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Biochemical Activity And GC-MS Analysis Of Fraction Of Ethylacetate Extract Of *Erythrina senegalensis* Stem Bark

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ABSTRACT
Bioassay and Gas Chromatography Mass-Spectrometry (GC-MS) analysis of fraction from ethylacetate extract of *Erythrina senegalensis* stem bark were examined. The bioassay indicates that, the fraction of the ethylacetate extract inhibits the growth some clinical isolates (ie medicinal properties). The GC-MS analysis of the fraction of the extract result showed the molecular formulas and the possible structures of the bioactive compounds which may be used in drugs preparation.
Keywords: *Erythrina senegalensis***,** bioactive compounds, GC – MS

INTRODUCTION

The practices of traditional medicine vary greatly from country to country, and from region to region, as they are influenced by factors such as culture, history, personal attitudes and philosophy (Bothon *et al.,* 2016). In most cases, their theory and application are quite different from those of conventional medicine (Adiaratou, 2008)

Erythrina senegalensis grows in West African tropical and sub-tropical areas. It is native to Senegal, Gambia, Guinea, Guinea-Bissau, Sierra Leone, Liberia, Mali, Burkina Faso, Ivory Coast, Niger, Ghana, Togo, Benin, Nigeria and Cameroon (Adiaratou *et al.,*).

Erythrina senegalensis (known as Minjiriya in hausa) belong to the family leguminasae (Fabaceae) Subfamily: Papilionoideae and its genus is *Erythrina* (Kumar *et al.,* 2008). It is 5 – 15 meter tall (Adiaratou and Ingvild, 2011). It has deeply fissured corky bark; the branches are armed with slightly hooked spines up to 10 mm long. The leaves are composed of three leaflets, each measuring (5 – 15) x (4 – 10) cm and having thorny stalk. The flowers appear in large group at the end of the branches. The flowers are bright red and 4 – 5 cm long. The fruits are bent, twisted and slightly hairy pod. It is constricted between seeds, which are bright red (Lewis, *et al.,* 2011).

Erythrina senegalensis was selected on the basis of ethno medical information indicating that the local communities use them. Tea from *Erythrina* flowers is regularly used as a relaxant in Mesoamerica (Kumar *et al*., 2010). *Erythrina senegalensis* is used in West African countries for almost same therapeutic indications. The ailments treated are bacterial, fungal, parasitic and viral diseases, gastrointestinal disorders, liver disorders, sexual asthenia, nervous disorders, sterility, eyes diseases and kidney pain (Witabouna, 2012).

The crude extract of *Erythrina senegalensis* stem bark shows bioactivities against some clinical isolate and was due to the presence of alkaloids, glycoside, reducing sugar, saponins, flavonoid, terpenoids and tannins which were found present in the plant extract (Gunda et al., 2015a). The ethylacetate crude extracts shows cytotoxic effect against brine shrimp using DPPH scavenging (Gunda, et al., 2015b). The *Erythrina senegalensis* stem bark crude extract also shows antioxidant activity (Gunda et al., 2015c). The present study focused on investigating bioactivities of one of the fractions of the ethylacetate extract of *Erythrina senegalensis* stem bark and determine the molecular mass and molecular formula of the phytochemical responsible for the inhibition of the clinical isolate.

EXPERIMENTATION

Preparation of plant extracts

The stem bark of *Erythrina senegalensis were* harvested in Biu, Borno state of Nigeria. The sample was air dried and grounded to powder using mortar and pistil. The powdered sample was extracted sequentially with n-hexane, ethylacetate, acetone, methanol and water using soxhlet extractor. The extract was concentrated and dried in an oven at 40^{o}C. Purification of the crude ethylacetate extract was attempted on silica gel (mesh 60-120) column chromatography using chloroform, acetone and ammonia in a ratio of 7:2:1 as eluting solvent as this solvent system gives a reasonable separation in TLC analysis.

Bioassay

Agar disc diffusion method was adopted as described by Paul (1992) Ramzi, *et al.,* (2009).Alo, *et al.,* (2012) and Taylor, *et al.,* (1998) to evaluate the activity of the fraction of the extracts on some clinical isolates (microorganism).

Gas Chromatography-Mass Spectrometry GC/MS analysis:

GC-MS model QP5050A SHIMADZU jabatan Kimia was used. The column size is 30 meter x 25mm ID x 25µm film thickness. The injection volume was 0.5nµL, injection temperature 250^{o}C and interface temperature is 250^{o}C. The auto-sampler injects 0.5 µl of the sample, injection in un-split mode. The carrier gas was helium, at a working constant flow rate of 1.5 mL/min. Mass spectra were recorded in electron impact mode at 70 eV; electron multiplier 2500V; ion source. Mass spectra data were acquired in the scan mode in m/z range 40-350 uma.

RESULTS AND DISCUSSION

The biological activity test carried out on the fraction (1) of ethylactate extract of *Erythrina senegalensis* stem bark is represented in table 1 below. Gas Chromatography-Mass Spectroscopy analysis carried out on the fraction of ethylacetate extract of *Erythrina senegalensis* stem bark is represented in Table 2. The identification of phytochemical compounds is based on the Peak area (%), Retention time (RT), Molecular weight, Molecular formula. Interpretation of mass spectrum (GC-MS) was conducted using the database of National Institute Standard and Technology (NIST) 08 spectral library collection).

Table 1: Antimicrobial Activities of the fraction of the ethylacetate extract of *Erythrina senegalensis* stem bark on Some Clinical Isolates (Extracts Concentrations are 200 mg/ml.). Zones of Inhibition is in mm.

Microorganism	F_1 of ethylacetate extracts	+C cipro/amp	-C distilled water
S. typhi	19	23	00
S. dysentarae	13	24	00
E. coli	16	26	00
S.aureaus	10	27	00
T. rubrum	8	25	00
A. niger	11	24	00
C. albicans	16	23	00

NOTE: Zone of inhibition ≥ 8 mm is sensitive while < 8 mm is resistant

KEY:

S. typhi = Salmonella typhi
S. dysentarae = Shigella dysentarae
E. coli = Escherichia coli
S. aureaus = Staphylococcus aureaus
T. rubrum = Trichophyton rubrum
A. niger = Aspergillus niger
C. albican = Candida albicans
- C = Negative control

+C = Positive control
amp = Amphotericin B
Cipro = Ciprofloxacin

Table 2: The volatile compounds identified in the fraction of ethyl acetate extract of *Erythrina senegalensis* stem bark.

Line No.	Ret. time	Peak area %	Molecular mass	Mol. formula
1	3.325	3.60	Acetic acid	$C_2H_4O_2$
2	5.142	0.69	1,2-ehanediol monoactate	$C_4H_8O_3$
3	7.608	1.11	2-(2-butoxyethoxy) ethanol	$C_8H1_8O_3$
4	8.483	0.24	(1-butylheptyl)-Benzene,	$C_{17}H2_8$
5	8.617	0.77	(2-ethoxy-1-methoxyethoxy)-ethene	$C_7H_{14}O_3$
6	9.425	11.51	2-[2-(2-methoxyethoxy)ethoxy]-ethanol	$C_7H_{16}O_4$
7	10.158	2.48	2-propanoic acid, oxybis(methyl-2,1-ethanediyl)ester	$C_{15}H_{28}O_2$
8	10.375	0.39	Ethanol, 2-[2-(2-methoxyethoxy)ethoxy acetate	C_9H1_8O5
9	10.858	0.39	(1-pentyloctyl) benzene	$C_{19}H_{32}$
10	11.125	0.31	(1,3,3-trimethylnonyl)benzene	$C_{18}H_30$
11	12.208	6.75	2-[2-(2-butoxyethoxy)ethoxy]ethanol	$C_{10}H_{22}O_4$
12	12.933	0.36	4-isopropyl-1,6-dimetyl-1,2,3.4,4a,7,8,8a-octahydro-1-naphthalenol	$C_{15}H_{26}O$
13	14.217	0.76	1-chloro-octadecane	$C_{18}H_{37}Cl$
14	14.842	0.66	2,3-dihydrobenzofuran	$C_8H_{18}O$
15	15.133	9.64	2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethanol	$C_9H_{20}O_5$
16	15.542	0.06	3-methoxy-phenol	$C_7H_8O_2$
17	16.108	0.47	2-[2-(2-methoxyethoxy)ethoxy ethanol	$C_9H_{18}O_5$
18	16.842	1.13	Hexadecanoic acid, 1,1-dimetykethyl ester	$C_{20}H_{40}O_2$
19	17.175	0.58	9-octyl-heptadecane	$C_{25}H_{52}$
20	17350	0.60	5-formylmethyl-6-hydroxy-3,3-dimetyl-6-vinyl-Bicycle[3.2.0]hepta-2-one,	$C_{13}H_{18}O_3$
21	17.875	0.50	4-hydroxy-3-methoxy-benzaldehyde	$C_8H_8O_3$
22	18.250	2.33	3,6,9,12,15-pentaoxanonadecan-1-ol	$C_{14}H_{30}O_6$
23	18.625	0.70	octacosane	$C_{19}H_{40}$
24	19.708	3.82	1,2-benzenedicarboxylic acid dibutyl ester	$C_{16}H_{22}O_4$
25	19.883	0.88	Octadecanoic acid butyl ester	$C_{22}H_{40}O_2$
26	20.225	0.17	2,6-dimethoxy-4-(2-propenyl)-phenol	$C_{11}H_{14}O_3$
27	21.142	0.07	2-(1-pentenyl)-(E)-furan	$C_9H_{12}O$
28	21.317	0.14	2,5,8,11,14-pentaoxohexadecan-16-ol	$C_{11}H_{24}O_6$
29	21.892	3.46	3,5-dimethoxyphenol	$C_8H_{10}O_3$
30	22.617	2.23	n-hexadecanoic acid	$C_{16}H_{32}O_2$
31	23.125	0.70	4,4,8-trimethyltricyclo[6.3.1.0(1,5]dodecane-2,9-diol	$C_{15}H_{26}O_2$
32	23.492	1.49	1-Eicosanol	$C_{20}H_{42}O$
33	24.292	0.56	3,6,9,12,15-pentaoxanonadecan-1-ol	$C_{14}H_{30}O_6$
34	24.742	1.33	n-pentadecanol	$C_{15}H_{32}O$
35	25.708	1.02	1,3-benzenediol	$C_6H_6O_2$
36	26.475	0.73	Octadecanoic acid	$C_{18}H_{36}O_2$
37	27.025	0.71	(Z)-9-octadecanoic acid	$C_{18}H_{34}O_2$
38	28.158	34.25	1,2-benzenedicarboxylic acid bis(2-ethylethyl) ester	$C_{24}H_{38}O_4$
39	29.750	1.15	1-heneicosanol	$C_{44}H_{21}O$

The bioactivity study shows that, the fraction of the ethylacetate extract *erythrina senegalensis* stem bark inhibits the growth of *S. Typhi, S. Dysentarae, E. Coli, S.aureaus, A. Niger and C. Albicans.* Mohammed, *et al*., (2010) also reported some antimicrobial activity of selected Indian folk medicinal plants against some clinical isolates (*S. aureus S. aureus* and *S. epidermidis K.pneumoniae,E. coli and I. Batatas*). So it is anticipated that plants can provide potential bioactive compounds for the development of new 'leads' to combat various disease.

GC-MS chromatogram of the fraction of the ethyl acetate extract of *Erythrina senegalensis* stem bark clearly shows 39 peaks indicating the presence of 39 phytochemical compounds. The identification of the phytochemical compounds was based on the peak area, retention time and molecular formula. The table 2 shows the compounds peak number, retention time, % area, name and molecular formula. The results reveal the presence of Acetic acid; 1,2-ehanediol monoactate; 2-(2-butoxyethoxy) ethanol; (1-butylheptyl)-Benzene; 2-[2-(2-methoxyethoxy)ethoxy]-ethanol (2-ethoxy-1-methoxyethoxy)-ethene; 2-propanoic acid, oxybis(methyl-2,1-ethanediyl)ester; Ethanol, 2-[2-(2-methoxyethoxy)ethoxy acetate; (1-pentyloctyl) benzene; (1,3,3-trimethylnonyl)benzene; 2-[2-(2-butoxyethoxy)ethoxy]ethanol; 4-isopropyl-1,6-dimetyl-1,2,3.4,4a,7,8,8a-octahydro-1-naphthalenol; 1-chloro-octadecane; 2,3-dihydrobenzofuran; 2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethanol; 3-methoxy-phenol, 2-[2-(2-methoxyethoxy)ethoxy ethanol; Hexadecanoic acid; 1,1-dimetykethyl ester; 9-octyl-heptadecane; 5-formylmethyl-6-hydroxy-3,3-dimetyl-6-vinyl-Bicycle[3.2.0]hepta-2-one, 4-hydroxy-3-methoxy-benzaldehyde; 3,6,9,12,15-pentaoxanonadecan-1-ol; octacosane, 1,2-benzenedicarboxylic acid dibutyl ester; Octadecanoic acid butyl ester; 2,6-dimethoxy-4-(2-propenyl)-phenol; 2-(1-pentenyl)-(E)-furan; 2,5,8,11,14-pentaoxohexadecan-16-ol; 3,5-dimethoxyphenol; n-hexadecanoic acid; 4,4,8-trimethyltricyclo[6.3.1.0(1,5]dodecane-2,9-diol; 1-Eicosanol; 3,6,9,12,15-pentaoxanonadecan-1-ol, n-pentadecanol;1,3-benzenediol, Octadecanoic acid; (Z)-9-octadecanoic acid; 1,2-benzenedicarboxylic acid bis(2-ethylethyl) ester and 1-heneicosanol. Bis(2-ethylhexyl) phthalate the major compound which has the biggest peak area (49.21%) has been isolated from a marine alga, *Sargassum weightii*, and apart from its plasticizing ability it was also found to have antibacterial effect on a number of bacteria (Sastry and Rao, 1995). Di(2-ethylhexyl) phthalate is not known to occur naturally. However, the natural occurrence of phthalates in a wide variety of plants is already in the literature (Rasika, *et al., 2015;* save *et al., 2015* and Azra *et al.*, 2012).) The principal route by which it enters the environment is via transport in air or via leaching from plastics and plasticizer plants or other sources such as sewage treatment plants, paper and textile mills and refuse incinerators (IARC, 2000).

The association of these volatile compounds (Bothon et al., 2016) in the *Erythrina senegalensis* stem bark justify the antioxidant, anticancer and antimicrobial reported earlier.

CONCLUSION

The presence of the phytochemicals observed from the GC–MS spectrum is thus responsible for the biological activity of the plant and thus justify their use in traditional medicine.

REFERENCES

Adiaratou T., Ingvild A., Annette T., Drisss D. and Berit S. P. (2008). Ethnopharmacological uses of *Erythrina Senegalensis*. A comparison of three areas in Mali abd a linr between traditional knowledge and moderm biological science. *Journal of Ethnobiology and Ethnomedicine*. **4**:6, **DOI:** 10.1186/1746-4269-4-6

Adiaratou Togola and Ingvild Austarheim (2011). Erythrina senegalensis A.rich [FABACEAE]., *nettredaktoren, Farmasi*. instituttkontoret@farmasi.uio.no, retrived on 06/06/2013.

Azra A, . Ekwenchi, M.M Dashak and Dildar D.A. A. (2012) Gas Chromatography-Mass Spectrometry (GC/MS) Analysis of Phthalate Isolates in n-Hexane Extract of *Azadirachta indica A. Juss* (Neem) Leaves. Journal of American Science. 8(12) 146 – 155.

Bothon, F. T. D.; J. Adovelande, F. Cazier, F. Avlessi1 and D. C. K. Sohounhloue1(2016); Phytochemical Analysis and Medecinal Evaluation of Hydro Ethanolic Extract of Two Varieties *Hibiscus Sabdariffa* Calyxes. International Journal of Green and Herbal Chemistry Vol.5, No.3, 349-354.

Gunda H. J., Adamu H. M., Chindo I. Y., Zanna H. K. and Ladi F. L. (2015a) Biochemical evaluation of of *Erythrina senegalensis* stem bark. *International journal of applied Research and* Technology 4(8): 44-48

Gunda H. J., Adamu H. M., and Chindo I. Y., (2016b). Cytotoxicity evaluation of erythrina senegalensis stem back using brine shrimp lethality assay. International journal of applied research and technology. 5(4): 37-40

Gunda H. J., Adamu H. M., Chindo I. Y., and Ushie A. O. (2015c). phytochemical and Antioxidant Analysis of *Erythrina senegalensis* stem bark. World Research Journal of Pharma Technology. 1(3): 1-6.

Kumar A., Lingadurai S., Jain A. and Barman N. R. (2010). *Erythrina variegate Linn*; A review on morphology, phytochemistry, and pharmacology aspect. *Plant Review.* 4(8): 147-152.

IARC (2000) Di(2-ethylhexyl) phthalate *Monographs* Volume 101: 149 - 284

Mohamed S.S.H.; Hansi P.D. and Kavitha T. (2010) Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants, International Journal of Pharma Sciences and Research (IJPSR) Vol.1(10), 430-434

Rasika C. T., Gayatri S. K., Swati M. D., Usha D.P. and Nirmala R. D. (2015). Isolation and Characterisation of 1, 2 Benzenedicarboxylic acid, bis (2 ethylhexyl) ester – Dioctyl Phthalate, a Bioactive Compound from *Ehretia laevis. Journal of Pharmacy Research 2012,5(6),3251-3252*

Save, S. A. Lokhande1, R. S. and Chowdhary A. S. (*2015)* Determination of 1, 2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester from the twigs of *Thevetia peruviana* as a Colwell Biomarker. *Journal of Innovations in Pharmaceuticals and Biological Sciences.* Vol 2 (3), 349-362.

Witabouna M. K., Kakou-N. E. S. and Mireille D., (2011) Assessing Sub-Saharan *Erythrina* for Efficacy: Traditional uses, Biological Activities and Phytochemistry. *Pakistan Journal of Biological Sciences, 14(1): 560-571.*

Appendix: GC-MS Spectrum of the extract