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Phytochemical Constituents and Antimicrobial Activity of Some Medicinal Plants Used for Treating Skin Diseases in Bosso Local Government, Niger State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author AA performed phytochemical screening and wrote the first draft of the manuscript. Author IFO designed, supervised the study and helped in the interpretation of the result. Authors DM and AI collected the samples, prepared the samples and performed the anti microbial activity. All authors approved the final version of the manuscript and gave consents for the publication of the paper in this journal.

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ABSTRACT

Traditional medicine practitioners TMPs have developed means of treating skin and soft tissue infections by using plant extract. In this study, four medicinal plants which are used for treating skin diseases were analysed, to determine their phytochemical constituents and antimicrobial activity. Extraction and phytochemical screening was done using standard analytical procedures. The antimicrobial assay was carried out using agar well diffusion method. Methanol extract of *Mitrarcapus villosus, Psidium guajava, Senna spectabilis* and *Anogeisus leocarpus* contained

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tannins, phenols, cardiac glycosides, flavonoids, saponins, stereoids and terpenoids. However, anthraquinones was present in only *Psidium guajava*. Phlobatanins was absent in only *Senna spectabilis*. alkaloids was absent in only *Mitrarcapus villosus*. All the plant extracts demonstrated antimicrobial activity against *Candida albican, Salmonella typhii, Bacillus subtillis, Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli.* Although *Senna spectabilis* extract shows no antimicrobial activity against *E. coli*. The extracts of *Mitrarcapus villosus* showed the highest activity against *Candida albican* (26.00 mm), *Salmonella typhii* (32.00 mm) and *E. coli* (15.00 mm) while *Anogeisus leocarpus* showed the highest activity against *Staphylococcus aureus* (18.00 mm) and *Pseudomonas aeruginosa* (18.00 mm). Extracts of *Psidium guajava* was also found to have the highest activity against *B. subtilis* (37.00 mm). In conclusion, the antimicrobial activity of *Psidium guajava* and *Mitrarcapus villosus* was comparable to standard drugs. All the plant contained important phytochemicals of therapeutic significance and also possessed antimicrobial activity.

Keywords: Phytochemicals; antimicrobial; skin; disease; plant.

1. INTRODUCTION

Nigeria vegetation has been considered to form a vital part of the natural wealth of the country [1]. It has been described as a reservoir of phytomedicines by [2]. Plants are generally endowed with various bioactive phytochemical compounds which includes; terpenoids, phenolic, lignins, tannins, flavonoids, coumarins, alkaloids to mention a few [3]. Studies have revealed that these phytochemicals possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial, and anti-viral activities [4,5,6,7]. Plants have been used traditionally to manage various ailments. Traditional medicine practitioners TMPs through ancient indigenous technology and by a series of "trial and error" over time have successively applied various plant parts to treat diverse sicknesses [1]. Ethnomedicine is the oldest means of treating diseases and Infections [4]. Recently, researchers have recognized ethnomedicine as the most viable method for identifying new drugs from plant or refocusing on those plant earlier reported for bioactive constituent [8]. Medicinal plants are sources of many powerful novel drugs, although the plant kingdom has not been fully exploited. Herbal medicine have provided affordable health care for over 80% of the world's population, especially rural people in the developing countries [9], making herbal medicines a promising choice over modern synthetic drugs. Since they are affordable, available and show minimum/no side effects hence are considered to be safe [10].

Bioactive medicinal agents extracted from plants either as crude extract or as purified products are being investigated for their potential to treat various disease and infections. Hundreds of medicinal plant species worldwide are used in the traditional medicine system for the treatment for skin diseases caused by bacteria and fungi [11]. Skin and mucosal infections, are popular among the rural settlers, due to lack of good hygiene practices, lack of proper sanitation and lack of clean water [12]. Skin diseases such as; wounds, furuncles, sepsis, atopic dermatitis, cellulitis, gas gangrene, acne, candidiasis is common among villagers and rural inhabitants, this skin and mucosal infection are caused by a variety of the microbes which include; *Staphylococcus aureus*, *Streptococcus pyogenes* (Group A haemolytic streptococcus), *Clostridium perfringes* and the bacteriodes group. Others are *Mycobacterium tuberculosis, Mycobacterium leprae, Neisseria gonorrhea, Pasturella tulurensis, Bacillus antracis and Pseudomonas aeruginosa.* The common fungi which cause skin infections are *Candida albicans, Candida neoformans, Epidermophyton flocossum, Trychophyton tonsurans, Melassezia furfur,* to mention a few [11].

Mitrarcapus villosus, Psidium guajava, Senna spectabilis and *Anogeisus leocarpus* are popular medicinal plants used by TMPs in Bosso, local government of Niger state, Nigeria for treating various skin diseases. Crude paste prepared by crushing fresh leaves of *Psidium guajava* Linn. is applied to wounds, cuts, ulcers, boils, skin and soft tissue infectious site, rheumatic places [13]. Decoction of fresh leafs of *Mitrarcapus villosus* is used for treating rheumatism, eczema, ulcer and other skin diseases by TMPs in Bosso, Niger State. Similarly, herbal preparations developed from leaves of *Senna spectabilis* and *Anogeisus leocarpus* are used to treat skin diseases and other bacterial infections [14]. Bioactive

compounds from plants have novel mechanisms of action against microorganism [9,15]. They are effective in the treatment of infectious and skin diseases while simultaneously mitigating many of the side effects that are often associated with synthetic orthodox antibiotics [16]. The clinical success of quinine and quinidine isolated from the *cinchona* tree bark and recently artemisin from *Artemisia annua* in the treatment of malaria have rekindled interest in the medicinal plants as potential of novel drugs. This study is aimed at determining the phytochemical and antimicrobial activity of *Psidium guajava, Mitracarpus villosus, Anogeisus leocarpus* and *Senna spectabilis* against *Candida albican, Salmonella typhii, Bacillus subtillis, Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli.* These medicinal plants are used for treating skin diseases by TMPs in Bosso Local Government Area of Niger State. *Candida* species, *Staphylococci, Streptococci, Pseudomonads*, *Bacilli* and *Escherichia coli* are among microorganism which are frequently isolated from skin and wound infection according to [17].

2. METHODOLOGY

2.1 Sampling and Sample Preparation

Fresh leaves of *Senna spectabilis, Psidium guajava, Anogeisus leocarpus* and aerial parts of *Mitracarpus villosus,* were collected from Kampala Village, Bosso Local Government Area of Niger State, Nigeria and where identified by the Taxonomist in the Department of Biology Federal University of Technology, Minna, Niger State, Nigeria. The samples were rinsed with clean water and air-dried for two weeks, and then pulverized using a mechanical grinder. Powered samples was kept separately in air-tight containers and stored for further analysis.

2.2 Extraction

200 g each of the pulverized plant was transfered into a thimble and extracted using methanol for 24 hours. Exhaustive extraction was achieved under Reflux with the aid of a Soxhlet apparatus. The extracts were then concentrated at 46℃ using a rotary evaporator. The methanol concentrate was evaporated to dryness on a water bath. The extracts were stored in airtight sample bottles and kept in the desiccators until required for further analysis.

2.3 Phytochemical Screening

The phytochemical screening was carried out according to the methods outlined in [18,19,20]. Briefly, the procedures include:

2.3.1 Test for alkaloids

2 ml of extracts were dissolved individually in 1% dilute hydrochloric acid and filtered. The following test were performed on the filtrate

2.3.1.1 Mayer's test

Filtrates were treated with few drops of Mayer's reagent (potassium mercuric iodide). Formation of a yellow cream precipitate indicated the presence of Alkaloids.

2.3.1.2 Wagner's test

Filtrates were treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/reddish brown precipitate indicated the presence of alkaloids.

2.3.1.3 Dragendoff test

To the filtrate, 1 ml of dragendoff's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

2.3.2 Test for phenolics and tannins

2.3.2.1 Ferric chloride test

To the extract 2 ml of 2% solution of $FeCl_3$ was added. A blue-green or black coloration indicated the presence of phenols and tannins.

2.3.2.2 Alkaline reagent test

To the extract, 2 ml of 5% sodium hydroxide solution were added, a yellow solution indicates the presence of flavonoids. On adding dilute hydrochloric acid the solution becomes colourless.

2.3.3 Test for anthraquinones

2.3.3.1 Borntrager's test (for free anthracene derivatives)

The powdered leaf (0.5 g) was taken in a test tube and 5 ml of chloroform was added and shaken for 5 min. The mixture was filtered and

the filtrate shaken with equal volume of 10% ammonia solution. A pink, red or violet colour in the aqueous layer after shaken indicates the presence of free anthraquinone.

2.3.3.2 Modified Borntrager's test (for combined anthracene derivatives)

One gram of the powdered leaves was boiled with 5 ml of 10% hydrochloric acid for 3 min. The hot solution was filtered in a test tube, cooled and extracted gently with 5 ml of benzene. The upper benzene layer was pipetted off and shaken gently in a test tube with half of its volume of 10% ammonium hydroxide solution. A rose pink to cherry red colour in the ammonia layer indicates the presence of anthraquinone.

2.3.4 Test for cardiac glycosides

2.3.4.1 Salkowski's test

Crude extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring which is the glycone portion of the glycoside.

2.3.4.2 Keller-kilani test

Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated H_2SO_4. A brown ring at the inter-phase indicated the presence of cardiac glycosides.

2.3.4.3 Bromine water test

Test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

2.3.5 Test for flavonoids

2.3.5.1 Shinoda's test

Few magnesium chips were added to 3 ml of the aqueous solution and 2 drops of dilute hydrochloric acid was added and warmed. A pink or red colour indicated the presence of flavonoids.

2.3.5.2 Alkaline reagent test

2 ml of 2% sodium hydroxide solution, was added to the test solution, a yellow colour which become colourless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

2.3.5.3 Lead acetate solution test

Few drops of 10% lead acetate solution was added to the test tube containing the extract, a yellow precipitate indicates the presence of flavonoids.

2.3.6 Test for phlobatanins

2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

2.3.7 Test for saponin

2.3.7.1 Frothing test

The powdered leaves (0.5 g) was placed in a test tube and 10 ml of distilled water was added and shaken vigorously for 30 seconds. It was then allowed to stand for 30 min and observed. Formation of honey comb froth indicated the presence of saponins.

2.3.8 Test for steroid

2.3.8.1 Liebermann's test

Crude extract was mixed with 2 ml of chloroform and 2 ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus.

2.3.8.2 Salwoski's test

To test tube, 3 drops of concentrated sulphuric acid was added to form a lower layer. Reddish-brown colour at the inter phase indicated the presence of steroidal ring.

2.3.9 Test for terpenoids

2.3.9.1 Lieberman-Burchard's reaction

To test tube, equal volume of acetic anhydride was added and gently mixed. Then 1 ml of concentrated H_2SO_4 was added down the side of the tube. The appearance of a brownish-red ring at the contact zone of the two liquids and a greenish colour in the separation layer indicated the presence of sterols and triterpenes.

2.4 Antimicrobial Activity

The antimicrobial activity of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtillis, Candida albican and Salmonella typhi* was determined using agar well diffusion method. The plant extracts were reconstituted using dimethyl sulphoxide (DMSO), 40 mg/ml concentrations was used for all the extracts and standards used as control. The Media used comprised of; Peptone-10 g, NaCl-10 g and Yeast extract 5 g, Agar 20 g in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37℃ for 18 hours. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hour old cultures and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of samples. The control wells were filled with nystatin for *Candida albican* and ampiclox for *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtillis and Salmonella typhi*. Cork boer of diameter 3mm was used in the study. All the plates were incubated at 37℃ for 24 h and the diameter of inhibition zones were noted. The tubes containing above media were prepared, autoclaved and respective concentrations of the samples were added. Each tube was inoculated with 18 h old cultures. A control tube with inoculums and without any sample was prepared along with a sile media tube as blank. The zones of inhibition were recorded and the percentage inhibition was also calculated.

3. RESULTS AND DISCUSSION

As shown in Table 1 the methanol extract of aerial part of *Mitrarcarpus villosus* contained phenols, tannins, cardiac glycoside, flavonoid, phlobatannins, saponins, steroid and terpenoids. However, alkaloids and anthraquinones are absent. From Table 2 the extract of *M. villosus* showed antimicrobial activity against all tested microorganism, the highest antimicrobial activity against *Candida albican* (26.00 mm)*, Salmonella typhii* (32.00 mm) *and E. coli* (15.00 mm) was shown by *M. villosus.* The zone inhibition of the extract against this microorganism was comparable to the standard drug used in this study. This is in agreement with the report of [21].

Table 1 reveals the presence of alkaloids, phenols, tannins, anthraquinones, cardiac glycosides, flavonoids, phlobatanins, saponins, steroid and terpinoids in the methanol extract of *Psidium guajava. Psidium guajava* contains all the tested phytochemicals. The result of the phytochemical screening is similar to the result of [17]. Paste produced by pounding fresh leaf of *Psidium guajava* is used by herbalist to treat skin and soft tissue infections [13]. From Table 2 methanol extract of the leaf of *Psidium guajava* showed antimicrobial activity against all tested microorganism, with the highest antimicrobial activity against *B. subtillis* (37.00 mm). This is similar to the finding of [15]. The antimicrobial activity of *P. guajava* extract against *C. albian, s. aureus* and *S. typhi* is significantly high and comparable to the standard drugs used in the study.

The phytochemical screening result of extract of *Anogeisus leocarpus* has presented in Table 1, reveals that alkaloid, phenols, tannins, cardiac glycoside, flavonoids, phlobatains, steroids , saponnins and terpenoids were present, however, anthraquinones were absent in the crude methanol extract of the plant. Table 2 presents the antimicrobial activity of the methanol extract of *Anogeisus leocarpus* against the tested organism, although the antimicrobial activity of *Anogeisus leocarpus* extract was generally low compared to other extracts, it is worth noting that the zone inhibition against *Staphylococcus aureus* is comparable to the standard drugs. This finding is in agreement with the report of [22]. Therefore justifying the use of extracts of the leaf of *Anogeisus leocarpus* in managing skin infections, as *staphylococcus aureus* is one of the most common bacterial isolated from skin infection. Furthermore, the antimicrobial activity of the methanol extract of the leaf of *Anogeissus leiocarpus* had a high activity against multi drug resistance *Staphylococcus aureus* [14].

From Table 1, *Senna spectabillis* methanol extract contains; alkaloid, phenol, tannis, cardiac glycoside, flavonoids, saponins, steroid and terpenoids. Antraquinone and phlobatanins were not detected in the extract. From Table 2, the extract has no activity against *E. coli*, however, the extract showed a remarkable antimicrobial activity against *S. aureus* (17.00 mm) which is comparable to standard drug used this study.

Table 1. Result of phytochemical components of methanol extract of *Senna spectabilis, Anogeisus leocarpus, Psidium guajava* and *Mitrarcapus villosus*

Phytochemicals	*Senna spectabilis*	*Anogeisus leocarpus*	*Psidium guajava*	*Mitracarpus villosus*
Alkaloid				
Dragendoff	+	+	+	-
Wagner	+	+	+	-
Mayer	+	+	+	-
Phenols and tannins				
Alkaline reagent	+	+	+	+
Ferric chloride	+	+	+	+
Anthraquinonne				
Free anthraquinonne	-	-	+	-
Combined anthraquinonne	-	-	+	-
Cardiac glycosides				
Keller-kilani test	+	+	+	+
Salkowski's test	+	+	+	+
Bromine water test	+	+	+	+
Flavonoid				
Shinoda test	+	+	+	+
Alkaline reagent	+	+	+	+
Lead Acetate Solution	+	+	+	+
Phlobatanins	-	+	+	+
Saponin				
Frothing test	+	+	+	+
Steroid				
Liebermann's test	+	+	+	+
Salkowski's test	+	+	+	+
Terpenoid				
Liebermann Burchard test	+	+	+	+

+ = Present/ - = Absent

Table 2. Result of antimicrobial screening of crude methanol extract of *Senna spectabilis, Anogeisus leocarpus, Psidium guajava* and *Mitrarcapus villosus* showing zones of inhibition in mm

Microorganism	*Senna spectabilis*	*Anogeisus leocarpus*	*Psidium guajava*	*Mitracarpus villosus*	Standard drugs (Nystatin/ Ampiclox)
C. albican	12.00	9.00	22.00	26.0	26.00
S. typhi	15.00	13.00	25.00	32.00	34.00
B. subtilis	17.00	12.00	37.00	12.00	27.00
S. aureus	17.00	18.00	17.00	15.00	17.00
E. coli	-	12.00	12.00	15.00	19.00
P. aeroginosa	15.00	18.00	12.00	14.00	25.00

Inhibition Zones measured in mm: - = implies no zone of inhibition,
Concentration of extract= 40 mg/ml, concentration of standard= 40 mg/ml

3.1 Discussion

The percentage yields of the extracts are *Anogeisus leocarpus (*9.5%*), Senna spectabilis* (13.2%), *Mitrarcarpus villosus (*11.1%) and *Psidium guajava* (12.9%) respectively. The overall results of the phytochemical screening of the methanol leaves extract of *Psidium guajava, Senna spectabilis, Anogeisus leocarpus,* and *Mitrarcapus villosu* as shown in Table 1 revealed the presence of alkaloids, phenols, cardiac glycosides, flavonoid, saponin, tannin, steroid and terpenoid in all the plant samples. However phlobatannins was absent in *Mitracarpus villosus* and anthraquinone was not present in all test samples. The therapeutic activities of plant are due to the presence of secondary metabolites (phytochemicals). In this study, tannins, phenols,

flavonoids, glycoside and alkaloid in synergy with other phytochemics are responsible for the antimicrobial activities of the methanol extract of these plants. Although the antimicrobial mechanism of phytochemicals varies; tannins have been reported to produce a permanent complex with proline-rich proteins, therefore inhibiting the cell protein synthesis. Tannins also react with proteins to produce the typical tanning effect which is vital in the treatment of inflamed or ulcerated tissues, burns, wounds and cuts [23]. Tannins are astringent in nature and places important roles as stable and potent antioxidants [24]. Adekunle and Ikumapayi, [25], reported that tannins act as an antifungal agent at higher concentrations by coagulating the protoplasm of the microorganism. Similarly, Harekrishna et al., [26], suggested that the possible mechanism of tannins may be to interfere with energy generation by uncoupling oxidative phosphorylation or they may interfere with glycoprotein of cell surface. While saponins a surfactant and natural glycosides, have also been reported to possess strong antifungal activities [21]. This strong antifungal activity has been linked to the formation of complexes with sterol in fungal plasma membrane leading to death by destruction of cellular semi-permeabile membrane. The interaction of saponins with cell membrane sterols is suggested as the major mechanism of the anti fungal activities of saponins [27]. Aboh [21], noted that the antifungal activities of the crude saponin from *M. villosus* at a concentration of 12.50 mg/ml against yeast, moulds and dermatophytes, fungi were comparable with the standard drugs fluconazole and ketoconazole at a concentration of 0.05 mg/ml. Saponins have also been reported to show high antimicrobial activities against wide range pathogens. Furthermore, saponins are anti-retroviral in invitro HIV studies, Shibatas [28] suggests a possible use of these plants as potential remedy for HIV infections. More so, they directly inhibit colon cancer and may also prevent cancer by protecting DNA from damage. They may also be cardio-protective because of their ability to lower cholesterol. Mann [14], remarked that methanol partitioned fraction of *Anogeissus leiocarpus* contained more potential antimicrobial agents against multi-resistant *Staphylococcus aureus* when compared with chloroform and ethyl acetate fraction.

Other phytochemicals such as; phenols, terpenoids, and flavonoids have also been reported to posses anti-bacterial properties [29,30,31]. The therapeutic activity of *Psidium guajava* is attributed to tannins, phenols and flavonoids, in particular, quercetin [31-33]. Quercetin-3-Oalpha-l-arabinopyranoside (guaijaverin) an active flavonoid isolated from *P. guajava* leaves inhibits the growth of *Streptococcus mutans* [34]. Also Gallocatechin isolated from the methanol extract of *P. guajava* leaves have high inhibitory activity against *E. coli* [35]. Berdy et al. [36], attributed the microbicidal activity of *Psidium guajava* to guajaverine and psydiolic acid. Mbuh et al. [37], investigated the antibacterial activity of the leaves extract of *P. guajava* and reported that the crude extracts inhibited the growth of *S. aureus, S. typhi, E. coli, B. subtilis, Shigella spp., P. mirabilis* and *K. pneumoniae* and that the methanol extract was more potent. In a similar study, Abdelrahim et al. [38], reported that the methanol extract was more effective in inhibiting the growth of *B. subtilis, S. aureus, E. coli* and *P. aeruginosa.*

4. CONCLUSION

The results obtained from this study shows that; *Senna spectabilis, Anogeisus leocarpus, Psidium guajava, Mitrarcapus villosus* contained important phytochemical that posses antimicrobial activities. This study therefore justifies the use of these plants by TMPs in Bosso, Niger state, Nigeria for treatment of skin diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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