



Original Research Article

Qualitative and Quantitative Phytochemical Screening of the Aqueous Leaf Extract of *Senna mimosoides* : Its Effect in *in vivo* Leukocyte mobilization induced by inflammatory stimulus

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ABSTRACT

Senna mimosoides formerly known as *Cassia mimosoides* belongs to the family Caesalpinacea and the genus senna. The leaf is used in folklore medicine for the treatment of oedema and breastmilk toxicity in neonates. The phytochemical analysis and *in vivo* leukocyte mobilization effect of *Senna mimosoides* were investigated. For the animal model experiment, a total of twenty five (25) Wistar albino rats of either sex used in *in vivo* leukocyte mobilization were grouped as follows. Rats in group A (control) were administered 0.2 ml of normal saline; rats in groups B, C and D were treated with 100, 250 and 500 mg/kg of the aqueous extract of *S. mimosoides* respectively while group E rats received 25 mg/kg of indomethacin a standard drug. The qualitative and quantitative phytochemical parameters carried out showed presence of 2.67 ± 0.0013 mg of flavonoids; 3.43 ± 0.0028 mg of alkaloids; 1.97 ± 0.0030 mg of saponin; 2.32 ± 0.0032 mg of terpenoids; 0.86 ± 0.0023 mg of steroid; 3.61 ± 0.0025 mg of phenol; 8.31 ± 0.0032 mg of reducing sugar; 4.75 ± 0.0034 mg of tannin; 1.61 ± 0.0031 mg of cyanide; 2.75 ± 0.0029 mg of glycoside and 4.68 ± 0.0033 mg of soluble carbohydrates for every 100 g of the extract. The total leukocyte count of the groups treated with different concentrations of extract increased in a dose dependent manner while the group treated with indomethacin decreased significantly ($p < 0.05$) when compared with control. The result showed that the extract significantly stimulated the mobilization of leukocyte. The findings of this study provide evidence that aqueous extract of this tested plant contains medicinally important bioactive compounds, justifying its use in folklore medicine for the treatment of diseases. It was also deduced from this study that the anti-inflammatory property of the extract is not at the level of leukocyte mobilization. However, the stimulation of leukocyte by the extract suggests that it may play a role in immune response.

Keywords

Senna mimosoides,
Phytochemicals,
Leukocyte
mobilization,
Indomethacin

Introduction

Senna mimosoides formerly known as *Cassia mimosoides* belongs to the family Caesalpinaceae and the genus Senna

(Young, 2000). It is an erect, sometimes diffuse, annual or perennial shrub that reproduces from the seeds. It has a pithy

stem which is woody at the base and is hairless or minutely hairy. The leaves are compound and alternate, with the petiole always bearing a gland below the bottom pair of leaflet. These leaflet are numerous, small, asymmetric and blunt at apex. The flowers are yellow and few. The fruit is a flat pubescent pod, about 6cm long and 5mm broad with 12-24 seeds. Generally, the leaves of some senna are useful in treating constipation, abdominal disorder, leprosy, skin diseases, leucoderma, splenomegaly, hepatothy, jaundice, helminthiasis, dyspepsia, cough, bronchitis, typhoid fever, anaemia, tumours (Joy *et al.*, 1998). The aqueous leaf extract of Sennamimosides has anti-inflammatory potential which it exhibited by stabilizing membrane, inhibiting phospholipase A2 activity, prostaglandin synthase activity (Ekwueme *et al.*, 2011).

Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibres to act as a defense system against diseases or more accurately, to protect against disease. Unlike vitamins and minerals, they have no nutritional value. They can however influence various body processes. They work together with nutrients and dietary fibre to protect the body against diseases, slow the aging process and reduce the risk of many diseases such as cancer, heart disease, stroke, high blood pressure (Igwenyi *et al.*, 2011). Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds (Krishnaiah *et al.*, 2009) and many more such as flavonoids, tannins and so on. The

medicinal values of plants lies in these bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al.*, 2007).

Inflammation is a complex pathophysiologic response of vascularised tissue to injury arising from various stimuli including thermal, chemical or physical damage, ischemia, infectious agents, antigen-antibody interactions and other biologic processes (Clark, 2002). Inflammation, a fundamental protective response, can be harmful in conditions such as life-threatening hyper-sensitive reactions to insect bite, drugs, toxins and in chronic diseases such as rheumatic arthritis, lung fibrosis and cancer. Inflammatory response is brought about or mediated by inflammatory mediators such as chemokines, cytokines, cell adhesion molecules, extracellular matrix proteins (Simon *et al.*, 2000) which when in excess are deleterious (Liu and Hong, 2002).

Leukocyte recruitment from circulation to a site inflammation is regulated by various mediators, including adhesion molecules. Activated leukocyte adhere to monocyte, neutrophils, basophils and T cells (Freedman and Loscalzo, 2002; Jawwieriet *et al.*, 2002). This phenomenon enables leukocyte to transmigrate into the subendothelial tissue. With activation of the immune system, various proinflammatory mediators may induce concomitant activation of platelets. Platelets contain various mediators that attract leukocytes (Gawazet *et al.*, 2005), and mRNA for several chemokines has identified in platelets. These chemokines, which not only attract leukocyte but also stimulate them and further activate other platelets have been shown to be important for the pathogenesis of chronic allergic dermatitis. Leukocyte interaction with vascular endothelium are highly orchestrated

processes that include the capture of free flowing leukocyte from the blood with subsequent leukocyte rolling, arrest, firm adhesion and ensuing diapedesis. This study was to justify the use of this plant in folklore medicine for the treatment of diseases. Moreover, to determine, if the anti-inflammatory potential of this plant, as shown by previous studies, was at the level of leukocyte mobilization.

Materials and Methods

Collection of plant material

The leaves of (*Senna mimosoides*) were collected from Ibagwa Roadside, Nsukka in Enugu state of Nigeria, during the months of July and August. The plant characterisation and identification was carried out by a taxonomist Mr P.O. Ugwuozor, in the Department of Botany, University of Nigeria, Nsukka.

Animal material

Wistar albino rats of either sex weighing between 130g and 250g, and fed on standard feeds, were obtained from the Animal House, Faculty of Biological Sciences, University of Nigeria, Nsukka. Mice of either sex weighing 13-35g were also obtained from the same source. These animals were given standard feeds for at least one week after purchase to acclimatize them before use.

Aqueous extraction

A known amount (2000g) of *Senna mimosoides* leaves was extracted with 8400ml of distilled water using cold maceration. It was then filtered first with calico and subsequently with glass wool and finally Whatmann No.4 filter paper. The filtrate was concentrated by lyophilisation.

A brown slurry-like substance was obtained and stored in the refrigerator for further investigation.

Determination of percentage yield

The percentage yield of the extract was calculated using the following formular

$$\% \text{ yield} = \frac{\text{mass of extract}}{\text{mass of leaves}} \times \frac{100}{1}$$

Assay of biological activity

Acute toxicity and lethality

Investigation on acute toxicity of the extract with estimation of the median lethal dose (LD₅₀) was carried out using Lorke's method (1983). Thirteen experimental animals (mice with weight range of 20g-30g) were used for the test. In the investigation, three groups of mice containing three mice each were administered 10-, 100- and 1000g/kg respectively of the aqueous extract intraperitoneally (ip). They were observed closely for 24 hr for lethality or any other behavioural response. Based on the result, further increased doses of 1500-, 2000-, 3000- and 5000 mg/kg were administered ip to four other mice respectively. They were also observed for 24 hr for any death or behavioural changes.

Phytochemical analysis

Phytochemical analysis of the organic extract was carried out according to the general method of Harbone (1998). Basic phytochemical screening was carried out using simple chemical tests to detect the presence of secondary plant constituents such as alkaloids, tannins, flavonoids, saponins, triterpenes, sterols, phenols, glycoside, reducing sugar and soluble carbohydrate in the sample. The methods

used were those outlined by Harbone (1998) except otherwise stated.

Qualitative phytochemical analysis

Test for Saponins

A 5.0ml aliquot of the extract was diluted with 20ml of deionized water, shaken vigorously and observed. Persistent foaming indicated the presence of saponins.

Test for Alkaloids

A known quantity of the extract, 0.1 mg was added to 6ml of dilute hydrochloric acid and boiled, after boiling, it was cooled and filtered. The filtrate was divided into three portions and subjected to the following tests.

To the first portion, 2 drops of Dragendorff's reagent were added. The formation of a red precipitate indicated the presence of alkaloids.

To the second portion, 2 drops of Meyer's reagent were added. A creamy white precipitate indicated the presence of alkaloids.

To the third portion, 2 drops of Wagner's reagent were added. A reddish-brown precipitate indicated the presence of alkaloids.

Test for Tannins

The extract, 1 ml was added to 10 ml of deionised water and then treated with 3 drops of ferric chloride. A greenish-brown precipitate indicated the presence of tannins.

Test for Flavonoides

A quantity of the extract was boiled in ethylacetate (10 ml) for 3 minutes, filtered and cooled. Then the filtrate (4 ml) was

shaken with 1ml of dilute ammonia solution. An intense yellow colouration indicated the presence of flavonoids.

Test for Terpenoids

Nine millilitre (9 ml) of ethanol was added to 1g of the extract and refluxed for a few minutes and filtered. The filtrate was concentrated down to 2.5 ml in a boiling water bath. Hot distilled water (5ml) was added to the concentrated solution; the mixture was allowed to stand for 1 hour and the waxy mater was filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. The chloroform extract was evaporated to dryness in a water bath and dissolved in 3 ml of concentrated sulphuric acid and then heated for 10 min in a water bath. A grey colour indicated the presence of terpenoids.

Test for Steroids

A known quantity of the test sample was extracted in the chloroform and filtered. The filtrate was mixed with 2 ml of conc. H₂SO₄ carefully so that the sulphuric acid formed a lower layer. A reddish-brown colour at the interphase indicated the presence of steroidal ring.

Test for Phenols

The test sample 0.1 g was added to 10 ml of distilled water. The solution was heated in a boiling water bath for 3 min and filtered. A 2 ml aliquot of the filtrate was placed in each of 3 test tubes. The filtrate in one of the test tubes was diluted with distilled water in the ratio 1:4. A blue or greenish colour indicated the presence of phenols.

Test for Glycosides

Dilute sulphuric acid (5 ml) was added to 0.1 g of the extract in a test tube and boiled

for 15 min in a water bath, then cooled and neutralized with 20% potassium hydroxide solution. Ten millilitre (10 ml) of a mixture of equal parts of Fehling's solution A and B was added and boiled for 5 min. A more dense brick red precipitate indicated the presence of glycoside.

Test for Reducing Sugar

Five millilitre (5 ml) of a mixture of equal parts of Fehling's solution A and B was added to 5 ml of extract and then heated in a water bath for 5 min. Brick red precipitate showed the presence of reducing sugar.

Test for Cyanide

Distilled water (15 ml) was added to 0.1 g of the extract in a test tube. An alkaline picrate paper was suspended over the mixture and held in place by rubber bung. The arrangement was allowed to stand for 18 hr at room temperature. Colour change from yellow to orange indicated the presence of cyanide.

Test for Soluble Carbohydrate (Molisch test)

The extract (0.1 g) was boiled with 2 ml of distilled water and filtered. To the filtrate, few drops of naphthol solution in ethanol (molisch's reagent) were added. Concentrated sulphuric acid in a Pasteur pipette was then gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicated the presence of carbohydrate.

Quantitative Phytochemical Analysis

Test for Saponins

The extract (1g) was macerated with 10 ml of petroleum ether and decanted into a beaker. Another 10 ml of the petroleum

ether was added into the beaker and the filtrate evaporated into dryness. The residue was dissolved in 6 ml of ethanol. The solution (2 ml) was put in a test tube and 2 ml of chromagen solution added into it. It was left to stand for 30 min and the absorbance was read at 550 nm.

Test for Alkaloids

The extract (1 g) was macerated with 20 ml of ethanol and 20% H₂SO₄ (1:1 v/v). The filtrate (1 ml) was added to 5 ml of 60% H₂SO₄. After 5 min, 5 ml of 0.5% formaldehyde in 60% H₂SO₄ was mixed with the mixture and allowed to stand for 3 hr. The absorbance was read at 565 nm.

Test for Tannins

The extract (1 g) was macerated with 50 ml of methanol and filtered. To the filtrate (5 ml), 0.3 ml of 0.1N ferric chloride in 0.1N HCl and 0.3 ml of 0.0008 M of potassium ferricyanide were added and the absorbance read at 720 nm.

Test for Flavonoids

The extract (1 g) was macerated with 20 ml of ethylacetate for 5 min and filtered. To the filtrate (5 ml), 5 ml of dilute ammonia was added and shaken for 5 min. The upper layer was collected and the absorbance read at 490 nm.

Test for Terpenoids

The extract (1 g) was macerated with 50 ml of ethanol and filtered. To the filtrate (2.5 ml), 2.5 ml of 5% aqueous phosphomolybdic acid solution was added and 2.5 ml of concentrated H₂SO₄ was gradually added and mixed. The mixture was left to stand for 30 min and then made up to 12.5 ml with ethanol. The absorbance was taken at 700 nm.

Test for Steroids

The extract (1 g) was macerated with 20 ml of ethanol and filtered. To the filtrate (2 ml), 2 ml of chromagen solution was added and the solution left to stand for 30 min. The absorbance was read at 550 nm.

Test for Glycosides

The extract (1 g) was macerated with 50 ml of distilled water and filtered. To the filtrate (1 ml), 4 ml of alkaline picrate solution was added. The mixture was boiled for 5 min and allowed to cool. The absorbance was read at 490 nm.

Test for Reducing Sugar

The extract (1 g) was macerated with 20 ml of distilled water and filtered. To 1 ml of the filtrate, 1 ml of alkaline copper reagent was added. The mixture was boiled for 5 min and allowed to cool. Then 1 ml of phosphomolybdic acid reagent and 2 ml of distilled water was added and the absorbance read at 420 nm.

Test for Soluble Carbohydrate

The extract (1 g) was macerated with 50 ml of distilled water and filtered. To the 1 ml of the filtrate, saturated aqueous solution of picric acid was added and absorbance read at 580 nm.

Test for Cyanide

The extract (1 g) was macerated with 50 ml of distilled water and then filtered. To 1 ml of the filtrate, 4 ml of alkaline picrate solution was added. The mixture was boiled for 5 min and allowed to cool. The absorbance was measured at 490 nm.

Test for Phenols

The extract (1 g) was macerated with 20 ml of 80% ethanol and then filtered. The filtrate (5 ml) was added to 0.5 ml of folinciocalteus reagent and allowed to stand for 30 min. Then 2 ml of 20% sodium carbonate was added and absorbance measured at 650 nm.

Effect of the extract on *in vivo* leukocyte mobilization

The effect of the extract on *in vivo* leukocyte migration induced by inflammatory stimulus was investigated using the method of Ribeiro *et al.*, (1991). One hour after oral administration of extract, each rat in the groups received intraperitoneal injections of 0.5ml of 3% (w/v) agar suspension in normal saline. Four hours later, the rats were sacrificed and the peritoneum washed with 5ml of 5% solution of EDTA in phosphate buffered saline (PBS). The peritoneal fluid was recovered and total and differential leukocyte counts (TLC and DLC) were performed on the perfusates.

Results and Discussion

Extraction of *Senna mimosoides*

Dried leaves of *Senna mimosoides* 2000g was subjected to cold aqueous extraction yielded 35.3%. The extract which was brown and slurry-like was used in all biological activity determination.

Phytochemical composition

Qualitative Phytochemical Composition

The result in Table 1 below indicated the presence of steroid, alkaloid and cyanide in mild concentration; saponins, flavonoids, terpenoids, phenols and soluble

carbohydrate in moderate amount while tannins and reducing sugar were present in abundance

Qualitative Phytochemical Composition

Table 2 below reveals the qualitative phytochemical composition present in the extract. It shows the degree of abundance of these phytochemicals in mg/100g of the extract is as follows; 4.68 ± 0.0033 of soluble carbohydrate, 4.75 ± 0.0034 of tannin, 1.61 ± 0.0031 of cyanide, 8.31 ± 0.0032 of reducing sugar, 1.97 ± 0.0030 of saponin, 0.86 ± 0.0023 of steroid, 2.67 ± 0.0013 of flavonoid, 3.43 ± 0.0028 of alkaloid, 2.75 ± 0.0029 of glycoside, 0.87 ± 0.0032 of terpenoid and 3.61 ± 0.0025 of phenol.

LD₅₀

In the investigation, there was no lethality or behavioural change in the three groups of mice that received 10, 100 and 1000 mg/kg of the extract. Based on this result, further increased doses of 1500, 2000, 3000 and 5000 mg/kg of the extract were administered to four other groups respectively. Those that received 3000 and 5000 mg/kg showed weakness and drowsiness. No death occurred within 24 hr of administration.

Result of effect of extract on in vivo leukocyte mobilization

Effect of the Extract on *in vivo* Leukocyte Migration

From the table below the extract of *Senna* leaves produced a significant increase ($p < 0.05$) in agar induced leukocyte mobilization into the peritoneum. The total leukocyte count of the groups treated with different concentrations of extract increased in a concentration dependent manner while that

of the group treated with indomethacin decreased significantly ($p < 0.05$) when compared with that of the control. Lymphocyte and neutrophils were the most mobilized leukocytes. Neutrophil mobilization was more in the group that received 100mg/kg of the extract and least in the group treated with 500mg/kg. The mobilization of lymphocyte increased gradually as the concentration of the extract increased.

Plants are known to contain a variety of secondary metabolites. These secondary metabolites or bioactive compounds produce definite physiological actions on the human system. According to Yadav and Agarwala, (2011), approximately 25 percent of all prescribed medicines today are substances derived from plants. Interestingly, many of these phytochemicals have been discovered and even isolated from a variety of medicinal plants. Regrettably, however, not many of them have been exploited for clinical use. It is important to comment that the presence or absence of any particular bioactive compounds fundamentally depends on the solvent of extraction and the plant part used for the extraction (Dai and Mumper, 2010). Phytochemical analysis of plants is predicated by the need for drug alternatives of plant origin, made imperative by the high cost of synthetic drugs. These secondary plant metabolites extractable by various solvents exhibit varied biochemical and pharmacological actions in animals when ingested (Nwoguet *et al.*, 2008). The leaf extract of *Senna* contains, glycosides, reducing sugars, saponins, triterpenes, flavonoids, tannin, soluble carbohydrates, cyanide, steroids, phenols and alkaloids (Table 1 and 2).

The flavonoid content of the aqueous extract of *Senna* as shown in Table 2 is (2.67 ± 0.0013 mg/100 g), which is more than that

found in species like *T officinale* (1.0 ± 0.02 mg/100 g), *A. indica* (0.62 ± 0.10 mg/100 g), *C. asiatica* (0.52 ± 0.20 mg/100 g), *E. officinalis* (0.55 ± 0.13 mg/100g), *H. rosa-sinensis* (0.40 ± 0.15 mg/100 g), *M. oleifera* (0.51 ± 0.18 mg/100 g), *I. cylindrica* (0.32 ± 0.16 mg/100 g), *R. Arvensi* (1.769 ± 0.022 mg/100 g), *C. lanatus* (0.562 ± 0.0013 mg /100 g), *E. ravens* (1.034 ± 0.008 mg/100 g), *F. critica* (0.9863 ± 0.0026 mg/100 g), *Garcinia kola* (2.041 mg/100 g), (Krishnaiah *et al.*, 2009). The presence of flavonoids in the leaf of *Senna* could account for its use as an anti-inflammatory agent (Ekwueme *et al.*, 2011). It also means that the plant could be used to prevent damage caused by free radicals in the body (Dweck and Mitchell, 2002) and for the treatment of diarrhoea (Schuier *et al.*, 2005), fever-reducing (antipyretic), pain-relieving (analgesic) and spasm-inhibiting (spasmolytic) activities and anticancer activities. Flavonoids exhibit dramatic effects on immune and inflammatory cells; these can be either immunosuppressant or immunostimulatory (Huang *et al.*, 2010). In some cases, the immunosuppressant effect is not caused by direct cytotoxicity of the flavonoids themselves. Some studies indicate that the effects are possible only when these cells are physiologically-activated.

Tannins are known to possess immuno stimulating activities. Well known Ayurvedic formulation, *Triphala churna* contains *Terminalia chebula*, *Terminalia belenica* and *Embliaofficialis*, which are rich in tannin and has been reported for its immunostimulating activity (Kumar and Subrahmanyam, 2013). The significant immunostimulant effect of the aqueous extracts of *Senna* leaves on cyclophosphamide induced myelo suppression may be attributed to the collective presence of saponins, sterols,

tannins and flavonoids in the extracts. A number of plants used in traditional medicines for rejuvenation therapy and chronic ailments have been shown to stimulate immune responses and several active substances have been isolated (Kumar and Subrahmanyam, 2013). Tables 1 and 2 show the presence of tannins in the leaves *S. m* (4.75 ± 0.0032). This is lesser than the tannin content in some plants like *A. indica* (9.1 ± 0.20), *C. asiatica* (10.3 ± 0.15), *E. officinalis* (11.2 ± 0.16), *H. rosa-sinensis* (8.5 ± 0.22), *I. Cylindrical* (9.3 ± 0.11), *M. oleifera* (9.2 ± 0.26) but higher than that of *G. kola* (0.342 ± 0.00). These plants are used in wound healing (Okwu and Josiah, 2006; Nayak *et al.*, 2007); treatment of dysentery, diarrhoea and urinary tract infection (Gohet *et al.*, 1995; Okwu and Josiah, 2006; Fahey, 2005). This suggests the possible potential of *Senna* leaves in the treatment of dysentery, diarrhoea, urinary tract infection and in wound healing.

The alkaloid content of the aqueous extract of *Senna* was found to be (3.43 ± 0.0029 mg/100 g) which is more than the alkaloid content in some plants like *Taraxacom officinale* (1.1 ± 0.03 mg/100 g), *C. asiatica* (0.31 ± 0.06 mg/100 g), *I. cylindrica* (0.45 ± 0.18 mg/100 g), *E. officinalis* (0.24 ± 0.03 mg/100 g), *A. indica* (0.52 ± 0.12 mg/100 g), *H. rosa - sinensis* (0.51 ± 0.16 mg/100 g), *E. ravens* (0.399 mg/100 g), *R. arvensis* (0.2579 ± 0.007 mg/100 g), *C. lanatus*, (0.176 ± 0.0013 mg/100 g), *F. critica* (0.226 ± 0.0068 mg/100 g), *C. lanatus* (0.176 mg/100 g) and *G. kola* (0.647 mg/100 g) (Krishnaiah *et al.*, 2009). The presence of alkaloids (Table 2) in the leaves of *Senna* means that the leaves could be used in hypertension treatment (Akinpelu and Onakoya, 2006; Raffauf, 1996 and Olaleye, 2007); prolonging the action of several hormones and acting as stimulants, pain reliever and tranquilizer.

Saponins are either triterpenoid or steroidal glycosides proven as important phytoconstituent with different pharmacological activities such as antiallergic, antiphlogostic, cytotoxic, antitumour, antiviral, immunomodulating, antihepatotoxic, molluscicidal and antifungal effects. There is evidence of the presence of saponins in traditional medicine preparations, where oral administrations might be expected to lead to hydrolysis of glycosides from terpenoids (and obviation of any toxicity associated with the intact molecule) (Musa *et al.*, 2011).

The saponin content of the aqueous extract of *Senna* was found to be (1.97 ± 0.003 mg/100 g) which is lesser when compared with the saponin content of *T. officinale* (2.95 ± 0.1 mg/100 g), *A. indica* (2.10 ± 0.13 mg/100 g), *C. asiatica* (2.20 ± 0.11 mg/100 g), *M. oleifera* (2.30 ± 0.04 mg/100 g), *H. rosa-sinensis* (2.00 ± 0.08 mg/100 g), *R. arvensis* (2.49 ± 0.02 mg/100 g), *C. lanatus* (2.52 ± 0.016 mg/100 g), *G. kola* (2.47 mg/100 g) and higher than that of *E. officinalis* (1.10 ± 0.05 mg/100 g) and *I. cylindrical* (1.4 ± 0.02 mg/100 g) *F. Critica* (0.82 ± 0.004 mg/100 g), *E. ravens* (1.61 ± 0.012 mg/100 g) (Krishnaiah *et al.*, 2009). The saponin content of *Senna* also might be responsible for its antiinflammatory properties (Ekwuemeet *et al.*, 2011) and for its immunomodulating effect as indicated above. The presence of saponins in *Sennaleaves* suggests that it could be used to treat hyperglycaemia (Sotheeswaranet *et al.*, 1998), to cleanse and purify blood, treat hypertension (Fahey, 2005), and might also have cholesterol binding properties, haemolytic activities (Okwu, 2004), wound healing properties and treatment of bleeding.

As shown in Table 3 there was a significant increase ($p < 0.05$) in agar induced

mobilization of leukocyte upon treatment with the extract. This increase was dose dependent. This shows that the agar suspension was able to create an injury that was responded to by the extract probably enhancing the proliferation of inflammatory mediators that increased the production of leukocytes which migrated to the site of injury.

From the result in Table 3, neutrophil was the most mobilized leukocyte agreeing with the report of Vega and Corbi (2006) which states that during injury, phagocytic cell increases in number in the blood stream and they are largely responsible for elevated white blood cell count during infection. The neutrophil stimulated by the extract then fight and eliminate the infectious agent using myeloperoxidase present in the primary granules, lactoferrin and gelatinase present in the secondary granules, to degrade extracellular matrix, digest phagocytosed material, exert antimicrobial activity and initiate inflammation (Dale *et al.*, 2008).

The increase in neutrophil by the extract shows its probable potential in recruiting DCs and monocyte that complete innate clearance of invading microbes and also initiate more specific adaptive immune responses (Vega and Corbi, 2006).

Furthermore, the mobilization of neutrophil by the extract suggests that the extract activates the generation of respiratory burst by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which generates ROS used in killing microbes (Pugaet *et al.*, 2012). Moreover, the extract can also mop up this ROS when they are produced in excess due to its antioxidant effect.

Table.1 Results of the Qualitative Analysis

S/N	Test	Observation	Inference
1	Saponins (Frothing test)	Persistence foaming	++
2	Tannins (Ferric chloride)	Greenish-brown precipitate	+++
3	Flavonoids (Ethylacetate test)	Intense yellow colouration	++
4	Terpenoids (Chloroform and acetic anhydride test)	A grey colouration	++
5	Steroid (Conc. H ₂ SO ₄)	Reddish brown colouration (Steroidal ring)	+
6	Phenols (Distilled water test)	Blue-Greenish colouration	++
7	Alkaloid		
i	Dragendorff's reagent	Red precipitate	+
ii	Mayer's reagent	Creamy-white precipitate	+
iii	Wagners reagent	Reddish-brown precipitate	+
8	Reducing sugar	Brick-red precipitate	+++
9	Soluble carbohydrate	Purple interfacial ring	++
10	Glycoside (Sulphuric acid test)	Brick-red precipitate	++
11	Cyanide	Orange colouration	+

+mild

++moderate

+++abundance

Table.2 Results of the Quantitative Analysis

Phytochemical(mg/100g)	Mean ± STD
Soluble carbohydrate	4.68 ± 0.0033
Tannin	4.75 ± 0.0034
Cyanide	1.61 ± 0.0031
Reducing sugar	8.31 ± 0.0032
Saponin	1.97 ± 0.0030
Steroid	0.86 ± 0.0023
Flavonoid	2.67 ± 0.0013
Alkaloid	3.43 ± 0.0028
Glycoside	2.75 ± 0.0029
Terpenoid	0.87 ± 0.0032
Phenol	7.44 ± 0025

Values represent mean ± standard deviation of triplicate sample

Table.3 The Median Lethal Dose of Aqueous Extract of the Leaf Extract of *S.mimosoides*

Phases	Dosages mg/kg body weight	Mortality	Behavioural Changes
Phase I			
Group 1	10	0/3	Nil
Group 2	100	0/3	Nil
Group 3	1000	0/3	Nil
Phase II			
Group 1	1500	0/3	Nil
Group 2	2000	0/3	Nil
Group 3	3000	0/3	Nil
Group 4	5000	0/3	Weakness and drowsiness

Table.4 The Effect of Aqueous Extract of *S.mimosoides* Leaves on In Vivo Leukocyte Migration in Rats

Treatment	Dose (mg/kg)	TLC (mms3)	Differential leucocyte mobilization (%)				
			N	E	M	B	L
A	-	2500 ± 86.60	10.50	-	-	-	89.50
B	100	3425 ± 53.11	16.75	-	-	-	83.25
C	250	3813±166.42	14.80	-	1	-	85.20
D	500	4488±196.12	13.50	-	-	-	85.49
E	25	1738±38.00	11.50	-	-	-	88.25

Key

N= Neutrophil; E= Eosinophil; M= Monocyte; B= Basophil; L= Lymphocyte

The fact that indomethacin used decreased leukocyte mobilization corresponds with former findings that high doses of indomethacin inhibit the accumulation of leukocytes (Bhattacharjee *et al.*, 1983).

These results show that the extract has immunostimulatory effect and could be used in boosting immune response

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