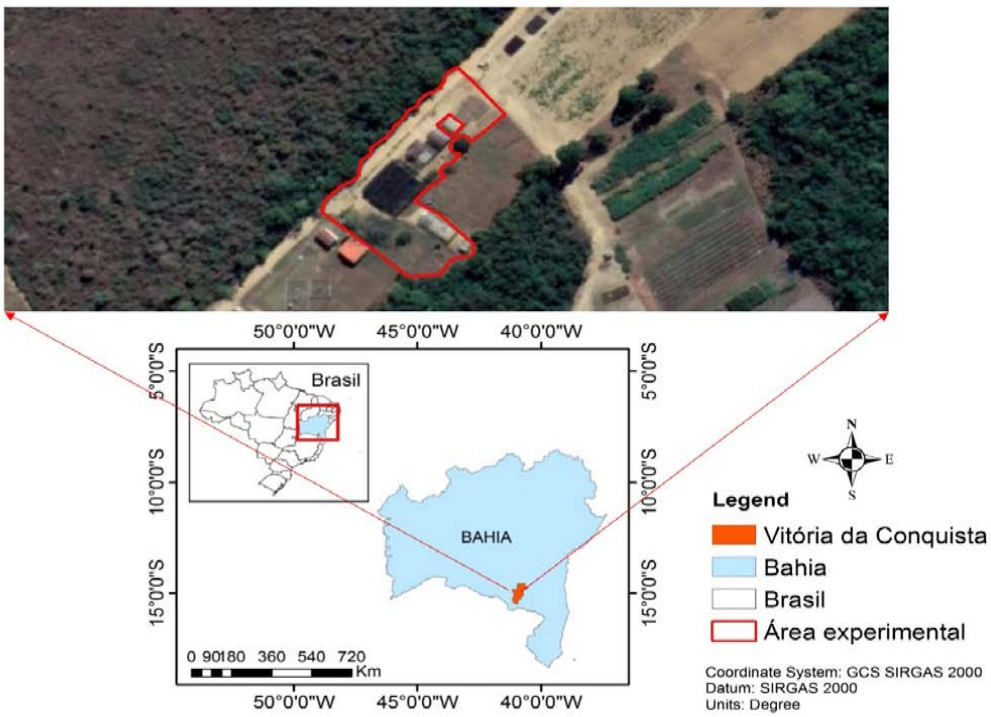

Figure 1: Location of the study area. Vitória da Conquista - BA, Northeast Brazil

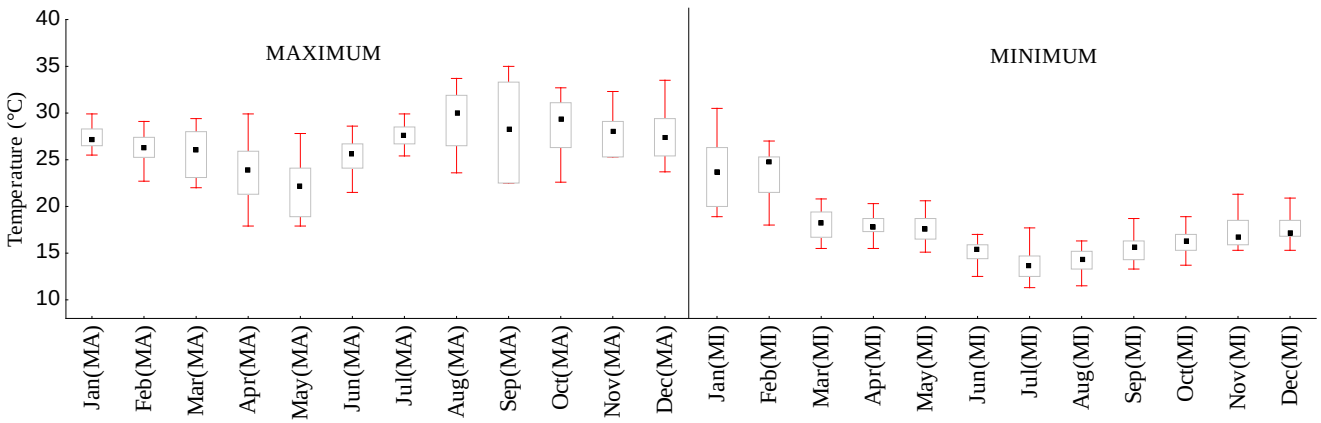

Figure 2: Maximum and minimum temperature for 2018 in the municipality of Vitória da Conquista - Bahia (INEMET)

The rainy season in the region runs from November to March. The total annual rainfall is around 700 mm, and the thermal averages show a maximum of 26.4 ° C and a minimum of 16.1 ° C, with an annual average of 20.2 ° C (Figure 02).

b) Experimental design, transplantation and cultivation conditions

In an entirely random experiment, young African mahogany plants were grown in pots containing washed sand and nutrient solution (Table 01) with NaCl in varying concentrations - 0, 20, 145, 270, 395 and 520 mM NaCl, whose concentrations equivalent to the

following levels of electrical conductivity: 1.0 • 3.38 • 15.14 • 29.90 • 42.61 • 53.60 dS m^{-1} with four repetitions, totaling 24 plots (Figure 03).

Figure 3: Young plants of *Khaya senegalensis* in the experimental units, representing the distribution of treatments in pots in the greenhouse

The seedlings were produced by the Instituto Brasileiro de Florestas, in conical model tubes, being acquired at 180 days of age. Seedlings were transplanted in pots with a volume of 15 L, previously filled with sand passed through a 0.005 mm mesh sieve. Small perforations at the base of the vessels allowed the collection of drainage water for further analysis of its chemical characteristics.

The amount of water applied was determined by the vessel capacity method, in which the water content of the sand was kept close to the field capacity. The monitoring of the soil water content was based on the daily verification of the weight of the pots.

The addition of NaCl to the nutrient solution, in varying concentrations, was done every 15 days. The electrical conductivity (dS m^{-1}) was monitored using a portable conductivity meter. In the treatment without addition of NaCl, the electrical conductivity was 1 dS m^{-1}. In the other treatments, there was an increase in electrical conductivity, due to the addition of NaCl to the nutrient solution, in varying concentrations.

Table 1: Ranges of concentration of nutrients in Hoagland and Arnon's solution (1950), with some modifications (COLMER; MUNNS; FLOWERS, 2005)

Hoagland and Arnon (1950)		
Nutrient	Atomic mass	mg L^{-1}
$N\text{-}NO_3^-$	14,00	196,0
$N\text{-}NH_4^+$	14,00	14,00
P	31,00	31,00
K	39,10	234,0
Ca	40,00	160,0
Mg	24,30	48,00
S	32,00	64,00
B	10,8	0,50
Cu	63,5	0,02
Fe	55,8	1,00
Mn	54,9	0,50
Mo	95,9	0,01
Zn	65,4	0,05

c) Plant height

Every 5 days, the height of the plants, in cm, was measured with the aid of a graduated ruler, placed parallel to the stem, measuring the height from the neck to the insertion of the last leaf (apical bud of the stem).

d) Relative growth rate

With the height results in mind, the relative growth rate (RGR) was evaluated using the following equation:

$$RGR = \frac{\left(\frac{Fh - Sh}{Sh}\right)}{t}$$

Where: Fh, represents the final height; Sh, the starting height; and t, the period considered.

e) Absolute growth rate

With the height results, a growth analysis was performed, in which the absolute growth rate (AGR) was determined.

$$AGR = \frac{(Fh - Sh)}{t}$$

Where: AGR represents the absolute growth rate; Fh, the final height; Sh, the starting height; and t, the length of the period considered.

f) Statistical analysis

The results were subjected to analysis of variance (ANOVA), Table 02, using the F test to compare the means, and regression analysis for the quantitative study of the characteristics evaluated, using the statistical program SISVAR 5.6 with subsequent regression analysis in the study. between each treatment without including the control, and analysis of main components in the absolute growth rate, and the control treatment was compared with the others by the Dunnet test ($p < 0.05$).

Table 2: Description of variance analysis table of the components used in the comparison of treatment averages

Causes of Variation	Degrees of freedom	Sum of Squares	Medium Squares	Calculated F
Treatments	I-1	SQTrat	QMTrat	QMTrat/ QMRes
Residue	I(J-1)	SQRes	QMRes	
Total	IJ-1	SQTotal		

Where:

$SQTotal = \sum_{i=1}^{I}\sum_{j=1}^{J} y_{ij}^2 - C$, where $C = \frac{\sum_{i=1}^{I}\sum_{j=1}^{J} y_{ij}^{\ 2}}{IJ}$

Measures the overall variation of all observations.

$$SQTrat = \frac{\sum_{i=1}^{I} y_i^2}{J} - C$$

Sum of squares of groups (treatments), associated exclusively with an effect of groups (salinity levels).

The sum of squares of the residues was obtained by difference:

$$SQRes = SQTotal - SQTrat$$

Sum of squares of residues, due exclusively to random error, measured within the groups (salinity levels).

$$QMtrat = \frac{SQtrat}{I - 1}$$

Being the square mean of the groups (treatment).

$$QMRes = \frac{SQRes}{I(J - 1)}$$

Being the square average of the residues (parameter attributed to effects not dimensioned in the treatments).

To find a significant difference between treatments (absolute / relative growth rate for the different levels of salinity), the F test was used, considering that if F calculated > F tabulated, the null hypothesis H_0 is rejected, that is, there is evidence of a significant difference between at least a pair of treatment averages, at the α level of signifi cance chosen, with a 5% probability. Otherwise, the H_0 null hypothesis is not rejected, that is, there is no evidence of a significant difference between the treatments, at the α level of significance chosen.

To generate the regression equations, the least squares method was used, being a mathematical optimization technique that seeks to find the best fit for a data set, trying to minimize the sum of the squares of the differences between the estimated value (regression equation) and the observed data of growth rate of the African mahogany culture, such differences being called residues and expressed mathematically by:

$$\sum_{i-1}^{n} e_i^2$$

Where: n = represents the number of observations, with the number of data sampled from the growth rate of the crop; e = difference between the real value of the growth rate data observed during the time period of the experiment (four months) and those estimated by the equation.

To measure the model's quality in relation to its ability to correctly estimate the values of the growth rate response variable (dependent variable) as a function of the tested salinity levels, independent variable (Growth rate x Electrical Conductivity) and as a function of the number of days after transplant - DAT (growth rate x DAT) the correlation coefficient R^2 was generated, determined by:

$$R^2 = 1 - \left(\frac{SQRes}{SQTot}\right)$$

Where: SQRes = sum of square of the residue; SQTot = total square sum.

The value of R^2 can take values from 0 to 1, and the higher the value of the correlation coefficient, the closer to real data are the data estimated by the regression equation model generated.

III. Results And Discussion

The F test at 5% probability ($p < 0.05$), in the ANOVA analysis table (Table 03), shows the significance of the evaluated characteristics, relative growth rate (RGR) and absolute growth rate. (AGR) of Khaya senegalensis plants, with statistical difference in RGR and similarity between treatments for AGR. The existing correlation for RGR in the different treatments generated linear regression: RGR = - 0.0014 E.C + 0.00111 (Figure 04. B), where the estimated data represent 99.74% of the observed data, demonstrating that young African mahogany plants show sensitivity in their daily growth when subjected to different concentrations of NaCl.

As can be seen in Figure 05.B, the plants subjected to electrical conductivity of 2 $dS.m^{-1}$ showed a daily growth of 0.01 cm whereas with the increase in the concentration of NaCl it registered one in the relative growth rate in around 0.004 cm per day for the treatment of 52 $dS.m^{-1}$ of E.C (Electrical Conductivity), generating a growth about 2.5 times less than the treatments of 2 and 52 $dS.m^{-1}$ respectively.

This behavior occurs due to the saline growth medium, which causes many adverse effects on plant development, due to the low osmotic potential of the soil solution (osmotic stress), effects of specific ions (salt stress), nutritional imbalances or a combination of these factors (SHRIVASTAVA; KUMAR, 2015).

Table 3: Analysis of variance (ANOVA) for Relative Growth Rate (RGR) and Absolute Growth Rate (AGR) of *Khaya senegalensis* plants

Variation sources	GL	SQ	QM (RGR)	F
Electric conductivity (E.C)	5	0,000112	0,000022	8,670*
Residue	18	0.000046	0.000003	
Total	23	0.000158		
CV (%)	21.34			
Variation sources	GL	SQ	QM (AGR)	F
Electric conductivity (E.C)	5	0,000112	0,000022	0.743^{NS}
Residue	18	1,684343	0,201358	
Total	23	2.018610		
CV (%)	21.34			

** significant ($p < 0.05$); ns = not significant; CV = Coefficient of variation; E.C = Electrical Conductivity.*

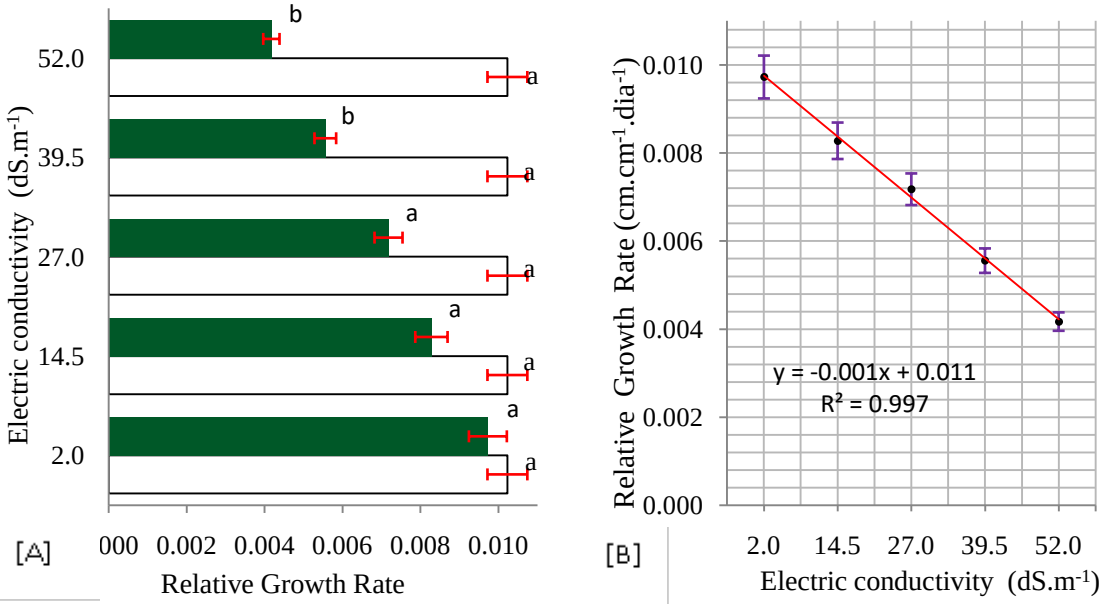

Figure 4: Relative growth rate of young plants of Khaya senegalensis at 120 DAT. [A] Behavior of the relative growth rate at different levels of salinity; [B] Variation in the relative growth rate at different levels of salinity compared to the control treatment. The bars in each column represent the variation of the standard error of the mean. Same letters in the paragraphs indicate that the analyzed variable does not differ from each other, by Dunnett's test ($p < 0.05$)

Table 4: Analysis of variance (ANOVA) of the height of young plants of *Khaya senegalensis* over the experimental period of 120 days for each electrical conductivity

Variation sources	GL	SQ	QM	F
DAT (Trat. 01,00 dS.m^{-1})	7	905.804688	129.400670	7.664*
Residue	24	405.207500	16.883646	
DAT (Trat. 03,38 dS.m^{-1})	7	933.973672	133.424810	3.701*
Residue	24	865.261875	36.052578	
DAT (Trat. 15,14 dS.m^{-1})	7	680.375000	97.196429	12.150*
Residue	24	192.000000	8.000000	
DAT (Trat. 29,90 dS.m^{-1})	7	757.913750	108.273393	14.690*
Residue	24	176.895000	7.370625	
DAT (Trat.42,61 dS.m^{-1})	7	578.078750	82.582679	1.505*
Residue	24	1316.520000	54.855000	
DAT (Trat. 53,60 dS.m^{-1})	7	507.595000	72.513571	2.087*
Residue	24	834.065000	34.752708	

** significant (p <0.05); ns = not significant; DAT = Days After Transplantation.*

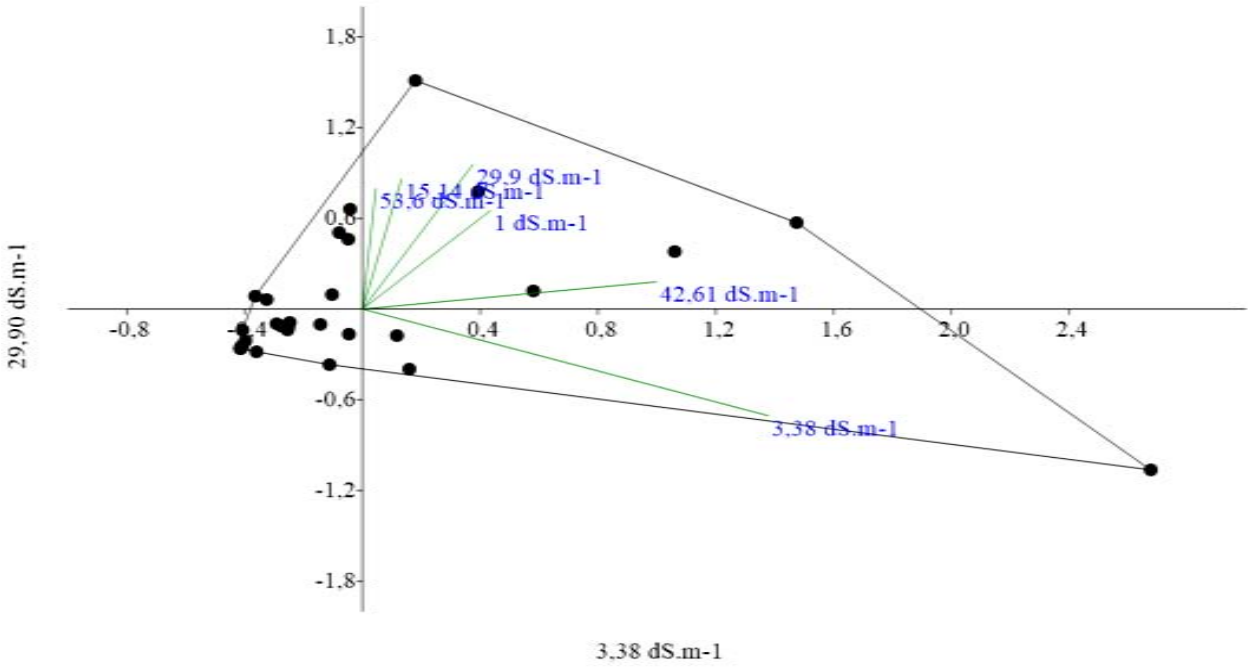

Figure 7: Electrical conductivities as main components for the analysis of the absolute growth rate (cm.day^{-1}) of young plants of *Khaya senegalensis* over the 120-day experimental period. E.C 3.38 dS.m^{-1} as component 1 explaining 45% of the variation in AGR; E.C 29.9 dS.m^{-1} as component 2 explaining 20% of the variation in AGR

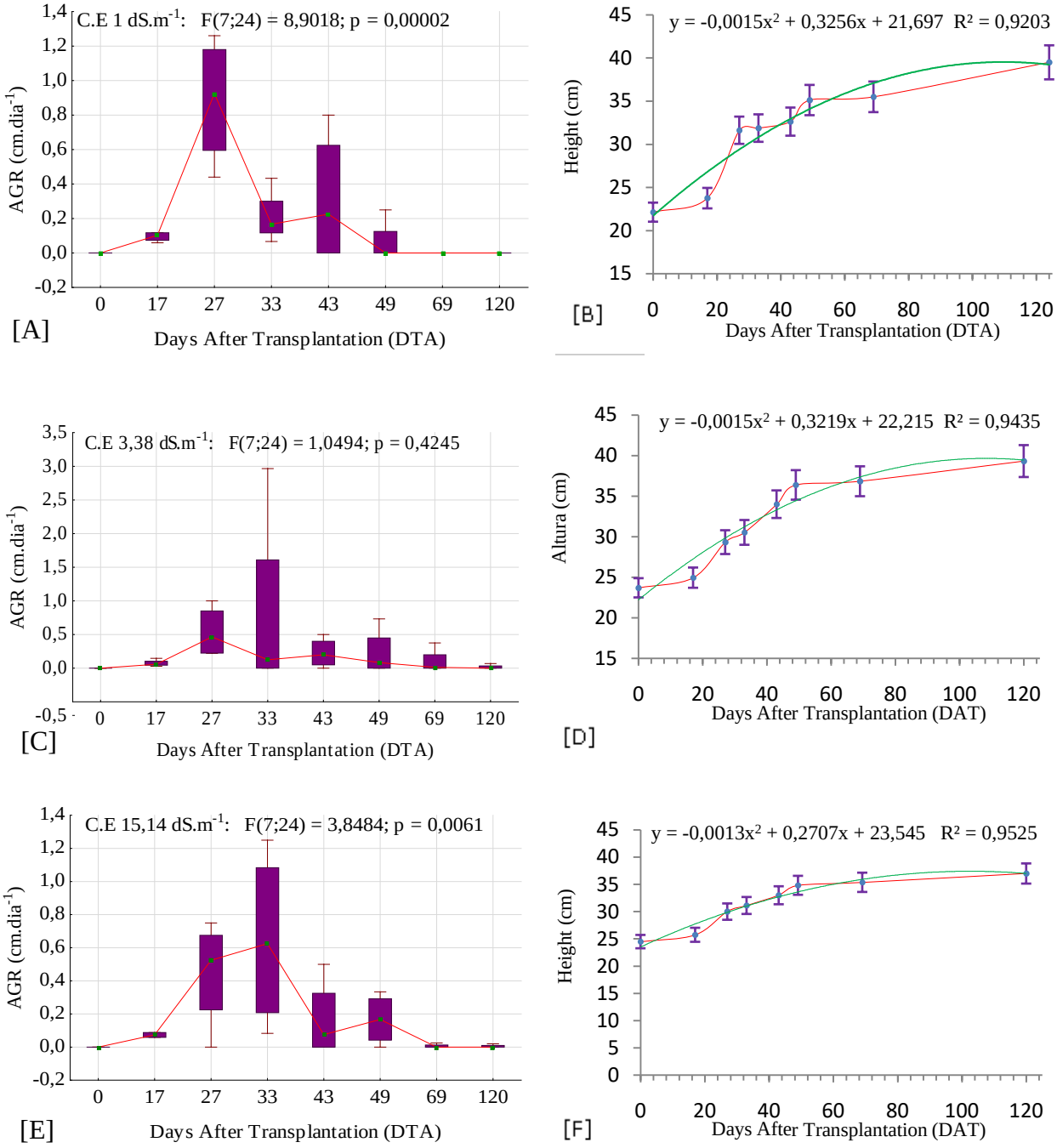

Figure 5: [A]; [C]; [E] Rate of absolute growth in cm. day-1 of young plants of Khaya senegalensis in treatments submitted to 1.0 electrical conductivity; 3.38 and 15.14 dS.m^{-1}, respectively, over the 120-day experimental period. [B]; [D]; [F] Linear height ratio in cm of young plants of *Khaya senegalensis* in treatments submitted to 1.0 electrical conductivity; 3.38 and 15.14 dS.m^{-1}, respectively, depending on the experimental period of 120 days

The decrease in the growth rate between treatments (Figure 04) is due first to a decrease in the water potential of the soil (osmotic phase) (LIMA et al., 2020), since the presence of salt in the sand substrate changes the kinetic energy of the water, reducing it. Later, a specific effect appears as a rapid increase in salt in the walls of the cell or cytoplasm, when the vacuoles can no longer sequester incoming salts (ionic phase), and decreases in the leaf and stem cause a reduction in all sizes of the aerial parts and at plant height (BERNSTEIN, 2019) (MUNNS; TESTER, 2008).

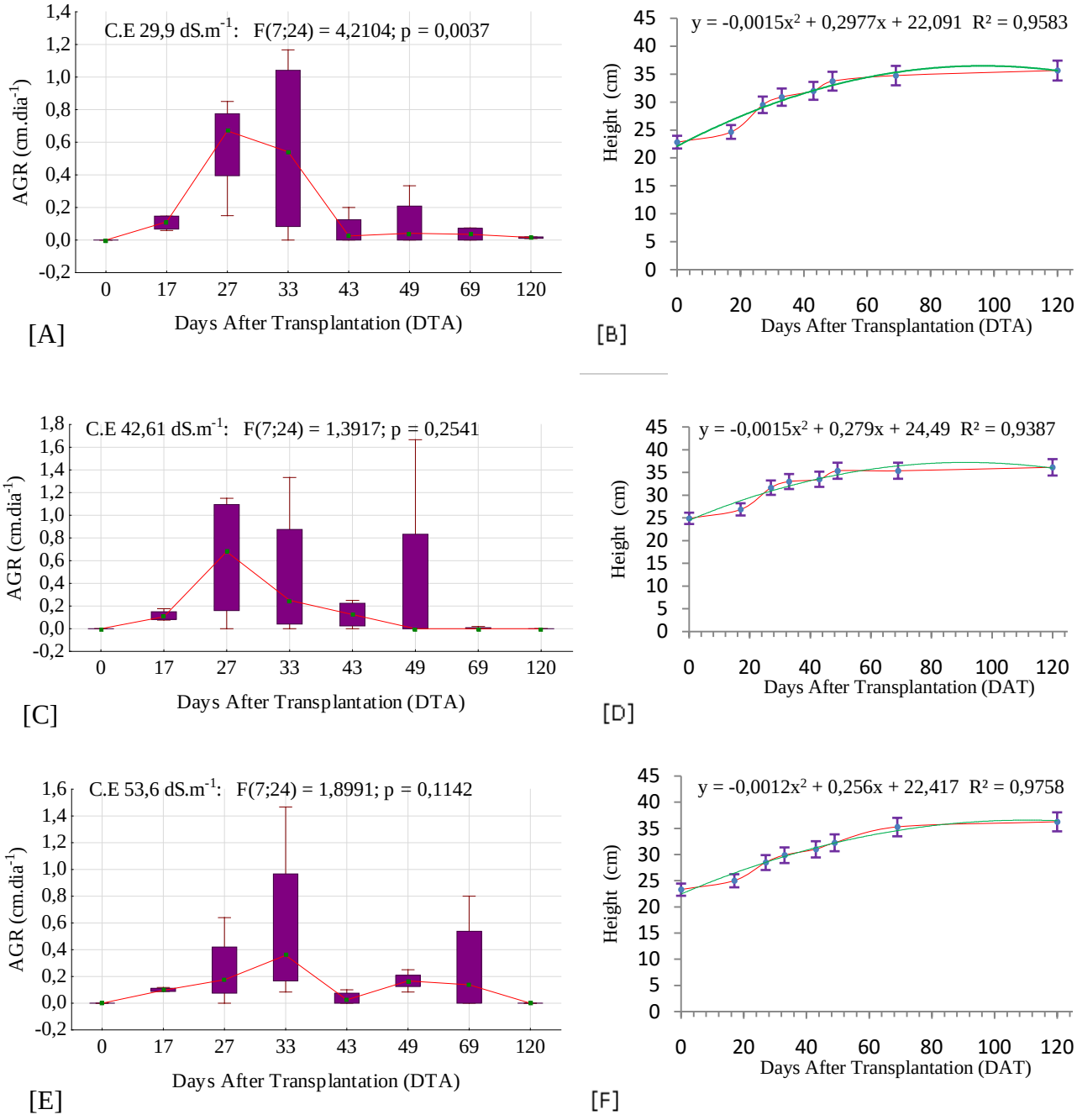

Figure 6: [A]; [C]; [E] Rate of absolute growth in cm. day-1 of young plants of *Khaya senegalensis* in treatments submitted to 29.9 electrical conductivity; 42.61 and 53.6 dS.m^{-1}, respectively, over the 120-day experimental period. [B]; [D]; [F] Linear height ratio in cm of young plants of Khaya senegalensis in treatments submitted to 29.9 electrical conductivity; 42.61 and 53.6 dS.m^{-1}, respectively, depending on the experimental period of 120 days

It is verified if the grouping of the points representing the absolute growth rate (Figure 05), through the analysis of the electrical conductivities of 3.38 dS.m-1 and 29.9 dS.m^{-1}, explaining 70% of the behavior, which indicates close values in the plotted points (Figure 05) showing that there was no difference for the absolute growth rate in the different electrical conductivities, a condition evidenced by the analysis of variance using the F test at 5% probability, which considered the hypothesis statistically valid. of nullity H_0 (Table 03).

In a study on the growth and physiological responses of tree species in salinized soil treated with correctives, (SOUSA et al., 2012) observed that young ironwood plants grown in saline-sodium soil, without correction, had their growth practically paralyzed, unlike

the behavior shown by the mahogany plants, which showed no reduction in their absolute growth rate with the increase in salinity, causing growth to be maintained (Figures 05 and 06, B; D; F), probably due to the application of the nutrient solution for all treatments.

In the different E.C treatments, there was no difference in plant height (Table 05), results also corroborated in the research by (SOUZA et al., 2017), who working with the concentration of macronutrients and sodium in mahogany seedlings submitted to salt stress , observed that there was no difference in growth in height for the different electrical conductivities.

In the E.Cs of 2; 14.5 and 27 $dS.m^{-1}$ there was no significant difference for the relative growth rate when compared to the treatment without the addition of sodium chloride (Figure 4.A), this behavior shows that mahogany plants have a tendency to tolerate salinity for the characteristic relative growth rate, since the change in RGR remains the same with the control treatment until an electrical conductivity of 27 $dS.m^{-1}$, representing a NaCl concentration about three times higher than most cultures tolerate in natural environments (TANG et al., 2015).

The fact that mahogany tolerates concentrations of up to 270 mM NaCl in the characteristic relative growth rate represents an osmotic adjustment carried out by plants that contribute to the maintenance of water balance and the preservation of the integrity of proteins, enzymes and cell membranes (RODRÍGUEZ et al. , 2005). However, the plants were unable to maintain this adjustment for salt concentrations between 39.5 and 52 mM NaCl, which may have occurred due to the very high absorption of salts by the plant cells, leading to ionic toxicity, causing cell death and consequently, the reduction in the relative growth rate for conductivities above 39.5 $dS.m^{-1}$.

Souza (2017) researching the species Swietenia macrophylla found that the increase in the level of salinity up to the electrical conductivity of 6.49 dS m^{-1} did not impair growth in height, however for the species of *Khaya senegalensis*, object of this study, the tolerance level is higher, as this only showed a difference between treatments and control for electrical conductivity from 39.5 $dS.m^{-1}$ (Figure 04.A), demonstrating that, in fact, for the characteristic growth rate relative, the effect of salts is not significant to the point of paralyzing growth (Figures 05 and 06, B; D; F).

Contrary to what is expected in plants subjected to salt stress, in which one of the most observed effects is the reduction of growth (PARIDA; DAS, 2005), in all treatments the plants of *Khaya senegalensis* did not show growth drop over 120 days of the trial period. In all NaCl concentrations it developed a statistically difference in height for the eight periods of time analyzed after 120 days, with a significant difference as seen in Table 04, reinforcing the fact that the species *Khaya senegalensis* adapted to the conditions of changes in conductivity electricity, provided by the different NaCl concentrations.

Table 5: Analysis of variance (ANOVA) for height of *Khaya senegalensis* plants in different electrical conductivities

Variation sources	GL	SQ	QM (RGR)	F
Electric conductivity (E.C)	5	73.157083	14.631417	0.507^{NS}
Residue	18	519.552500	28.864028	
Total	23	592.709583		
CV (%)	37.04			

** significant ($p < 0.05$); ns = not significant; CV = Coefficient of variation; E.C = Electrical Conductivity.*

In a study on the physiological mechanisms of ornamental plants of *Eugenia myrtifolia L* in saline conditions, (ACOSTA-MOTOS et al., 2015) explain that the ability of plants to control the concentration of salt in their aerial parts, either by the accumulation of salt in the roots, by the reduction in the rates of salt uptake and / or by the controlled translocation to the leaves, constitutes an important survival mechanism of plants under saline conditions. These mechanisms may be present in african mahogany plants, since they showed little significant difference in growth for different concentrations of salts.

In the absolute growth rate over the experimental period, a growth of about 0.1 cm per day was observed for all treatments until the seventeenth day after the implantation of the seedlings in the substrate (Figures 05 and 06, A; C; E), growth rate is measured from the height difference between the first and the last leaf insertion branch. With 17 days of plant implantation in the experiment, there was a significant increase in the growth rate from 0.1 to about 0.4 cm per day in all treatments, occurring due to the application of the first nutrient solution that provided the plants, among other nutrients, Ca^{2+}, supplementation that under salt stress has been reported to improve the stability of cell membranes through their interaction with phosphates and proteins in the membranes, thus strengthening their stability (HONG-BO; LI-YE; MING-AN , 2008).

The application of the nutrient solution caused the growth rate to fluctuate throughout the experimental period, so that, whenever there was an application, the growth rate increased and decreased again over the period of time until the application of the next solution (Figures 05 and 06, A; C; E). Pitann et al. (2011) state that the lower growth rate in grain legumes under saline stress is attributed to the lack of acidification of the cell

wall, which plays a key role in stimulating growth by activating dependent cell wall loosening enzymes pH, involved in the growth and increase of cells (RAYLE; CLELAND, 1992).

Although there is no statistical difference between the treatments in the AGR (Table 03), the effect of an increase in the absolute growth rate after the application of the nutrient solution was more expressive for the treatments with lower NaCl contents, which can be observed for the control treatment that presented 0.8 cm per day in growth, while plants submitted to an electrical conductivity of 52 $dS.m^{-1}$ showed a growth of 0.35 cm per day.

IV. Conclusions

- The absolute growth rate in the different electrical conductivities are statistically equal, a condition evidenced by the analysis of variance, using the F test at 5% probability, which considered statistically valid the H_0 null hypothesis and by grouping the representative points the absolute growth rate, through the analysis of the electrical conductivities of 3.38 $dS.m^{-1}$ and 29.9 $dS.m^{-1}$, explaining 70% of the behavior, indicating close values in the plotted points;
- Throughout the experimental period, the absolute growth rate was implemented around 0.1 cm per day for all treatments until the seventeenth day after the installation of the seedlings on the sand substrate;
- The existing correlation for RGR in the different treatments generated linear regression:

$$RGR = - 0{,}0014\ E.C + 0{,}00111$$

Where the estimated data represent 99.74% of the observed data, demonstrating that young african mahogany plants show sensitivity in their daily growth, when subjected to different concentrations of NaCl.

- All NaCl concentrations developed a statistically difference in height as a function of the time periods analyzed after 120 days, however, in the different treatments of E.C there was no difference in plant height, reinforcing the fact that the species *Khaya senegalensis* adapted to conditions of changes in electrical conductivity, provided by different concentrations of NaCl, due in part to the use of nutrient solution;
- The application of the nutrient solution caused the growth rate to fluctuate throughout the experimental period, so that, whenever there was an application, the growth rate increased again decreasing over the period of time until the application of the next solution.

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Kinetic Characterization of a Protease Isolated from Crude Fresh Latex of Plantain (*Musa Paradisiaca*) Plant

By Olabisi Olufunmilayo Ogunrinola, Olusegun Omolade Fajana & Olaseni Pascal Oresanya
Lagos State University

Abstract- The plant latex consist of biologically active compounds useful for diverse health benefits and could be a potential source of unique proteases. Proteases play key roles in the regulation of biological processes in plants and currently have become a vital part of the food and feed industry. In order to understand the underlying mechanisms of action of unique plant latex, the present study isolate and characterized a protease enzyme from the crude latex of plantain plant. Crude latex collected from different parts (leaves, stem, trunk and branches) of the plantain plant was centrifuge to separate the supernatant from any particles. The supernatant was used as the crude latex extract for the research. A protease enzyme was isolated from the crude latex extract and the effect of pH, temperature, incubation time, kinetic study of the protease activity as well as protein were determined spectrophoto-metrically. The enzyme protease was found to have a 9.84 mg/ml of protein with a specific activity of 3.06 unit/mg protein respectively.

Keywords: *protease, proteolytic, plantain and latex.*

GJSFR-C Classification: *FOR Code: 060199*

Strictly as per the compliance and regulations of:

Kinetic Characterization of a Protease Isolated from Crude Fresh Latex of Plantain (*Musa Paradisiaca*) Plant

Olabisi Olufunmilayo Ogunrinola [α], Olusegun Omolade Fajana [σ] & Olaseni Pascal Oresanya [ρ]

Abstract- The plant latex consist of biologically active compounds useful for diverse health benefits and could be a potential source of unique proteases. Proteases play key roles in the regulation of biological processes in plants and currently have become a vital part of the food and feed industry. In order to understand the underlying mechanisms of action of unique plant latex, the present study isolate and characterized a protease enzyme from the crude latex of plantain plant. Crude latex collected from different parts (leaves, stem, trunk and branches) of the plantain plant was centrifuge to separate the supernatant from any particles. The supernatant was used as the crude latex extract for the research. A protease enzyme was isolated from the crude latex extract and the effect of pH, temperature, incubation time, kinetic study of the protease activity as well as protein were determined spectrophotometrically. The enzyme protease was found to have a 9.84 mg/ml of protein with a specific activity of 3.06 unit/mg protein respectively. The enzyme exhibited its highest activity at a temperature of 35^{0}C, and showed optimum proteolytic activity at pH of 7.0. The protease enzyme isolated from the latex of plantain was also found to be optimally active at 120 seconds. The proteolytic activity of the enzyme revealed that the K_m and V_{max} represent 0.051 mgml^{-1} 0.976 mmoles min^{-1}ml^{-1} respectively. These results highlighted the characteristics of plantain latex protease that revealed it as a good source for neutral protease which might be of useful application in biotechnological industries.

Keywords: protease, proteolytic, plantain and latex.

I. Introduction

Plant latex is a sap milky fluid that is secreted from laticiferous tissues (stems, roots, leaves and fruits) which is discharge from the point of tissue damage and functions in protein processing, digestion, growth, reproduction, apoptosis, senescence, defense against herbivores and pathogens, etc and excretion of waste metabolites [1-4]. Traditionally, plant latex have been utilized as therapeutic agents to treat different types of ailments [5]. Latex serves as anthelmintic, insecticidal, anti-inflammatory, antioxidant, anti-cancer activities, antiparasitic, anticoagulant and also used in fishing, veterinary and human medicine, as well as biofuel [6]. The latex ethno-pharmacological properties is due to the presence of phytoconstituents such as terpenoids, alkaloids, gums, and cardenolides as well as proteins and enzymes such as chitinases, glucosidases and proteases [1, 7].

Proteases, are proteolytic enzymes that catalyse the cleavage of the peptide bond in the protein to give peptides and amino acids by hydrolysis significant to food digestion and intracellular protein turnover [7-9]. The protein substrates specifically break either from the N-terminus (aminopeptidases) or C-terminus (carboxypeptidases) and/or in the middle of the protein molecule (endopeptidases) respectively by protease [8-9]. Proteases are classified as metallo, serine, acidic, carboxyl, alkaline and neutral based on structures and/or properties of the active site [10]. Proteases are found in the plant and animal as well as bacteria and viruses [11]. Apart from plant latex proteases function to defend the plant against pests/insect, it is also involved in hemostatisis (coagulation), wound healing, tissue remodelling, DNA replication, cell proliferation, cell death, cell-cycle progression, and immune responses [5, 7, 8, 12]. Plant proteases has also emerge as useful therapeutic agents in the treatment and management of debilitating conditions including sepsis, chronic inflammatory disorders, cystic fibrosis, retinal disorders, and psoriasis [7, 8, 12, 13]. They have applications in food processing, detergent, pharmaceutical and other chemical industries [10, 14-15]. Proteolytic enzymes from plant latexes are of widespread interest due to their involvement in various physiological functions and economic benefits. They receive added attention due to broad substrate specificity and activity in a wide range of pH, temperature, in the presence of organic compounds and other additives [16].

In the last few years, proteases from different plant latex have been the object of consideration with most studied belong to the family of cysteine or serine or aspartate endopeptidases family [17]. Cysteine protease in the latex of papaya (*Carica papaya*) and wild fig (*Ficus virgatalatex*) has shown high toxicity to caterpillars of herbivorous insects [1, 6, 18-19], while plant serine protease known as subtilases has been isolated from several plants showed to be involved in many metabolic functions like hypersensitive response, symbiosis, microsporogenesis, signal transduction and differentiation, senescence and protein degradation/ processing [20-21]. Plantain (*Musa paradisiaca*) latex

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from the smooth stem was reported to have a high level of proteolytic activity [22], but the kinetic characterization of latex from different parts of the plantain has not been investigated.

Plantain, a perennial crop with a short gestation period is available for harvest year round in tropics and sub-tropics region of the World. It has a significant economic activity for income generation for both large scale and small farmers in the area of food production [23-24]. Plantain has medicinal applications in bronchitis, dysentery, ulcers, diabetics and culinary uses [23]. Considering the search for novel proteases from medicinal plants multidrug resistance and toxicity associated with the existing remedies, there is need to clarify the type of protease isolated from crude plantain parts latex. Therefore, this study was conducted to isolate and characterized the crude protease from the latex of plantain.

II. Materials and Methods

a) Materials

The plantain plants used for the research was from Ojo - Area of Lagos State, Western Nigeria. All chemicals and reagents used were of analytical reagent grade.

b) Methods

i. *Preparation of crude plant latex extract*

Latex was collected early in the morning from different parts of the plantain (leaves, stem, trunk and branches) by nipping the leaves near the stem and incision of the trunk and branches so as to allow the milk to drain into a clean glass tube. 10 ml of latex collected was measured into a glass measuring cylinder and equal volume of distilled water was added to the crude extract. The mixture was centrifuged at 5000 rpm for 20 minutes at 4°C. The resulting supernatant was collected and used as the crude enzyme extract for further investigation of the protease activities.

c) Biochemical Analysis

i. *Protein determination*

Protein concentration was quantitatively analysed by modified methods of Layne [25] and Aitken and Learmonth [26] using the Biuret method and BSA as standard.

ii. *Protease assay*

Protease assay was performed using a modification of Kunitz case inolytic assay as described by Janssen *et al*. [27] as described in Raimi *et al*. [28] and Raimi *et al*. [29]. Briefly, 0.5 ml of the crude enzyme source was added to 2.0 ml of 0.5% casein in 50 mM phosphate buffer (pH 7.4). The reaction mixture was incubated at 37 °C and terminated after 30 min by adding 3.0 ml of 5% TCA. The solution was kept for additional 30 min at room temp and then centrifuged. The absorbance of the supernatant was read at 280 nm by using UV/Visible Spectrophotometer (Model SM 755s), a product of Surgienfield Instrument, Zhejiang, China (Mainland). One unit of TCA soluble casein hydrolysis product was defined as an increase of 0.1 in absorbance at 280 nm.

d) Analysis of proteolytic activity

i. *Effect of temperature on protease activity*

The effect of temperature on the enzyme activity was carried out at a temperature ranging from 15°C to 55°C with an interval of 5°C. Modified Kunitz caseinolytic assay method as described for enzyme assay was used.

ii. *Effect of pH on protease activity*

This was carried out following the modified Kunitz caseinolytic assay method as described for enzyme protease assay but the pH was varied for the reaction mixture between a range of 5.0 and 11.0 with an interval of 1.0 using 50 mM phosphate buffer (pH 7.5).

iii. *Effect of incubation time on protease activity*

The incubation time effect on the protease activity was determined by incubating the assay at different time ranging from zero (0) to 300 seconds with an interval of 100 seconds.

iv. *Assessment of kinetic constants (K_m and V_{max}) of the protease enzyme*

The enzyme activities were assayed at various concentrations of substrate (casein) in a reaction volume of 2.2 ml incubated for 30 min at 37°C and terminated using 3.0 ml of 5% TCA. The Michaelis-Menten constant (K_m) and maximum reaction velocity (V_{max}) of the purified enzyme were determined from the Lineweaver-Burk plot.

III. Results

a) Protein determination

The fresh plantain crude extract of the latex contained 9.84 mg/ml of protein with a specific activity of 3.05 unit/mg protein as shown in Table 1.

b) Proteolytic activity

The crude latex protease enzyme demonstrated maximum proteolytic activity at a temperature of 35°C and then gradually decreased until it reached a temperature of 55°C (Fig. 1). At optimum pH 7.0 (Fig. 2) the protease enzymes was found to be active and its activity decreased to approximately pH 11. The effect of incubation on the enzyme activity (Fig. 3) shows that the crude latex protease enzyme is optimally active at 120 seconds. Figure 4 depicted the kinetic constants of the latex protease enzyme based on a Lineweaver-Burk plot of the protease and substrate – casein. The K_m and V_{max} values were found to be 0.051mg ml^{-1} and 0.976 mmoles $min^{-1}ml^{-1}$ respectively.

IV. Discussion

Plant latex, compose of various types of protease enzymes which occupy a pivotal locus with respect to their applications in both physiological and commercial fields [10, 15, 30]. The activity of these enzymes depends on the plant source, extraction and purification methods [30]. In this study, the units of protease activity present per milligram of crude plantain (*Musa paradisiaca*) latex from various parts revealed that it's a rich source of protease which agrees with the report of Awoyinka and Shokunbi [22]. The high latex protease activity suggests that it is not a waste product but a crucial parts of plant resistance [31].

Temperature and pH plays an important role on an enzyme-catalyzed reaction. The stability of proteases from crude latex of plantain (*Musa paradisiaca*) parts were determined by incubation at various times, temperatures and pH. The observed optimum incubation time, temperature and pH suggest that the plantain crude latex is very stable. This is similar to the report of Antao and Macloata [20]; Domsalla and Melzig [17] and Macalood *et al*. [3], even though, the optimum temperature of plantain is lower to that of papaya (*Carica papaya Linnaeus*). This result suggest that the isolated protease from crude plantain latex with neutral pH might be neutral protease [32] which might be the mechanism of action for its wide range of applications in therapeutic [24, 33], food and brewing industries [34]. This is in agreement with other researchers that have isolated neutral protease from different sources [28-29, 35-36, 37]. The decrease of activity after optimum temperature may be due to the alteration of the structure of the protease [38].

For a fixed enzyme concentration, the best substrate for the enzyme activity depends on low K_m value (strong substrate binding or high affinity) and high V_{max} value (high catalytic efficiency) [39]. The low K_m and high V_{max} values observed in this study, revealed that the crude latex protease enzyme has high affinity for casein as a substrate which is similar to the previous reports of Sharma *et al*. [39] and Raimi *et al*. [28].

In conclusion, the kinetic characterization of a protease isolated from crude fresh latex of plantain (*Musa paradisiaca*) plant parts was evaluated in this study. The study revealed the presence of neutral protease enzyme in the crude latex of the plantain which can be exploited commercially as a useful application in biotechnological industries.

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Table 1: The total protease enzyme activity from the isolated crude latex of plantain

	Crude extract
Volume (ml)	10
Total protein concentration (mg/ml)	9.84
Total protein (mg)	98.4
Protein concentration (Units/ml)	30
Specific activity (Units/mg protein)	3.06
Total Enzyme Activity (units)	300
Yield (%)	100

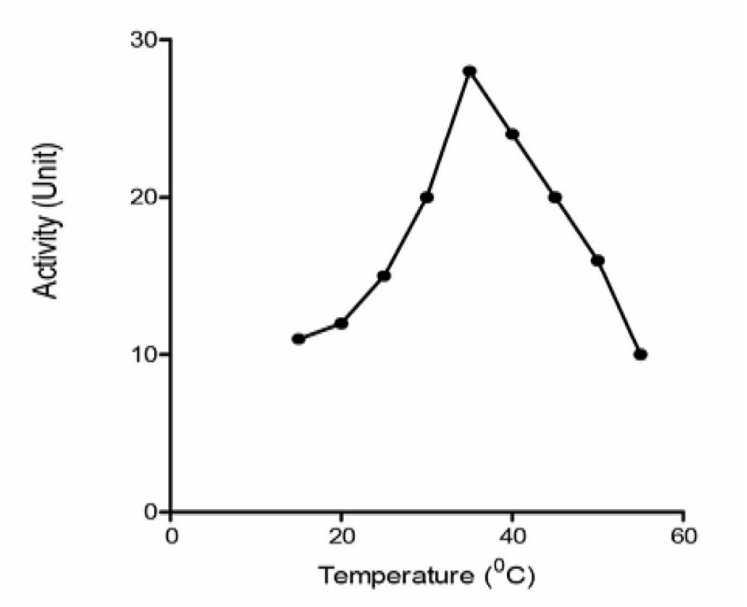

Figure 1: Effect of temperature (^{0}C) on protease enzyme activity (Unit) from crude plantain latex

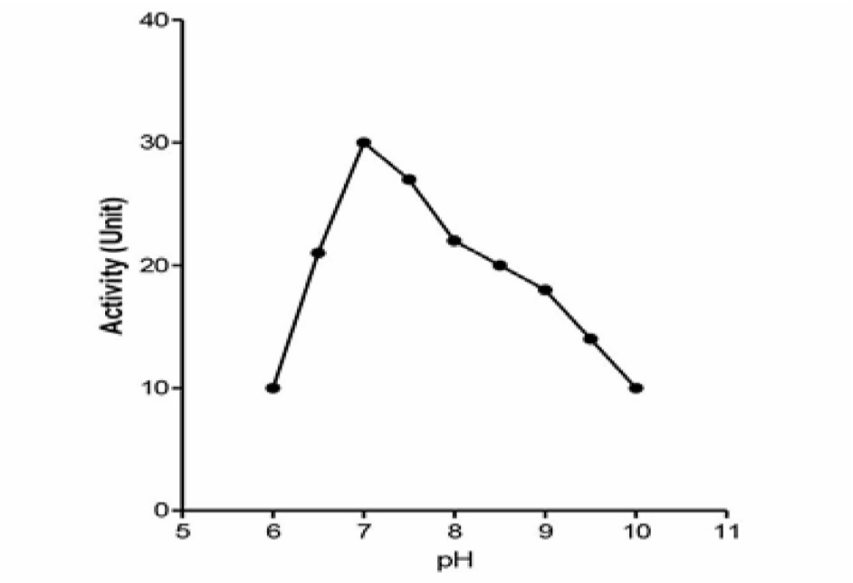

Figure 2: Effect of pH on protease enzyme activity (Unit) from crude plantain latex activity

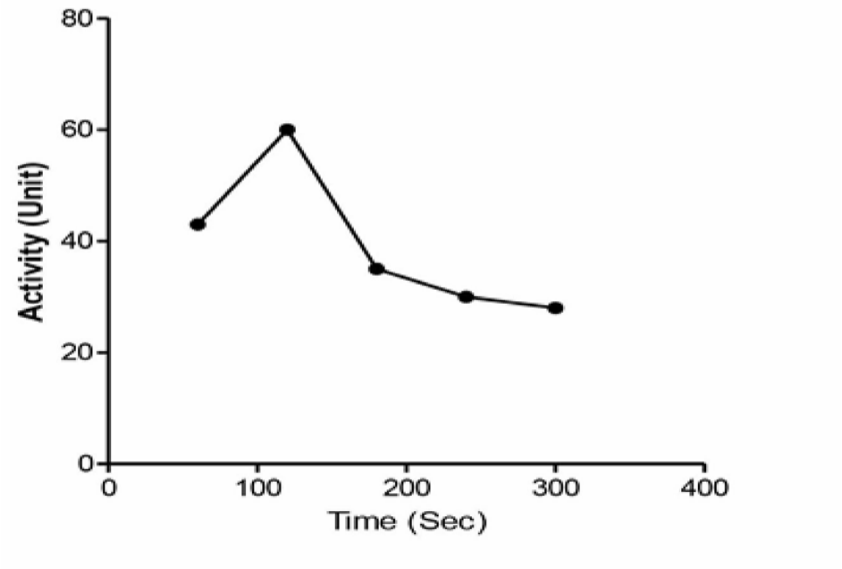

Figure 3: Effect of time on enzyme Effect of time (Sec) on protease enzyme activity (Unit) from crude plantain latex activity

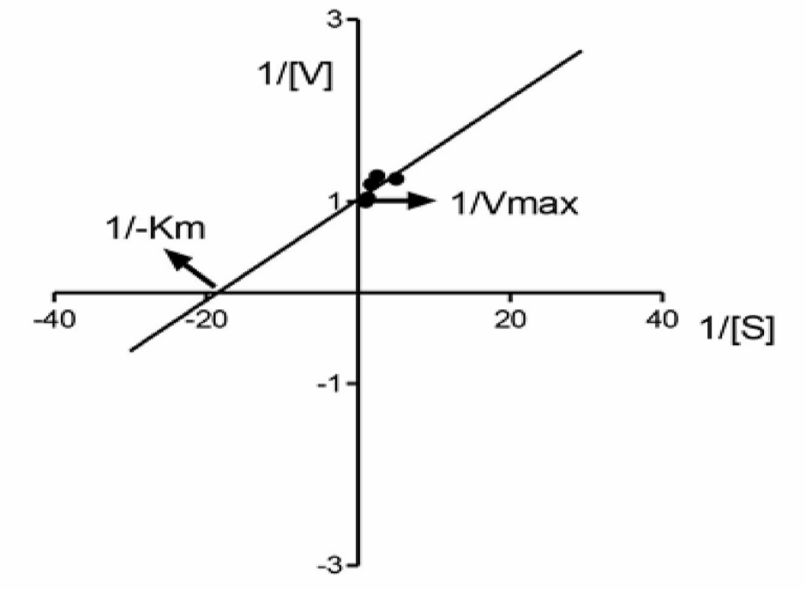

Figure 4: Line-weaver-Burk plot showing the K_m and V_{max} of the protease enzyme from crude plantain latex

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GC-MS Evaluation of the Phytoconstituents of the Ethanolic and *N*-Hexane Extracts of the whole Plant of *Cleome Rutidosperma*

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Abstract- Plants are the source of a great variety of phytochemicals, the valuable properties of which have been utilized by human being for centuries ever since the dawn of human civilization. Man has employed therapeutic plants as the greatest bio-resource of remedies for folk and contemporary treatments. Most plant species used as folklore medicine are claimed to retain the capacity to cure or mitigate some health disorders. *Cleome rutidosperma* has been reported for treatments as anti-inflammatory, antimicrobial, etc. agents. The present investigation was aimed to explore the phytoconstituents of the ethanolic and *n*-hexane extracts of the entire plant of *Cleome rutidosperma* employing the GC-MS technique. The extracts were analyzed with Thermo-Finnigan Trace GC Ultra (Waltham, MA, USA) equipped with a splitless injector, coupled to an ion trap mass spectrometer (MS) (Polaris Q) coupled to a Xcalibur data software processor.

Keywords: *phytoconstituents, GC-MS, ethanolic and n-hexane extracts, cleome rutidosperma.*

GJSFR-C Classification: *FOR Code: 069999*

Strictly as per the compliance and regulations of:

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Keywords: phytoconstituents, GC-MS, ethanolic and n-hexane extracts, cleome rutidosperma.

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I. Introduction

The exploitation of plants retaining therapeutic benefits has been a consistent practice by man all through history (Chen, *et al*; 2016). Plants have continued to be invaluable organic sources of therapeutic constituents for folk and contemporary treatments, pharmaceutical intermediates, food supplements and chemical entities for synthetic remedies (Alamgir, 2017; Dias, *et al;* 2012). There are more than 1300 healing plants utilized in Europe, of which 90 % are collected from wild resources; in the United States, around 118 of the top 150 medical preparations are naturally based (Balunas & Kinghorn, 2005). Moreover, up to 80 % of those in the developing nations are entirely reliant on herbal remedies for their major healthcare, and over 25 % of recommended medications in industrialized nations are derivative of wild plant species (Alves & Rosa, 2005; Hamilton, 2004). Through the growing request for herbal preparations, natural health products, and secondary metabolites of therapeutic plants, the application of curative plants is rising swiftly all over the sphere (Nalawade, *et al*; 2003; Cole, *et al*; 2007). Pharmaceutical plants are inexpensive, freely accessible and have insignificant side effects. The strength, therapeutic and organoleptic properties of these natural products have been attributed to the occurrence of bioactive phyto-constituents. Hence there is a need to ascertain, isolate, characterize, quantify and validate these compounds which are frequently desirable by pharmaceutical establishments for the manufacture of novel therapeutic remedies for the management of several health disorders (Wadood, 2013; Altemimi, *et al.,* 2017). *Cleome rutidosperma* DC (Family, Cleomaceae),

generally recognized as Fringed Spider Flower (FSF), is an annual herbaceous, low growing plant reaching up to 100 cm tall. It is native to Tropical Africa, found in Guinea, Nigeria, Cameroons, Zaire and Angola. It thrives in waste grounds, humid or grassy places with trifoliate leaves and small blue, violet with whitish patches of flowers (Ghosh, *et al.,* 2019). It has naturalized in diverse areas of Asia, Australia, America and the West Indies (Acevedo-Rodriguez & Strong, 2012). The ethnomedicinal applications of *Cleome rutidosperma* have been documented and these have been accredited to the essential phytochemical constituents confined in them. Phytochemical evaluation of *Cleome rutidosperma* indicated the existence of tannins, lipids, amino acids, flavonoids, cardiac glycosides, alkaloids, steroids, saponins, terpenoids, polyphenols, phlobatannins, pentose and reducing sugars (Ibezim & Ugoeze, 2017; Ghosh, *et al*; 2019). Extracts of the entire plant or its parts have anti-convulsant, anti-inflammatory, anti-stimulant, anti-scorbutic, rubefacient, anti-diarrheal, vesicant and carminative properties. Anti-oxidant, anti-plasmodial, analgesic, anti-microbial, diuretic, laxative, anthelmintic and anti-diabetic activities have well been documented (Bose, *et al*; 2006; Bose, *et al*; 2007; Chakraborty, *et al*., 2010; Bose, *et al.,* 2017; Ibezim & Ugoeze; Akah & Nwambie, 1993). Solvent extraction is an essential approach in the isolation of these phytoconstituents in their original form. The extraction and yield of plant metabolites frequently hinge on the plant part as well as the nature, combination and concentration of extraction solvent (Ibezim & Ugoeze, 2017). Thus, diverse extraction solvent defines the presence of various phytoconstituents to be anticipated in an extract based on the variation in the solubility of the various phytochemical compounds existent in them. The subsequent extract with its bioactive constituent, in turn, defines the activity to be associated with the plant, thus, agreeing with the previous report that the phytochemical investigation of crude ethanolic extract of several parts of *Cleome rutidosperma* revealed different extents of steroids, saponins, triterpenoids and reducing sugars (Ibezim & Ugoeze, 2017). These were accountable for the detected microbiological activity related to the plant extract. It was reported that the methanol, chloroform and petroleum ether crude extracts of *Cleome rutidosperma* displayed substantial analgesic and depressed locomotor activity (Bose, *et al*; 2004). Also, petroleum ether, chloroform, methanol and aqueous extracts of *Cleome rutidosperma* has been verified to have wound healing property (Mondal & Suresh, 2012).

In recent times, a detailed, modern analysis of bioactive, non-nutritive components of plants are undertaken in the isolation, identification and quantification of new therapeutic compounds of medicinal importance from plants for specific diseases (Altemimi, *et al;* 2017).

Gas chromatography-mass spectrometry (GC-MS) is a hyphenated analytical approach applied in the identification of various constituents contained by a test sample (Gabriella, *et al*; 2016). GC-MS can provide meaningful information for thermally stable, low molecular weight, volatile components (Kanimathi, *et al*; 2019). Qualitative confirmation of the divergent bioactive complexes from the chloroform and methanol crude extracts of Cleome species seeds employing GC-MS showed diverse categories of high and low molecular weight chemical entities with variable amounts (Kanimathi, *et al*; 2019). Their identification and characterization were based on their elution order in HP-5MS column. Although, there are reports on the GC-MS analysis of Cleome spp. extracts and the phytoconstituents present, but there is a paucity of information on their medicinal properties. Therefore, the present study employed GC-MS in the exploration of the phytoconstituents present in the ethanolic and *n*-hexane extracts of *Cleome rutidosperma* whole plant.

II. Materials and Methods

a) Chemicals and reagents

The following reagents were used as procured and were of analytical grades: Ethanol (96 %), *n*-hexane (99 %) (Merck, Germany).

b) Collection of plant the sample

The whole plant of *Cleome rutidosperma* was collected from the premises of the University of Port Harcourt, Choba, Port Harcourt, Nigeria. The plant was identified and authenticated by a Taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt. The reference specimen was prepared and deposited in the University of Port Harcourt central herbarium with a voucher specimen no. UPH/3/107

c) Pre-extraction treatment of sample

The plant sample was washed with purified water, air-dried in the shade (2 - 3 weeks), pulverized into a coarse powder (Binatone, China).

d) Extraction of plant material

The method of Bose, *et al*., 2007 was involved with minor adjustment. The powdered plant material was extracted with 96 % ethanol and *n*-hexane successively by Soxhlet extractor at 78 and 69° C respectively. The respective extracts were concentrated to a semi-solid paste with the aid of a rotatory-evaporator. Each residue was transferred into a 50 ml beaker and further dried with the aid of nitrogen gas. The dried extracts were stored separately in air-tight universal bottles and labelled appropriately for GC-MS analysis.

e) GC-MS analysis

Extracts were analyzed with Thermo-Finnigan Trace GC Ultra (Waltham, MA, USA) equipped with a

splitless injector, coupled to an ion trap mass spectrometer (MS) (Polaris Q) and coupled to a Xcalibur data software processor. Chromatographic separation was done with a HP-5MS capillary column of 30 m length × 0.25 mm i.d. × 0.25 *μm* film thickness (Agilent J & W Scientific Co., Folsom, CA, USA). The oven temperature was automated, which was initially held at 80 °C for 5 min, and was increased to 200°C at a rate of 20 °C/min, held for 5 min and then raised to 280°C at a rate of 10 °C/min and held for 2 min. The flow rate of the carrier gas (Helium, 99.99 % purity) was kept constant at 1.18 mL/min. Splitless injection mode at an injection temperature of 250°C was carried out at a pressure of 79.5 kPa. The linear velocity and total flow were 10.0 cm/s and 32.7 mL/min, respectively. The interface line and ion source temperatures were 260°C and 250°C, respectively.

III. Results and Discussion

a) GC-MS Analysis

Figures 1 and 2 show the GC-MS spectra of ethanolic and *n*-hexane extracts of the whole plant of *Cleome rutidosperma* respectively. The phytochemicals identified and their retention times, molecular weight, molecular formula and percentage peak area (as percentage composition) are presented in Tables 1 and 2. Their identification was based on the National Institute of Standards and Technology (NIST) library similarity index. The GC-MS analysis of the ethanolic and hexane extracts displayed the existence of 22 and 25 compounds correspondingly. These compounds were mostly esters, fatty acids, sterols, diketones and alcohols.

i. *GC-MS Analysis of ethanolic extract of Cleome rutidosperma*

Amongst the 22 compounds identified in the ethanolic extract of *Cleome rutidosperma*, thirteen of them were predominant. These were phthalic acid, di(2-propylpentyl) ester (22.5 %), 8-Isopropyl-5-methyl-5, 6,7,8-tetrahydro-2,4-quinazolinedione (14.82 %), *n*-hexadecanoic acid (14.78 %), 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (Phytol) (9.96 %), 9,9-Dimethoxybicyclo[3.3.1] nona-2, 4-dione (5.5 %), 1, 2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (5.19 %), (R)-(-)-14-Methyl-8-hexadecyn-1-ol (4.24 %), (1S, 15S)-Bicyclo[13.1.0] hexadecane-2-one (3.45 %), hexadecanoic acid ethyl ester (2.97 %), retinal (vitamin A aldehyde) (2.54 %), 3-Methyl-2-(2-oxopropyl)furan (2.25 %), 1-hexyl-2-nitrocyclohexane (2.13 %) and tetradecanoic acid, 12-methyl ester (1.88 %). The structures of these compounds are presented in Figure 3. Most of these phytoconstituents have been found to be bioactive against some pathogens and illnesses. For instance, phthalic acid, di (2-propylpentyl) ester has been reported to exhibit strong anti-bacterial and anti-fungi properties (Osuntokun *et al.,* 2017; Osuntokun and Gamberini, 2019). Also, 8-Isopropyl-5-methyl-5,6,7,8-tetrahydro-2,4-quinazolinedione as a derivative of quinazolinone is likely to possess a broad spectrum of biological actives such as antibacterial, antifungal, anticonvulsant, anti-inflammatory, anti-HIV, anticancer and analgesic activities (Jafari, *et al.,* 2016). Jafari, *et al*., 2016, stated that quinazolines and quinazolinone derivatives have presented promises of antimicrobial and cytotoxic activities. The compound *n*-hexadecanoic acid (palmitic acid) has been used successfully for skin and anti-inflammatory purposes (Aparna, *et al.,* 2012; Korbecki & Bajdak-Rusinek, 2019). Phytol or 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol is an acyclic diterpene alcohol, a key constituent of chlorophyll and a common precursor for the assembly of synthetic forms of vitamin E and vitamin K1. It is an antifungal and antimalarial agent which is very active against *Salmonella typhi* (Morah & Apebende, 2018). In addition, it is also recognized to have anti-ulcer, antioxidant, anti-inflammatory and diuretic properties (Soyingbe, *et al*, 2013). Likewise, phytol has been made known to modulate transcription in cells via transcription factors PPAR-alpha and retinoid X receptor (RXR) and as well as a regulator of lipid metabolism (Adnan, *et al*., 2019). No biological activity have been reported for 9, 9-Dimethoxybicyclo [3.3.1] nona-2, 4-dione. Govindappa, *et al*., 2014 reported 1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester to have revealed robust α-Glucosidase inhibition and *in-vivo* hypoglycemic outcome, while 3-Methyl-2-(2-oxopropyl) furan – a furan derivative is antipyretic, anti-inflammatory and hepato-protective (Borthakur, *et al.,* 2020). The phytochemical (R)-(-)-14-Methyl-8-hexadecyn-1-ol, is an alkynol. Although, there is no precise documentation on its bioactivity, but, it has been described as amongst predominant phytochemicals in plant extracts responsible for pharmacological actions like antibacterial, anti-inflammatory, diabetics, anti-histaminic, hepatoprotective, hypocholesterolemic (Sellamuthu, *et al.,* 2009; Vinay, *et al.,* 2014). Retinal, also recognized as vitamin A -aldehyde – an oxidized metabolite of retinol, with a polyene chromophore, being the most vital chemical for animal vision (Tsin, *et al.,* 2018) was also found in the whole plant of *Cleome rutidosperma*.

ii. *GC-MS Analysis of n-hexane extract of Cleome rutidosperma*

Eleven (11) phytochemicals were predominant amongst the 25 compounds identified in the *n*-hexane extract. The following phyto-compounds were also found in the ethanolic extract and have been discussed, they are - (R)-(-)-14-Methyl-8-hexadecyn-1-ol (22.91 %), hexadecanoic acid ethyl ester (14.52 %), 1-hexyl-2-nitrocyclohexane (4.97 %) and 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol (Phytol) (4.30 %). The other compounds found in the *n*-hexane extract were 2-methyl-5-5-

diphenyl-4-(methylthio)imidazole (18.22 %), linoleic acid ethyl ester (8.11 %), phthalic acid, isobutyl octadecylester (3.92 %), 4-fluoro-1-methyl-5-carboxylic acid ethyl ester (3.69 %), octadecanoic acid 17-methyl-methyl ester (3.08 %), Estra-1,3,5(10)-trien-17β-ol (2.25 %) and 2, 4-dimethyl-7-oxo-4,7-dihydro-triazolo (3, 2,-c)triazine (1.84 %). The phytochemical 2-methyl-5-5-diphenyl-4-(methylthio) imidazole is an imidazole derivative. Imidazole and its derivatives are reported to be biologically active and have found use in the management of health disorders for their anti-fungal and anti-bacterial effects (Zampieri, *et al.,* 2007; Shingalapur, *et al.,* 2009; Sharma, *et al.,* 2009), anti-inflammatory and analgesic activity (Puratchikodya & Doble, 2007; Achar, *et al*., 2010), anti-tubercular influence (Gupta, *et al*., 2004; Shingalapur, *et al*., 2009; Jyoti, *et al*., 2009), anti-depressant actions (Hadizadeh, *et al.*, 2008), anticancer activity (Congiu, *et al*., 2008; Ozkay, *et al*., 2010; Refaat, *et al.,* 2010), antiviral effects (Sharma, *et al*., 2009; Tonelli, *et al*., 2010) and anti-leishmanial activity (Shalini, *et al.,* 2010). The imidazole derivative constituted over 18 % of the *n*-hexane plant extract – being the second most predominant phytochemical found. Linoleic acid ethyl ester or Ethyl linoleate (ELA) - an essential fatty acid was also found. ELA is used as a vital constituent in many cosmetics, primarily due to its anti-inflammatory properties (National Center for Advancing Translational Sciences, NCATS). ELA is employed to aid neutrophils in the release of the reactive oxygen species (ROS) due to excess of bacteria and thus inhibits hyper-keratinization induced by lack of linoleic acid (Alarcon, *et al*., 2020). In addition it has been found to be used as parenteral injection for the treatment of ailments where there is a high plasma-cholesterol level of the blood (Feng, *et al*., 2020). 4-fluoro-1-methyl-5-carboxylic acid ethyl ester was reported by Hussein, *et al.*, (2016) to have anti-protozoal and anti-mycobacterial.

GC-MS identification of phytochemicals in ethanolic and n-hexane extracts

The MS fragmentation patterns of key phytochemicals in the extracts were identified using the NIST library. Below are selected MS obtained for some compounds present in both ethanolic and *n*-hexane extracts, matched with that of the NIST library (Figures 4a – 7b).

IV. Conclusion

This study showed the presence of biologically active components in *Cleome rutidosperma* through GC-MS analysis. Bioactives such as phthalic acid, di(2-propylpentyl) ester, 8-Isopropyl-5-methyl-5,6,7,8-tetrahydro-2,4-quinazolinedione, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, 2-methyl-5-5-diphenyl-4-(methylthio)imidazole, 4-fluoro-1-methyl-5-carboxylic acid ethyl ester and others were identified by the GC-MS profiling of both ethanolic and *n*-hexane extracts of *Cleome rutidosperma*. Our findings has justified its use in traditional and herbal therapy. More bioactive compounds were identified through the *n*-hexane extractant. The myriads of bioactives existing in the investigated plant could have synergistic effect and this may boost its therapeutic ability and could also be utilized for the amelioration of different health disorders. Therefore, *Cleome rutidosperma* has high medicinal potentials.

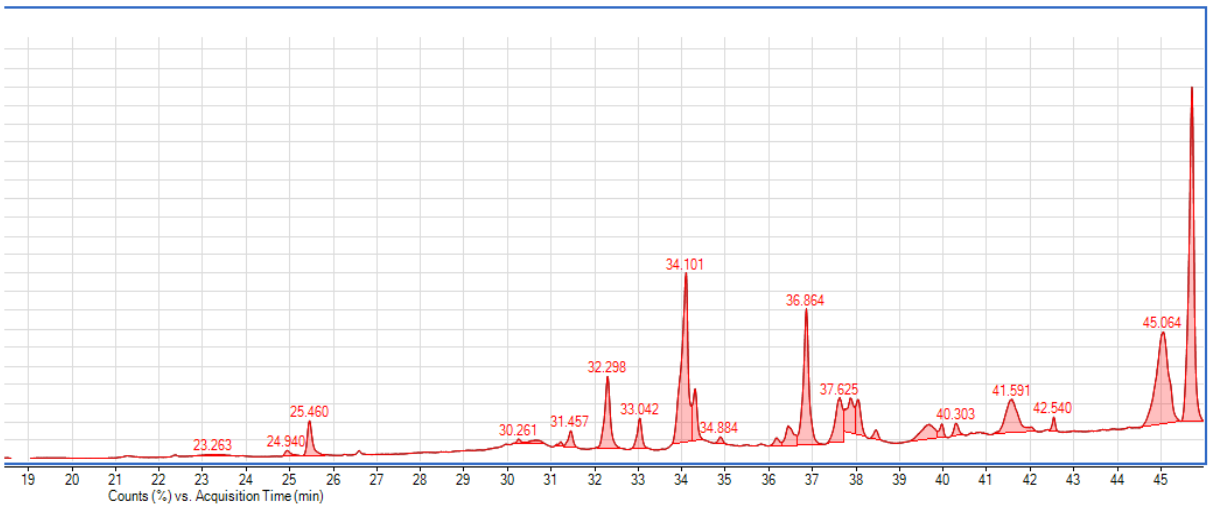


Figure 1: GC-MS chromatogram of ethanolic extract of *Cleome rutidosperma* whole plant

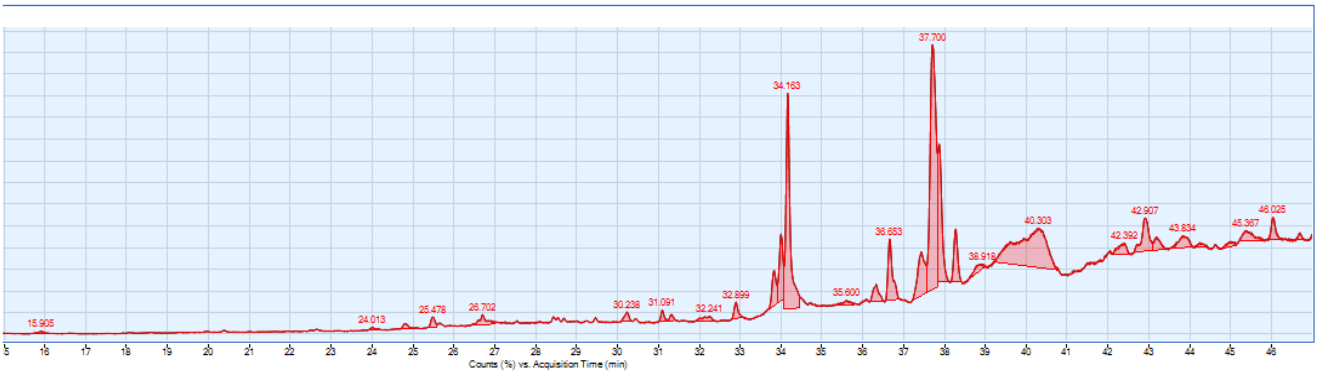
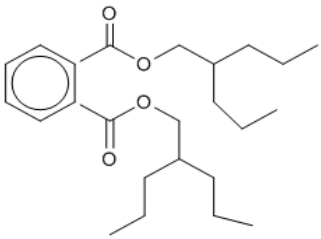
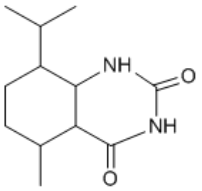


Figure 2: GC-MS chromatogram of hexane extract of *Cleome rutidosperma* whole plant



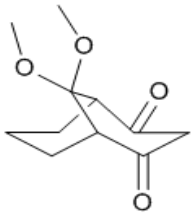
1

Phthalic acid, di (2-propylpentyl) ester



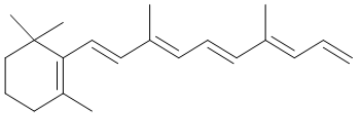
2

8-Isopropyl-5-methyl-5, 6, 7, 8-tetrahydro-2, 4-quinazolinedione



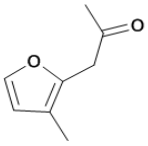
3

9, 9-Dimethoxybicyclo [3.3.1] nona-2, 4-dione



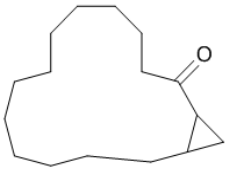
4

Retinal (Vitamin A aldehyde)



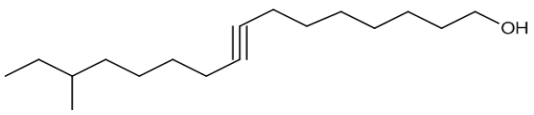
5

3-Methyl-2-(2-oxopropyl) furan hexadecane-2-one



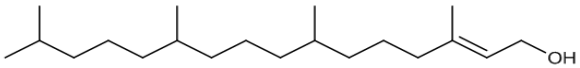
6

(1S, 15S)-Bicyclo [13.1.0]



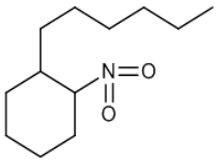
7

(R)- (-)-14-Methyl-8-hexadecyn-1-ol



8

3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (Phytol)



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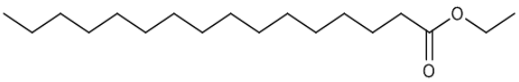
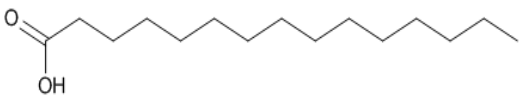
1-Hexyl-2-nitrocyclohexane



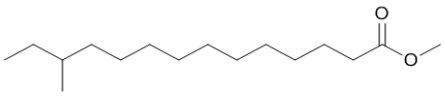
10

Hexadecanoic acid, ethyl ester



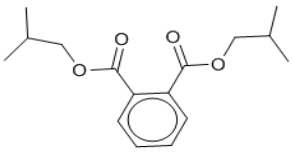
11

n-Hexadecanoic acid



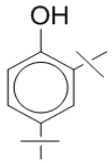
12

Tetradecanoic acid, 12-methyl ester



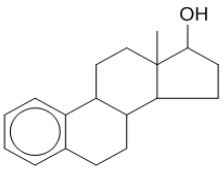
13

1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester



14

2, 4-bis(1,1-dimethylethyl)-Phenol



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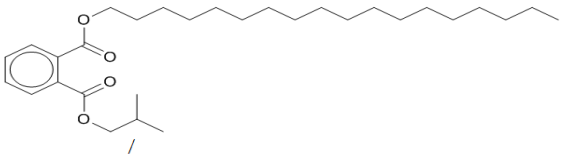

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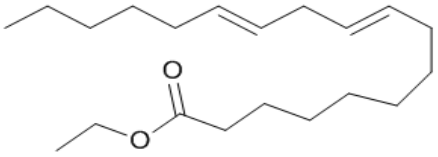
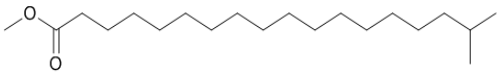


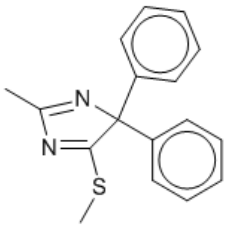
17

Linoleic acid ethyl ester



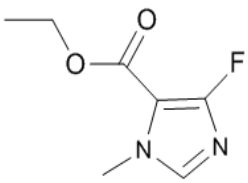
18

Octadecanoic acid, 17-methyl ester



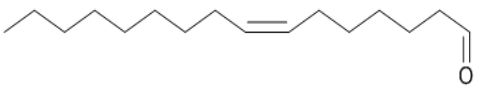
19

2-Methyl-5, 5-diphenyl-4-(methythio)imidazole



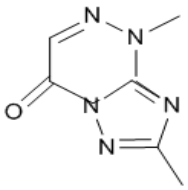
20

4-Fluoro-1-methyl-5-carboxylic acid, ethyl ester



21

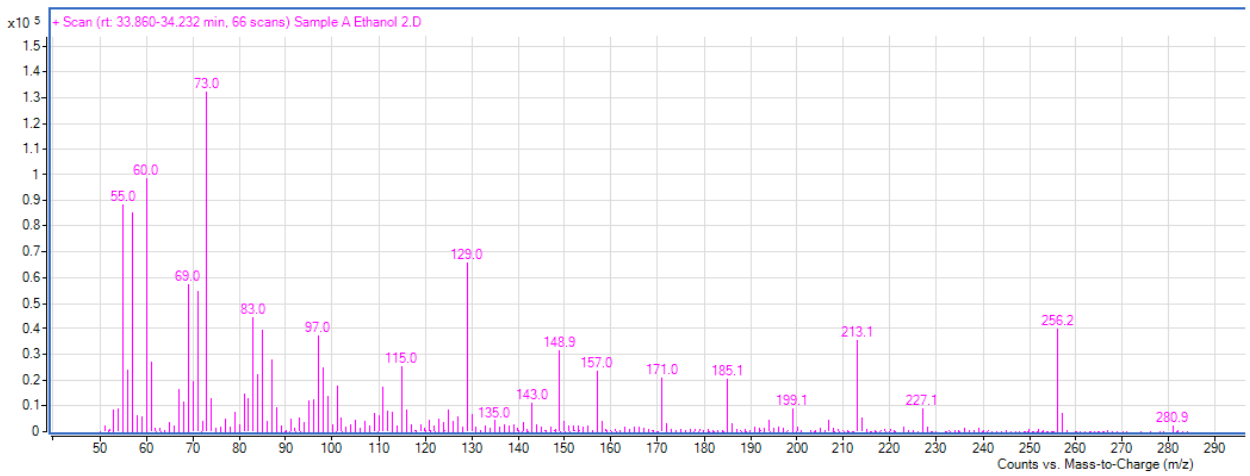
7- Hexadecenal, (Z) -



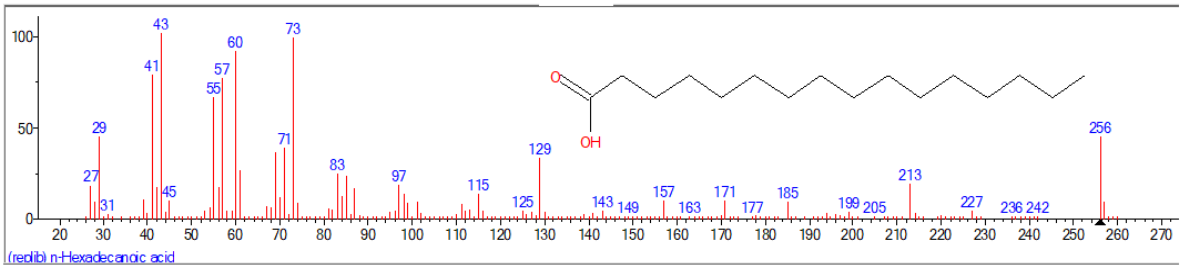
22

2, 4-Dimethyl-7-oxo-4, 7-dihydro-triazolo (3,2-c)triazine

Figure 3: Chemical Structures of major constituents identified in ethanolic and hexane extracts of *Cleome rutidosperma* whole plant using GC-MS

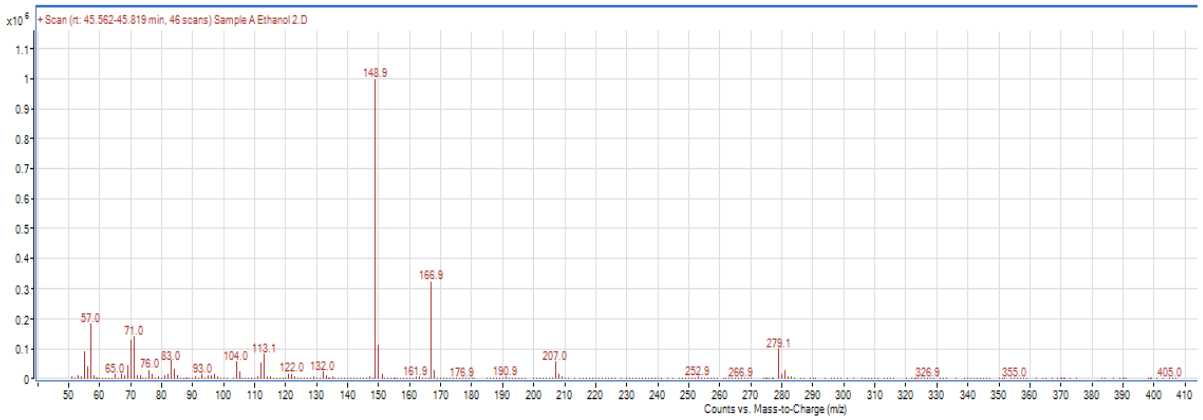


(a)

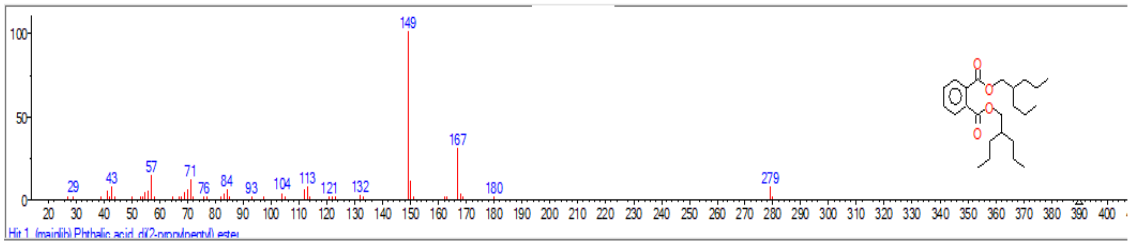


(b)

Figure 4: Mass spectra of n-hexedecanoic acid (a) in ethanolic extract (b) NIST library

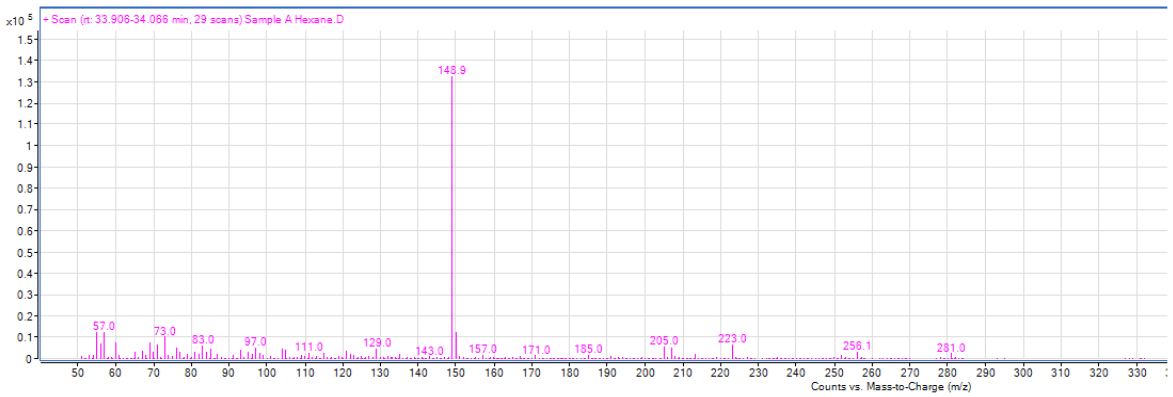


(a)

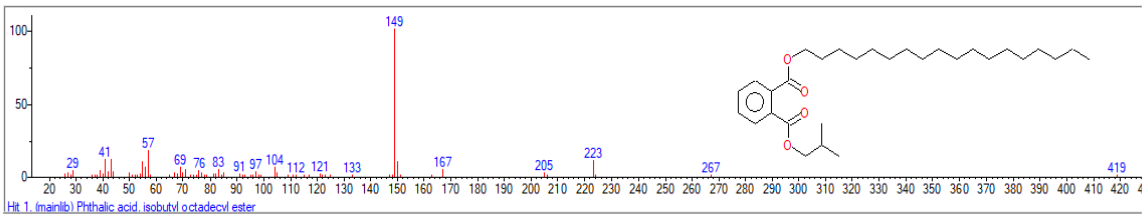


(b)

Figure 5: Mass spectra of Phthalic acid, di (2-propylpentyl) ester (a) in ethanolic extract (b) NIST library

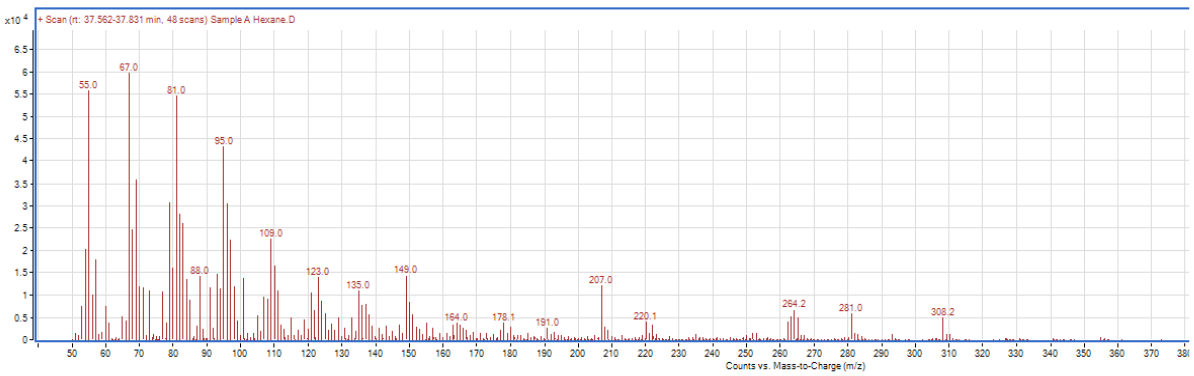


(a)

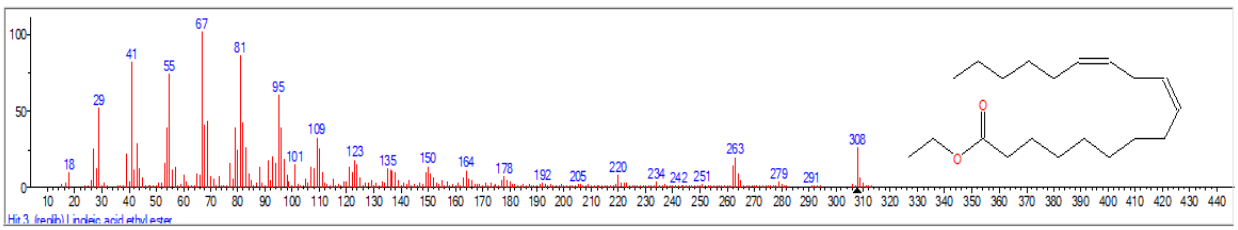


(b)

Figure 6: Mass spectra of Phthalic acid, isobutyl octadecyl ester (a) in hexane extract (b) NIST library



(a)



(b)

Figure 7: Mass spectra of Linoleic acid ethyl ester (a) in hexane extract (b) NIST library

Table 1: Compounds identified in ethanolic plant extract by GC-MS

S/N	RT	Name of compound	M. formula	M.wt	Peak Area (%)
1	23.263	S(+)-1-Cyano-2-methyl-azetidine	$C_5H_8N_2$	96.068	0.35
2	24.94	2, 5-bis(1, 1-dimethylethyl)-Phenol	$C_{14}H_{22}O$	206.167	0.45
3	25.46	2, 4- bis(1, 1-dimethylethyl)-Phenol	$C_{14}H_{22}O$	206.167	2.32
4	30.261	6, 10, 14-Trimethyl-pentadecan-2-ol	$C_{18}H_{38}O$	207.292	0.27
5	30.667	Carbamic acid, hydroxyl-, ethyl ester	$C_3H_7NO_3$	105.043	0.42
6	31.228	14-Heptadecenal	$C_{17}H_{32}O$	252.245	0.23
7	32.298	1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.152	5.19
8	33.042	Tetradecanoic acid, 12-methyl ester	$C_{16}H_{32}O_2$	256.240	1.88
9	34.100	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.240	14.78
10	34.306	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.271	2.97
11	36.183	2-Methyl –E-7-octadecene	$C_{19}H_{38}$	266.297	0.57
12	36.447	1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}NO_2$	213.173	2.13
13	36.864	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (Phytol)	$C_{20}H_{40}O$	296.308	9.96
14	37.625	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	252.245	4.24
15	37.883	(1S, 15S)-Bicyclo[13.1.0] hexadecane-2-one	$C_{16}H_{28}O$	236.214	3.45
16	38.054	3-Methyl-2-(2-oxopropyl)furan	$C_8H_{10}O_2$	138.068	2.25
17	38.461	3-Acetoxypentadecane	$C_{17}H_{34}O_2$	270.256	0.51
18	39.685	Retinal (Vitamin A aldehyde)	$C_{20}H_{28}O$	284.214	2.54
19	40.303	7-Hexadecanal, (Z)	$C_{16}H_{30}O$	238.230	0.73
20	41.591	9,9-Dimethoxybicyclo[3.3.1] nona-2, 4-dione	$C_{11}H_{16}O_4$	212.105	5.50
21	45.064	8-Isopropyl-5-methyl-5, 6,7,8-tetrahydro-2,4-quinazolinedione	$C_{12}H_{18}N_2O_2$	222.137	14.82
22	45.716	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	390.277	22.05

Table 2: Compounds identified in hexane plant extract by GC-MS

S/N	RT	Name of compound	M. formula	Mw.t	Peak Area (%)
1	15.905	2-Methyl-1-undecanol	$C_{12}H_{26}O$	186.198	0.02
2	24.808	2-Hexyl-1-octanol	$C_{14}H_{30}O$	214.230	0.38
3	25.478	Phenol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.167	0.69
4	26.702	2-Methyl-E-7-octadecene	$C_{19}H_{38}$	266.297	1.21
5	30.238	2-Hexyl-1-octanol	$C_{14}H_{30}O$	214.230	0.65

6	31.091	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	$C_{20}H_{40}O$	296.308	0.53
7	32.241	3-Methyl-2-(2-oxopropyl)furan	$C_8H_{10}O_2$	138.068	0.66
8	32.899	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242.261	0.98
9	33.832	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	212.105	2.25
10	33.998	Phthalic acid, isobutyl octadecyl ester	$C_{30}H_{50}O_4$	474.371	3.92
11	34.163	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.272	14.51
12	36.326	1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}NO_2$	213.173	1.48
13	36.653	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.308	4.30
14	37.419	1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}NO_2$	213.173	4.97
15	37.700	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	252.245	22.91
16	37.871	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.272	8.11
17	38.272	Octadecanoic acid, 17-methyl-, methyl ester	$C_{20}H_{40}O_2$	312.303	3.08
18	38.918	Acetic acid, trifluoro-, 3,7-dimethyloctyl ester	$C_{12}H_{21}F_3O_2$	254.149	0.93
19	40.303	2-Methyl-5,5-diphenyl-4-(methylthio)imidazole	$C_{17}H_{16}N_2S$	280.103	18.22
20	42.392	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238.230	1.38
21	42.907	4-Fluoro-1-methyl-5-carboxylic acid, ethyl ester	$C_7H_9FN_2O_2$	172.065	3.68
22	43.193	exo-1,2-O-Ethylidene-α-d-erythrofuranose	$C_6H_{10}O_4$	146.058	1.29
23	43.834	2,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c) triazine	$C_6H_7N_5O$	165.065	1.84
24	44.228	Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5-(2-propenyl)-, [1R-(6-endo,7-exo,8-syn)]-	$C_{21}H_{24}O_7$	388.152	0.28
25	45.001	Phenol, 4-(1,1,3,3-tetramethylbutyl)-	$C_{14}H_{22}O$	206.167	0.53

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Conflict of interest

The authors declare no conflict of interest

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ASSOCIATE OF SCIENCE FRONTIER RESEARCH COUNCIL

ASSOCIATE OF SCIENCE FRONTIER RESEARCH COUNCIL is the membership of Global Journals awarded to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

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

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Career | Credibility | Reputation

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john [AT] globaljournals [DOT] org.

Career | Credibility | Reputation

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Associate	Fellow	Research Group	Basic
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Preferred Author Guidelines

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

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

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

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

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

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2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
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7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

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

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Plagiarism is not acceptable in Global Journals submissions at all.

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- Writings
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- Printed material
- Graphic representations
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2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

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

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

Acknowledgments

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

Declaration of funding sources

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

Preparing your Manuscript

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

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

Manuscript Style Instruction (Optional)

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

Structure and Format of Manuscript

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

A research paper must include:

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

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

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

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

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

Format Structure

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

All manuscripts submitted to Global Journals should include:

Title

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

Author details

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

Abstract

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

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

Keywords

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

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

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

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

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

Numerical Methods

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

Abbreviations

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

Formulas and equations

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

Tables, Figures, and Figure Legends

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

Figures

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

Preparation of Eletronic Figures for Publication

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

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

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

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final points:

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

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

The discussion section:

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

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

General style:

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

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

Mistakes to avoid:

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

Title page:

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

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

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

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

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

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

Approach:

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

Introduction:

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

The following approach can create a valuable beginning:

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

Approach:

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

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

Procedures (methods and materials):

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

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

Materials:

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

Methods:

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

Approach:

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

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

What to keep away from:

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

Results:

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

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

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

Content:

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

What to stay away from:

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

Approach:

As always, use past tense when you submit 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 section.

Figures and tables:

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

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an 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 implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

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

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

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

Approach:

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

Describe generally acknowledged facts and main beliefs in present tense.

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

Index

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