https://chemclassjournal.com/
ChemClass Journal Vol. 8 Issue 1 (2024); 291-302
e-ISSN:0198-2734 p-ISSN:0198-5680
doi.org/10.33003/chemclas_2024_0801/025

Phytochemical and In-vivo Acute Toxicological Investigations of Leaf extracts of *Sesbania cannabina* plant

Suraj Abdulkarim[1] and Abubakar Lawal*[2]

[1]Department of Basic and Applied Science, College of Science and Technology Hassan Usman Katsina Polytechnic, P.M.B. 2052 Katsina, Nigeria
[2]Department of Pure and Industrial Chemistry, Umaru Musa Yar'adua University, P.M.B. 2218 Dutsin-ma Road Katsina, Nigeria.

***Corresponding Author's Email: abubakarlawal360@yahoo.com**

Abstract

Over the years, the increase in resistance and side effects of pharmaceutical drugs have encouraged more research on plants and their diverse secondary metabolites which led to the production of various kinds of medicines. *Sesbania cannabina* is a plant traditionally known for treatment of inflammation and other skin related illnesses in rural areas. For these reasons, Phytochemical study was carried out to investigate the aqueous, methanol and acetonitrile leaf extracts of the plant for their phytochemical content and *in-vivo* toxicological effects. The Preliminary phytochemical screening of each extract using standard methods revealed the presence of alkaloids, flavonoids, glycosides, saponins, saponin glycoside, steroids, tannins, and volatile oils in either one, two or all the three extracts. The LD_{50} of the extracts were above 3000 mg/kg per average body weight of the tested Wistar albino rats. Therefore, the obtained results justify the edibility, nutraceutical and medicinal properties of *Sesbania cannabina* leaves as raw material for production of numerous medicines especially anti-inflammatory drugs due to the presence of antioxidants such as flavonoids.

Keywords: Anti-inflammatory, In-vivo Acute Toxicological Investigation, Nutraceutical food components, Phytochemical screening, *Sesbania cannabina*, Wistar albino rats

Introduction

Plants are the major source of energy for the animal kingdom including the human beings [1]. Even though, plants have to tolerate stress conditions to improve their survival rates against stress factors that include drought, salinity, nutritional deficiency, adverse climate condition, pests [2], and pollutants such as pesticides [3-16]. These conditions make the plants to exhibit various defensive abilities to be able to survive in their respective environments [2]. Consequently, production of numerous phytochemicals by plants is among main strategies to withstand such environmental challenges as revealed by Lawal and Dangoggo [17]. The phytochemicals are secondary metabolites that are not directly involved in the development, reproduction and growth of the plant but serves mostly as defense molecules or mechanisms [18]. Others are for pollinatory

benefits where they produce sweet scent to attract insects for reproductory purpose [19].

In addition, nutritional and medicinal properties of plants as local herbs are known for many ages for curing various kinds of ailments [20]. However, the important scientific basis behind the use of plants as medicines came to light only recently due to scientific advancements in research and instrumental analysis [21]. Thus, this has been a major development in medicine because of the increasing demands for more effective drugs sourced from plant materials [22]. This is mainly due to the current widespread belief that the green medicine is safe, more dependable and without side effects compared to the synthetic drugs [23].

Other major types of food items produced by plants include spices, which are used in seasoning of foods [24]. Also, the plant materials composed of compounds having medicinal properties are called nutraceuticals [25]. These nutraceuticals are food components possessing biological or medicinal properties that help to improve fitness of human health [26]. Such biological or medicinal properties include antioxidant, anti-inflammatory, anti-mutagenic, anti-hypersensitive, anti-tumor, cardio protective, and anticancer [25].

Besides, the biological or medicinal properties of nutraceuticals can only be possible with the presence of phytochemicals that include alkaloids, tannins, saponins, flavonoids, glycosides, cardiac glycosides, Saponins, volatile oils and steroids.

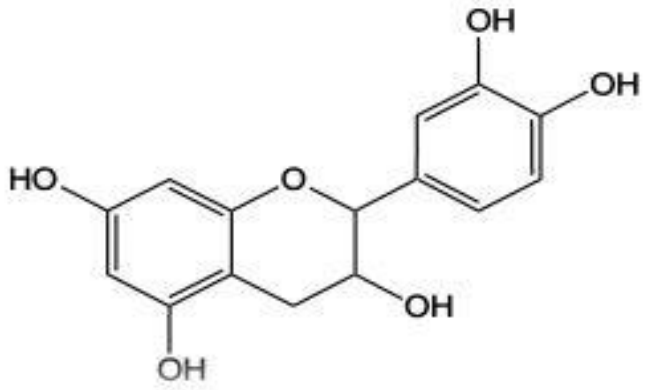
Sesbania Cannabina (Retz.) Poir. belongs to the family of *Fabaceae* plant, known in English as Corkwood and Zamarke in Hausa. The plant is naturalized bioregions of Indomalaya (South-Eastern part of Asia) and Australasian region and mostly inhabitant of urban ruderal, derived grassland as well as natural grassland, which is locally used as herb, fodder and fuelwood (Swetha, 2012) [27]. Moreover, the plant is having excellent structural morphology that gives it the ability to withstand all forms of environmental and climatic conditions. Also, the documentation of Nitisha and Girjesh [28] revealed that the morphological characteristics (mean ± standard deviation) range for the three different accessions of Sesbania cannabina plant include; Plant height (170.00 ± 0.00 to 200.00 ± 2.88 cm), Stem girth (3.62 ± 0.10 to 3.96±0.05 cm), Internodes length (5.65 ± 0.17 to7.64 ± 0.20 cm), No. of leaves/plant (32.20 ± 1.28 to 39.50 ± 0.50), Days to Initiation of first flower (33 to 65 days), Seed length (2.90 ± 0.10 to 3.86 ± 0.97mm), Seed breadth (1.92±0.04 to 2.00 ± 0.00 mm), Seed coat colour (Light brown with black patches - Deep brown & shiny). However, Plate 1 shows the leaves and branches of Sesbania cannabina.

Plate 1: *Sesbania cannabina* plant showing its leaves and branches.

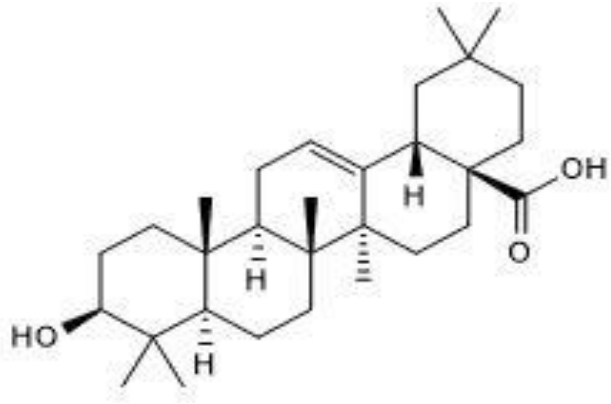
The plant suspected to contain important phytochemicals because of its traditional/ethno-medicinal uses for the treatment of inflammation and other skin related illnesses in the rural areas of in Katsina and other states of Nigeria. The pharmacognosy properties of the plant was also documented [29] based on the comparative pharmacognostic and in-vitro antioxidant assessment of *Sesbania cannabina*, *Sesbania sesban* and *Sesbania grandiflora* linn.

Besides, some of the compounds reportedly isolated and characterized from the plant includes the following:

Leucocyanidin (I) and Oleanolic acid (II) among others as illustrated, respectively.



(I)



(II)

Leucocyanidin is mainly used for the management of diabetes [30], while Oleanolic acid is used for the treatment of various disorders in human [31].

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Therefore, the aim and objectives of this studies is to perform qualitative phytochemical screening in aqueous, methanol and acetonitrile extracts of *Sesbania cannabina* leaves as well as the acute (LD_{50}) toxicological assessment of the leaf's extracts using Wistar albino rats as experimental animals. And the study is hoped to create awareness to larger population on the medicinal and edible benefits of consuming *Sesbania cannabina* leaves and also serving as reference guide for future studies.

Materials and Methods

Sampling and treatment

Fresh leaves of *Sesbania cannabina* were obtained from the campus of Umaru Musa Yar'adua University Katsina (UMYUK), Nigeria and transported to the Botany unit of Biology Department of UMYUK for identification. The sample was washed and shade dried at room temperature for a period of 14 days before it was blended to powder and preserved in an airtight container.

Sample Preparation Method

The sample extraction was carried out by adopting the technique reported by Lawal and Muhammad [32]. Briefly, 50 g of the blended sample was transferred into a 250 cm^3 conical flasks, already containing 100 cm^3 of distilled water. The mixture was shaken vigorously at a specific time interval to obtain a homogenous solution and optimum condition for maximum extraction. Then, the flask was tightly covered and set aside at room temperature for 24 hours. Resulting solution was filtered and evaporated to dryness over a steam bath. The obtained extract was labelled as 'crude aqueous extract of *Sesbania cannabina*. Therefore, same procedure was repeated for methanol followed by acetonitrile extraction. The obtained extracts were also labelled 'crude methanol and crude acetonitrile extracts of *Sesbania cannabina*, respectively.

Preliminary qualitative screening

Phytochemicals (secondary metabolites) are the medicinal contents of most plant materials (Ezzat *et al.*, 2019), which were investigated for their presence (+) or absence (-) during the qualitative screening. A change in colour or the formation of precipitate was taken as positive (+) or negative (-) to the various tests carried out in accordance to the documentation of Lawal and Dangoggo [17].

Preliminarily, 5 g each of the aqueous, methanol and acetonitrile crude extract was respectively transferred into 100 cm^3 conical flasks and 50 cm^3 each of aqueous, methanol and acetonitrile solvents were used to dissolve the solutes respectively. Each mixture was then shaken vigorously to obtain a homogeneous mixture. Each was set apart for the following test:

Test for alkaloids

Dilute HCl_3 (1 cm^3) was added to 3 cm^3 of aqueous extract solution in a test-tube. The content was shaken, filtered and 2 cm^3 of the filtrate was treated with few drops of Mayer's reagent slowly along the side of the tube. Formation of white or creamy precipitate indicates the presence of alkaloids.

Test for flavonoids

Dilute NaOH (1 cm^3) was transferred into a test-tube containing 3 cm^3 of the aqueous extract solution. Yellowish colouration of the solution indicates the presence of flavonoids.

Test for glycosides

The solution of aqueous extract (5 cm^3) was mixed with 2.5 cm^3 of 50 % H_2SO_4 in a test-tube. The mixture was heated for 15 mins and allowed to cool down before neutralizing it with dilute NaOH, followed by addition of 5 cm^3 of Fehling's solutions mixture (A and B) and heated again. Appearance of brick-red precipitate indicates the presence of glycosides.

Test for cardiac glycosides

The test-tubes were occupied with 2 cm^3 of the aqueous extract solution. The content was mixed with 2 cm^3 of acetic acid containing traces of $FeCl_3$ and 2 cm^3 of Conc. H_2SO_4. A blue-layer appearance indicates the presence of cardiac glycosides.

Test for saponin glycosides

Fehling solutions (2.5 cm^3) mixture (A and B) was mixed with 2.5 cm^3 of the aqueous extract solution in a test-tube. The formation of bluish-green precipitate indicates the presence of saponin glycosides.

Test for steroids

Aqueous extract (1 cm^3) solution was mixed with 2 cm^3 of chloroform. Then, 2 cm^3 of conc. H_2SO_4 was carefully added to form a lower layer. Appearance of a reddish brown colour at the interface indicates the presence of steroids.

Test for volatile oils

Two drops of diluted HCl were added to 5 cm^3 of aqueous extract solution in a test-tube and shaken. Formation of white precipitate confirmed the presence of volatile oils.

Test for tannins

Aqueous extract (1 cm^3) solution was treated with 5 drops of freshly prepared dilute KOH. Presence of dirty-white precipitate indicates the presence of tannins.

Test for saponins

Formation of large quantity of froths that lasted for 30 mins while strongly shaken the 5 cm^3 of the aqueous extract solution in a test tube confirmed the presence of saponins.

Therefore, the same procedure was repeated for methanol and acetonitrile extracts.

Acute (LD_{50}) Toxicological Studies

The LD_{50} toxicological studies were respectively carried out on the aqueous, methanolic and acetonitrile Leaf extracts of *Sesbania cannabina*. Even though, majority of the traditional herbs are consumed in aqueous medium [33, 34]. So, it is important to compare and employ other extracting solvents to achieve the best result. Twelve (12) Wistar albino rats weighing averagely 119 g were grouped into 4 groups of 3 rats/cage labelled O, X, Y and Z. Each group member was administered with the prepared standard solution (1 cm^3) dose of 3000 mg/kg of the aqueous, methanolic and acetonitrile extracts of Sesbania *cannabina* leaves per average body weight of the test animals, respectively. The observed changes in behavioral attitude such as mortality, salivation, Itching, coloration, freedom, apatite loss, lack of anxiety and movement toward the corners after administration of the standard solutions were recorded within the period of 48 hours [35].

Results and Discussion

Table 1 shows the results of the phytochemicals or phytochemical contents of aqueous, methanolic and acetonitrile leaf extracts of *Sesbania cannabina*

Table 1: Results of the phytochemicals present/absent in leaf extracts of *Sesbania cannabina* leaves**.**

Phytochemicals	Aqueous extract	Methanol extract	Acetonitrile extract
Alkaloids	+	+	+
Tannins	+	+	-
Saponins	+	+	-
Flavonoids	+	+	+
Glycosides	-	+	+
Cardiac glycosides	-	-	-
Saponin glycosides	+	+	+
Volatile oils	+	+	-
Steroids	+	+	-

Key: +, present; -, absence.

The highlighted results of phytochemical constituents of the aqueous, methanol and acetonitrile extracts of *Sesbania cannabina* leaf extracts of indicates the presence of several

secondary metabolites in the plant, except for cardiac glycosides which was absent in all the 3 extracts. This supports the findings of Agidew [36]. However, the methanolic extract revealed significant quality of phytochemicals, a result which supports the findings of Dhawan and Gupta [37]. They reported that the extraction potential of methanol followed by aqueous and acetonitrile extract on leaves of *Daturametel* plant. The alkaloids were found in the aqueous, methanol and acetonitrile extracts which supports their medicinal applications. The report agrees with the one of Khalil *et al.* [38] for the Characterization of methanolic extracts in Agarwood leaves. Tannins were found in both the aqueous and methanolic extracts while absent in the acetonitrile extract.

Thus, *Sesbania cannabina* leaves extract will also be good for wounds healing since they contain tannins, which is in accordance with the report of Liu *et al.* [39]. The saponins were found in the aqueous and methanolic extracts while absent in the acetonitrile extract. Saponins are responsible for defensive against microbes causing diseases [40]. Flavonoids were present in the aqueous, methanol and acetonitrile extracts. Thus, flavonoids protect against inflammation, allergies, microbial infections and platelets aggregation in accordance with the report of Lawal *et al.* [41]. Glycosides were observed in acetonitrile and methanol extract but absent in the aqueous extract. However, consumption of glycosides in foods or medicines provides medicinal benefits such as antioxidant, antidiabetic, cardioprotective and even anticancer activities [42].

The aqueous and methanol extracts were observed to contain volatile oils while absent in acetonitrile extract. Thus, volatile oils in *Sesbania cannabina* leaves could prevents high blood pressure and microbial infections as supported by Ramsey *et al.* [43]. Likewise. steroids were observed in aqueous and methanol extracts but were absent in acetonitrile extracts. This result is in agreement with the report of Karthikeyan *et al.* [44] who showed that steroids were observed in methanolic extract of *Leucasaspera* plant. Moreover, plants containing steroids supports production of reproductive hormones in the body as documented by Zubeldia-Brenner *et al.* [45].

Secondly, the acute toxicity (behavioral attitudes and mortality) results for the administered dose concentrations of *Sesbania cannabina* leaves extract per average body weight of the Wistar albino rats are presented in Table 2.

Table 2. Toxicity of aqueous, methanol and acetonitrile extracts of *Sesbania cannabina* leaves

		Change in Behavioral Attitude							
Group	**Dose (**mg/Kg**)**	**Mortality**	**Salivation**	**Itching**	**Coloration**	**Freedom**	**Apatite**	**Lack of Anxiety**	**Movement toward corners**
O	0	-	-	-	-	+	+	-	-
X	3000	-	-	-	-	+	+	-	-
Y	3000	-	-	-	-	+	+	-	-
Z	3000	-	-	-	-	+	+	-	-

Key: +, present; -, absent; O, Distilled water; X, Aqueous extract; Y, Methanolic extract; Z, Acetonitrile extract

The results of the toxicological studies shown above for the respectively administered 3000 mg/kg extracts of aqueous, methanol and acetonitrile for *Sesbania cannabina* leaves proved that LD_{50} of the plant extract is above 3000 mg/Kg per average body weight of the tested Wistar albino rats. This is because the tested animals showed no mortality and signs of all the changes in behavioral attitudes but having freedom and apatite after 72 hours of administering the groups of tested animals with 3000 mg/Kg extracts of aqueous, methanol and acetonitrile for *Sesbania cannabina* leaves, respectively. This is in accordance with the findings of Porwal *et al.* [46] based on the evaluation of acute and subacute oral toxicity induced by ethanolic extract of *Marsdenia tenacissima* leaves in the experimental rats.

Conclusion

The fresh leaves of Sesbania *cannabina* obtained from the campus of Umaru Musa Yar'adua University Katsina (UMYUK) were successfully screened qualitatively for the presence of phytochemicals in the extracts of aqueous, methanol and acetonitrile, as well as their In-vivo acute toxicological investigations. Therefore, the obtained results justify the edibility and nutraceutical properties of the *Sesbania cannabina* leaves.

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