Original Research Article

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

Abstract

Herbal remedies have been used for the treatment of various diseases as they contain phytochemicals with useful pharmacological activities. *Stachytarpheta angustifolia* has been used traditionally as anti-ulceranti-diarrhoea, anti-hypertensive, anti-fever, anti-helminth, anti-bacteria and remedy for diabetes. 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 thus obtained was then screened for phytochemicals, free radical scavenging activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), protein denaturation inhibition using bovine serum albumin (BSA), anti-inflammatory activity using carrageenan-induced paw edema and analgesic activity using hot plate. Results of the methanol extract 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 percentage radical inhibition of the extract was 71.93 and 70.80% at 500 and 250 µg/ml compared to Vitamin C and Vitamin E at 92.56 and 84.17%, respectively. The percentage anti-denaturation activity of the extract was dose-dependent (31.58 %) but higher than Aspirin (28.32%). % inhibition of paw edema of the extract was 79.10 % compared to Aspirin (78.33%). The percentage analgesic of the extract was dose-independent (62.01%) compared to Aspirin (59.15%). The study found that the extract was effective compared to standard drugs.

Keywords: ~~*Phytochemicals, Antioxidant, Anti-Inflammatory, Analgesic.*~~

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INTRODUCTION

Natural products still served humankind as the source of all foods and plants provides several prophylaxis and therapeutic agents [1]. Traditional medicinal practices are an 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 [13]. 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* [14].It has been used as an abortifacient, emmenagogue, sedative, anti-hypertensive, and anti-fever [15].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 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 [7].

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2.2 Phytochemical Screening

The qualitative phytochemical screening was carried out according to the method by [2]. The quantitative analysis of the plant extract was carried out using the methods of Maurya and Singh [16-17] for total phenolic content, Rajeev *et al.,*[18] for tannins, Zengnin *et al*., [19] for flavonoids, Raheleh *et al*., [20] for alkaloids, Thimmaiah, [21] for quinones, Vianna *et al*., [22]for coumarins, Thakur and Sahani [23]for terpenoids, Attarde *et al*., [24]for steroids, Sofowora [25]for cardiac glycosides, Uematsu *et al*., [26]for saponins, and Tofighi *et al*., [27]for total glycosides content.

2.3 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.

2.4 *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.

2.5 *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. 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.

2.6 *In Vivo* Analgesic Activity

Evaluation of analgesic activity of the extract was carried out using hot plate method [31,32]. 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. 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 [31]. 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 figure 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.

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

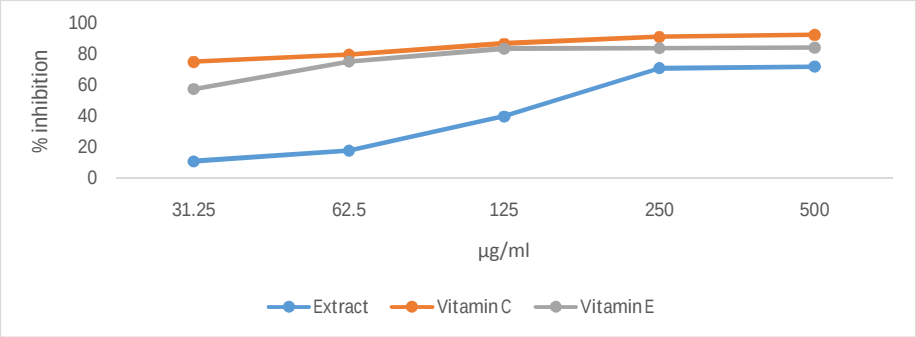

Figure 1: DPPH Radical Scavenging Activity

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 figure 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.

3.1.4 *In vivo* Anti-inflammation (Carrageenan-induced rat paw oedema)

Anti-edematogenic activities of the methanol extract of *S. angustifolia* are presented in figure 3. Injection of carrageenan into the hind paw of rats produced a time-dependent increase in paw size with the peaked

at the 5th hr. Pre-treatment with Aspirin which served as a reference drug produced time-dependent significant inhibition of edema 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.

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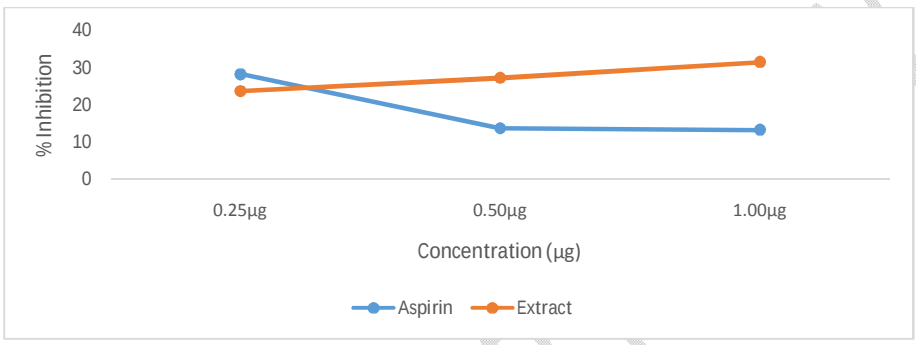

Figure 2: % Anti-Denaturation Activity of *S. angustifolia*Extract

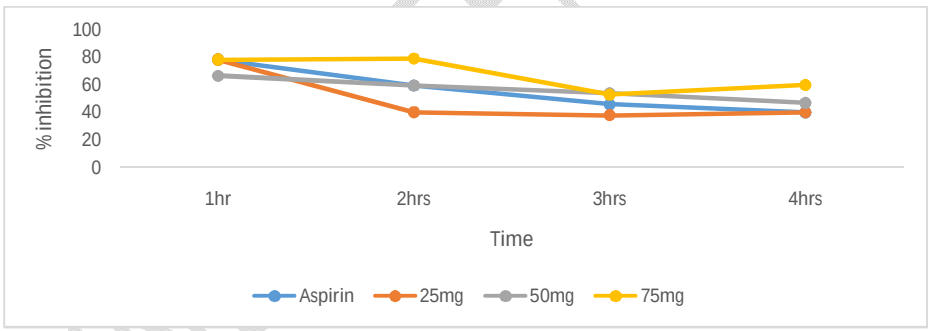

Figure 3: Anti-inflammatory (Paw Edema) Effect of *S. angustifolia* Methanol Extract

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 figure 4.

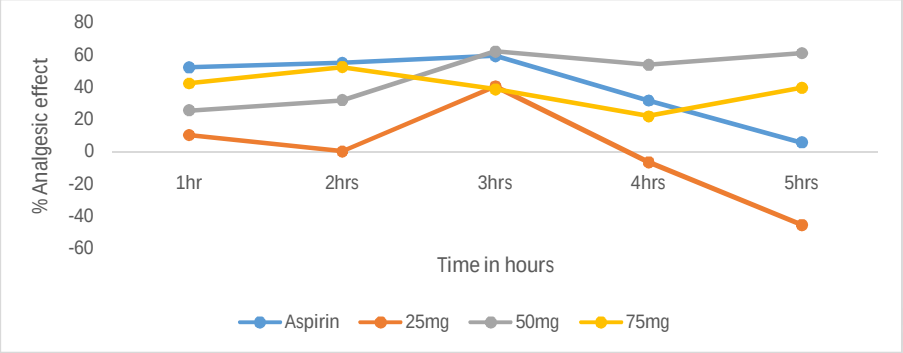

Figure 4: % Analgesic Effect of *S. angustifolia* Methanol Extract

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~~ ofMohammed *et al.,*[33] and the results of Enwuru *et al*., [34] ~~also~~ whoaffirmed the presence of saponins as the major active secondary metabolite.

These secondary metabolites are reported to possess several biological and therapeutic properties [35]. Tannins have been ascribed to have complex metal ions, scavenge radicals, reduce reactive oxygen species and form tight complexes with a wide variety of proteins and polysaccharides [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 [37]. Phenolic compounds derived from natural sources have been linked to antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, antihypertensive, cardioprotective, anti-arthritic and antimicrobial activities [38]. Naturally occurring terpenoids often exhibit a variety of biological activities such as anti-inflammatory, anti-HIV, anti-tumour- promoting and antimycobacterial activities [39].

Flavonoids have the ability to induce human protective enzyme systems which have protective effects against many infectious and degenerative diseases such as cardiovascular diseases, cancers, and other age-related diseases [40]. Plant steroids possess many interesting medicinal activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, sex hormone, antihelminthic, cytotoxic and cardiotonic activities [41]. 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. This result is in accordance with [42]. that antioxidant activity of *S. angustifolia* extract against DPPH is dose-dependent. The activity was comparable to that of vitamin C and vitamin E (92.56 and 84.27%). Linked to the of phenolic, flavonoid, alkaloid, and terpenoid

compounds in the extract, since they can readily donate hydrogen atom to the radical [43, 42] 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.

Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation [44]. The *in vitro* anti-inflammatory activity of the *S. angustifolia* extract have shown protein protective capabilities with (31.58 %) higher than Aspirin (28.32 %). Similarly, *in vivo* anti-inflammatory activity of the *S. angustifolia* extract carrageenan paw edema 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 flavonoids [44], glycosides, steroids [45], saponins [37], and phenols [38], 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 [46].

The analgesic property of the extract has shown 62.01 % maximum effect as 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 cytokine production and prostaglandin known to elevate the pain threshold of animals [47, 48]. These data suggest that the extract may probably provide the basis for the folk use of the plant as an analgesic agent [49].

4. CONCLUSION

Findings of this study have identified the presence of several phytochemicals present in the methanol extract of *S. angustifolia* with potent antioxidant, anti-inflammatory and analgesic properties. This research supported the ethnomedicinal claims of therapeutic efficacy of the extract in the management of pain and inflammatory conditions.

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