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Comparative Phytochemical and Anti-microbial Studies of Leaf, Stem, Root of *Spathodea companulata*

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Authors' contributions

This work was carried out in collaboration among all authors. Author CEA designed the study, supervised the work and wrote part of the literature. Author QMO carried out the analysis. Authors CJOA and NLU wrote the literature and edited the work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To compare the phytochemicals and anti bacterial activities of leaf, stem and root extracts of *Spathodea companulata.*
Methodology: The leaf, stem and root of *Spathodea companulata* were collected, washed, air-dried, ground and each extracted with water, methanol, ethyl acetate and n-hexane. The extracts were analysed for the presence of phytochemicals. Antimicrobial analysis was also carried out on the extracts.
Results: Glycosides were present in all the extracts except stem aqueous extract. Steroids were found absent in most of the extracts except methanol stem and n-hexane root extracts. Saponins were found in methanol, aqueous and ethyl acetate extracts of the root and stem, and in methanol and aqueous leaf extracts. Alkaloids were present in methanol and aqueous extracts of the leaf and root, and in ethyl acetate and n- hexane extracts of the stem. Quantity of Alkaloids and tannins were higher in leaf, while flavonoids and glycosides were higher in the stem and the roots

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contained higher amount of saponins. The extraction solvent polarities played important role in the type of metabolites extracted. The stem extracts were found to be most active against the seven test organisms used.

Conclusion: More work should be done on the isolation and identification of compounds responsible for some of the pharmacological effects of the plant parts and the subsequent development of the compounds in formulation of drugs.

Keywords: Spathodea companulata; phytochemicals; antimicrobial; leaf; stem; root.

1. INTRODUCTION

Plants contribution in various fields such as medical, pharmaceutical and food industry brings a lot of benefits to human capital. Especially in the medical field, plants are major contributors for medicinal drugs as it itself is rich in various medicinal agents [1]. Plants continue to play a key role in health care systems in Africa, indeed up to 80% of people rely on traditional medicine (TM) for their primarily health care [2]. The use of plants for therapeutic purposes, whether in the treatment or prevention of diseases, is one of the oldest human medicinal practices and several traditional clinical procedures have been registered using many plant species [3]. *Spathodea campanulata* P. Beauv is commonly known as the African tulip tree, fountain tree, pichkari, Nandi flame or Flame of the forest, ímí éwū = goat's nose(NIGERIA, IGBO), native of West African tropical forests, a member of *Bignoniaceae* is a large, upright, 50 to 60-foot tree has a dense, 40-foot-wide crown and one-and-one-half-foot-long, pinnately-compound, evergreen leaves composed of four-inch leaflets [4]. The plant is widely distributed in Nigeria and other West African countries, and is reputedly used for epilepsy and convulsion control, against kidney disease, urethritis and as antidote against animal poisons. Decoctions of the plant stem are also employed against eczemas, fungal skin disease, herpes, stomach ache, diarrhea. Wound healing, enemas. Its leaf decoction is used in the treatment of pain, inflammation, constipation and dysentery. *S. campanulata* flowers and bark are used to treat fever, convulsion, bacterial infections, HIV, poor blood circulation, gastro-intestinal diseases, respiratory ailments, genital-urinary system disorders, filaria, gonorrhea, epilepsy and mental disorders. The flowers which are used as diuretic and anti-inflammatory; the leaves help in curing kidney diseases, urethra inflammations and animal poisoning [5,6]. Flowers of *S. campanulata* development is unique as inner whorls develop immersed in a fluid called water calyces and the fluid has been demonstrated to possess antimicrobial activities [7]. Traditionally, the seeds, bark and leaves are used for swollen cheeks, cleaning of new born babies and antimalarial, mulluscicidal, antioxidant [8]. Different parts of *Spathodea campanulata* such as flowers, leaves, stem, bark and roots have been reported for possessing anti-inflammatory, analgesic, cytotoxic, anti-diabetic and anticonvulsant activity. Phytochemical screening showed the presence of various secondary metabolites like alkaloids, tannins, flavonoids, glycosides and sterols [9,10]. The leaves possess analgesic and anti-inflammatory activities. The ethanolic extract of leaf and flower shows antimicrobial properties. The roots peel contains iridoid glucoside and phenolic derivatives p-hydroxy-benzoic acid and methyl p-hydroxy-benzoate [11]. Hexane fraction of methanol leaf extract of *S. campanulata* has been reported to have very high antioxidant activity [12]. *S. campanulata* extracts have also been reported to be a promising biolarvicides [13]. Aqueous methanolic extract of the stem bark of *S. campanulata* has also been found to have hypoglycemic and antihyperglycemic effects [14]. The result of anti-cataract activity of *Spathodea campanulata* flower bud exudates against cataractogenesis using rat lenses revealed that exudate elicited its anti-cataract potential through its anti-oxidant activities [15]. The results of the evaluation of total phenolic content and antioxidant activity of different solvent extracts of leaf material of *Spathodea campanulata* P. Beauv. and investigation of their proliferation inhibition potential against EAC cell line revealed the presence of remarkable antioxidant and anticancer properties in the ethanolic extract of *S. campanulata* leaf. Ethanol (70%) could be the suitable solvent to recover the polyphenolic compounds with high antioxidant and anticancer potentials from *S. campanulata* leaf [16]. The result of In vitro free radical scavenging activity of leaves of *Spathodea campanulata* p. Beauv clearly indicated that leaves of *Spathodea campanulata* is an effective antioxidant and also showed superoxide and nitric oxide radicals scavenging activity [17]. The flower of

Spathodea campanulata dye was used to dye silk at optimized dyeing conditions, using combination of mordant and evaluate the resultant colour fastness of the dyed samples to washing, rubbing, perspiration and light. However; the colour fastness of the test samples exhibited excellent fastness to washing with different mordants and some extracts shown good results after mordanting [18]. Some compounds like: Myristic, palmitic, Palmitoleic, stearic, oleic, Arachidic, Gondoic, Behenic, Erucic, Lignoceric acids have been found to be present in *S. campanulata* [19]. The decoction of the root of *S. campanulata* have showed inhibitory activity on 7 *M. ulcerans* strains isolates with MIC mean value of 25 μg/mL [20]. The aim of this work is to compare the phytochemicals present in the leaf, stem and root extracts of *Spathodea companulata*, check the effect of polarity of solvents used on the extraction of the metabolites and compare the antimicrobial activities of the extracts.

2. METHODOLOGY

2.1 Sample Collection and Preparation of Samples

Leaf, stem and root samples of *Spathodea companulata* were collected from Ozubulu in Ekwusigo LGA of Anambra State, Nigeria and identified by two taxonomists in Botany Deparment of Nnamdi Azikiwe University, Awka. The samples were washed with distilled water, air-dried, ground and stored in air-tight container.

2.2 Extraction

In 200 ml of each of the four solvents – water, methanol, ethyl acetate and n-hexane, 20 g each of the ground plant leaf, stem and root was soaked. Each of the four solutions was shaken and the mixtures were left to stand at room temperature for 48 hours with intermittent stirring. At the end of 48 hours, the solutions were filtered. The filtrates were collected and concentrated using a rotary evaporator. The solvents used were selected based on the differences in their polarities – water(protic polar) > methanol(protic polar) > ethyl acetate(semipolar, aprotic polar) > n-hexane(non polar).

2.3 Phytochemical Analysis

Qualitative analyses were carried out using the standard methods described by Edeoga et al. [21], Raaman [22], Harborne [23], Mera et al. [24], Gul et al. [25], Banu and Cathrine [26], Sivanandham [27], Roghini and Vijayalakshmi [28], De Silva et al. [29], Gupta et al. [30] to ascertain the presence of phytochemicals, such as tannins, alkaloids, flavonoids, steroids, saponins, glycosides in the extracts.

2.4 Test for Presence of Alkaloids

2.4.1 Wagner's reagent test

In a test tube, 1 ml of the filtrate was added, followed by addition of 1 ml of Wagner's reagent (1.27 g of iodide and 2 g of potassium iodide are weighed, mixed and dissolved in 30 ml distilled water and made up to 100 ml with distilled water). The solution was mixed properly and the colour change was observed. A reddish brown precipitate indicated presence of alkaloid.

2.4.2 Meyer's reagent test

In a test tube, 1 ml of filtrate was added, followed by addition of 1 ml of Meyer's reagent (1.4 of mercuric chloride in 60 ml distilled water and 4.5 g of potassium iodide in 20 ml distilled water) in the test tube. The solution was mixed properly and the colour change was observed. A cream colour /precipitate indicated presence of alkaloid.

2.5 Test for the Presence of Steroids

Liebermann-Burchard's test was used to test for steroids. In 1 ml of each extract, 0.5 ml of acetic anhydride was added and cooled. This was later mixed with 0.5 mL of chloroform and 1 ml of concentrated sulphuric acid was carefully added using a pipette. There was the formation of a reddish brown ring which indicated the presence of steroids.

2.6 Test for the Presence of Flavonoids

2.6.1 Ammonium test

In 4 ml of filtrate, 1 ml of dilute ammonia solution was added and shaken. The layers were allowed to separate and the yellow colour in the ammoniacal layer indicated the presence of flavonoids.

2.6.2 Aluminum chloride test

In 4 ml of the filtrate, 1 ml of 1% aluminum chloride solution was added and shaken. The layers were allowed to separate and the yellow

colour in the aluminum chloride layer indicated presence of flavonoids.

2.6.3 Test for the presence of saponins

In 10 ml of the filtrate, 5 ml of distilled water was added and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for emulsion.

2.7 Test for the Presence of Tannins

2.7.1 Ferric chloride test

To 3 ml of the filtrate in the test tube, few drops of ferric chloride were added. A greenish black precipitate indicated the presence of Phenolic Compounds.

2.7.2 Lead acetate test

A few drops of lead acetate were added to 3 ml of the filtrate in a test tube. A cream precipitate appeared showing presence of Phenolic Compounds.

2.8 Test for Glycosides (Borntrager's Test)

To 0.5 g of each of the extracts, concentrated hydrochloric acid was added, hydrolysed for 2 hours on a water bath, filtered and the hydrolysate was obtained. To 2 ml of the hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added. Presence of glycosides was indicated by pink colour.

2.9 Quantitative Determination of the Phytochemical Constituents of the Plant Samples

2.9.1 Alkaloids determination

Into a 250 ml beaker, 5 g of the powdered sample and 200 ml of 10% acetic acid in ethanol were added. The mixture was stood for four (4) hours at room temperature (25°C). Thereafter, the mixture was filtered through Whatmann filter paper No. 42. The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia hydroxide solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the residue on the filter paper is the alkaloid, which is dried in the oven at 80°C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed.

$$\% \text{ weight of Alkaloid} = \frac{W2 - W1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where,

W_1 = weight of filter paper
W_2 = weight of filter paper + alkaloid precipitate (residue)

2.9.2 Flavonoids determination

With 100 ml of 80% aqueous methanol, 10 g of the plant sample was extracted at room temperature. The whole solution was filtered through Whatmann filter paper No. 42 (125mm). The filtrate was transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

$$\% \text{ Flavonoid} = \frac{W2 - W1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where,

W_1= Weight of crucible
W_2= Weight of crucible + Flavonoid extract (residue)

2.10 Determination of Saponins

Into a conical flask, 20 g of the sample and 100 cm^3 of 20% aqueous ethanol were added. The mixture was heated over a hot water bath for 4 hours with continuous sliming at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in water bath. After evaporation, the sample was dried in oven to a constant weight. The saponin content was calculated in percentage.

$$\% \text{ Saponin} = \frac{W2 - W1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where,

W_1= Weight of filter paper
W_2= Weight of filter paper + Saponin extract (residue)

2.11 Tannin Determination

Into a 50 ml plastic bottle, 500 mg of the sample was added. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml of volumetric flask and made up the mark. Then 5 ml of the filtrate was added into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

$$\% \text{ Tannin} = \frac{An}{As} \times C \times \frac{100}{W} \times \frac{Vf}{Vn}$$

An= Absorbance of test sample
As= Absorbance of standard solution
C= Concentration of standard solution
W= Weight of sample
Vf= Total volume of extract
Vn= Volume of extract analyzed

2.12 Glycosides Determination

In 1 ml of 2% solution of 3,5-dinitro salicylic acid in methanol and 1 ml of 5% aqueous NaOH, 1 ml of each of the extracts was added. The mixture was boiled for 2 minutes (until brick-red precipitate was observed), filtered and dried in an oven at 50°C. The cardiac glycoside was calculated in percentage.

2.13 Antimicrobial Activities of Leaf Stem and Root Extracts of *S. companulata*

Antimicrobial activities of the extracts of leaf, stem and root of *Spathodea companulata* was determined using a modified Kirby-Bauer as described by Kowti et al. [31], Balouiri et al. [32] disc diffusion method.

2.13.1 Media used

Nutrient agar for bacteria and Sabouraud dextrose agar for fungus.

2.13.2 Test micro organisms

Gram positive bacterium- *Staphylococcus aureus*, Gram negative bacteria- *Escherichia coli, Salmonella typhimurium, Vibrio cholera, Pseudomonas aeruginosa, Klebsiella pneumonia* and fungus- *Candida albicans* were clinical strains obtained from the stock culture of Microbiology Laboratory Awka.

2.14 Preparation of Culture Media

Nutrient Agar was prepared by dissolving 12 g of nutrient agar in 1 L of water contained in a sterile conical flask. The resulting mixture was properly shaken, covered with cotton wool and foil. The flask was sterilized by autoclaving for 15 minutes, allowed to cool, poured into sterile Petri dishes, allowed to set and used in culturing *S. typhimurium, V. cholera, P. aeruginosa and K. pneumonia, E. coli, S. aureus*. Sarbaround dextrose agar (SDA) was prepared by soaking 65 g of SDA in 1 L of water contained in a sterile conical flask for 10 minutes. The mixture was shaken and sterilized by autoclaving for 15 minutes at 120°C, cooled to 45°C. The mixture was poured into sterile Petri dishes, allowed to gel and used in culturing *C. albicans*.

2.15 Method

A sterile cork-borer was used to bore holes of 5 mm in diameter in the media in the Petri dish. The holes were labeled according to the extract used, and the Petri dish was also labeled according to the microbes tested for. A wire loop was sterilized on a flame and used to collect 100 µ of the *S. aureus*, *E. coli, S. typhimurium, V. cholera, P. aeruginosa, K. pneumonia*, *C. albicans* and were inoculated on the labeled Petri dish. The organism was distributed evenly on the media using streak method. The extracts were tested using 10 mm sterilized filter paper discs. Discs were impregnated with 250 µg/ml of each of the extracts and placed into inoculated plates. The plates were allowed to stand at 4°C for 2 hours before incubation with the test microbial agents. Nutrient agar was incubated for 24 hours at 37°C while the Sabouraud dextrose agar was incubated for 3 days at 25°C. Water was absorbed into the disk from the agar. The antimicrobial began to diffuse into the surrounding agar and inhibited germination and growth of the test microorganism. The rate of diffusion through the agar was not as rapid as the rate of extraction of the antimicrobial out of

the disk, therefore the concentration of antimicrobial was highest closest to the disk and a logarithmic reduction in concentration occurred as the distance from the disk increased and then the diameters of inhibition growth zones were measured in millimeters.

3. RESULTS AND DISCUSSION

The result of qualitative phytochemical analysis (Tables 1-3) showed that the methanol leaf extract of *S. cmpanulata* contained alkaloids, saponins, tannins and glycosides. The presence of tannins in the leaf extract could be the basis to the therapeutic use of the leaf as an antidote to animal poisoning and antioxidant. Onah [12] also revealed that methanol leaf extract of *S. companulata* showed antioxidant property, other researchers also reported that methanol(polar) extract has a high effectiveness as antioxidants, with these reports it is not surprising that the plant is used as an antioxidant [8,12,16,17,33,34]. The absence of flavonoids in the methanol extract is in line with report of Pulipati et al. [11] that methanol leaf extract of *S. companulata* contained no flavonoids. The methanol stem extract contained all metabolites except alkaloids and steroids. The presence of tannins in the methanol leaf, stem and root extracts is in line with the report of Ghasemzadeh et al. [35], that methanol(polar) was most effective in the extraction of phenolic compounds and still confirmed the use of most parts of the plant as antioxidants. Also the use of stem as anti diarrheal agent could be as a result of the presence of tannins in the methanol stem extract [10,6,33]. The absence of tannins in the n-hexane leaf, stem and root extracts and its presence in the methanol extracts is in line with the previous work done by Thavamoney et al. [36] that the solubility of phenolic compounds increased with increasing solvent polarity, though water extract being the most polar solvent did not contain any phenolic compound. The methanol root extract contained all the metabolites except the steroids which is in line with the report that steroid is hydrophobic compound, and so not surprisingly insoluble in methanol [37]. Both aqueous leaf and root extracts contained saponins, alkaloids and glycosides while the aqueous stem extract contained only the saponins. The presence of saponins in all the aqueous extracts is understandable since water has been reported to be one of the most common extraction solvents for saponins [38]. The cytotoxic, hepatoprotective and hypoglycemic activities of the plant could also be associated with the presence of saponin [10,38,39]. The ethylacetate leaf extract contained flavonoids, tannins and glycosides. The presence of tannins and flavonoids showed the basis of ethnomedicinal use of the leaf as antioxidant since flavonoids and tannins were reported to have shown high antioxidant activities [16,40,41,42]. Alkaloids, saponins, flavonoids, glycosides were present in the ethyl acetate stem extract. The ethyl acetate root extract contained saponins, flavonoids and glycosides. The presence of flavonoids in all the ethyl acetate extracts is in line with the report that medium polar solvents like ethyl acetate can be used in the extraction of flavonoids [43,44]. The n-hexane leaf extract contained only the glycosides. Alkaloids and glycosides were present in the n-hexane stem extract. Steroids and glycosides were found in the n-hexane root extract. The polarity of solvent used played important role in the extraction of the metabolites example, tannins were present in all methanol extracts but were absent in all n-hexane extracts. On comparing the phytochemicals found in the methanol extract in all the plant parts, the leaf extract contained the least number of phytochemicals while the stem and root contained the same number of the metabolites. The stem aqueous extract contained the least number of the metabolites while the leaf and the root contained the same number of the phytochemicals. The stem ethyl acetate extract contained most of the metabolites while the leaf and the root contained the same number of the metabolites. The leaf n-hexane extract only contained one metabolite while the stem and root extracts contained three metabolites each. Glycosides were found in most of the extracts except the stem aqueous extract. The quantitative phytochemical analysis (Table 4) of the plant parts revealed that alkaloids and tannins were significantly higher in the leaf (1.98 and 2.00% respectively) when compared with the stem and the root. Flavonoids and glycosides (3.06 and 2.03% respectively) were also higher in the stem when compared with leaf and the root. Saponins were higher in the root when compared with the leaf and the stem. The antimicrobial analysis (Tables 5-7) carried out the plant parts revealed that methanol leaf extract exhibited antimicrobial activities against *E. coli, S. aureus, C. albicans, V. cholera, P. aeruginosa and K. pneumonia*. The higher quantity of tannins and alkaloids found in the leaf of the plant could be responsible for the extract's activity against the six organisms [45,46]. Other leaf extracts showed no activities against the test organisms.

The methanol stem extract showed activities against *S. aureus, V. cholera, P. aeruginosa* and *K. pneumonia*, ethyl acetate stem extract revealed some activities against *E. coli, C. albicans, S. typhi, P. aeruginosa*. The aqueous stem showed activities against only *P. aeruginosa*, and n-hexane extract showed activity only against *E. coli*. These activities could be linked to the higher flavonoids and glycosides quantity found in stem of the plant and the use of stem in treatment of diarrhea, and the antioxidant property exhibited by the stem as reported by Heim et al. [47,10,48]. The methanol extract of the plant showed activities against *E. coli, S. aureus, V. cholera, and P. aeruginosa*, the ethyl acetate root extract only showed activity against *C. albican*. The higher saponin quantity found in the root could be the basis of the activities of the root against the five test organisms [49,50,51,52,53,54].

Table 1. Result of qualitative phytochemical analysis of leaf extract of *S. companulata*

Solvent	Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Glycosides
Methanol	+	+	-	-	+	+
Water	+	+	-	-	-	+
Ethyl acetate	-	-	+	-	+	+
N-hexane	-	-	-	-	-	+

Table 2. Result of qualitative phytochemical analysis of leaf extract of *S. companulata*

Solvent	Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Glycosides
methanol	-	+	+	-	+	+
Water	-	+	-	-	-	-
Ethyl acetate	+	+	+	-	-	+
N-hexane	+	-	-	-	-	+

Table 3. Result of qualitative phytochemical analysis of root extract of *S. companulata*

Solvent	Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Glycosides
methanol	+	+	+	-	+	+
Water	+	+	-	-	-	+
Ethyl acetate	-	+	+	-	-	+
N-hexane	-	-	-	+	-	+

+ = present, - = absent

Table 4. Result of quantitative phytochemical analysis of root extract of *S. companulata*

Phytochemicals (%)	Leaf	Stem	Root
Alkaloids	1.98	0.61	1.00
Saponin	0.61	0.61	3.06
Flavonoids	1.58	3.06	1.34
Tannin	2.00	0.10	1.36
Glycosides	0.34	2.03	0.34

Table 5. Result of diameter of zone of inhibition (mm) for the 250 µg/ml of leaf extract of *S. companulata*

Organism	Methanol	Water	Ethyl acetate	N-hexane
E. coli	4 mm	-	-	-
S. aureus	5 mm	-	-	-
C. albicans	7.2 mm	-	-	-
S. typhimurium	-	5.3 mm	-	-
V. cholera	3.1 mm	-	-	-
K. pheumonia	6 mm	-	-	-
P. aeruginosa	5 mm	-	-	-

Table 6. Result of diameter of zone of inhibition (mm) for the 250 µg/ml of stem extract of *S. companulata*

Organism	Methanol	Water	Ethyl acetate	N-hexane
E. coli	-	-	3 mm	6 mm
S. aureus	5 mm	-	-	-
C. albicans	-	-	4 mm	-
S. typhimurium	-	-	3 mm	-
V. cholera	3.1 mm	-	-	-
K. pheumonia	6 mm	-	-	-
P. aeruginosa	3 mm	3.2 mm	3 mm	-

Table 7. Result of diameter of zone of inhibition (mm) for the 250 µg/ml of root extract of *S. companulata*

Organism	Methanol	Water	Ethyl acetate	N-hexane
E. coli	-	-	-	-
S. aureus	5 mm	-	-	-
C. albicans	-	-	4 mm	-
S. typhimurium	-	-	-	-
V. cholera	3 mm	-	-	5 mm
K. pheumonia	-	-	-	-
P. aeruginosa	3 mm	-	-	-

- = No activity

4. CONCLUSSION

The extracts of *S. companulata* have been found to contain many phytochemicals and were also active against some pathogens. The polarities of solvents used affected the metabolites extracted, and the pharmacological action of some of the metabolites found in different parts of the plant also confirmed the ethnomedicinal uses of the plant. There is need to isolate and identify the compounds responsible for some of the pharmacological actions of the plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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