

Phytochemical and Antimicrobial Properties of African Peach (*Sarcocephalus Latifolius*)



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Abstract

This study includes phytochemical screening, quantification of secondary metabolites, thin-layer chromatography (TLC) and antimicrobial assay of African peach (*Sarcocephalus latifolius*). The phytochemical analysis revealed the presence of alkaloid, flavonoid and saponin at various quantities with saponin more abundant amongst others like tannin, glycoside and phenols that are present. All the extracts were tested for antimicrobial activity with ethanol extract and water extract exhibiting a higher activity against the reference strains. It exhibits a higher sensitivity than the standard antibiotic erythromycin which was used as control.

Keywords: Chromatography; Phytomedicines; African peach

Introduction

Nigeria is blessed with a vast distribution of vegetations and different plants-based medicines used traditionally, which means plants can now be a “natural chemical factory” for the earth. These plant parts are used for the treatment and management of diseases and disorders especially in pre-historic days. Despite medicinal importance, they are also useful as ornamental, flavouring, additives, and preservatives [1-3]. According to the WHO [4], most of them have been found to contain therapeutic benefits and are also precursors for the synthesis of useful drugs; such plants are termed medicinal plants. The aim of this research is to investigate those bio-active components responsible for the medicinal properties observed in *Sarcocephalus latifolius* as used by the local people of Bekwarra community in Cross River state of Nigeria and to test for its antimicrobial properties.

Materials and Methods

Sample collection and preparation

The leaves of *Sarcocephalus latifolius* were collected from its natural habitat in Bekwarra Local Government Area of Cross River State, Nigeria. The sample was air-dried for two weeks under shade then milled into fine powder using a milling machine.

Extraction

The extraction was done using a solute-solvent ratio of 1:10. This was done by soaking 20g of the samples in 200ml of solvent (n-hexane, ethanol and water) for four (4) days with frequent

agitation in order to get the leaves extract. The resulting mixture was filtered using a filter paper and concentrated by allowing the solvent (n-hexane) evaporate in an open air and ethanol was concentrated using the rotary evaporator and water extract was concentrated in a water bath at 60°C. The extract was kept in a refrigerator until required for testing.

Phytochemical screening of extracts

A standard preliminary screening of each extract (aqueous extracts, n-hexane extracts, and ethanol extracts) of *Sarcocephalus latifolius* was performed as reported by [5-7] the test was carried out as follows:

Detection of alkaloids

Wagner's test: About 10mg of extract was taken and few drops of Wagner's reagent were added, and the formation of a reddish-brown precipitate indicates the presence of alkaloids.

Detection of saponins

0.5mg of extract was diluted with 20ml distilled water and shaken well in a graduated cylinder for 15min. The formation of foam to a length of 1cm indicated the presence of saponins.

Detection of flavonoids

10mg of extract was taken and few drops of 10% lead acetate solution were added. Appearance of yellow colour precipitate indicates the presence of flavonoids.

Detection of tannins

5mg of extract was taken and 0.5ml of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins.

Detection of glycosides

0.5mg of extract was dissolved in 1ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

Detection of steroids and Sterols

5mg of extract was dissolved in 2ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound, in the extract.

Detection of anthraquinones

About 0.5g of the extract was boiled with 2ml of 10% HCl for few minutes in a water bath. The resultant solution was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH₃ solution was added to the mixture and heated. Formation of rose-pink colour indicated the presence of anthraquinones on both extracts.

Detection of phenols

5.0mg of extract was dissolved in 0.5ml of 20% sulphuric acid solution. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue which indicates the presence of phenols.

Detection of terpenoids

0.2g of the extract was mixed with 2ml of chloroform and 3ml of concentrated H₂SO₄ was carefully added to form a layer. A reddish-brown interface was formed which indicates a positive result for the presence of terpenoids.

Quantitative determination of phytochemicals

The quantity of phytochemicals in *S.latifolius* were determined and quantified following standard procedures.

Determination of total alkaloids

Standard method according to Dey & Chaudhuri [8], and Obadoni & Ochuko [9], 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution could settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

$$\text{Percentage yield of alkaloid} = (W2 / W1) * 100 \quad (1)$$

Where, W2 = Weight of dried end product; W1= Weight of powdered sample taken for the analysis

Determination of total flavonoids

5 grams of plant sample was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was filtered through a Whatman No 1 filter paper into a pre weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed according to Harborne, [10],

$$\text{Percentage of flavonoid} = (W2 / W1) * 100 \quad (2)$$

Where, W2 = Weight of water bath dried product; W1= Weight of powdered sample taken for test

Determination of saponin

Estimation of saponin was done according to slightly modified standard method by Van-Burden and Robinton [11]. Ten grams of powder sample was taken in 250ml conical flask and 100ml of 20% ethanol was added to it. The mixture was heated in a hot water bath of 55°C for 5 hours with continuous stirring. The mixture was filtrated through Whatman paper number 1 and the supernatant liquid was separated. The solid residue was mixed with 20% ethanol and heated in a similar way for about 5 hours. The solution was filtered and mixed with previously filtered solution. The combined filtered solution was placed on a hot water bath of 90°C and heated still the volume was reduced to 20% of its initial volume. The concentrated sample was transferred into a 250ml separating funnel and 10ml of diethyl ether was added to it and shaken vigorously. The aqueous layer was separated carefully after setting down the solution. The purification process repeated. 60ml of n-butanol extracts were washed twice with 10ml of 5% aqueous NaCl solution. The remaining solution was heated in a water bath at 50°C until the solvent evaporates and the solution turns into semi dried form. The sample was then dried in an oven. This saponin content was calculated by the following equation:

$$\text{Percentage yield of saponin} = (W2 / W1) * 100 \quad (3)$$

Where, W2 = Weight of oven dried end product; W1= Weight of powdered sample taken for test.

Antimicrobial analysis

Test Micro-organism

The organisms: *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, were gotten from the Microbiology laboratory, Federal University Wukari, Taraba state Nigeria.

Sterilization of materials

All glass wares used in this study were washed with detergent, rinsed, and sterilized in a dry ventilated oven at 160°C for 2 hours.

All media were sterilized by autoclaving at a temperature of 121°C and 15 psi for 15 minutes. The scalpel, cork borer, inoculating needle were sterilized by dipping them into 70% ethanol and passing them over a Bunsen burner flame until red hot.

Media preparation

Mueller Hinton Agar (MHA) was prepared according to the manufacturer's instruction. 38g of the powdered MHA medium was dissolved in 1L of sterile distilled water and sterilized by autoclaving at 121°C at 15 psi for 15 min and allowed to cool before pouring gently into 100 sterile Petri dishes. The Petri dishes that contained the medium were incubated for 24 hours at room temperature (37°C) to check for sterility before use as described by Cheesebrough [12].

Antimicrobial activity

Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of anti-bacterial activities of the plant samples [13]. A sterile swab was used to evenly distribute bacterial culture over the appropriate medium (Mueller Hinton Agar). Muller Hinton agar was prepared as per the instructions by the manufacturer. The plates could dry for 15 minutes before use in the test. Once the media solidified then it was then inoculated with the bacteria species. The media was then punched with 6mm diameter hole and was filled with extract; a

pipette was used to place 30µl of the extract into the well. A total of two extracts was used on a bacterial species; with a total of three plates used for each extract including. The positive control was the same on all isolate. The plates were incubated at 37°C for 24 hours after which they were examined for inhibition zones. A ruler was used to measure the inhibition zone.

Results and Discussion

Discussion

Qualitative phytochemical analysis

As shown in table 1, the qualitative analysis of the aqueous (water) extract of *S.latifolius* exhibited positive results for eight phytochemicals. Five phytochemical test presents in the hexane extract and seven phytochemicals were detected positive in the ethanol extract of the plant. Alkaloid, saponin, flavonoid, tannin, glycoside, steroid, phenol, and terpenoid were detected present in aqueous (water) extract except anthraquinones which was found absent. All were present in hexane extract except saponin, steroid, anthraquinone and terpenes. While only terpenes and anthraquinones were found absent in ethanol extract. According to Evans [14], these phytochemicals detected are the major constituent of any medicine used for the treatment of diseases and they are also responsible for the characteristic odour, pungencies and colour in the plant.

Table 1: Phytochemical analysis of *sarcocephalus latifolius* leaves extract

Phytochemicals	Aqueous Extract	Hexane Extract	Ethanol Extract
Alkaloids	+	+	+
Saponins	+	-	+
Flavonoids	++	+	+
Tannins	++	+	+
Glycosides	++	+	++
Steroids	+	-	+
Anthraquinones	-	-	-
Phenols	+	+	+
Terpenes	+	-	-

+ = Detected, - = Not detected, ++=Intensively present

Quantitative phytochemical analysis

The result of the quantitative analysis carried out on the leaves of *S.latifolius* (Table 2) shows that the plant leave is more abundant with saponin followed by flavonoid, alkaloid is markedly found in a trace amount compared to saponin. Different phytochemicals have been found to possess different medicinal property. For example, alkaloid protect against chronic diseases, saponin has been found to protect against hypercholesterolemia. The abundant nature of saponins in this plant could be responsible for the phytoanticipins or phytoprotectant nature of the plant alongside the antimicrobial

activity observed in these plants as backed up by Lacaille-Dubois et al. [15] and Yoshiki et al. [16] (Table 3 & Figure 1).

Thin layer chromatography

Table 3 presents the results of the thin layer chromatography carried out on the leaves extract of *S.latifolius*. Chromatographic profiles of crude extracts obtained through different solvents were similar. The visualization of chromatographic profiles for each extraction technique and solvent used permit to evaluate the qualitative and quantitative variations in secondary metabolite content [17]. In addition, these data present compound profiles

related to the biological effects and medicinal use (Table 4) from the tables, the solvent system with the best component resolution is a mixture of hexane and ethyl acetate in a ratio of 5:1 because

it shows more number of components and so can be used for the extraction.

Table 2: Quantitative Phytochemical analysis of *Sarcocephalus latifolius*.

S/No	Phytochemicals	Percentage Composition
1	Alkaloids	5.24
2	Flavonoids	25.18
3	Saponins	80

Table 3: Thin-layer Chromatography analysis (TLC).

Samples	Solvent System	Number of Components	Retention Factor
Aqueous(water) extract	n-hexane:ethyl acetate (1:1)	Nil	-----
	n-hexane:ethyl acetate (5:1)	5	0.22, 0.88, 0.79, 0.95, 0.98
	n-hexane:ethyl acetate (7:3)	3	0.35, 0.87, 0.97
Hexane extract	n-hexane:ethyl acetate (1:1)	2	0.62, 0.97
	n-hexane:ethyl acetate (5:1)	5	0.14, 0.23, 0.31, 0.46, 0.53
	n-hexane:ethyl acetate (7:3)	6	0.10, 0.20, 0.32, 0.68, 0.77, 0.94

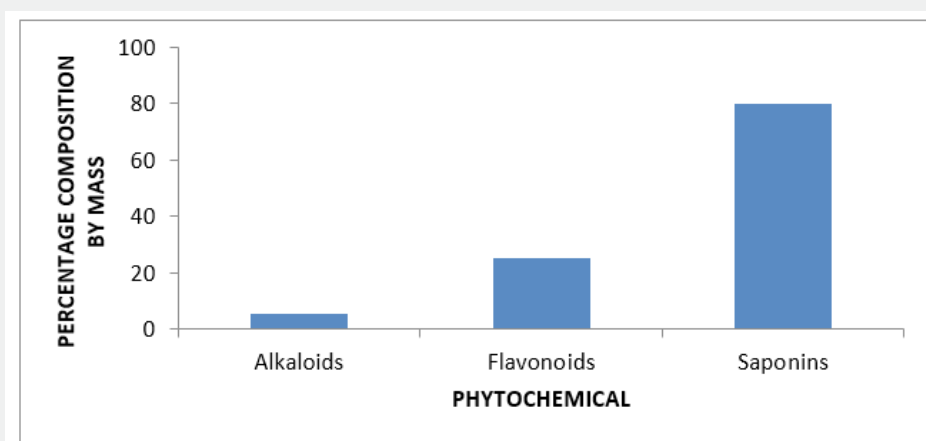


Figure 1: A chart showing the quantification of the phytochemicals.

Antimicrobial analysis

Table 4 depicts the results of the antimicrobial analysis of *S.latifolius* leaves extract and Figure 2 shows the zones of inhibitions. The aqueous (water) and solvent (hexane and ethanol) extract of *S.latifolius* was used for the antibacterial assays. The ethanol extract shows the highest inhibitory activity against the tested microorganisms (*Escherichia coli*, *salmonella typhi* and *pseudomonas aeruginosa*) with inhibition zones ranging between 12.33 and 20.67mm. This was followed by the aqueous (water) extract with inhibition zones ranging from 10 and 18.67mm. then the hexane extract was not active against *S.typhi* and *P.aeruginosa* but only active against *E.coli* with an inhibition zone of 9.67mm.

In general, the plant is found to possess a strong antibacterial effect since it showed activities even higher than the standard antibiotic (erythromycin) which was used in this study as a positive control drug. Erythromycin was active against all the tested microorganisms with an inhibition zone ranging from 15.67mm and 18.33mm. (Table 4) this means the ethanol extract of *S.latifolius* can be preferable since its zone of inhibition is more than that of the erythromycin in this study. This finding indicates a possible use of such extracts as an alternative drug and against nosocomial infections (e.g. urinary tract infections, respiratory system infections, dermatitis, and gastrointestinal infections), typhoid fevers and malaria. Such antimicrobial properties can be attributed to the presence of phenols, tannins, and flavonoids [18] (Figures 3-5).

Table 4: Antimicrobial Screening.

Test Organisms	Water Extract	Hexane Extract	Ethanol Extract	Erythromycin (Control)
<i>E.coli</i>	10.00±1.00	9.67±1.53	12.33±2.52	15.67±1.15
<i>S.typhi</i>	18.67±1.15	0	0	16±1
<i>P. aeruginosa</i>	11.67±1.53	0	20.67±3.05	18.33±0.58

Value represents mean±Standard deviation of three replicates.

There is significant difference between means at P (< 0.05).

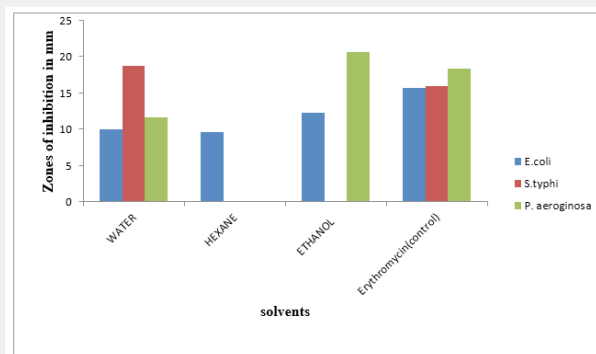


Figure 2: A chart showing the zones of inhibition of the various extract of *S. latifolius*.

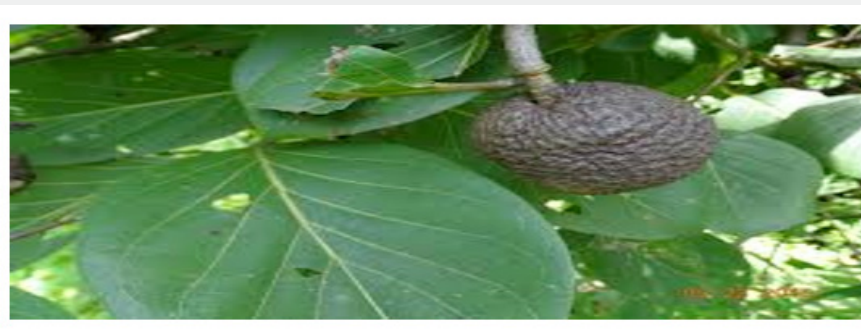


Figure 3: A pictorial view/identification of *Sarcoccephalus latifolius*.

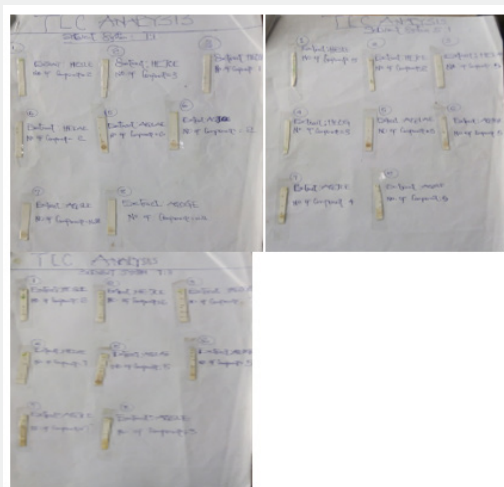


Figure 4: TLC plates.



Figure 5: Antimicrobial analysis (plates).

Conclusion

This present investigation affirms the folkloric use of African peach (*Sarcocephalus latifolius*) as a phytomedicine which can be harnessed to drugs because of its high action against *Salmonella typhi* and other disease associated organisms. More research is therefore open from the data obtained during this study on the areas of extraction and elucidation of those bioactive chemical responsible for the medicinal properties of this plant.

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