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(RESEARCH ARTICLE)

Phytochemical screening and antibacterial activity of *Aspilia africana* on some gastrointestinal tract pathogens

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Abstract

The main aim of this study was to determine the phytochemical constituents and antibacterial activities of the leaf extracts of *A. africana.* The powdered leaf of *A. africana* was extracted using 70% methanol and partitioned into n-hexane, chloroform, ethyl acetate and aqueous methanol fractions. The extract and fractions were phytochemically screened and agar well dilution technique was used to evaluate the antibacterial activity against some gastrointestinal tract pathogens (*Escherichia coli, Staphylococcus aureus, Shigella dysentriae, Salmonella typhi, Salmonella paratyphi* A*, Salmonella paratyphi* B *and Salmonella paratyphi* C). The phytochemical analysis of *A. africana* showed the presence of alkaloids, terpenoids, tannins, flavonoids, anthraquinones and saponins in the methanol extract but varied across the fractions. The methanol extract and fractions inhibited the growth of gastrointestinal tract pathogens but their effectiveness varied with the concentrations. The methanol crude extract showed the best antibacterial activity with zone of inhibition ranging from 15-18 mm followed by aqueous soluble methanol fraction (10-15 mm) and ethylacetaate fraction (9-13 mm) respectively. The n-hexane and chloroform fraction showed no activity. Generally, the diameter zone of inhibition increased with increased extract concentrations. The antibacterial activity of the various extracts of the leaf was comparable to the reference antibiotics, though the antibiotics showed better inhibitory property ($p<0.05$) in some cases on the test isolates than the leaf extracts. The study showed that the leaves of *A. africana* possessed inhibitory properties due to the detected phytochemicals, thus validates the traditional uses of the plant for the treatment of gastrointestinal tract infections.

Keywords: Phytochemical; Gastrointestinal; Antibacterial; Inhibition; *Aspilia africana*

1. Introduction

A bacterial gastrointestinal infection (GIT) is a common illness worldwide and has a considerable effect on the public health of communities. In developing countries, it is a major cause of death claiming about two million lives each year among children <5years of age [1]. Gastrointestinal infections contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programs. Resistance to antimicrobial agents is a major global public health problem that requires action across all government sectors and society partly due to indiscriminate use of antibiotics [2, 3]. The microbial resistance to most antibiotics available, high cost of treatment, negative side effects of allopathic and the emerging GIT infections have necessitated more research for novel, efficient, safe and cost effective therapeutic compounds from plants to tackle this problem. There are several reports in the scientific literature describing the antimicrobial properties of crude extracts prepared from plants [3-6]. *Aspillia africana* C.D. Adams (*Asteraceae*) is a semi woody herb occurring throughout the regions of the savannah and

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tropical Africa on wastelands [7]. In Nigeria, it is commonly known as "yunyun" by the Yorubas, "Orangila" by the Igbos, "Tozalin" by Hausas [8]. It is a semi woody herb from perennial woody root stock. The height varies from 60cm to 200cm depending on the amount of rainfall in the zone. The leaves are crowded in capitular heads and the plant possesses bright yellow star shaped petals hence it is commonly known as "wild sunflower" [9]. The ethnomedicinal uses of these plants are documented below: It is used in traditional medicine to stop bleeding from wounds, clean the surfaces of sores, treatment of rheumatic pains, bee and scorpion stings and for removal of opacities and foreign bodies from the eyes [10]. Decoction of the leaves is used to wash the face and eyes to relieve feverish headache [11, 12]. The leaves infusion is also taken to assist women in childbirth and also to increase milk flow in the mother. A root decoction is taken as oral contraceptive, cough syrup for children and for the treatment of tuberculosis [13]. In South-eastern Nigeria, leaves of this plant is claimed to be effective in the treatment of stomach ache and bleeding gastric ulcers, especially when taken as an aqueous decoction [14]. The present research was therefore, undertaken to validate the possible antibacterial effects of the plant and to correlate these activities with the traditional use.

2. Material and methods

2.1. Collection and identification of the plant

Fresh leaves of *A. africana* were collected from Odu hills at Adavi eba, Adavi LGA Kogi State. The plant was identified at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria where voucher number 1146 was deposited.

2.2. Extract preparation

The leaves were carefully washed under running water and air-dried at room temperature and then pulverized into fine powder. About 300g of the powdered leaves were macerated with 1.5 liters of 70% methanol for 72 hours. The extract was filtered using a muslin cloth and subsequently evaporated using a rotary evaporator. The semi- dried extract was weighed, placed in sterile sample bottles and stored in a refrigerator until required for use as reported by Tiwari *et al*. [15].

2.3. Solvent partioning of crude extract

The methanol crude extract was undertaken for solvent-solvent partitioning by using the methods employed by Emran *et al*. [16]. The crude extract was successively partitionedby using solvents of increasing polarity in the following order; n-hexane, chloroform and ethylacetate in a separating funnel. Resulting fractions of the crude extract were evaporated to dryness using rotary evaporator at 40 ℃. All the concentrated fractions were weighed and stored at 4 ℃ in air tight containers till further analysis. The fractions were coded and labeled as AA1-01 to AA1-04. About one gram (1g) of each extract and fraction were dissolved in 5 ml of 50% dimethylsulphoxide (DMSO) to make 200mg/mlstock solution from which was serially diluted to give concentrations of 100 mg/ml, 50 mg/ml and 25mg/ml.

2.4. Preliminary phytochemical screening

The plant extracts were phytochemically screened for the qualitative detection of alkaloid, flavanoids, terpenoids, tannins, anthraquinones and saponins using standard procedures as described by Tiwari *et al.* [15] and Sofowora [17].

2.5. Antibacterial assay

2.5.1. Test organisms

Clinical isolates of *Escherichia coli, Staphylococcus aureus, Shigella dysentriae, Salmonella typhi, Salmonella paratyphi* A*, Salmonella paratyphi* B *and Salmonella paratyphi C* were obtained from the Microbiological laboratory of Aminu Kano teaching Hospital, Kano for the susceptibility tests. The organisms were used after the identity was confirmed at the Department of Microbiology, Bayero University, Kano. The stock culture was maintained on Nutrient agar slant at 40 ℃ in the refrigerator.

2.5.2. Innocula standardization

The standardization of inoculum was carried out as described by the National Committee for Clinical Laboratory Standards [18]. Active culture for experiments were prepared by transferring a loopful of cells from the stock culture to sterile Mueller-Hintonagar (MHA) and then incubated for 24 hours at 37 ℃. Few colonies of the overnight growth of

the isolates to be tested were dispersed in sterile normal saline to form a turbid culture suspension that matched 0.5 McFarland turbidity.

2.5.3. Antibacterial susceptibility test

The sensitivity of the crude extracts and fractions were determined using the agar well diffusion method as described by Abdulsalami *et al.* [19]. The prepared bacterial suspension equivalent to 0.5 McFarland standard was swabbed onto the surface of sterile Mueller-Hinton agar medium in a sterile Petri-dish. A sterile 6 mm diameter cork borer was used to bore wells into the agar medium. The wells were then filled up with approximately 0.1 ml of the crude extract and fractions (AA1and AA1-01 to AA1-04) at concentrations of 25, 50 and 100 mg/ml taking care to prevent spillage onto the surface ofthe agar medium. A positive control, 0.1ml amoxicilin (100µg/ml) was used as standard reference antibiotic while 0.1 ml 50% DMSO dispensed into one of the wells served as the negative control. The plates were allowed to stand on the laboratory bench for 1 hour to allow proper diffusion of the extract into the medium after which the plates were incubated at 37 ℃ for 24 hours, and thereafter the plates were observed for zones of inhibition and measured in millimeter using a transparent ruler. The analysis was done in triplicates.

2.6. Statistical analyses

The statistical analyses were carried out using statistical package for social sciences (SPSS- computer package). Data from three independent replicates on the antibacterial activities of *A. Africana* were subjected to one-way analysis of variance (ANOVA) at $p<0.05$ level of significance for comparison of the extract activities.

3. Results and discussion

3.1. Physical characteristics of extracts and fractions

The percentage yield and appearance of the methanol crude exracts and fractions of *A. africana* are presented in Table 1. Among all the solvents used for partitioning, aqueous methanol soluble fraction was found to have the highest extractive yield 21.77 g (56.78%) followed by ethylacetate soluble fraction 5.03 g (13.11%) (Table 1). This shows that the plant materials were more soluble in polar solvents as compared to the non-polar solvents.

Table 1 Physical characteristics of extracts and fractions obtained from *A. africana*

Extracts/Fractions	Code	Weight (g)	Yield (%w/w)	Appearance
Methanol extract (Crude)	AA1	38.34	12.78	Black gummy mass
n-Hexane soluble fraction	AA1-01	4.81	12.54	Blackish oily mass
Chloroform soluble fraction	AA1-02	3.43	8.95	Blackish oily mass
Ethylacetate soluble fraction	AA1-03	5.03	13.11	Black gummy mass
Aqueous methanol soluble fraction	AA1-04	21.77	56.78	Black powdery mass

3.2. Phytochemical constituents of extracts and fractions

The phytochemical screening carried out on the methanol extract and fractions showed the presence of bioactive constituents. The phytochemical characters of the four different fractions of *A. africana* (AA) are summarized in Table 2. The results showed the presence of alkaloids, terpenoids, tannins, flavonoids, anthraquinones and saponins in the methanol crude extract and the aqueous methanol soluble fraction. The presence of these phytochemicals varied in the other fractions. The medicinal properties of Plant materials result from the secondary metabolites present in the Plant which may be attributed to a single compound or a combination of the metabolites. The phytochemical analysis of *A. africana* showed the presence of alkaloids, terpenoids, tannins, flavonoids, anthraquinones and saponins distributed in the crude extract but varied across the fractions. The result obtained in this study is consistent with the works of Oko and Akiang [20-23]. The observed differences in phytochemicals detected in the fractions may be attributed to the variation in the distribution of active principles according to their affinity for the solvent used in fractionation. However, the presence of these secondary metabolites in the leaves of *Aspilia africana* is an indication of its potency as a medicinal plant material. Alkaloids have been used as a stimulant of the central nervous system, topical anaesthetic in ophthalmology, analgesic and anti-malarial [23, 24]. Terpenoids have also been reported to be active against bacteria [25]. Tannins have been reported as having astringent activities which helps to hasten wound

healing and treat inflammations [22]. Saponins have the ability to precipitate and coagulate red blood cells, they are characteristically able to form foams in aqueous solutions, bring about hemolytic activity, have bitterness properties as well as cause cholesterol binding [22, 26]. The high saponin contents of *Aspilia africana* leaf justify its use to treat wounds and stop bleeding. Flavonoids have been reported to be useful against an array of microorganisms [27]. Anthraquinones represent a major class of glycosides, they have demonstrated potential therapeutic uses as antibacterial, antiviral, antifungal as well as antioxidant, anti-inflammatory and cytotoxic agents [28].

Table 2 Phytochemical constituents of extracts and fractions of *A. africana*

Fractions	Alkaloids	Terpenoid	Flavonoid	Tannins	Anthraquinones	Saponin
AA1	+	+	+	+	+	+
AA1-01	-	-	-	-	-	+
AA1-02	-	-	-	-	-	+
AA1-03	+	-	+	+	+	+
AA1-04	+	+	+	+	+	+

(+) indicates presence (-) indicates absence

3.3. Antibacterial activity of extracts and fractions

The antibacterial potential of the crude extract and different fractions represented by the mean zone of inhibition (mm) of different concentration against the test bacteria are presented in Table 3. The crude extract and fractions showed antibacterial activity except the n-hexane and chloroform fraction. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. The present investigation showed that the crude extract and fractions inhibited the growth of gastrointestinal tract pathogens but their effectiveness varied with the extract and fractions. The methanol extract showed the best antibacterial activity with zone of inhibition ranging from 15-18 mm, indicating a synergistic activity of the phytochemicals present. All the organisms tested showed different form of susceptibility but *E. coli* and *S. typhi* were most sensitive to the extract at all the concentrations ranging from 13-18 mm. The other organisms showed resistance to the organism at the lowest concentration of 25mg/ml. The ethylacetate fraction exhibited a good activity on all the organisms at the highest concentration of 100mg/ml causing 9-13 mm diameter sizes of zones of inhibition against the bacteria. The aqueous methanol soluble fraction also recorded maximum antibacterial activity on all the organisms at a concentration of 50mg/ml and 100mg/ml with inhibition zone sizes ranging from 10-15 mm. n-hexane and chloroform fraction showed no activity. This could be due to the fact that the active components present in the plant were more soluble in the polar solvents than the non-polar solvents. Generally, the diameter zone of inhibition increased with increased extract concentrations. Similar results have also been obtained by other researchers, Anibijuwon *et al.* [29] reported that the ethanol extract of *A. africana* possesses antibacterial activities against some pathogenic organisms of clinical origin. Moreover, the study corroborates with the work of Ezeigbo *et al.* [21] who demonstrated *in-vitro* antibacterial effect of the leaves of *A. africana o*n some clinical isolates incriminated in gastrointestinal tract infection. However the reference standard antibiotics showed better inhibitory property ($p<0.05$) on the test isolates than the leaf extract (Table 3). This is confirmed by the reports of Oluduro *et al.* [30] that conventional antibiotics drugs are more active than the plant extract ostensibly due to the chemical synthesis of the pure compounds of the antibiotics.

Table 3 Zones of inhibition (mm) of the different concentrations of the leaf fractions of *A. africana* on the test bacteria

Fractions	Conc. (mg/ml)	E.C	S.A	S.D	S.T	S.Pa	S.Pb	S.Pc
AA1	100	18	12	11	18	13	13	14
	50	16	10	9	14	13	12	13
	25	15	-	-	13	-	-	-
AA1-01	100	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-
AA1-02	100	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-
AA1-03	100	12	9	12	11	11	11	13
	50	-	-	12	-	-	-	-
	25	-	-	-	-	-	-	-
AA1-04	100	14	14	13	13	14	14	15
	50	11	11	10	12	11	11	12
	25	-	-	-	-	-	-	-
Control	100	33	35	25	33	19	35	21

*Inhibition zone (mm) includes the diameter of disc (6 mm). E.C = *Escherichia coli*, S.A= *Staphylococcus aureus*, S.D= *Shigella dysentriae*, S.T= *Salmonella typhi*, S.Pa= *Salmonella paratyphi* A, S.Pb= *Salmonella paratyphi* B and S.Pc= *Salmonella paratyphi* C.

4. Conclusion

The presence of these bioactive compounds as revealed by the phytochemical screening of the leaf extract of *A. africana*, may be responsible for the potent antibacterial activity of the plant. The present investigation therefore, provides the scientific backings and justifies the traditional claim of the plant and its immense potential in the treatment of bacteria that are implicated in either cholera, diarrheoa, dysentery or other gastrointestinal disorders. It is recommended that further work on the isolation and characterization of the active ingredients in the crude extract of *A. africana* be advocated.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

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