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The Antimicrobial and Phytochemical Analysis of the Leaves of *Aspilia africana* on Clinical Isolates

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DAA and ICE designed the study, performed the statistical analysis, wrote the protocol and handled the literature searches. Author ORE wrote the first draft of the manuscript and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The uses of medicinal plants for treatment of various infections in traditional communities have been an age-long practice. This provides the rationale to study medicinal plant extracts as a possible source of alternative therapy against infections. The current study was undertaken to evaluate the phytochemical and antimicrobial properties of *Aspilia africana*. The antimicrobial activity and minimum inhibitory concentration (MIC) of the extracts of *A. africana* were evaluated against eight organisms-*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans, Aspergillus niger, Penicillum* spp and *Fusarium* spp. The ethanolic and aqueous extracts were obtained by standard methods. Antimicrobial activity was conducted using a modified agar well diffusion method. The phytochemical screening and analysis carried out in this study showed that the plant extracts contains alkaloids (6.350±0.84), saponins (2.260±0.15), flavonoids (2.006±0.11), tannins (0.881±0.04) and phenols (0.109±0.02). The result showed that ethanolic extract of *A. africana* exerted antimicrobial effect on the test organisms at 25 mg/ml, 50 mg/ml and 100 mg/ml concentrations, while the hot aqueous extract exerted

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antimicrobial effect at 100 mg/ml only on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The ethanolic extract of *A. Africana* showed the highest antimicrobial activity with diameter of zone of inhibition of 3.35 mm to 17.9 mm at 100 mg/concentration. The minimum inhibitory concentration (MIC) of the ethanolic extracts was at a concentration of 25 mg/ml. The antimicrobial activity of the extract could be enhanced if the components are purified. This plant therefore holds a promising potential source of new drug for treating infections caused by these clinical pathogens.

Keywords: Phytochemical screening; antimicrobial activity; Aspilia africana; clinical isolates.

1. INTRODUCTION

Medicinal plants have globally been used for the treatment of various infections in traditional communities. Hugo and Russell [1] estimated that over 80% of African population use herbal regimen for treatment and control of diseases. Over 50% of all modern chemical drugs are of natural plant product origin, and is essential in drug development programs of pharmaceutical industry. The expensive costs of some antibiotics and their setbacks in clinical treatments, provides the rational for studying medicinal plant extracts as a possible source of alternative therapy against infections. The primary benefit of using herbal drugs is that they are relatively safer and cheaper than their synthetic alternatives [2]. In addition, herbal medicine is a complex mixture of different phytochemicals acting by different mechanisms, which makes it difficult for pathogens to develop resistance [3].

In Nigeria and other parts of the world, many plants are used in herbal medicine to cure diseases and heal injuries. *A. africana,* is one of such plants. *A. africana* (pers) C.D. Adams (Fig. 1) [4] is a perennial herb varying in height from 60cm to about 1.5m, depending on rainfall and soil fertility. It is a common weed of field crops in West Africa and sometimes found in fallow land, especially, the forest zones. The plant is a weed grazed by cattle and sheep and is mostly used in Nigeria as food for rabbits. *A. africana* is widely used in medicinal practice for its ability to stop bleeding, even from a severe artery, as well as promote rapid healing of wounds and sores and for the management of problems related to cardiovascular diseases [5,6]. The extracts of the leaf exhibit differential anti-bacterial activities on both Gram-positive and Gram-negative bacteria species [7]. In Tanganyika, the root decoction of *A. africana* is taken for tuberculosis [7]. The leaf infusion is used in treating cough and related ailments in children and can also be mixed with clay as a medicine for stomach trouble [8]. It has been reported that the plant is effective against malaria infection [9]. Traditional midwives administer the leaf and stem extract of *Aspilia africana* as enema to pregnant women to quicken and ease delivery [10]. Eweka and Eweka [11] also reported anti-ulcer effects of *Aspilia africana*. Phytochemical screening of *Aspilia africana* revealed the presence of alkaloids, saponins, tannins, flavonoids, resins, sterols, terpenoids and carbohydrates [7]. This study evaluated the phytochemical constituents and antimicrobial potency of *Aspilia africana* on some clinical isolates with a view of providing clues to aid other scientists who may use this plant for other purposes.

Fig. 1. *Aspilia africana* (pers) C.D. adams [4]

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Fresh leaf samples of *A. africana* were harvested from the Botanical Garden of the Biology Department, Abia State Polytechnic, Aba. A plant taxonomist in the Department identified and authenticated the leaves as *A. africana* with voucher number BGBD 103.

2.2 Extraction of Plant Materials

The methods of Oyagade et al. [12] were employed in the preparation of the plant extracts. The leaves of *A. africana* were plucked, rinsed with tap water and air-dried at room temperature for 3-4 days. The dried leaves were pulverized

using a milling machine to obtain fine powder. Soaking method was adopted for both ethanol and aqueous extraction. 50 g of finely grounded leaf of the plant materials was suspended in 250 mL of 95% ethanol and hot distilled water respectively for 48 hours, to achieve pure dissolution and extraction of the sample. The samples were filtered using Whatman's No 1 filter paper and the filtrate obtained was concentrated in water bath at 40℃ for about 12-14 hours.

2.3 Phytochemical Screening of the Leaves of *A. africana*

The aqueous extracts of *A. africana* were subjected to qualitative and quantitative screening for chemical constituents using standard procedures [13,14].

2.4 Source of Test Organisms

Pure culture of test organisms used in this study; *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Candida balbicans, Aspergillus niger, Penicillium* spp and *Fusarium* spp were obtained from the Department of Microbiology, Abia State Polytechnic, Aba, and sub-cultured in nutrient broth and sabouraud agar and incubated for 24-48 hours before use. Isolates were identified by carrying out morphological and biochemical tests which include Gram staining, catalase, coagulase, oxidase, citrate utilization, indole and sugar fermentation [15].

2.5 Antimicrobial Sensitivity Testing

The antimicrobial sensitivity test was carried out using the methods modified by Isu and Onyeagba [16]. Twenty milliliter of molten sterile nutrient agar and potato dextrose agar were poured into Petri dishes respectively. After solidification overnight, broth cultures of bacteria were introduced into the surface of the sterile nutrient agar plate and a sterile glass spreader was used for even distribution. Holes were made aseptically with a 5 mm diameter sterile cork borer and 0.1 mL of the test solution of different concentrations was introduced into the well. The potato dextrose agar plates used for fungi were agar-welled using 5 mm cork borer. Inside the well, different concentrations of the different extractions (ethanol and water) were dropped. The extract was allowed to diffuse into the medium for 1 hour. The bacteria plates were incubated for 24 hours at 37℃ and the fungi plates incubated for 2-5 days at room temperature. The plates containing the controls were incubated also. The plates were later examined for zones of inhibition, which indicated the degree of susceptibility of the test organisms.

2.6 Determination of Minimum Inhibition Concentration (MIC)

Minimum inhibitory concentration of *A. africana* extract was determined using agar-well techniques. Media plates containing varying concentrations of 6.25%-50% of the water and ethanol extracts respectively were incubated at 37℃ for 24 hours. The lowest concentration of the various extracts causing complete inhibition of the bacterial and fungal growth was taken as the minimum inhibitory concentration (MIC).

2.7 Statistical Analysis

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. The statistical tools employed were descriptive statistics and Analysis of Variance (ANOVA) [17].

3. RESULTS

The phytochemical screening of *A. africana* leaf is shown in Table 1. The qualitative analysis using the aqueous extract of this leaf revealed that alkaloids were high in concentration; flavonoids and saponins were moderately concentrated while tannins and phenols were low in concentration.

Table 1. Qualitative screening of the phytochemical constituents of the leaf of *A. africana* using aqueous extract

Phytochemicals	Concentration
Alkaloids	+++
Flavonoids	++
Saponins	++
Tannins	+
Phenols	+

Key: +++ = high concentration
++ = moderate concentration
+ = low concentration

Table 2 shows the quantitative composition of the phytochemicals obtained in this study. Alkaloids have percentage concentration of 6.350±0.84, followed by saponins with 2.260±0.15 while the least was phenols with percentage concentration of 0.109±0.02.

Table 2. Quantitative screening of the phytochemical constituents of the leaf of *A. africana* using aqueous extract

Phytochemicals	Percentage concentration (%)
Alkaloids	6.350±0.84
Saponins	2.260±0.15
Flavonoids	2.006±0.11
Tannins	0.881±0.04
Phenols	0.109±0.02

Table 3 shows the mean zones of inhibition of the ethanolic and aqueous extracts of *A. africana* using antibiotics as control. The ethanolic extract inhibited more (3.4 mm- 17.9 mm) than the aqueous extract (4.5 mm - 5.3 mm). *Pseudomonas aeruginosa* has the highest zones of inhibition of 17.9 mm, followed by *Staphylococcus aureus* with 16.5 mm zone of inhibition while the least zone of inhibition occurred in *Aspergillus niger* (3.4 mm). The extracts (aqueous and ethanolic) had no zone of inhibition on *Fusarium* spp. Among the antibiotics that served as control, Gentamycin had a zone of inhibition between 8.0 mm – 12.0 mm while Nystatin had 7.0 mm - 14.0 mm on the fungi test organisms.

The minimum inhibitory concentration (MIC) of *A. africana* extracts on the test organisms is shown on Table 4. Using the ethanolic extract, MIC was effective on *Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa* at the least minimum inhibition of 25% respectively. *Candida albicans* was inhibited at 50%, *Aspergillus niger* and *Penicillium* spp at 100% respectively while *Fusarium* spp had no inhibition. For the aqueous extract, inhibition only occurred at 100% for *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively.

4. DISCUSSION

The phytochemical analysis of *A. africana* showed that the leaf is very rich in alkaloid, flavonoids, saponins, tannins and phenols. The qualitative analysis using the aqueous extract of this leaf revealed that alkaloids were high in concentration; flavonoids and saponins were moderately concentrated while tannins and phenols were in low concentration. Alkaloids have percentage concentration of 6.350±0.84, followed by saponins with 2.260±0.15 while the least was phenols with percentage concentration of 0.109±0.02.

Similar results were obtained by other authors [6,7,18], however there are minor differences which could be as a result of methods of extraction and plant parts used. Sofowora [19] reported that age of plant and the season of

Table 3. Mean zone of inhibition of ethanolic and aqueous extracts of *A. africana*

Test organism	Ethanolic inhibition (mm)	Aqueous inhibition (mm)	Control A (Gentamycin)	Control B (Penicillin)	Control C (Nystatin)
Staphylococcus aureus	16.5	4.5	8.0	11.0	
Escherichia coli	12.0	-	9.0	8.0	
Salmonella typhi	10.1	-	11.0	9.0	
Pseudomonas aeruginosa	17.9	5.3	12.0	9.0	
Candida albicans	9.8	-			14.0
Aspergillus niger	3.4	-			11.0
Penicillium spp	5.2	-			11.0
Fusarium spp	-	-			7.0

Table 4. The Minimum Inhibitory Concentration (MIC) of *A. africana* extract on test organisms

Microbes	Ethanol extract (%)	Aqueous extract (%)
Staphylococcus aureus	25	100
E. coli	25	-
Salmonella typhi	25	-
Pseudomonas aeruginosa	25	100
Candida albicans	50	-
Aspergillus niger	100	-
Penicillium spp	100	-
Fusarium spp	-	-

harvest determine the amount of bioactive ingredients. The presence of these phytochemicals as observes in this study is the proper evidence to regard *A. africana* as a medicinal plant [19,20]. This explains why the leaf of *A. africana* is used by tradomedical practitioners to stop bleeding, cure wounds, allergies, rheumatism, inflammation and ulcers [7,11]. Alkaloids are stimulants and act by prolonging the action of several hormones [21]. Saponin-containing plants are important for their haemolytic, expectorative, anti-inflammatory and immune-stimulating activities [22]. Beyond these, saponin demonstrates antimicrobial properties particularly against fungi, bacteria and protozoa [23]. George et al. [24] observed that the blood stream becomes toxic when injected with saponin because of its reaction with enzymes, but when administered orally, it becomes comparatively harmless. Flavonoids on the other hand have antioxidant activity and have become popular in many health-promoting activities such as anti-allergic, anti-cancer, anti-inflammatory and antiviral effects.

In the antimicrobial sensitivity test, ethanolic extract of *A. africana* exhibited an outstanding antimicrobial activity against *Pseudomonas aeruginosa* with an inhibiting effect of 17.9 mm at 100 mg/mL concentration higher than the others at the same concentration. The aqueous extract did not exert much antimicrobial effect on the organisms. It exhibited a zone of inhibition of 5.3 mm at 100 mg/mL for *Pseudomonas aeruginosa.* This might be possible due to the failure of the active ingredient to dissolve sufficiently in water. The superior antimicrobial activity of the ethanolic extract over the aqueous, on the test organisms justified the principle observed in herbal practitioners' preference for using local gin as extraction agent. It may be possible that the bioactive substances that were less soluble in water were dissolve by the solvent [12].

A better antimicrobial effectiveness was obtained for the ethanolic extracts of the plant samples at 25-100 mg/mL concentrations compared to the activity of some selected antibiotics (Gentamycin and Penicillin) of the same concentration. Among the antibiotics that served as control, Gentamycin had a zone of inhibition between 8.0 mm – 12.0 mm while Nystatin had 7.0 mm - 14.0 mm on the fungal test organisms. The sensitivity of the test organisms to the extract of *A. africana* in this study corresponds with the work of Adeniyi and Odufowora [6] that showed the extracts of *A. africana* to possess a broad spectrum anti-bacterial activity against both Gram-positive and Gram-negative bacteria. However the antifungal drug (Nystatin) was more effective than the plant extract at the same concentration. This is confirmed by the reports of Esimone et al. [25] that constitutional antifungal drugs are more active than the plant extract.

5. CONCLUSION

A. africana holds a promising potential source of new drug for treating infections caused by clinical pathogens. The anti- bacterial activity of the extract could be enhanced if the components are purified. Therefore further research should be carried out to know the effect of different solvents in extracting the bioactive constituent of *A. africana*. Research laboratories are therefore enjoined to corroborate and collaborate with traditional herbal practitioners; while the traditional healers provide preliminary information on the uses of medicinal plants based on their historic knowledge, the scientific basis for the efficacy of the extracts and proper advice can be given on how the drugs should be prepared and administered.

DISCLAIMER

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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