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

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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.

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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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