Advanced Journal of Chemistry-Section B
Natural Products and Medical Chemistry

Journal homepage: http://www.ajchem-b.com/

Original Research Article

Phytochemical Screening and the Effect of Methanolic Leaves Extract of *Senna mimosoides* on Inflammatory Stimulus-Induced Leukocyte Mobilization (*In-vivo*)

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ARTICLE INFO

Article history
Submitted: 2022-01-05
Revised: 2022-01-30
Accepted: 2022-02-27
Available online: 2022-03-07
Manuscript ID: AJCB-2201-1105
DOI: 10.22034/ajcb.2022.323175.1105

KEYWORDS
Senna mimosoides;
anti-inflammatory;
leukocytes mobilization;
methanolic extract;
phytochemistry.

ABSTRACT

In traditional medicine, the leaves of *Senna mimosoides*, has been used to cure oedema and breastfeeding poisoning in newborns. The animal model experiment included a total of twenty-five (25) Wistar albino rats of both sexes, with A (control) receiving 0.2 ml of normal saline. B, C and D received 100, 250 and 500 mg/kg of the methanolic extract respectively, and E receiving 25 mk/kg of the indomethacin standard drug. Preliminary phytochemical screening of the *Senna mimisoides* methanolic leaves extract indicated the presence of ten (10) different secondary metabolites, including; carbohydrates, glycosides, tannins, reducing sugars, phenols, steroids, terpenoids, saponins, alkaloids and flavonoids. When compared to the control, the total leukocyte count of the groups treated with various dosages of extracts increased in a dose-dependent manner, whereas the group treated with indomethacin fell dramatically. The extract dramatically increased leukocyte mobilization, according to the findings. The results of this investigation showed that the methanolic leaves extract of *Senna mimosoides* contains medically relevant bioactive components, indicating that it can be used in traditional medicine to treat ailments. The anti-inflammatory property of the extract is not at the level of leuckocyte mobilization. According to this study, however, the extract's ability to stimulate leukocytes implies that it may have a function in immunological response.

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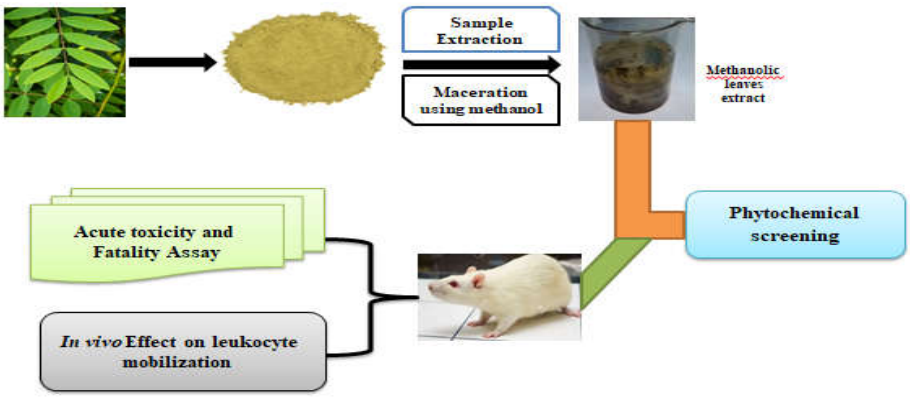
GRAPHICAL ABSTRACT



1 Introduction

Senna mimosoides linn, commonly known as Cassia mimosoides, is a member of the Caesal pinaeae family and can be found in the senna genus. It is an erect, occasionally widespread annual or perennial shrub that reproduces by seed. It has a hairless or minutely hair pithy stem that is woody at the base. The leaves as shown in figures 1 and 2, are complex and alternating, with a gland below the bottom pair of leaflets on the petriole. These leaflets are tiny, asymmetric and blunt at the apex. The blooms are yellow, and there are only a few of them. The fruit is a flat pubescent pod with 12-24 seeds that is roughly 6 cm length and 5 mm wide.

In general, the leaves of some senna plants can be used to cures several diseases such as constipation, gastrointestinal disorders, leprosy, skin illnesses, cough, bronchitis, typhoid fever, anemia and tumors [19] are all treated with the leaves of some senna. *Senna mimosaides* methanolic leaf extract possess anti-inflammatory properties, as evidenced by membrane stabilization inhibition of phospholipase A2 activity, and inhibition of prostaglandin synthase activity [9].

Fig.1: leaves of *Senna mimosaides*

Fig.2: Grounded leaves of *Senna mimosaides*

Phytochemicals are natural bioactive substance found in plants such as vegetables, fruits, flowers, leaves and roots that work as antibiotics against a variety of human infections but lack the nutritional value of vitamins and minerals [5]. However, they have an impact on a number of bodily functions. They function in tendem with nutrients and dietary fiber to protect the body from disease, slow aging process and lower the reisk of a variety of ailments, like cancer, heart disease, stroke and high blood pressure [17]. In accordance to their roles in plant

metabolism, Primary and secondary phytochemical constituents are classified into groups; Common sugars, amino acids, proteins and chlorophyll are the plant primary metabolites, whereas, alkaloids, tannins, terpenoids, flavonoids, reducing sugars, phenolic compounds and other chemicals make up the secondary constituents of plant as shown in fig. 3. Plants show medicinal and therapeutic potentials because of the accumulation of bioactive phytochemicals contents in many plant parts, and these chemical substances play a vital role in the physiological function of the human body [6].

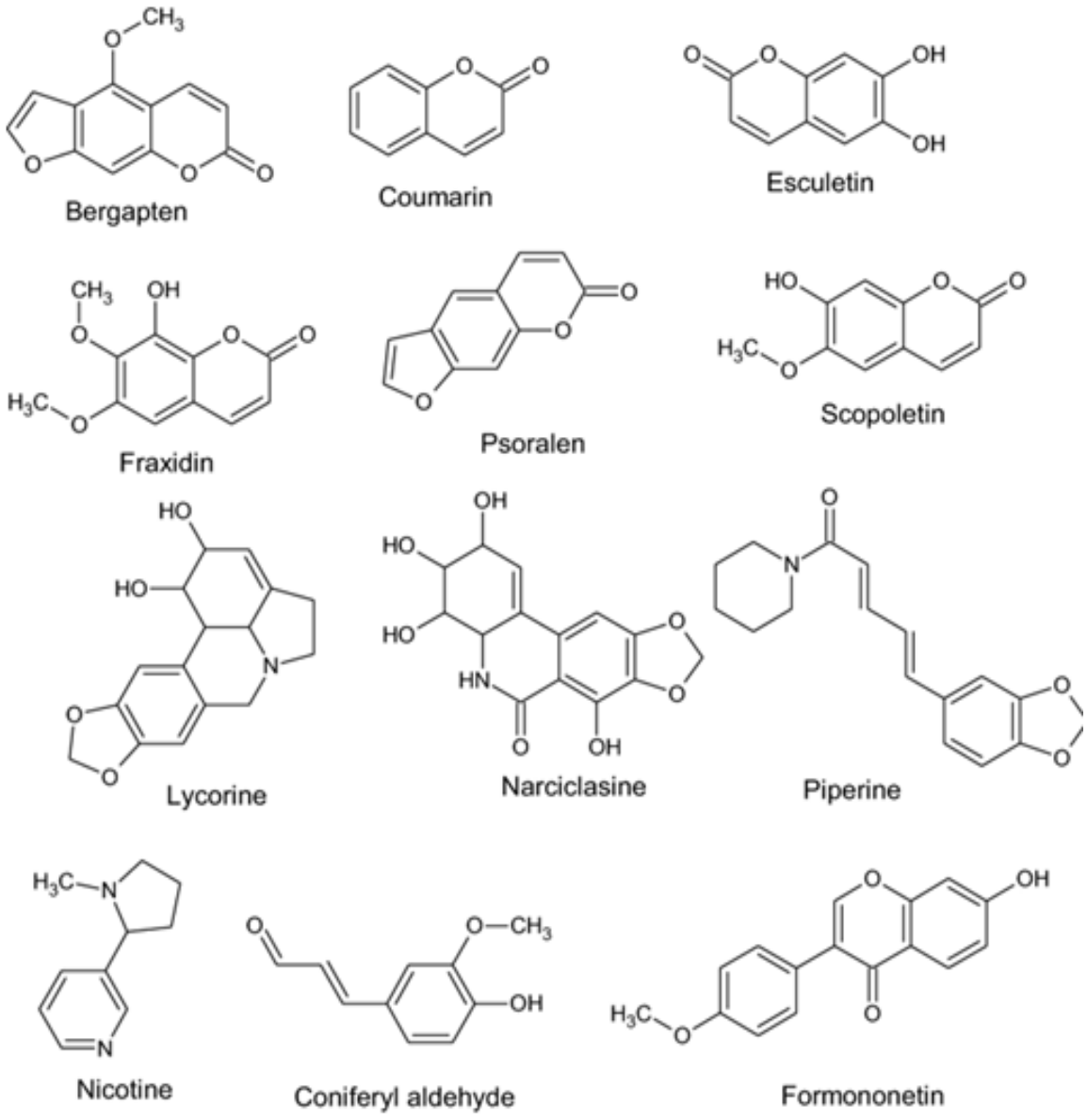


Fig. 3: Some secondary metabolites from plants.

Inflammation is a multifaceted pathophysiologic reaction of vascularized tissue to injury caused by variety stimuli such as thermal, chemical or physical damage, ischemia, infectious agents, antigen-antibody interactions, and other biological processes [5, 22].

Various mediators, including adhesion molecules, control leukocyte migration from the circulation to an inflammatory location. Monocytes, neutrophils, basophils, and T cells attach to activated leukocytes [12, 18]. This phenomenon allows leukocytes to cross the endothelium barrier and enter the sub-endothelial tissue. When the immune system is activated, Platelet activation may be induced by a number of pro-inflammatory substances. Platelets include a variety of mediators that attract leukocytes [14], and platelets have been found to have mRNA for many chemokines. These chemokines have been linked to the development of chronic allergic dermatitis, as they not only attract leukocytes but also excite them and activate other platelets. The capture of free-flowing leukocytes from the circulation, followed by leukocyte rolling, arrest, solid attachment, and eventual diapedesis, are all carefully coordinated events. The purpose of this study was to support the usage of this herb in traditional medicine to treat ailments. Furthermore, to see if the plant's anti-inflammatory effect, as demonstrated in earlier studies, was at the level of leukocyte mobilization.

2 Experimental

2.1 Plant collection and identification

Senna mimosoides leaves were obtained from the roadside in Batagarawa, Katsina State, Nigeria. The plant was identified and authenticated at Al-Qalam University in Katsina, Nigeria's Department of Biological Sciences.

2.2 Animal sample

A total of twenty-five (25) Wistar albino rats of both sexes, weighing between 120 g and 200 g and 10 – 30 g, were purchased and acclimatized for one week on regular meals before being used.

2.3 Sample Extraction

The powdered leaves sample (figure 2) (780 g) of *Senna mimosoides* was macerated with methanol for 72 hours using cold maceration method. The mixture was stirred using magnetic stirrer, for 24 hours, in order to dissolve the powders, it was then decanted, filtered using Whatmann No.4 filter paper and concentrated on rotavapor (R110) at 40 ℃ to obtain the crude methanol extract. Lyophilization was used to concentrate the filtrate. For further study, a brown slurry-like substance was collected and put in the refrigerator. The following formula was used to compute the % yield of the extract [37]:

$$\% \text{ yield} = \frac{mass\ of\ extract}{mass\ of\ sample} \times 100$$

2.4Assay for acute toxicity and fatality in bio logical activities

Lorke's approach [23] was used to investigate the extract's acute toxicity and to estimate the median lethal dose (LD_{50}). The test involved thirteen experimental animals (mice weighing between 20 and 30 grams). Three groups of rats, each with three rats, were given administered with 10, 100, and 1000g/kg of methanolic extract intraperitoneally in the study (ip). For the next 24 hours, they were closely observed for sign of lethality or any other abnormal behavior. As a result of the findings, four additional mice were given ip dosages of 1500, 2000, 3000 and 5000 mg/kg respectively. They were also monitored for 24 hours to see if they died or changed their behavior. 2.5 Qualitative Phytochemical analysis for the identification of the constituents, standard analy tical techniques as described were used by [4, 15, 34, 37].

2.6 Quantitative Phytochemical Analysis

For the amounts of the constituents, analysis wa s performed on the powdered sample using conv entional analytical procedures as described [15].

2.7 Effect on leukocyte mobilization *in vivo*

Using the method of [31], the *Senna mimosoides* methanolic leaves extract's effect on *in vivo* leukocyte migration produced by inflammatory s timuli was examined. Each rat in the groups received intra-peritoneal injection of 0.5 ml of 3 percent (w/v) agar suspension in normal saline one hour after the extract was administered orally. The rats were euthanized four hours later, and the peritoneum was rinsed with 5ml of a 5% EDTA solution in phosphate buffered sal ine (PBS). After recovering the peritoneal fluid, total and differential leukocyte counts (TLC and DLC) were performed on the perfusates.

3 Results

Table 1: Results of Qualitative Phytochemical Screening of the methanolic extract of *Senna mimosoides* leaves

S/No	Phytochemical(s)	Inference
1	Glycoside	++
2	Carbohydrates	++
3	Reducing sugars	+++
4	Alkaloids	+
5	Phenols	++
6	Steroids	+
7	Terpenoids	++
8	Flavonoids	++
9	Tannins	+++
10	Saponins	++

Key: (+) = fairly present, (++) = moderately present, (+++) = highly present

Table 2: Results of Quantitative Phytochemical Screening of the methanolic extract of *Senna mimosoides* leaves

Phytochemical(s)	Concentration (mg/kg)
Glycoside	2. 54 ± 0.029
Carbohydrates	3.54 ± 0.032
Reducing sugars	6.32 ± 0.029
Alkaloids	3.23 ± 0.021
Phenols	5.43 ± 0.025
Steroids	1.32 ± 0.034
Terpenoids	0.85 ± 0.025
Flavonoids	2.23 ± 0.021
Tannins	3.67 ± 0.033
Saponins	2.32 ± 0.028

Each value is a mean of three determinations.

Table 3: Effect of the methanolic leaf extract of *Senna mimosoides* on *in vivo* leukocyte migration in rats

Treatment	Dose (mg/kg)	TLC	Differential leucocyte mobilization (%)				
			N	E	M	B	L
A	-	3500 ± 54.87	12.49	-	-	-	68.60
B	100	2425 ± 43.22	15.54	-	-	-	56.34
C	250	3315 ± 87.83	13.40	-	1	-	67.20
D	500	3488 ± 98.56	12.40	-	-	-	75.43
E	25	2738 ± 32.33	10.30	-	-	-	72.24

Key: (N= neutrophile, E= Eosinophil, M=monocyte, B=basophil, L=Lymphocyte)

4 Discussion

4.1 Qualitative Phytochemical Screening

Grounded leaves of *Senna mimosoides* was subjected to cold maceration for three days using methanol to obtain 37 % yield of the extract.

The presence of different secondary metabolites such as alkaloid, glycosides, saponins, tannins, te rpenoids, reducing sugars, flavonoids, carbohydr ates, steroids, and phenols in the methanolic lea ves extract of *Senna mimosoides* were tested usin g standard procedures. These are the key secondary metabolites that are responsible for the therapeutic properties of the plant. Furthermore, the extract was subjected to additional analytical assays for phytochemical constituent quantification. The results are presented in **Table 1**.

The table further showed that, alkaloids and steroids are fairly present, tannins and reducing sugars are highly present, while saponins, flavonoids, terpenoids, phenols, carbohydrates and glycosides are moderately present.

4.2 Quantitative Phytochemical Analysis

The amount of the phytochemicals which were found in the methanolic leaf extract of *Senna mimosoides* (fairly present {+}, moderately present {++} and highly present {+++}) was quantitatively determined by standard procedures. Among the ten (10) secondary metabolites present in the methanolic leaf extract of *Senna mimosoides*, reducing sugars (6.32 ± 0.029) was highest, while terpenoids (0.85 ± 0.025) showed the lowest, as indicated in **Table 2**.

4.3 Effect of extract on *in vivo* leukocyte mobilization

The methanolic extract of *Senna mimosoides* leaves increased agar-induced leukocyte utilization into peritoneum significantly (p 0.05). When compared to the control, the overall leukocyte count of the groups. The growth of cells treated with varied doses of the extract was concentration dependent. But that of the group treated with 500 mg/kg, lymphocytes and neutrophils were the most mobilized leukocytes, while lymphocytes and neutrophils were the least mobilized. As the concentration of the extract grew, lymphocyte mobilization improved gradually. Secondly, metabolites are known to be present in plants, these are bioactive chemicals, and have specific physiological effects on the human body. Plant-derived compounds account for about 25 % of all prescribed medicines today, according to [36]. Many of these phytochemicals have been found and isolated from a range of medicinal plants, which, but only few have been to use medically. As shown in Table 3, treatment with the extract resulted in a significant increase (p 0.05) in agar-induced leukocyte mobilization. This was a dose-dependent increase. This indicates that the agar suspension was able to cause an injury that the extract responded to by increasing the production of inflammatory mediators, which in turn raised the number of leukocytes. According table 3, neutrophils were the most mobilized leukocytes, which is consistent with a research by [7, 32].

4.4 Acute Toxicity (LD_{50}) of *Senna mimosoides* leaf Methanolic Extract

The three groups of rats administered with 10, 100 and 1000 mg/kg of the methanolic leaves extract of *Senna Mimosoides*, showed no mortality or behavioral changes during the study. Based on these findings, four further groups received increasing doses of the extract of 1500, 2000, 3000 and 5000 mg/kg respectively. Weakness and tiredness were observed in those administered with doses of 3000 and 5000 mg/kg of the extract. Within 20 hours of administration, no one died. According to [22], the LD_{50} was determined from doses given in two stages.

In the acute toxicity test, the LD_{50} of *Senna mimo soides* leaves extract was shown to be larger than 5000 mg/kg.

4.5 Phytochemistry

The presence of any given bioactive chemical is mostly determined by the extraction solvent and the plant portion employed [6]. Secondary metabolites extracted by various solvents have been proven to possess a variety of biochemical and pharmacological effects in animals [26]. Glycosides, reducing sugars, saponins, terpenoids, flavonoids, tannins, carbohydrates, steroids, phenols, and alkaloids were found to be present in the methanolic leaves extract of *Senna mimosoides* (Table 1 and 2).

Secondary metabolites, which are chemical compounds responsible for biological activity, are commonly found in plants with biological activities. Phytochemical analysis of *Senna mimosoides* leaves methanolic extract reveals the presence of saponin, which are steroid or triterpenoid glycosides that have a bitter or astringent taste, foaming capabilities, and a hemolytic impact on red blood cells [16, 20]. Saponins have both therapeutic (cholesterol-lowering) and detrimental (cytotoxic permeabilization of the gut) qualities, as well as biological actions that are structural dependant [15]. Saponins cause a drop in blood pressure. It is effective in cardiovascular disease because it prevents cholesterol reabsorption.

Steroids were determined to be present. Because of their interaction with substances like sex hormones, steroidal compounds are important and interesting in pharmacy [21]. Because the steroidal structure could serve as a potent starting material in the synthesis of these hormones, the leaves of *Senna mimosoides* may be useful as a vegetable for expectant mothers or breast-feeding mothers to ensure their hormonal balance, which has been used in some countries.

Flavonoids are polyphenolic compounds that are water soluble and hence belong to the polyphenol family. Flavonoids, like carotenes, are responsible for the color of fruits, vegetables, and herbs. Flavonoids have anti-allergic, anti-cancer, anti-oxidant, anti-inflammatory, anti-thrombotic, vasoprotective, tumor inhibitory, and anti-viral properties in addition to antioxidant activity. Flavonoids' influence on arachidonic acid metabolism has been linked to these effects. Some flavonoid-rich plants are diuretics, while others are antispasmodic and have antimicrobial properties.

Alkaloids are extremely significant in medicine, as they make up the majority of the most valuable medications. Animals are affected physiologically by them [28]. The importance of tannin in wound healing has been established discovered that tannin has anti-diabetic properties [27, 30]. When consumed internally, the presence of phenol in the leaves of *Senna mimosoides* acts as an antiseptic and lowers inflammation. When applied to the skin, these bioactive substances cause irritation [30]. Glycosides have a powerful and direct effect on the heart, helping to sustain its strength and rate of contraction when it is failing [19, 22].

5 Conclusions

The presence of ten (10) different secondary metabolites, including carbohydrates, glycosides, tannins, reducing sugars, phenols, steroids, terpenoids, saponins, alkaloids, and flavonoids was discovered during preliminary phytochemical screening of the *Senna mimisoides* methanolic leaves extract. Reducing sugars (6.32 ± 0.029) were the most abundant secondary metabolites in the methanolic leaf extract of *Senna mimosoides*, while terpenoids (0.85 ± 0.025) were the least abundant. The extract significantly increased leukocyte mobilization. The results of the anti-inflammatory evaluation showed that the extract does not extend to leuckocyte mobilization. However, the extract's capacity to excite leukocytes suggests that it may play a role in immune response, according to this study.

Acknowledgements

The authors would like to express their gratitude to the staff and laboratory technologists Department of Chemistry, Al-Qalam University Katsina, Katsina State. Nigeria, for giving the resources that allowed this study to be completed.

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HOW TO CITE THIS ARTICLE
AbdulRazaq Tukur, Nasiru Malam Musa, Jimoh Tajuddeen Abdullahi, James D Habila and Martha Omotola Bamidele, Phytochemical Screening and the Effect of Methanolic Leaves Extract of Senna mimosoides on Inflammatory Stimulus-Induced Leukocyte Mobilization (In-vivo), , Ad. J. Chem. B, 4 (2022) 29-38.
DOI: 10.22034/ajcb.2022.323175.1105
URL: http://www.ajchem-b.com/article_146039.html