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Antioxidant, Anti-inflammatory and Analgesic Properties of *Stachytarpheta angustifolia* Mill Vahl (Verbenaceae) Methanol Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Herbal remedies have been used for the treatment of various diseases as they contain phytochemicals with useful pharmacological activities. The plant *Stachytarpheta angustifolia* has been used traditionally as anti-ulcer, anti-diarrhoea, anti-hypertensive, anti-fever, anti-helminth, anti-bacteria and antidiabetics. This study was aimed at evaluating the antioxidant, anti-inflammatory, and analgesic properties of *Stachytarpheta angustifolia.* The whole plant was extracted with Methanol using Soxhlet apparatus. The extract obtained was screened for phytochemicals, free radical scavenging activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), *in vitro* anti-inflammatory (protein denaturation inhibition) activity using bovine serum albumin (BSA), *in vivo* anti-inflammatory activity using carrageenan-induced paw edema and analgesic activity using hot plate methods.

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Results of the phytochemical analysis showed the presence of Saponins and Alkaloids in copious amounts, flavonoids, terpenoids, glycosides and quinones in moderate amounts; while, phenol, tannins, steroids, and coumarins were present in low amounts. The free radical scavenging activity of the extract was 71.93 and 70.80% at concentrations of 500 and 250 µg/ml compared to Vitamin C and Vitamin E (92.56 and 84.17%), respectively. The *in vitro* anti-inflammatory activity of the extract was dose-dependent (31.58%) and higher than Aspirin (28.32%). The *in vivo* anti-inflammatory activity of the extract was 79.10% compared to Aspirin (78.33%). The analgesic activity of the extract was (62.01%) compared to Aspirin (59.15%). The study found that the extract possessed various phytoconstituents that can be used for medicinal purposes. Hence, the extract may serve as a source of drugs for the management of pain and inflammatory conditions.

Keywords: Medicinal plants; phytoconstituents; protein denaturation; paw edema.

1. INTRODUCTION

Natural products still served humankind as the source of all foods and plants provide several prophylaxis and therapeutic agents [1]. "Traditional medicinal practices are integral part of complementary or alternative medicine" [2]. "The World Health Organization (WHO) estimates that around 80% of the population in Africa uses traditional medicines, with about 85% of traditional medicine involving the use of plant extracts" [3]. A wide variety of herbal remedies have traditionally been used for diseases in Nigeria [4].

"Medicinal plants are widely used for the research of new drugs as they represent a rich source of compounds with pharmacological properties" [5]. "Herbal remedies have many traditional claims and are employed in the treatment of diseases of diverse origins as they contain active constituents with useful physiological and pharmacological activities" [6]. "These medicinal plants are enriched with phytochemicals such as tannins, saponins, flavonoids, essential oils and alkaloids seem to have therapeutic properties, and are used in the traditional system of medicine for the management of various ailments" [7]. "The phytochemicals have several biological properties which include antioxidant, analgesic, anti-inflammatory, anti-diarrhea, anti-ulcer, and anticancer activities, among others" [8].

The genus *Stachyterpheta* Vahl (*Verbenaceae*), known as "gervão" in English includes about 100 species widely distributed in tropical and subtropical America with few members in tropical Asia, Africa and Oceania [9]. "This genus is represented by three species in West Africa and in Nigeria: *S. cayannensis* (Rich.) Vahl., *S. indica* (Linn.) Vahl. and *S. angustifolia* (Mill.) Vahl." [10]. "Various chemical constituents have been reported in the genus, including; flavones and flavonoids, saturated hydrocarbons, phenols, terpenes steroids, quinones, and fatty acids such as stearic, oleic, and palmitic acids" [11].

"S. angustifolia is a seasonal plant growing mostly along the banks of rivers, streams, and in farmlands during the rainy seasons, especially in southern Nigeria" [6]. "The leaves of *S. angustifolia* are used for the relief of sprain by rubbing the juice on the affected part and the aerial part of the whole plant is boiled and taken as a remedy against diarrhoea, Intestinal parasite, and skin ulcer" [12]. "The decoction of the whole plant is taken as an antihelmintic agent, while the infusion of the plant mixed with the patron is taken as a remedy against gonorrhoea, syphilis, and other related venerable infectious diseases" [12]. "The leaf from the plant is boiled and taken as a remedy against diabetes in the northern part and the alcohol extract of the leaf has been reported to show some antimicrobial activities against *Mycobacterium tuberculosis, Staphylococcus aureus, and Escherichia coli"* [13]. It has been used as an abortifacient, emmenagogue, sedative, anti-hypertensive, and anti-fever [14]. Thus, this study was conducted to evaluate the analgesic and anti-inflammatory properties of the methanol extract of *S. angustifolia.*

2. MATERIALS AND METHODS

2.1 Plant Sample Collection and Extraction

The whole plant (roots, stem and leaves) of *Stachytarpheta angustifolia* was harvested, washed, and was identified using Virtual Botanic Garden (VIRBOGA) Dataset Identifier (Identification number 788). The plant was air dried under laboratory conditions for two weeks. The extract was prepared according to the

Institute of Medical Research (IMR) procedure. The dried powder was filled in the porous cellulose thimble and subjected to soxhlet extraction using 99.8% methanol for 12 hours at 65^{o}C, followed by filtration through a Whatman No. 1 filter paper. The methanol extract obtained was concentrated to dryness at 45^{o}C using a rotary evaporator under reduced pressure and the extract was weighed and then stored at 4^{o}C for further use [15].

2.2 Experimental Animals

Thirty adult Albino rats weighing 200--250 g were used in the study. The rats were housed in cages of 5 rats each and allowed acclimatization to laboratory status for one week before the experiments commenced [16]. The rats were maintained at room temperature and with a 12h light/12h dark cycle and allowed *ad libitum* access to feed and water.

2.3 Phytochemical Screening

The qualitative phytochemical screening was carried out according to the method by [17]. Total phenolic content was determined by adding 0.5ml of extract to 2.5ml of 10-fold diluted Folin Ciocalteu Reagent, 2ml of 7.5% of sodium carbonate and allowed to stand for 30 minutes. Garlic acid was used as standard and the absorbance was measured at 760nm [18]. Tannin content was evaluated by adding 0.5ml of extract to 3.5% sodium carbonate solution, 7.5ml of distilled water and 0.5ml of 10-fold diluted Folin Ciocalteu reagent and allowed to stand for 30min. Garlic acid was used as standard and the absorbance was measured at 725nm [19]. Flavonoid content was determined by adding 50µg/ml of extract to 75µl of sodium nitrite ($NaNO_2$ 5%) solution, 0.5ml of aluminium chloride ($AlCl_3$, 100g/l) and 0.5ml of sodium hydroxide (NaOH, 4%). Rutin was used as standard and the absorbance was measured at 510nm [20]. The alkaloid content was determined by adding 1ml of the extract to separating funnel and mixed with 5ml Bromocresol green solution in 5ml of phosphate buffer were. The formed mixture was extracted further with 10ml of chloroform and the absorbance of the chloroform complex was measured at 470nm [21]. Quinone content was determined by adding 1g of sample in 5ml of chilled phosphate buffer. The supernatant was collected by centrifugation at 2000rpm for 30min at 4^{0}C. 3 ml of buffer, 3ml of standard catechol and 1.5ml of the extract supernatant was added in 4ml of TCA (Trichloro acetic acid) reagent. The precipitate was filtered and the absorbance was measured at 400nm [22].

Coumarin content was determined by dissolving 10mg of the extract in methanol:acetone (1:1 v/v). The absorbance was measure at 327nm using Esculin was used as standard [23]. Terpenoids content was determined by adding 200µl of extract (1 mg/ml) to 1.5ml of chloroform vortexed and brought to rest for 3min then 100µl of Conc. Sulphuric acid was added and incubated at room temperature for 1.5–2hr in the dark. The precipitated formed was dissolved in 1.5ml of 95% Methanol and the absorbance was measured at 538 nm using linalool as standard [24]. Steroid content was determined by adding 1ml of the extract (1 mg/ml) to 4ml of chloroform then 2ml of Liebermann–Burchard (LB) reagent and stands for 15min in the dark. The absorbance was measure at 640nm with Beta-Sitosterol as standard [25]. Saponin content was determined by adding 2ml of each extract to 1ml of reagent A (p-anisaldehyde 0.5% in ethyl acetate 99.5%) and 1 ml of reagent B (H_2SO_4 50% in Ethyl acetate 50%) and vortex. The mixture was stirred and incubated at 60^{0}C for 10min in a water bath. The solutions were cooled at room temperature for 10min and the absorbance of the colour-developed solution were recorded at 430nm using digitonin as standard [26]. Glycosides content was determined by dissolving 1mg of extract in 8ml of distilled water and 1ml of 12.5% lead acetate solution and filtered. 5ml of the filtrate was transferred to a volumetric flask, 1ml of 4.77% Na_2HPO_4 solution was added, and made up to 10ml with distilled water and filtered. 10ml of Baljet's reagent was added to 1ml of the clear filtrate and the mixture was allowed to stand for an hr and diluted with 20ml of distilled water. The absorbance was measured at 495nm using digitoxin as standard [27].

2.4 Determination Free Radical Scavenging Activity

The free radical scavenging activity of the methanol extracts was measured using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay according to the method of Jain et al. [28]. A solution of 0.2 mM DPPH in methanol was prepared. 1.0 mL of this solution was mixed with 3 mL of extract in methanol containing 0.001-0.2 mg/mL of the extract. The mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance was

measured at 517 nm. Ascorbic acid and Vitamin E were used as the reference standards.

2.5 *In-vitro* Anti-inflammatory Activity

The anti-inflammatory activity of the plant extracts was determined using a modified version of the bovine serum albumin (BSA) assay reported by Williams et al*.,* [29]. BSA solution (0.4%, w/v) was prepared in Tris Buffered Saline (one tablet is dissolved in 15 mL of deionized water to yield 0.05M Tris and 0.15M sodium chloride, pH 7.6 at 25^{o}C). The pH of the buffer was adjusted to 6.4 with glacial acetic acid. Respective aliquots of 5.0 µL, 10 µL and 20 µL representing concentrations of 0.25 µg/mL, 0.50 µg/mL and 1.00 µg/mL of the stock solutions was added to test tubes containing 1 mL of 0.4%, w/v BSA buffer solution. Both negative (methanol) and positive (Aspirin) controls were assayed in a similar manner. The solutions were then heated in a water bath at 72°C for 10 minutes and cooled for 20 minutes under laboratory conditions. The turbidity of the solutions was measured at 660 nm in a Spectrophotometer using air as blank.

2.6 *In-vivo* Anti-Inflammatory Activity

The anti-inflammatory activity of the *S. angustifolia* extract was determined using the method of Omodamiro et al., [30]. The rats were randomly assigned to four groups of 5 animals each per group. Group 1 was negative control treated with normal saline, group 2 was positive control and treated with Aspirin 50mg/kg, and groups 3, 4, and 5 treated with the methanol extract of the *S. angustifolia* at dosages of 25, 50, and 75 mg/kg, respectively, which was calculated using Ratio and Proportion Method [31]. The animals were pre-treated for an hour before they were injected with 0.1ml of 1% Carrageenan solution into the sub-plantar region of the left hind paw. The paw volume was measured with a vainer calliper at 1-hour intervals for 4 hours. Reduction in the paw volume compared to the control group was considered as anti-inflammatory response.

2.7 *In vivo* Analgesic Activity

Evaluation of analgesic activity of the extract was carried out using hot plate method [32]. The rats were randomly assigned to four groups of 5 animals each per group. Group 1 was negative control treated with normal saline, group 2 was positive control treated with Aspirin 6gmg/kg, group 3, 4 and 5 treated with the methanol extract of the *S. angustifolia* at dosages of 25, 50 and 75 mg/kg, respectively, which was calculated using Ratio and Proportion Method [31]. The rats were placed on a hot plate maintained at 55°C within the restrained. The reaction time (in seconds) or latency period was determined as the time taken for the rats to react to the thermal pain by licking their paws or jumping according to [32]. The reaction time was recorded 60 min after the administration of the treatments.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Phytochemical analysis

The result of the preliminary phytochemical screening of the methanol extract of *S. angustifolia* in Table 1 shows the presence of phenol, tannins, flavonoids, steroids, terpenoids, saponins, glycosides, alkaloids, coumarins, and quinones with their percentage compositions in Table 1.

3.1.2 Radical DPPH scavenging assay

The result of the radical DPPH scavenging assay is presented in Fig. 1. The percentage (%) inhibition of the methanol extract of *S. angustifolia* was 70.80% and 71.93% at higher concentrations of 250 and 500 µg/ml as compared to standards vitamin C (75.09, 79.71, 86.81, 91.10 and 92.56) and vitamin E (57.44, 75.2, 83.47, 83.94 and 84.27) at concentrations of 31.25, 62.5, 125, 250 and 500 µg/ml, respectively.

3.1.3 *In vitro* anti-inflammation (anti-denaturation) activity

The anti-denaturation activity using the BSA protein denaturation of the methanol extract of *S. angustifolia* is shown in Fig. 2. The result revealed that the extract possessed 23.71, 27.29, and 31.58 % inhibition of protein denaturation at concentrations of 0.25, 0.50, and 1.00 1.00µg/ml, respectively. Whereas, Aspirin possessed 28.32, 13.79, and 13.34 % inhibition of protein denaturation at the same concentrations.

Table 1. Qualitative phytochemical analysis of the methanol extract of *S. angustifolia*

Phytochemicals	Qualitative composition	Quantitative composition (g/100 mg)
Tannins	+	19.11±0.63
Saponins	+	473.59±16.49
Flavonoids	+	78.83±6.81
Glycosides	+	76.00±02.18
Quinones	+	84.18±0.33
Phenols	+	36.78±1.23
Terpenoids	+	103.00±2.00
Cardiac glycosides	-	-
Coumarins	+	34.52±0.11
Anthraquinones	-	-
Steroids	+	35.78±5.04
Phlobatannins	-	-
Chalcones	-	-
Alkaloids	+	457.33±7.77
Anthocyanines	-	-

Results are expressed in mean ± SD (n = 3) + = Present, - = Absent

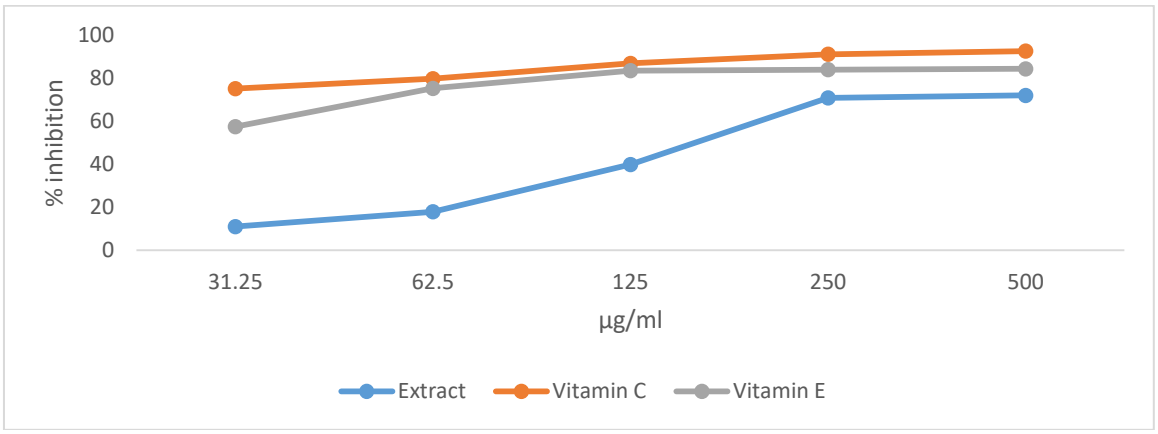

Fig. 1. DPPH radical scavenging activity

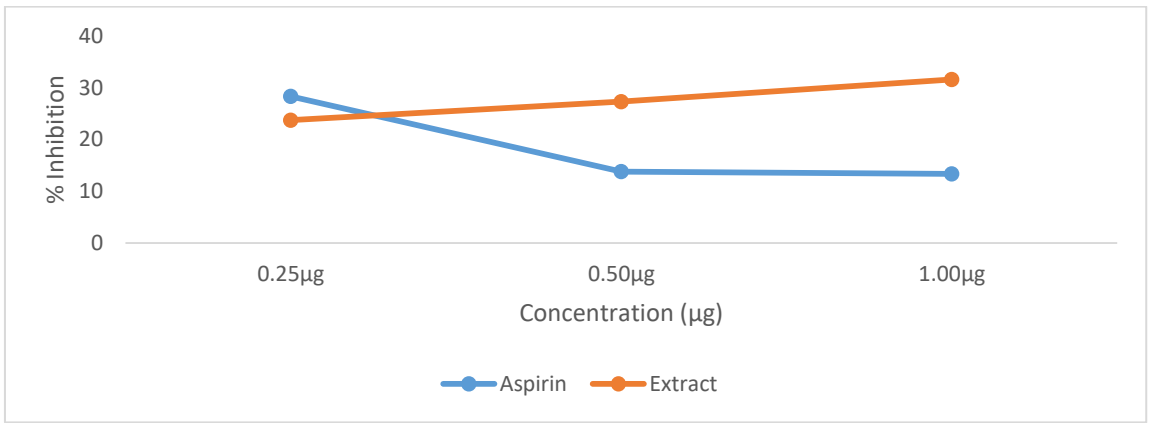

Fig. 2. % anti-denaturation activity of *S. angustifolia* extract

3.1.4 *In vivo* anti-inflammation (carrageenan-induced inflamed paw)

Anti-edematogenic activities of the methanol extract of *S. angustifolia* are presented in Fig. 3. Injection of carrageenan into the hind paw of rats produced a time-dependent increase in paw size with peaked at the 5th hr. Pre-treatment with Aspirin which served as a reference drug produced time-dependent significant inhibition of edema (inflamed paw) formation with a peak effect of 78.33% inhibition at the 1st hr and decreased to 40.00% after the 4th hr of carrageenan induction. Similarly, oral administration of the methanol extract of *S. angustifolia* (25, 50, and 75 mg) produced dose-related and time-dependent inhibition with the low dose (25 mg) possessing the same effect as Aspirin producing a significant inhibition of edema formation with a peak effect of 78.33% inhibition at the1st hr and decreased to 40.00% after the 4th hr of carrageenan induction. However, the highest dose (75 mg) possesses a significant inhibition with a peak effect of 79.10% at the 2nd hr and 60.00% at the 4th hr of carrageenan induction thereby suppressing inflammation with a long time effect.

3.1.5 Analgesic effect (hot-plate test)

In the hot-plate test, Aspirin (a non-selective cyclooxygenase inhibitor) produced a significant (52.16%) analgesic effect from an hr after administration and attain a maximum effect (59.15%) at the 3rd hr, then lost its effect to 05.62%. Oral administration of the methanol extract of *S. angustifolia* at different doses (25, 50, and 75 mg) produced no significant effect at low dosage but produced significant (62.01%) analgesic effect with medium dose at the 3rd hr to 60.91% at the 5th hr. However, the highest dose of methanol extract of *S. angustifolia* produced a significant (52.46%) analgesic effect at the 2nd hr then decreased significantly to 39.63% at the 5th hr as shown in Fig. 4.

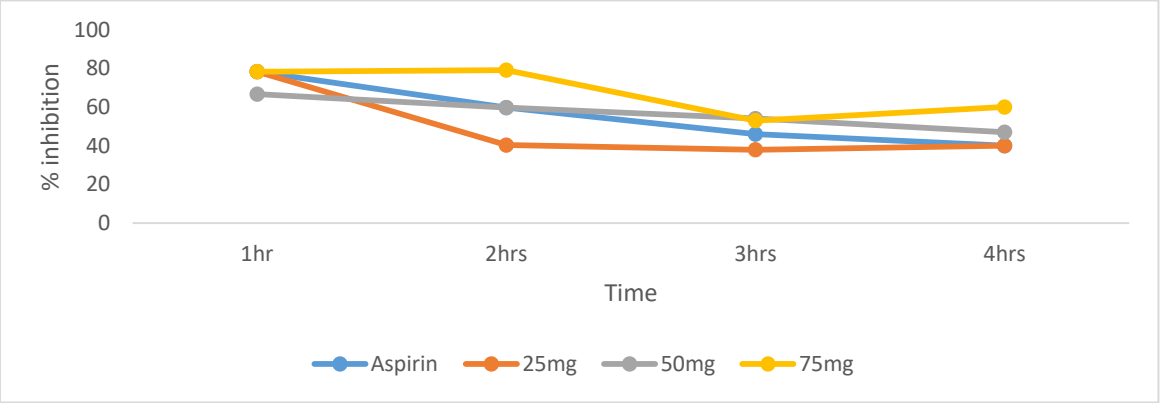

Fig. 3. Anti-inflammatory (inflamed paw) effect of *S. angustifolia* methanol extract

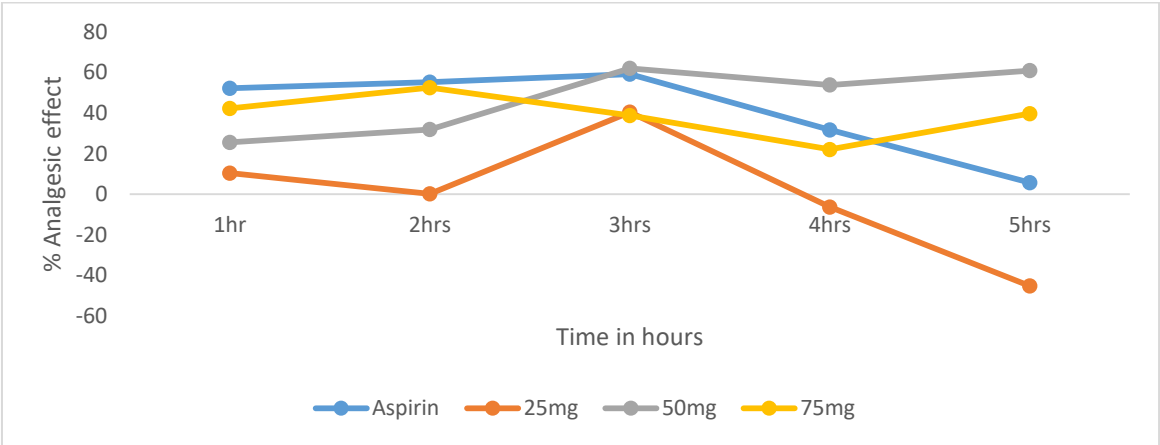

Fig. 4. % Analgesic effect of *S. angustifolia* methanol extract

3.2 Discussion

This study was aimed at evaluating the phytochemical, antioxidant, anti-inflammatory and analgesic properties of the methanol extract of *S. angustifolia* whole plant which is used traditionally as a remedy against diarrhoea, intestinal parasite, pain reliever and diabetes. Hence, the interest in its pharmacological properties especially its efficacy. The methanol extract of *S. angustifolia* in this study showed the presence of phenol, tannins, flavonoids, steroids, terpenoids, saponins, glycosides, alkaloids, coumarins, and quinones with saponins and alkaloids possessing the highest amounts (33.85 and 32.69 %), respectively. This result confirmed previous findings by Mohammed et al*.,* [33] and the result of Enwuru et al., [34] also affirmed the presence of saponins as the major active secondary metabolite in *S. angustifolia.*

These secondary metabolites are reported to possess several biological and therapeutic properties [35]. The numerous chemical compounds in plants having medicinal values include alkaloids, amino acids, amines and carboxylic acid derivatives, anthranoids, carbohydrates, glycosides, flavanoids, minerals, vitamins and inorganic compounds, peptidoglycans, polyphenol and its derivatives, saponins, and so on [36]. Alkaloids have been investigated for their antidiabetic activity and show free radical scavenging action. Glycosides are mainly involved in the restoration of pancreatic β cells and insulin secretion. Flavonoids have numerous medicinal effects including antidiabetic properties and free radical scavenging. Polyphenols are used for treatmnt of conditions such as cancer, liver cirrhosis, chronic renal disease, chronic obstructive lung disease, diabetes and Alzheimer's disease which have been linked to antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, antihypertensive, cardioprotective, anti-arthritic and antimicrobial activities [37,36]. Saponins exhibit a biological role and medicinal properties such as hemolytic factor, anti-inflammatory, antibacterial, antifungal, antiviral, anticancer, cytotoxic and exhibit cholesterol-lowering action in animals and humans [38]. Plant steroids possess many interesting medicinal activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, sex hormone, antihelminthic, cytotoxic and cardiotonic activities [39]. These metabolites are most likely to be linked to the biological activities of *S. angustifolia*.

The methanol extract of *S. angustifolia* possessed substantial dose-dependent antioxidant activity against DPPH (71.93) at 500 µg/ml. The activity was comparable to that of vitamin C and vitamin E (92.56 and 84.27%). This effect may link to the presence of phenolic, flavonoid, alkaloid, and terpenoid compounds in the extract, since they can readily donate hydrogen atom to the radical [40] to neutralize it. Therefore, the plant extracts could be used as source of natural antioxidant for prevention and treatment of diseases associated with oxidative stress.

Tissue protein denaturation is one of the main causes of inflammation [41]. The *in vitro* anti-inflammatory activity of the *S. angustifolia* extract has shown protein denaturation protective capability (31.58 %) higher than Aspirin (28.32 %). Similarly, *in vivo* anti-inflammatory activity of the *S. angustifolia* extract carrageenan paw edema (inflamed paw**)** was higher (60 %) inhibition than Aspirin (40 %) indicating that the extracts have potential to be used or as a source of anti-inflammatory agents. This anti-inflammatory property exhibited by this extract could be due to the presence of saponins [38], phenolics, flavonoids, alkaloids, and terpenoids in the extract suggesting that the anti-inflammatory activity may be due to the inhibition of inflammatory mediators, such as histamine, serotonin, prostaglandins and bradykinin released during inflammation [42].

The analgesic property of the extract has shown 62.01% maximum effect comparable to Aspirin (9.15 %). The increased reaction time of the pretreated rats with the extract in the hot plate model may be due to the presence of the phytochemicals which were shown to inhibit both inflammatory and neuropathic pain through mechanisms involving the inhibition of arachidonic acid discharge and prostaglandin synthesis known to elevate the pain threshold of animals [42]. These data suggest that the extract may provide the basis for the folk use of the plant as an analgesic agent.

4. CONCLUSION

The findings of this study have identified the presence of several phytoconstituents present in the methanol extract of *S. angustifolia*. The extract possessed potent antioxidant, anti-inflammatory and analgesic properties. Therefore, this research supported the ethnomedicinal claims of the therapeutic efficacy

of the extract in the management of pain and inflammatory conditions. Further studies will be required for investigations of the fractions of *S. angustifolia* in order to isolate potential lead anti-inflammatory and analgesic compounds.

ETHICAL APPROVAL

All experimental procedures were made according to Standard Operating Procedure for Institutional Animal Ethics Committee (IAEC).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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