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Antimicrobial, antioxidant and quantitative phytochemical evaluation of the dichloromethane and methanolic leaf extracts of *Solenostemon monostychus* P. Beauv

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Abstract

Solenostemon monostachyus P. Beauv (Lamiaceae family) is an important herb that is widespread in West and Central Africa.The leaves are used in various decoctions traditionally to treat diseases most especially microbial infections associated with the foot. In this study, the phytochemicals (qualitative and quantitative) of the plant were determined. The antimicrobial and antioxidant activities of the plant were also evaluated by standard methods. Results showed alkaloids, tannins, flavonoids, saponins phenolics in the methanolic extract of *Solenostemon monostachyus.* Cardiac glycosides and steroids absent in the methanolic extract were found present in the dichloromethane extract. The quantitative phytochemicals recorded were alkaloids (18.05 ± 0.87%), saponins (11.3 ± 0.56 %), tannins (3.3 ± 0.19 GAE). The antioxidant activity testing showed that the methanolic extract has higher DPPH scavenging ability over dichloromethane extract and standard ascorbic acid. In the antimicrobial activity testing, the cup-plate diffusion method was used and the result showed that the dichloromethane extract inhibited the growth of *Bacillus species* at concentrations 100 mg/ml, 50 mg/ml, 20 mg/ml and 10 mg/ml while methanolic extract inhibited the growth of *bacillus species* only at 100 mg/ml with 5 mm zone of inhibition. However, both methanol and dichloromethane extract showed no antimicrobial activity on the other test organisms such as *Psudomonas aeruginosa, Escherichia coli and Staphylococcus aureus.* In conclusion both methanolic and dichloromethane extracts of *Solenostemon monostachyus* are potential sources of antimicrobial and antioxidant.

Keywords: *Solenostemon monostachyus*; Quantitative analysis; Antimicrobial and antioxidant activities evaluations

1. Introduction

Over many centuries, plants have devised many mechanisms to repel attack by predators like insects and fungi by synthesizing different biochemical compounds. Many of these compounds have proved useful in the management of different human diseases. Phytochemicals are secondary metabolites found in plants with no nutritive value. They are known to have biological activities such as antioxidation [1]. The treatment of infectious diseases caused by resistant bacterial strains, represent one of the main challenges of medicine today [2]. The relative unavailability of medicines in developing countries and the appearance of widespread multiresistant bacterial strains let the effect of these diseases particularly large and considerable [3, 4]. In the search for new alternatives to treat these infections, many researchers have been looking for novel compounds derived from natural products to replace, or be used in combination with conventional antibiotics [2].

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In this modern world with emerging new technologies, new pathogenic infections are also emerging. Antimicrobial compounds have drawn attention of many pharmacologists and are now becoming one of the major areas of research. It is well known that plants are a major rich source of antimicrobial compounds. The antimicrobial properties of such plants remain unrevealed [5]. One such plant is *Solenostemon monostachyus*. *Solenostemon monostachyus* P. Beauv (Lamiaceae family) is an important herb that is widespread in West and Central Africa. It occurs as an annual weed in anthropogenic habitats and rocky savannahs. It is slightly succulent, aromatic and grows up to 100 cm tall [6]. The leaves are eaten as food, and the plant has been cultivated for this purpose. *Solenostemon monostachyus* has numerous medicinal uses. The leaf is considered as a sedative, it improves digestion and is applied internally to treat colic, and cough [7]. The leaves have been reported to treat dysmenorrheal, haematuria, female sterility, rheumatism, foot infections and snakebites [8]. To screen and quantify the phytochemicals as well as evaluate the antimicrobial and antioxidant properties of the leaf extracts of *Solenostemon monostychus*. Research has also shown that the leaves possess antimicrobial activity.

2. Material and methods

2.1. Reagents and Chemicals

Methanol, Dichloromethane, glacial acetic acid, n-butanol, sodium hydroxide, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl radical all other chemicals were of analytical grade.

2.2. Organisms Used

Gram-positive organisms used in the study were *Staphylococcus aureus* and *Bacillus species*. Gram-negative organisms used were *Escherichia coli* and *psudomonas aeruginosa* as gram-positive organisms.

2.3. Sample Collection and Preparation

The leaves of *Solenostemon monostychus* used for this study were collected from the Niger Delta University Botanical garden. Plant was authenticated at the Department of Pharmacognosy, Faculty of pharmacy, Niger Delta University, Bayelsa state. The leaves were washed with distilled water and allowed to dry at room temperature for about a week interval then were further dried in the laboratory oven at a temperature of 40°C for 30 minutes and were crushed and blended with an electronic blender into coarse powder particles.

2.4. Extraction of Plant Materials

200 grams of the powdered plant materials were carefully weighed using the analytical balance and were transferred into a clean 1500 ml reagent bottle containing methanol (1100 ml) and allowed to stand for 72 hours with frequent agitations. The mixture was filtered and the solvent removed by evaporation over a hot water bath. Similar procedure was carried out for the dichloromethane extract.

2.5. Phytochemical Screening

The methods described in Trease and Evans [9] was adopted.

2.5.1. Estimation of alkaloid content

The total alkaloid content was estimated using the method described by [10], a 0.5 g of the pulverized *Solenostemon monostychus* was immersed in 200 mL of 10% acetic acid in ethanol. The mixture was allowed to stand for 4 hr at room temperature. It was subsequently filtered and the filtrate was concentrated using a water bath at 55 ^{o}C to a quarter of its original volume. Concentrated ammonium hydroxide was added in single drops until completion of the precipitation process. The solution was then washed with dilute ammonium hydroxide and filtered again. The residue obtained was first dried and then weighed. The alkaloid content was calculated using the equation:

% Alkaloid = Weight of precipitate / Weight of original sample ×100

2.5.2. Saponin Determination

The method used was that of [11]. The sample *Solenostemon monostychus* was ground and 20 g was put into a conical flask and 20% aqueous ethanol (100 ml) was added. The sample was heated over a hot water bath for 4 hr with continuous stirring at about 55 ^{o}C. The mixture was filtered and the residue re-extracted with another 20% ethanol (200 ml). The concentrate was transferred into a 250 ml separatory funnel and diethyl ether (20 ml) was added and

shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and n-butanol (60 ml) was added. The combined n-butanol extracts were washed twice with 5% aqueous sodium chloride (2 x 10 ml). The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight; the saponin content was calculated thus:

% Saponins contents = Weight of residue /Weight of sample ×100

2.5.3. Estimation of Tannin content

The total tannin content was estimated using the Folin - Ciocalteu method of [12]. Distilled water (7.5 ml) was added to a tube containing 0.1ml of *Solenostemon monostychus, a* 0.5 ml of Folin-Ciocalteu phenol reagent and 1 ml of 35 % Na_2CO_3 solution was then added. The whole solution was made up to 10 ml with distilled water. The mixture was vortexed and kept at room temperature for 30 min. The absorbance was read at 725 nm using a spectrophotometer. The total tannin content was expressed as mg/g GAE equivalent.

2.6. DPPH radical scavenging assay

DPPH radical scavenging activity of *Solenostemon monostychus* extracts were determined according to the method described by [13] with some modifications. A preparation of 1 ml of 0.135 mM DPPH in methanol was mixed with 1ml of various concentrations (1 – 7 mg/ml) of the plant extracts and vitamin C. The mixture was left in the dark at room temperature for 30 min after being vortexed. The absorbance of the mixture was then measured spectrophotometrically at 517 nm. Vitamin C was used as standard. The DPPH radical scavenging activity was calculated from the equation:

DPPH radical scavenging activity = Abs control - Abs sample/ Abs control ×100

2.7. Antibacterial activity assay

2.7.1. Test microorganisms

For antimicrobial studies: The test organisms (*Staphylococcus aureus, KlebsiellaPnuemoniae*, *Pseudomonas Aeruginosa* and *Escherichia coli*) were procured from the Medical Microbiology and Parasitology Department, Niger Delta University, Bayelsa State and stored at -20 °C for further studies.

2.7.2. Assay

The antibacterial potential test was carried out using the agar disc diffusion method [14]. Negative controls were prepared by using the same solvents employed to dissolve the samples. Inhibition zones were measured and compared with the standard reference antibiotic amoxycillin. Each extract was subjected to serial dilution by using dimethyl sulphoxide (DMSO) as a solvent to give 100 mg/ml, 50 mg/ml, 20 mg/ml, and 10 mg/ml solutions of as *Solenostemon monostychus*. The concentration of amoxycillin standard used for this study was at 10 µg/ml. Each prepared concentration of the different extracts was tested for its antimicrobial activity against the test organisms (*Staphylococcus aureus, Bacillus species, Pseudomonas aeruginosa and Escherichia coli)* on nutrient agar plates using disc diffusion method. Whatman (No. 1), sterile filter paper discs (6 mm diameter) were impregnated with methanol and dichloromethane extracts of *Solenostemon monostychus* and placed on the inoculated agar. All the plates were incubated at 37 °C for 24 h. Evaluation of antibacterial activity was measured showing the diameter of the zones of inhibition against the tested bacteria. Each method in this experiment was replicated three times.

3. Results

Table 1 Phytochemical screening of methanolic and dichloromethane extracts of *Solenostemon monostychus*.

S/No.	Solvent	Methanol	Dichloromethane
	Sample	**Leaves**	**Leaves**
1	Saponins	+++	-
2	Phenolics	++	-
3	Alkaloids	+++	-
4	Flavonoids	+++	-
5	Tannins	++	-
6	Steroids	-	++
7	Cardiac glycosides	-	++

Key: - = absent; + = present; +++ indicates present in large amount.

Table 2 Quantitative phytochemical Analysis of *Solenostemonmonostychus*.

Phytochemicals	**Quantitative value**
Alkaloids	18.05± 0.87%
Tannins	3.3 ± 0.19 mg/GAE
Saponins	11.3 ± 0.56 %

Table 3 Zone of Inhibition of methanolic extract of *Solenostemonmonostychus* (mm).

Drug concentrations (mg/ml)	***Bacillus species.* (mm)**	***Escherichia Coli* (mm)**	***Pseudomonas aeruginosa* (mm)**	***Staphylococcus aureus* (mm)**
100	5	-	-	-
50	-	-	-	-
20	-	-	-	-
10	-	-	-	-
Amoxycillin10 µg/ml	-	-	-	-

Table 4 Zone of Inhibition of dichloromethane extract of *Solenostemonmonostychus* (mm).

Drug concentrations (mg/ml)	***Bacillus species* (mm)**	***Escherichia Coli* (mm)**	***Pseudomonas aeruginosa* (mm)**	***Staphylococcus aureus* (mm)**
100	8	-	-	-
50	6	-	-	-
20	6	-	-	-
10	5	-	-	-
Amoxycillin10 µg/ml	20	18	15	21

Key - = No zone of inhibition

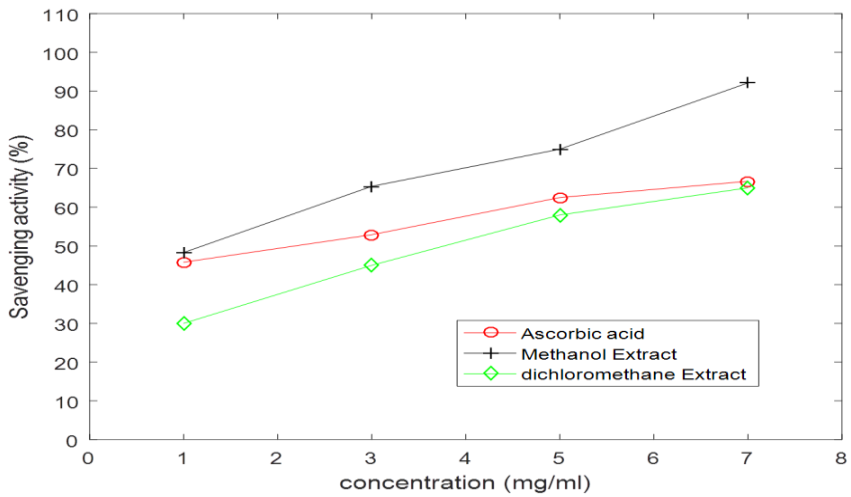

Figure 1 A graph showing the DPPH scavenging activity of methanolic and dichloromethane extracts of *Solenostemon monostychus*.

4. Discussion

Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer. [15]. All secondary metabolite components displayed antioxidant and antimicrobial properties through different biological mechanisms. Most of the secondary metabolite components were isolated and identified in the polar plant crude extracts

The biochemical screening of methanolic and dichloromethane crude extracts from dry leaf samples of *Solenostemon monostychus* used in this study revealed that the crude extracts contained phytochemicals (Table 1). The methanolic extract showed the presence of alkaloids, flavonoids, tannins, phenolics and saponins. The dichloromethane extract revealed the presence of steroids and cardiac glycosides. Therefore, the detected different bioactive compounds in different crude extracts from *Solenostemon monostychus* may be responsible for the antioxidant and antimicrobial activities. Several reports are available on flavonoid groups which exhibited high potential biological activities such as antioxidant, antimicrobial and anticancer [16]. These results corresponds with the results reported by [17]. The quantitative phytochemical determination of the leaves of *Solenostemon monostychus* shows high concentrations of alkaloids and saponins of 18.05 ± 0.87 and 11.3 ± 0.56 % of extract respectively as showed in table 2, and low concentrations of tannins (3.30 ± 0.19 mg/GAE).

The methanolic and dichloromethane extracts showed antimicrobial activity against *Bacillus species, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The dichloromethane extracts at concentrations of 100 mg/ml, 50 mg/ml, 2 mg/ml and 10 mg/ml were active against Bacillus species with zones of inhibitions of 8, 6, 6, 5 mm respectively while no action of dichloromethane extract against *Pseudomonas aeruginosa*, *Escherichia coli and Staphylococcus aureus* at the same concentrations results are showed in table 4. The methanolic extract of *Solenostemon monostychus* leaves showed antimicrobial activity against bacillus species only at a concentration of 100 mg/ml with a zone of inhibition of 5 mm. Alkaloids and phenolics in plants are responsible for antimicrobial action, the presence of these phytochemicals in *Solenostemon monostychus* may be responsible for its significant antimicrobial actions. Hence, the plant can be a source of drug for the treatment of infectious diseases. These results are in line with the recent work of [18] who also reported antimicrobial properties of *Datura motel* extracts.

The antioxidant activity through free radical scavenging activity of DPPH method of the methanolic and dichloromethane extracts of *Solenostemon monostychus* dry leaves at 1-9 mg/mL concentrations was determined and compared with ascorbic acid as standard (Fig. 1). The principle of antioxidant activity is their interaction to produce a stable compound. The role of DPPH method is that the antioxidants react with the stable free radical. During the free radical reaction, DPPH radical is converted into a non-radical with colour change. The rate of colour change gradually decreases to indicate the scavenging potentials of the sample antioxidant. The crude extracts of *Solenostemon monostychus* contain flavonoid, saponins, tannins, phenolics and aromatic compounds. All these bioactive compounds were able to discolour DPPH solution by their hydrogen donating ability as revealed in figure 1 [19].

5. Conclusion

The result obtained shows that *Solenostemon monostychus* leaf has phytochemicals such as phenolics, flavonoids, saponins, alkaloids, cardiac glycosides, and tannins. The presence of alkaloids, phenolic, and flavonoids may be responsible for the antimicrobial and antioxidant properties. It could be concluded that *Solenostemon monostychus* leaf extracts is a potential source of active ingredients that could be used as antimicrobial and antioxidant.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of intedest

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