

Phytochemical Analysis and Biological Activity of Some Sudanese Medicinal Plants

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Abstract

Review Article

The plants under investigations (*Boscia senegalensis*, *Boswellia papyrifera*, *Cadaba glandulosa*, *Aristolochia bracteolata* and *Nymphaea lotus*) were used in folkloric medicine in Sudan to cure some diseases. The results indicated a moderate presence of alkaloids on *B. senegalensis* and *A. bracteolata* and weak presence for *N. lotus*, *C. glandulosa* and *B. papyrifera*. Tannins were detected highly presence for *B. senegalensis*, *N. lotus* and moderate presence for other plants. Also flavonoids represented moderate presence for *N. lotus* and weakly presence for other plants. Remarkable cytotoxicity revealed for *B. senegalensis* with high value equal to 1.975 µg/ml. On the other hand *B. papyrifera*, *N. lotus*, *C. glandulosa* and *A. bracteolata* represented cytotoxicity equal 14.96, 316.22, 635.1 and > 1000 µg/ml respectively.

Keywords: Phytochemical Analysis Biological Activity Medicinal Plants.

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INTRODUCTION

Plants are utilized as therapeutic agents since time out of mind in both organized and unorganized forms [1]. The healing properties of the many herbal medicines are recognized in many ancient cultures. The term of medicinal plants includes various sorts of plants utilized in herbalism and a few of those plants have a medicinal activity. Herbs are utilized in many domains, including medicine, nutrition, flavorings, beverages, dyeing, repellents, fragrances and cosmetics [2]. And Traditional herbal medicine as a serious African socio-cultural heritage, obviously alive for several many 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 healthcare delivery, not only in Africa but also to various extents in all countries of the world [3]. 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 [4]. Sudan has been home to indigenous civilization, like 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 therefore the neighboring cultures (mainly Egyptian and West African cultures) have strongly influenced Sudanese culture. However, there's a good range of practices, which fall into the umbrella of traditional medicine [5], this encourage their use for the remedy of variety of diseases without supervision. Increased popularity and scarcity of scientific studies on the safety of these plants and their phytoconstituents have raised questions about their toxicity and adverse effects [6]. *B. papyrifera* plant (family Burseraceae, Fig 1) common name is Tarag trag - Rut-Rut, Gafal was distributed in Sudan in Blue Nile State (Jebel Elgarrie area, Ingasana and the border with Ethiopia), Southern Kordofan State in the Nuba Mountains. And Western Darfur, from Jebel Marra towards the west through Capitol Hill catenas of Zalingi to the south of Elgeneina until the border with the Republic of Chad [7]. It was claimed to have many medicinal applications, it produce a gum resin that is known as olibagum (frankincense). This natural resin has long been utilized in Ayurvedic and traditional Chinese medicine to treat a spread of health aspects [8] like inflammatory and arthritic diseases [9]. Several compounds isolated from these resins have growth inhibitory activity against cancer cells [10]. Moreover, boswellic acids can activate additional pathways in cancer cells. For example, boswellic acids can inhibit nuclear factor-κB and STATs activities in tumor cells [11]. *Boswellia* species volatile oils are the

most commonly used oils in aromatherapy. Chemical profile of those oils were analyzed and studied for his or her anti-tumor properties [12]. *Nymphaea Lotus* plant has a common name Soutab, Umm Ban geiga (Fig 2). It is aquatic pubescent herbs with submerged prostrate rhizomes, widespread throughout central and southern Sudan [13]. It is utilized in traditional medicine system as an aphrodisiac, anodyne, astringent, cardiotoxic, sedative, demulcent, analgesic and as anti-inflammatory agent [14]. The leaves of lotus are traditionally used for the treatment of haematemesis, haematuria, metrorrhagia, hyperlipidaemia, fever and inflammatory skin conditions [15]. Many biological activities, including anticancer and antiviral has been attributed to Gallic and ellagic acid which are widely present in *N. lotus* [16]. In addition, the plant *Aristolochia bracteolata* which common name is Um galagel. (Family Aristolochiaceae Fig 3). It is a shrub or small tree [17]. It covers an expansive region in Sudan with a wide distribution in lowland plains and water catchment areas [18]. It is traditionally used as a remedy for scorpion and snake bites, pain, tumor, malaria and fever [18]. In African ethnomedicine, it is widely used as anti-inflammatory and anticancer [19]. The plant is known as “worm killer” due to supposed anthelmintic activity and trypanocidal effect. Furthermore, the *A. bracteolata* possesses potent anti-allergic, antibacterial and antifungal activities [20].



Fig-1: Boswellia papyrifera

Nymphaea Lotus plant has a common name Soutab, Umm Ban geiga (Fig 2). It is aquatic pubescent herbs with submerged prostrate rhizomes, widespread throughout central and southern Sudan [4]. It is used in traditional medicine system as an aphrodisiac, anodyne, astringent, cardiotoxic, sedative, demulcent, analgesic and as anti-inflammatory agent [14]. The leaves of lotus are traditionally used for the treatment of haematemesis, haematuria, metrorrhagia, hyperlipidaemia, fever and inflammatory skin conditions. Many biological activities, including anticancer and antiviral has been attributed to Gallic and ellagic acid which are widely present in *N. lotus* [16].



Fig-2: Nymphaea lotus

In addition, the plant *Aristolochia bracteolata* which common name is Um galagel. (Family Aristolochiaceae Fig 3). It is a shrub or small tree [17].

It covers an expansive region in Sudan with a wide distribution in lowland plains and water catchment areas [18]. It is traditionally used as a remedy for scorpion and snake bites, pain, tumor, malaria and fever [18]. In African ethnomedicine, it is widely used as anti-inflammatory and anticancer [19]. The plant is known as “worm killer” due to supposed anthelmintic activity and trypanocidal effect. Furthermore, the *A. bracteolata* possesses potent anti-allergic, antibacterial and antifungal activities [20].



Fig-3: Aristolochia bracteolata

Boscia senegalensis (Fig 4) which features a common name Elcrasan – Elmekheat belong to family Capparacea. It is a shrubs or small trees, The plant occurs across area that in recent decades has faced more hunger than the other within the world—the vast swath of Sahel and Sahara savannas stretching from Mauritania, Senegal, and Mali all the thanks to southeastern Egypt, Sudan, Ethiopia, Somalia, Kenya [21] and western Sudan [22]. It's usually eaten as a food with oil and salt. Alternatively, seeds are ground to flour which is consumed within the sort of kisra, flat thin bread popular in Sudan or Asida, an area sort of porridge. The taste of the ultimate product is often improved by blending with millet or sorghum flour [21].

The leaves are wont to protect stored food against parasites [23]. Consistent with the African folk medicine, an infusion of leaves is employed to get rid of intestinal parasites from camels. Leaves mixed with millet flour taken each morning on an empty stomach for anthelmintic; dried leaves or dried bark are taken for schist osmosis. Infusion of the leaves is employed as eyewash, pruritus of the attention thanks to syphilis and to relief intestinal pain [24]. The seeds of *B. senegalensis* are a valuable source of glucocapparin. This component which presents a stimulating anti-hyperglycemic effect might be associated with the normal use of the seeds in Chad against type 2 diabetes. However, the cytotoxicity effect acknowledged suggests that further investigations extended to would be needed to form the glucocapparin a possible anti-diabetic drug [25]. One active principle in *Boscia* has been identified as a glucocapparin, a sulfonated glucose which exhibited not only hypoglycemic effect, but also cytotoxicity [26].



Fig-4: Bosciaseneglansis

Also *Cadaba glandulosa* may be a common name Elsrah. (Family Capparaceae Fig 5). It is a Shrub 0.3–1.5 m tall. The leaves are used for the treatment of hemorrhoids and tract infections [27]. Leaves, seeds and roots of the plant are widely used as an anthelmintic, antiscorbutic, antiseptic, cardiac stimulant, carminative, febrifuge sudorific, anticonvulsant, antidi- arrhoeal, and also are wont to treat skin diseases [28]. Several studies had shown that *Artemia salina* lethality may be a general bioassay which is a superb method for preliminary investigations of toxicity so as to screen of biologically active compounds [29]. The *Artemia salina* assay has been established as a secure, practical and economic method for the determination of the bioactivity of synthetic compounds [9]. However is studied so as to reveal new anticancer compounds [30]. Therefore this study aimed to gauge the cytotoxicity of plant extracts as a replacement potential source of natural anti – tumor agent.



Fig-5: Cadabaglandulosa

Plant materials

The Plants under investigations (*Cadaba glandulosa*, *Boswellia papyrifera*, *Aristolochia bracteolata*, *Boscia senegalensis*), Leaves and lotus whole plant were Collected in April 2017 from Gabrat ALSHEAKH and Kadogly in South Kordofan and from Khartoum region. The plants were taxonomically identified by Mr. Yahya, Medicinal and Aromatic Plants Institute, National Center for Research, and Dr. Manal Abdalla, Department of Botany, Faculty of Science and Technology, Omdurman Islamic University.

Preparation of Extract

Plant parts (leaves and *Nymphaealotus* whole plant) 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 before they were handled according to Harborne [30], The methanol solvent was used to extract secondary metabolite from leaves of plants and whole plant for *N.lotus* used in this study. 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.

Qualitative phytochemical Analysis

Phytochemical screening for the identification of major groups of chemical constituents using standard procedures [30]. The phytochemical compounds which tested were tannins, saponins, flavonoids, terpenoids, Steroids, Alkaloids and phenolic compound.

Chromatographic Separation

TLC plates were prepared by using colloid F254 type G 60 as a stationary phase consistent with Harborne [30]. Variety of developing systems were prepared and lots of trials were administered so as to achieve the foremost suitable solvent systems that give good separation.

Brine Shrimp Lethality Test

Brine shrimp lethality bio-assay was administered to research the cytotoxicity of plants extract. Brine shrimp (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, and then 5, 50 and 500 μ l of every solution were transferred into vials like 10, 100 1000 μ g / ml, respectively. Each dosage was tested in triplicates. The vials 9 for every test) and one control containing 500 μ l of the solvent were allowed to evaporate to dryness in 48h at temperature. Ten larvae of *A. Salina* leach (taken 48 – 72 h after the initiation of hatching) were added to every vial and therefore the final volume of the answer 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 reinforce the solubility of the plant extract [32]. LC50 values were determined at 95% confidence intervals by analyzing the info on a computer loaded with a Finney program [33]. The LC50 values of the brine shrimps obtained for the studied plant extracts were recorded. Etoposide, the reference medicine, was used as a positive control with LC50 (7.46.).

STATISTICAL ANALYSIS

LC50 values were determined at 95% confidence intervals by analyzing the info on a computer loaded with a Finney Program [33].

RESULTS AND DISCUSSION

Phytochemical Studies

The methanol solvent which resulted in a better extractability was supported by the report of Harborne [31], who had stated that more polar plant metabolites were isolated from plant materials when alcohol or water were used for extraction. However, this does not exclude the other possibility that different extractable quantities may also depend on the type of plant part used, or in other ways the variations in extractability may be due to the nature of the solvent and the plant parts used. Methanol extracts of leaves and whole plant parts have sticky and solid consistencies, and green, dark green and dark brown colors. The solvent gave, generally, higher exorability with *C.glandulosa* than other plants (Table 1). The presence or absence of some secondary plants products were tested by procedures described by Harbone [31]. The results indicated a moderate presence of alkaloids on *B. seneglansis* and *A. bracteulata* and weak presence for *N. lotus*, *C. glandulosa* and *B. papyriferae* (Table 2). Alkaloids and saponins were detected particularly in the methanolic extract of *B. papyrifera* [34]. Tannins were detected highly presence for *B. seneglansis*, *N. lotus* and moderate presence for other plants. Capparaceae family, showed moderate to abundant presence of alkaloids. No scientific reports are there on the chemical composition or biological activity of any species belonging to these genera, although some novel alkaloids have been isolated from fruits and aerial parts of some Capparaceae [35]. Also flavonoids represented moderate presence for *N. lotus* and weakly presence for other plants. It is reported that the lotus leaves are rich in flavonoids [36]. And alkaloids [37] and several flavonoids have been isolated [36]. Also triterpene showed weak presence for all plants used.

Table-1: Consistencies, color and extractabilities parts of all plants extracted with methanol

No	Plants	Plant part	Consistency	Color	Extractability%
1	<i>Aristolochia bracteulata</i>	Leaves	Solid	Dark green	8
2	<i>Boswellia papyrifera</i>	Leaves	Sticky	Dark brown	6.3
3	<i>Boscia seneglansis</i>	Leaves	Solid	Dark green	7.1
4	<i>Cadaba glandulosa</i>	Leaves	Solid	Green	10.3
5	<i>Nymphaea Lotus</i>	Whole plant	Solid	Dark brown	8.2

The seeds and leaves of *B. seneglansis* were characterized by the presence of alkaloids, saponins and tannins [38].

In addition to, steroids were weakly presence in all plants, which was in disagreement with what was reported by Periyasamy and Mahalingam [15]. When they used methanol extracts of *A. bracteolata*, a moderate presence of steroids were showed. Also all plants revealed negative results for saponin, phenolic compound and triterpens. Saponine and triterpens were

exhibited positive results when roots extract of *A.bracteulata* was used. Newman, et al. [39] And Jayanthi, et al. [40] reported that *N. lotus* have highly content of terpenoids, tannins and alkaloids. Phenolic compounds of *Boscia seneglansis*, especially flavonoids, Kampferol, quercetin and their derivatives proved to be effective against numerous cancer cell lines [14]. Mono terpenes in *B. papyrifera* have been reported to exert a wide variety of biological effects including antitumor activities [42].

Table-2: Phytochemical screening test for the secondary products of plants

Compound	Reagents Used	Aristolochia bracteulata	Boswellia papyrifera	Boscia senegalensis	Cadaba glandulosa	Nymphaea lotus
Alkaloid	Dragendorffs	++	+	++	+	+
Phenolic compound	KOH	-	-	-	-	-
Saponin	Frothing	-	-	-	-	-
Tannine	Ferric Chloride	++	++	+++	++	+++
Flavonoid	magnesium +HCL	+	+	++	+	++
Steroid	Chloroform+H ₂ SO ₄ Con	+	+	+	+	+
Triterpene	Chloroform+H ₂ SO ₄ Con	+	+	+	+	+

Keys:

- +++ = High presence.
 ++ = Moderate presence.
 + = Weak presence.
 - = Absent.

Separation of the Methanolic extracts Using TLC

TLC separation of extracts was carried out in order to identify the different constituents of the extracts with respect to their R_f values and colors of spots. The data obtained were compared with those from available literature, with similar experimental conditions.

The first group which consist of toluene: ethyl acetate: diethyl amine (70: 20: 10) gave five spots for Boscia senegalensis extract with dark blue color under UV (365 nm) while they gave orange color after the use of Dragendorff as spray reagent. On the other hand, Aristolochia bracteulata extract gave negative result with dark blue color under UV (365 nm) and were color less after the spray reagent (Table3).

Also Cadaba glandulosa extract gave six spots under UV (365nm) changed to dark orange color with the suitable reagent. Positive results were obtained for Boswellia papyrifera and Nymphaea lotus extracts using

both UV and spray reagent. The R_f Values for all previous spots reagent were between .07 to .92, these color indicated presence of alkaloid.

The second group which consisted of chloroform: glacial acetic acids: methanol: water (64: 32: 12: 8) was applied for all plant extracts (Table 4). The extracts of all plants did not give spots under UV and after spray with reagent.

The third solvent system which consisted of ethyl acetate: formic acid: glacial acid: water (100: 11: 11: 26) gave four spots for both Nymphaea lotus and Boswellia papyrifera extract with yellow colors with KOH as spray reagent. Also it gave two spots for Cadaba glandulosa, Aristolochia bracteulata and Boscia senegalensis extracts with reagent and blue color under UV. The R_f values for all previous spots reagent between .06 to 0.95. These colors indicated the presence of flavonoids (Table 5).

Table-3: TLC separation of plants extract for alkaloids representing florescence, color and R_f values of the spots obtained by using a solvent system of toluene: ethyl acetate: diethyl amine (70: 20: 10) and sprayed with Dragendorff.

Plants	Color .UV 365	Color with reagent	R _f					
A.bracteulata	Blue and Red dark	ellow/orange	-	-	-	-	-	-
B.papyrifera			0.38	0.43	0.48	-	-	-
B.senegalensis			0.12	0.43	0.60	0.73	0.86	-
C.glandulosa			0.18	0.28	0.50	0.60	0.79	0.85
N.lotus			0.07	0.89	0.92	- s	-	-

Table-4: TLC separation of plant extracts for flavonoids representing, color and R_f values of the spots obtained by using a solvent system ethyl acetate: formic acid: glacial acid: water (100: 11: 11: 26) and sprayed with KOH in water (10 %)

Plants	Color .UV 365 nm	Color with reagent	R. F					
A.bracteulata	Blue dark	Yellow/brown	0.06	0.12	-	-	-	-
B.papyrifera	Blue		0.10	0.31	0.75	0.95	-	-
B.senegalensis	Blue dark		0.10	0.82	-	-	-	-
C.glandulosa	Blue		0.12	0.95	-	-	-	-
N.lotus	Red dark		0.06	0.68	0.81	0.88	-	-

The fourth solvent system which consisted of ethyl acetate: methanol: water (100: 135: 10) (Table 5) gave two spots for both B. senegalensis and A. bracteulata extracts, on the other hand C.glandulosa and B.papyrifera extracts represented three spots with blue

green color. The last one was N.lotus exhibited four spots. All spots with blue and green color with Berlin blue as spray reagent and blue color under UV. This color showed R_f values between.08-.87 and indicated presence of tannins.

The last one consists of toluene: ethyl acetate: ferric acid (50: 40: 10) (Table 7) was used for detection of phenol compounds (Table 6). The chromatographic separation gave positive results for *B.senegalensis* and *B.papyrifera* extracts with five spots, while

A.bracteolata revealed negative result. Also *N.lotus* revealed two spots. However, with the use of spray reagent, brown and pale brown colors were detected (Rf. Values between .10 - .63).

Table-5: TLC separation of plant extracts for tannins representing florescence, color and Rf values by using a solvent system ethyl acetate: methanol: water (100: 135: 10) and spray reagents: Berlin blue

Plants	Color .UV 365 nm	Color with reagent	Rf value				
<i>A.bracteolata</i>	Blue dark	Blue/green	0.18	0.37	-	-	-
<i>B.papyrifera</i>	Blue		0.25	0.60	0.87	-	-
<i>B.senegalensis</i>	Blue dark		0.12	0.31	-	-	-
<i>C.glandulosa</i>	Blue		0.08	0.40	0.70	-	-
<i>N.lotus</i>	Red dark		0.10	0.47	0.56	0.87	-

Table-6: TLC separation of plant extracts for saponins representing florescence, color and Rf values by using a solvent system chloroform: glacial acetic acids: methanol: water (64: 32: 12: 8 and spray reagent: Vanillin -Sulphuric acid

Plants	Color .UV 365 nm	Color with reagent	Rf
<i>A.bracteolata</i>	Blue dark	Color less	No. Spots
<i>B.papyrifera</i>	Blue		
<i>B.senegalensis</i>	Blue dark		
<i>C.glandulosa</i>	Blue		
<i>N.lotus</i>	Blue dark		

Table-7: TLC separation of plant extracts for phenolic compound representing florescence, color and Rf values by using a solvent system: Toluene: ethyl acetate: ferric acid: (50: 40:10). And spray reagents: ferric Chloride in water (0.5%)

Plants	Color .UV 365 nm	Color with reagent	R _f value				
<i>A.bracteolata</i>	Blue brown	Color less	-	-	-	-	-
<i>B.papyrifera</i>		Brown	0.08	0.20	0.30	0.52	0.58
<i>B.senegalensis</i>			0.09	0.16	0.33	0.48	0.56
<i>C.glandulosa</i>			0.10	0.18	0.30	0.57	-
<i>N.lotus</i>			0.08	0.24	-	-	-

Brine Shrimp Lethality Test

The importance of the cytotoxicity from the fact that it is linked with the discovery of anticancer compounds [43]. From a pharmacological point of view, an honest relationship has been found with the *Artemia salina* lethality test to detect anti-tumoral compounds in terrestrial plant extracts [44]. 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 worth of this bioassay as a pre- screening tool for antitumor drug research [45]. Not only that there's direct correlation between *Artemia salina* toxicity and 9 KB (human nasopharyngeal carcinoma) cytotoxicity ($p = 0.036$ and $kappa = 0.56$). The *Artemia salina* test was getting used as a prescreen for a panel of six human solid tumor cell lines at the cell culture laboratory of the Purdue Cancer Center [46]. This is an internationally accepted bioassay for screening of antitumor compounds [47]. In this regard, an easy bioassay was used for screening purposes [48]. Thus brine shrimp larvae (brine shrimp nauplii) has been used target organism to detect bioactive compounds in plant extract and toxicity to the present crustacean features a good correlation with anti-tumor activities in man [33] since the brine shrimp responds similarly to the corresponding mammalian system [49]. According to the method described by Meyer et al. [32], methanol

extracts were used to determine its cytotoxicity against brine shrimp larvae (LC50) after 24 hours: The results of this study are classified as: LC50 less than 20 $\mu\text{g} / \text{ml}$ was considered as highly toxic, LC50 from 20 to 100 $\mu\text{g} / \text{ml}$ as toxic, LC50 from 100 to 500 $\mu\text{g} / \text{ml}$ as moderately toxic and from 500 to 1000 $\mu\text{g} / \text{ml}$ was weakly toxic according to Padmaja et al. [22]. However, meyer [32]. Considered the LC50 values > 1000 $\mu\text{g} / \text{ml}$ as non – toxic or safe. The brine shrimp lethality test revealed the cytotoxicity effects of plants extracts. The crude extract of plant materials were showed considerable results (Table 8). The highly effect against brine shrimp larvae was shown by *B. senegalensis* extract (1. 97 $\mu\text{g}/\text{ml}$). Also *B.papyrifera* extract exhibited high result equal to (14.96 $\mu\text{g}/\text{ml}$). On the other hand *N.lotus* and *C.glandulosa* revealed moderate values equal to 316.228 $\mu\text{g}/\text{ml}$ and 635.130 $\mu\text{g}/\text{ml}$ respectively. However, the *A. bracteolata* detected nontoxic or safe result, however this is disagree with which was reported by Thirumal et al. [17] who assess the potential antitumor activity of *A. bracteolata*, several root's extracts were tested against cutaneous melanoma cell line. The petroleum ether extracts notably reduced cell survival. Aristolochic acids are known to be toxic and a rodent carcinogen, in addition to their carcinogenicity, aristolochic acids are also highly nephrotoxic agents [51]. Although the *Artemia salina* lethality assay is quite inadequate regarding the elucidation of the mechanism of action,

it's very useful to assess the bioactivity of the plant extracts. The first time that the aerial parts of Cleome, family Capparaceae, extracts and the purified compounds exhibited significant cytotoxic activity (LC₅₀ values < 100 µg/ml) using brine shrimp lethality assay and are considered as a source of natural agents

that would be used as anti-proliferative, antitumor and will provide results in interesting pharmaceuticals of plant origin [52]. This a clear indication of first time achievement of results which were not preceded by any other ones reported in the available literature.

Table-8: Brine shrimp bioassay results of plant extracts

Plants	Part use	LC ₅₀ µg / mL
A. bracteolate	Leaves	>1000
B. paprifera	Leaves	14.963
B. Senegalensis	Leaves	1.975
C. glandulosa	Leaves	635.130
N. lotus	Whole plant	316.228

Keys: LC₅₀> 20 µg / ml = highly toxic, 20 -100 µg / ml as toxic, 100 -500 µg /ml moderately toxic, > 1000 µg / ml weakly toxic.

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