Asian Journal of Research in Medical and Pharmaceutical Sciences

3(1): 1-7, 2018; Article no.AJRIMPS.38600
ISSN: 2457-0745

Preliminary Phytochemical Screening and Analgesic Activity of the Methanol Extract of *Solenostemon monostachyus* (Whole Plant)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors APO and OI conceived and designed the study. Author BOA sourced for the plant material. Authors BOA and OHU performed all experiments and data analysis, while author BOA prepared the manuscript which was approved by all authors.

Article Information

DOI: 10.9734/AJRIMPS/2018/38600
Editor(s):
(1) Somdet Srichairatanakool, Professor, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200,Thailand.
Reviewers:
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Complete Peer review History: http://www.sciencedomain.org/review-history/23339

Short Research Article

Received 2nd December 2017
Accepted 18th February 2018
Published 26th February 2018

ABSTRACT

Background & Aim: *Solenostemon monostachyus* is an important herb that is used in the treatment of various ailments such as pain, cough, asthma and arthritis amongst the Bini people of Edo State, Nigeria. Hence this study, designed to evaluate the methanol extract of the whole plant of *Solenostemon monostachyus* in the management of pain.
Experimental: 220 g of the pulverized plant was first defatted by extracting with hexane (to remove the nonpolar constituents) using a Soxhlet apparatus, after which methanol was used to extract the defatted plant material. The percentage yield of the hexane and methanol extracts were 3.32% and 10.18% respectively. The methanol extract was then subjected to phytochemical

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screening, acute toxicity and antinociceptive tests (acetic acid-induced and hot plate models).
Results: Phytochemical screening of the methanol extract revealed the presence of glycosides, saponins, flavonoids, steroids, terpenoids and alkaloids. The methanol extract caused a significant ($p < 0.05$) dose-dependent reduction in pain induced by acetic acid (peripherally mediated) and hot plate (centrally mediated). These effects were comparable to the effects of the standard drugs (Acetylsalicylic acid 100 mg/kg and Pentazocine 10 mg/kg) used in the study.
Conclusion: This study has confirmed the presence of useful phytochemicals in the methanol extract of the plant material. These phytochemicals could either serve as templates for the synthesis of drugs or as drugs themselves. Also, the study validated the ethnomedicinal use of the whole plant of *Solenostemon monostachyus* as well as it's polar constituents in the methanol extract, for the treatment of pain.

Keywords: Solenostemon monostachyus; phytochemicals; methanol; analgesic.

1. INTRODUCTION

Pain which usually is associated with actual or potential tissue damage is an unpleasant sensation (sensory and emotional) [1] and may be classified as acute or chronic pain [2,3]. Pain could be peripherally or centrally mediated and is frequently associated with increased respiratory rate, blood pressure and dilated pupils or fatigue, loss of libido and depression mode respectively [4]. Due to lack of funds by rural dwellers to purchase orthodox drugs for the treatment of various disease conditions and to visit health care centres in their various communities, they have sought to use herbal products. These products which are usually made from herbs collected in their communities enjoy high accessibility, acceptability and use by them when compared to orthodox drugs. An example of such herb frequently used is *Solenostemon monostachyus* commonly called Monkey's potato (English); ẹbẹ kpu ahiẹmẹn (Bini); ariophe (Urhobo); ironopolo (Yoruba); sankwo (Hausa) and ntorikwot (Efik) [5,6].

Solenostemon monostachyus (P. Beauv.) Briq. which belongs to the family Lamiaceae is commonly found in West and Central Africa, growing in rocky savannahs and anthropogenic habitats. The leaves are simple, opposite and petiolated. The stem is pubescent and erect. The herb which may be annual or perennial grows up to 100 cm in height and it is slightly succulent and aromatic [7]. Ethnomedicinally, the plant is used as a sedative, stomachic and to treat convulsions, tuberculosis, fever, headache, cough, dysmenorrhoea, haematuria, fermale sterility, rheumatism, snakebites, foot infections and eyesight problems [6,7]. Biologically, the plant has been reported to have antioxidant, antihypertensive, antimicrobial, antiulcer, anti-inflammatory and analgesic activities [8]. Phytochemically, terpenoids, flavonoids, coumarins and polyphenols have been reported to be present in the plant [8–11]. Other constituents reported to be present are water, proteins, lipids, glucids, calcium phosphate and essential oils [12,13]. Essential oil of the leaf was reported to contain β-pinene, oct–1–en–3-ol, β-caryophyllene, octan–3-ol, and (E, E)–α-farnesene [13].

In this study, unlike in Okokon et al., 2016; we determined the phytochemical constituents and evaluated both the acute toxicity and analgesic properties of the methanol extract of the whole plant of *S. monostachyus* by first defatting with hexane (to remove non polar constituents), so as to enable us determine whether the polar constituents in the plant has analgesic activity. The effect of location and extraction method on the phytochemicals present in the plant material was also noted.

2. MATERIALS AND METHODS

2.1 Plant Material and Collection

The fresh whole plant of *S. monostachyus* was collected from an open field in Ikpoba - Okha Local Government Area of Benin City, Edo state, Nigeria. The plant was identified and authenticated by Dr. Emmanuel Izaka Aigbokhan of the Department of Plant Biology and Biotechnology, University of Benin. A herbarium voucher specimen with number UBN/ PCG/1659 was deposited in the herbarium of the Department of Pharmacognosy, University of Benin, Benin City, Nigeria.

2.2 Extraction

The whole plant of *S. monostachyus* was first rinsed under running tap water and air - dried at

room temperature. The dried materials were reduced to powder form using a mortar and pestle. 220 g of the pulverized plant was defatted with hexane using a Soxhlet apparatus, after which methanol was used for extraction. The extracts were concentrated by evaporation in evapourating dishes on a thermostatically controlled water bath at 40°C. The methanol extract was used for qualitative phytochemical screening, acute toxicity and antinociceptive tests.

2.3 Phytochemical Screening

Phytochemical screening of the methanol extract of *S. monostachyus* was carried out by employing standard procedures and tests [14, 15], to detect the presence or absence of chemical constituents such as glycosides, saponins, alkaloids, triterpenoids, steroids, tannins, phenolics and flavonoids.

2.4 Pharmacological Tests

2.4.1 Animal

Albino mice (20 – 32 g) of both sexes were used in this study. The animals were kept in different plastic cages at environmentally controlled room temperature in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. The animals were allowed to acclimatize in the animal house for two weeks and were fed with standard pelleted feed (Premier Feed Mills Co. Ltd). Water was allowed *ad libitum.* They were fasted for 18 hours prior to the experiment where necessary. This research was carried out in-line with internationally recognized protocols involving the use of laboratory animals. Administration of the extract was done orally via an oral gastric gavage.

2.4.2 Acute oral toxicity test

Twenty - four (24) albino mice were divided into four (4) groups (A-D) of 6 animals per group. The first group, group A (control) received 10 mL/kg of 5% Tween-80 solution while groups B, C and D were administered 1, 2 and 5 g/kg of the methanol extract respectively. Deviation in the general behaviour associated with the administration of the plant extract in mice such as weakness, aggressiveness, food refusal, diarrhea, discharge from eyes, ears, noisy breathing and mortality were monitored for 24 hr (immediate effects) after dosing and then for two weeks (14 days), for delayed effects post drug administration [16].

2.4.3 Anti – nociceptive tests

2.4.3.1 Acetic acid induced writhing model

Twenty - five (25) albino mice were randomly divided into five groups (A-E) of five mice per group. Group A received 10 mL/kg of 5% Tween-80 solution (control), groups B, C and D received 125. 250 and 500 mg/kg of the plant extract respectively, while group E received 100 mg/kg of acetylsalicylic acid (Aspirin; reference). Fifty minutes after administration of the drug and extract, 0.7% glacial acetic acid (10 mL/kg) was given intraperitoneally (i.p) to all the mice to induce pain characterized by abdominal constrictions or writhes. The number of writhes observed in each mouse was counted for 30 minutes and recorded [17].

2.4.3.2 Hot plate model

Twenty - five (25) albino mice were randomly grouped into five groups of five mice each. Each of the mice was placed on a hot plate maintained at a temperature of 55 ± 0.2^0C and the pain reaction time (PRT) or latency period was determined with a stopwatch. This was recorded and it represented the time taken for the mice to react to the pain stimulus. The response to pain stimulus considered include; jumping, raising and licking of hind foot. This served as control pain reaction time (0 min). The mice were treated as follows: Group A received 10 mL/kg of 5% Tween-80 solution (control), groups B, C and D received 125, 250 and 500 mg/kg of the plant extract respectively, while group E received 10 mg/kg pentazocine (reference). Thirty (30) minutes after the drug and extract administration, the pain reaction time for each mouse was again determined and recorded using the same method described above. This was repeated at 60, 90, 120 and 150 minutes after the drug and extract administration.

2.5 Data Analysis and Presentation

Data are presented as bar graph and tables. The results were expressed as Mean ± SEM (Standard Error of Mean). Statistical analysis was carried out using one-way analysis of variance (ANOVA) with Dunnett's post hoc test. n represents the number of animals (replicates) per group.

3. RESULTS

3.1 Phytochemical Screening

Phytochemical screening of the methanol extract revealed the presence of glycosides, saponins, flavonoids, steroids, triterpenes and alkaloids (Table 1). These are valuable chemical constituents that play crucial roles in plants and animals.

Table 1. Phytochemical constituents present in the methanol extract of *S. monostachyus*

S/no.	Phyto constituents	Status
1	Glycosides	+ve
2	Saponins	+ve
3	Flavonoids	+ve
4	Phenolics	-ve
5	Tannins	-ve
6	Steroids	+ve
7	Triterpenes	+ve
8	Alkaloids	+ve

Key: +ve means present and -ve means absent.

3.2 Acute Toxicity Test

At the end of the acute toxicity study, no deviation from normal behavior was observed and there was no death at the end of 14 days. Based on results obtained from the pilot study, therapeutic doses equivalent to $^1/_{40}{}^{th}$, $^1/_{20}{}^{th}$ and $^1/_{10}{}^{th}$ of the highest dose tested (5 g/kg), were chosen for evaluation of the analgesic effect of the plant extract.

3.3 Anti – nociceptive Test

This includes results obtained in the acetic acid-induced writhing and hot plate models.

3.3.1 Acetic acid - induced writhing model

The result of the analgesic activity of the methanol extract of *S. monostachyus* is shown in Fig. 1. Here, the extract caused a dose–dependent reduction in the number of writhes produced by acetic acid. The reference drug, Aspirin, had a better activity when compared with that of the extract.

3.3.2 Hot plate model

The result of the methanol extract in the hot plate model is shown in Table 2. The extract and reference drug (pentazocine) was observed to cause an increase in the pain reaction times of mice used in this pain model.

4. DISCUSSION

From ancient times, plants have been of great importance to man and animals by their provision of food, shelter and drugs. As drugs, man has utilized the healing potentials of plants to treat a wide range of ailments ranging from minor to severe [18]. These healing potentials of plants are usually due to the presence of important and medicinally useful secondary plant constituents (phytochemicals or chemical constituents) which may be present in either the leaves,

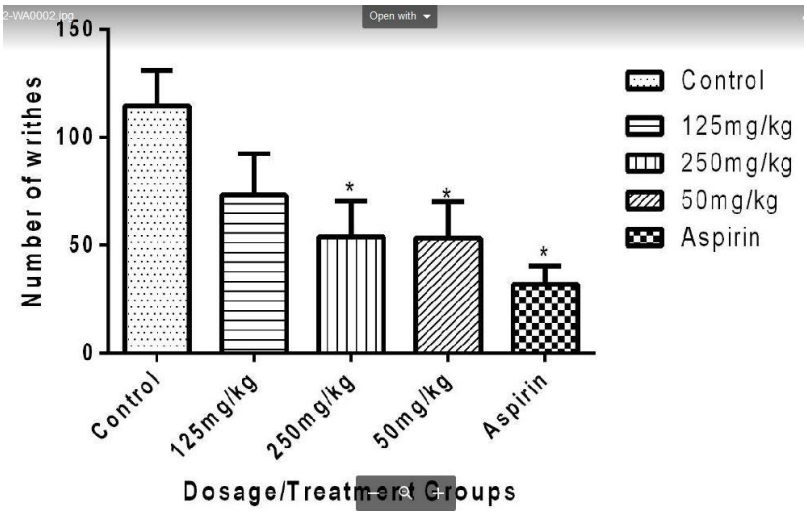

Fig. 1. Effect of methanol extract of *S. monostachyus* on the number of writhes produced by acetic acid

** p < 0.05 and ** p < 0.01, significantly different when compared to control. n = 5.*

Table 2. Effect of methanol extract of *S. monostachyus* on latency periods in mice

Treatment groups	Pain reaction time (Sec)					
	0 min	30 min	60 min	90 min	120 min	150 min
5% Tween-80 (10 mL/kg)	2.32 ±0.16	2.46 ± 0.26	2.34 ± 0.22	2.54 ± 0.33	2.52 ± 0.10	2.58 ± 0.12
125 mg/kg Extract	2.28 ± 0.26	2.46 ± 0.32	2.68 ± 0.21	2.74 ± 0.24	2.68 ± 0.19	2.54 ± 0.19
250 mg/kg Extract	2.08 ± 0.18	2.44 ± 0.14	2.58 ± 0.12	2.62 ± 0.18	3.02 ± 0.26*	1.98 ± 0.07
500 mg/kg Extract	1.88 ± 0.06	2.82 ± 0.14*	3.70 ± 0.20**	4.16 ± 0.36**	2.90 ± 0.18*	2.32 ± 0.20
Pentazocine	2.22 ± 0.10	3.20 ± 0.55*	3.46 ± 0.26*	3.42 ± 0.21*	2.96 ± 0.20	2.80 ± 0.28

Values are Mean ± SEM. p < 0.05, significantly different when compared to control (0 min). n = 5.*

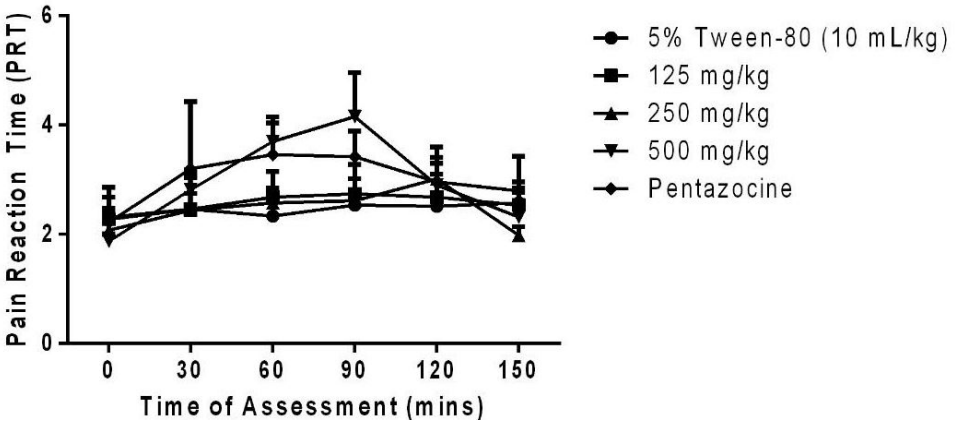

Fig. 2. Linear graph showing mean+/-SD of tween 80, different doses of methanol extract and Pentazocine

barks (stems or roots), roots, fruits, flowers or seeds. Examples of these chemical constituents are glycosides, saponins, tannins, flavonoids, terpenoids, steroids and alkaloids. The presence of these chemical constituents in *S. monostachyus* thus confirmed the plant as medicinal, like in the report of Okokon et al. 2016. However, unlike in the report of Okokon et al. 2016, polyphenolics and tannins were found to be absent, while alkaloids were present in the whole plant of *S. monostachyus*. The absence and presence of these phytochemicals may be attributed to the influence of environmental factors such as temperature, rainfall, humidity, altitude, soil characteristics, amongst others on the growth of *S. monostachyus* [19]. Thus, the place of collection (location) of *S. monostachyus* may affect the type of chemical constituents present in the plant material. The usefulness of these phytochemicals (glycosides, saponins, flavonoids, triterpenoids and alkaloids) as medicinal agents, are well documented [8,20, 21]. Unlike in the report of Okokon et al., 2016 were the plant material was extracted using cold method of extraction, this study showed that the phytochemicals in this plant material are thermostable, as extraction with hot method (Soxhlet apparatus) resulted in no loss of phytochemicals and their activity.

The acute toxicity test revealed the methanol extract to be practically nontoxic, as no toxic effect and death was observed after administration of the extract up to 5 g/kg [22]. Thus, the extract may be considered safe for acute usage.

In the acetic acid-induced writhes model, it was observed that the methanol extract caused a reduction in the number of writhes produced by acetic acid. This reduction was observed to be significantly ($p < 0.05$) different from control, for the 250 and 500 mg/kg doses of the extract. The reference drug (Aspirin) was significantly ($p < 0.01$) different from control and had a better activity than the extract (Fig. 1). These results

thus confirm the usefulness of the methanol plant extract in the treatment of peripherally mediated pain which is usually induced by the use of acetic acid. In the hot plate model, which is usually used to evaluate the usefulness of any agent in the treatment of centrally mediated analgesia, the methanol extract caused an increase in the pain reaction times (latency periods) of the mice, which is indicative of its usefulness in the treatment of centrally mediated analgesia. The 250 mg/kg extract only caused significant ($p < 0.05$) effect at 120 min after drug administration while the 500 mg/kg extract caused significant ($p < 0.05$) effect at 30 min, which persisted up to 120 min (Table 2). This effect of the 500 mg/kg extract was better and more pronounced than that of the control, 125 and 250 mg/kg extract. The reference drug (pentazocine) caused significant ($p < 0.05$) effect at 30 mins, and persisted up to 90 mins, when compared to control. This, therefore, indicates that, the methanol extract at a dose of 500 mg/kg, had better analgesic activity with longer duration of action when compared with the reference drug. Thus, the extract is suitable for centrally mediated analgesia and confirms *S. monostachyus*, one of the flowering plant species belonging to the Lamiaceae family, to have analgesic activity. The family Lamiaceae is known to contain plant species that have been evaluated for its' analgesic activities [23,24]. Examples of such plant species are *Leonurus cardiaca* L. [25], *Eremostachys laciniata* [26] and *Solenostemon scutellarioides* [27], amongst others.

5. CONCLUSION

It is worth mentioning that, like other studies reported on the plant *Solenostemon monostachyus*, this study confirmed the presence of useful phytochemicals in the plant. However, unlike these other studies, this study reported the presence of these phytochemicals in the methanol extract of the plant material, after first defatting with hexane. Also, these phytochemicals were shown to be affected by location and found to be thermostable, as extraction using a hot method resulted in no loss of phytochemicals and its' activity. These phytochemicals could either serve as templates for the synthesis of drugs or as drugs themselves. The study also validated the ethnomedicinal use of the whole plant of *S. monostachyus* as well as its' polar constituents (in the methanol extract) for the treatment of pain.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval for the use of laboratory animals was obtained from the Ethics committee, Faculty of Pharmacy, University of Benin, Benin City, Nigeria (EC/FP/017/06).

ACKNOWLEDGEMENT

The technical help of Dr. O. Edosuyi of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria is appreciated and thankfully acknowledged.

COMPETING INTEREST

We declare that no competing interest exists.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23339