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Antimicrobial Activity of *Senna alata* and *Phyllanthus amarus*

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Abstract: *Senna alata* and *Phyllanthus amarus* were evaluated *in-vitro* for their antimicrobial effectiveness on *Escherichia coli, Staphylococcus aureus*, *Salmonella typhimurium*, *Aspergillus niger, Aspergillus flavus* and *Candida albicans*. Ethanol and water extracts of *S. alata* and *P. amarus* were comparatively and selectively effective on test organisms. The best inhibitory zone was recorded in water extract of *S. alata* against *A. niger* with 27.2 mm while 10.1 mm was recorded as the least zone of inhibition against *S. typhimurium*. *P. amarus* ethanol and water extracts recorded the best antibacterial activity against *S. aureus* (20.2 mm) and *E. coli* (15.3 mm) while the best antifungal ethanol extract of *P. amarus* was recorded for *A. niger* (18.2 mm). Thus, both *S. alata* and *P. amarus* are good antimicrobial agents against bacterial and fungal pathogens of human and plants.

Key words: Antifungal · Antibacterial · Ethanol Extract · *Senna Alata* · *Phyllanthus Amarus*

INTRODUCTION

There has been an increasing interest world wide on therapeutic values of natural products. It is believed that the cure to any debilitating human ailments and diseases may be found among the world's flora in natural pharmacy [1]. Today natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action. A special feature of higher plants is their capacity to produce a large number of secondary metabolites [2]. Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases [3, 4]. In Nigeria various plant parts are used in treatment of different ailments with remarkable success. Among these enormous numbers of these medicinal plants are members of the genus *Senna* and the genus *Phyllanthus*. According to Odugbemi and Akinsulire [5], the genus *Senna* has six species with medicinal value but three have been reported to have antifungal activities which are *Senna tora, Senna orcidentalis* and *Senna alata* [6].

Senna alata is an ornamental flowering plant which is mostly used as antimicrobial agent [7]. The juice of fresh leaves of *Senna alata* is universally recognized by local healers as a remedy for parasitic skin disease and is used in the treatment of many eruptive and pustular skin condition by simply rubbing the crushed leaves either alone or mixed with oil on the skin [6]. Root decoction is taken in Nigerian and Guinea Bissau to regulate menstrual flow. Decoction with rock salt and other dry medicinal plants is taken in Nigeria thrice weekly on an empty stomach for effective treatment of chronic gonorrhea [7]. *Phyllanthus amarus* has been reported to have general medicinal application. The plant has a number of uses in Ivory Coast as anti-fever and to reduce sore throat pains while in Congo the sap of the plant is used to treat otitis and applied topically to maturate furuncles and abscesses [7]. Infusion of leaves is used in Kenya to relieve stomach pains and in southwestern Nigeria for haemorrhoids [7].
The significant of drugs derived from plants cannot be over emphasized with the recent trend of high percentage of resistance of microorganisms to the present day antibiotics [8]. Effort has been intensified by researcher towards a search for more sources of antimicrobial agents. Local medicinal plants provide a source of new possible anti microbial drugs such as anti viral, anti parasitic drugs and anti fungal [9]. Thus, it is necessary to evaluate the antimicrobial potential use of these two tropical plants *P. amarus* and *S. alata*.

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MATERIAL AND METHODS

Extraction of Plant Samples: Fresh leaves of *Senna alata* and *Phyllanthus amarus* were collected from the premises of the University of Ibadan and authenticated at the Department of Botany Herbarium, University of Ibadan, Nigeria. The leaves of the two herbal plants were plucked and air dried for two weeks. The dried leaves were milled into fine powder using a milling machine (will mx-391N) after which they were weighed accordingly using a weighing balance. Aqueous extraction was carried out by weighing fifty grams of powdered samples into conical flask after which 250 ml of sterile distilled water was added into each flask and then covered with aluminium foil. The flasks were allowed to stand for seventy two (72) hours at room temperature with occasional stirring at interval. The mixture was filtered using Muslim cloth and finally centrifuged at 5000 rpm for 30 minutes. The clear liquid was collected into a pre-weighed labelled evaporating dish. The clear liquid in the dish was concentrated at about 90°C for evaporation of water. The dish with the extract were re-weighed and then transferred to well labelled sterile bottles. Extracts were then stored at 4°C in refrigerator until needed. Ethanol extraction was also carried out. Fifty grams of powdered leaves were emptied into a fractionating column after which 250 ml of ethanol was added and the Soxhlet extraction was set up for four hours. The ethanol extract of the two leaves obtained were subjected to distillation by heating in a water bath at 60°C and the extracts aseptically transferred into a pre-weighed labelled sterile bottles. The pH of the water and ethanol extracts were determined.

Phytochemical Analysis of Plant Samples: Phytochemical analysis was carried out on powdered leaf samples of the plant in order to determine the presence of secondary metabolites (Tannins, Phenol, Chalones, Phylobatanmins, Steroids, Saponins, Flavonoids, Alkaloids and Anthraquinones) by means of some specific chemical reactions using the method of Harborne [10].

Antimicrobial Activity: Antimicrobial activity was carried out using agar well diffusion according to the method of Hirasawa *et al.* [11]. Antimicrobial activity of *Senna alata* and *Phyllanthus amarus* extracts were evaluated on the following test organisms; *Escherichia coli, Staphylococcus aureus*, *Salmonella typhimurium* and *Candida albicans*) obtained from Medical Microbiology Unit of University College Hospital, Ibadan, Oyo State, Nigeria, while *Aspergillus niger* and *Aspergillus flavus* were obtained from Plant Pathology Unit of Department of Botany, University of Ibadan. The extracts were separately passed through membrane Millipore filter (2µm) prior antimicrobial activity experiment to ascertain that the extracts are free from microorganisms. The experiment was replicated and arranged in completely randomized design.

Determination of Antibacterial Activity of Plant Extracts: The bacterial suspension were cultured separately in buffered peptone water for 24 hours after which 0.2 ml of each suspension was mixed with 25 ml of Tryptone Soya agar in a sterile Petri dishes. The agar was allowed to solidify; the already sterilized cork borer of 5 mm diameter was then used to bore well in the solidified inoculated agar. Equal amount of the extracts and sterile water (as control) was dispensed into the well using sterile pipette and incubated at 37°C for 18-24 hours thereafter the plates were examined for zone of inhibition.

Determination of Antifungal Activity of Plant Extracts: Sterile distilled water was dispensed into a fresh culture of the different fungi on a slant of well-formed spores and shake vigorously to make fungi spores suspension. *Candida albicans* suspension was made in buffered peptone water with 48 hours of incubation at 25°C. The suspension of 0.1 ml was mixed with 25 ml of sterile potato dextrose agar, poured in sterile petri dishes and allowed to solidify. A sterilized cork borer of 5 mm in diameter was used to bore well in the solidified agar. Equal amount of extracts and sterile water (as control) was dispensed into the bored media and incubated at 25°C for 72 hours. It was later observed for zone of inhibition.

Data Analysis: The data collected were analyzed using the analysis of variance (Student-Newman-Keuls Test) procedures of SAS (version 9.1 of 2009, SAS Institute, Cary, NC). The least significant different (LSD) test at 0.05 level of significant was used to compare means for zone of inhibition (mm).

RESULTS

Phytochemical Analysis and pH of Plant Extracts: The results of the phytochemical analysis showed that *Senna alata* contains steroids, saponins, phenol and phylobatannins while the phytochemical analysis of *Phyllanthus amarus* contain steroids, flavonoid, alkaloids, tannins, phylobatannins and phenol (Table 1).

Table 1: Phytochemical constituent of *Senna alata* and *Phyllanthus amarus*

Secondary Metabolites	*Senna alata*	*Phyllanthus amarus*
Flavonoid	-	+
Anthraquinones	-	-
Steroids	+	+
Alkaloids	-	+
Saponins	+	-
Tannins	-	+
Phenol	+	+
Chalcones	-	-
Phylobatannins	+	+

+ Present, - Absent

Table 2: pH of ethanol and water extracts of *Senna alata and phyllanthus amarus*

Extract	State	Colour	pH
Senna alata (Aqueous extract)	Liquid	Mud brown	5.42
Senna alata (Ethanolic extract)	Liquid	Dark green	6.00
Phyllanthus amarus (Aqueous extract)	Liquid	Brown	3.58
Phyllanthus amarus (Ethanolic extract)	Liquid	Dark green	4.10

Table 3: Zone of inhibition (mm) of plant extracts against test microorganism

	Senna alata		*Phyllanthus amarus*	
Organism	Ethanol extract (mm)	Water extract (mm)	Ethanol extract (mm)	Water extract (mm)
Staphylococcus aureus	20.1 ± 0.1^{c}	18.2 ± 0.3^{c}	20.2 ± 0.2^{a}	20.1 ± 0.1^{a}
Escherichia coli	17.2 ± 0.3^{e}	10.2 ± 0.2^{e}	15.3 ± 0.4^{d}	15.3 ± 0.1^{c}
Salmonella typhimurium	12.1 ± 0.1^{f}	10.1 ± 0.1^{e}	15.1 ± 0.1^{d}	20.1 ± 0.1^{a}
Aspergillus niger	25.2 ± 0.3^{a}	27.2 ± 0.2^{a}	18.2 ± 0.3^{b}	16.3 ± 0.4^{b}
Candida albicans	18.2 ± 0.2^{d}	14.1 ± 0.1^{d}	10.2 ± 0.3^{e}	16.2 ± 0.2^{b}
Aspergillus flavus	22.1 ± 0.1^{b}	20.1 ± 0.1^{b}	17.1 ± 0.1^{c}	15.2 ± 0.2^{c}

The pH of the ethanol and aqueous extracts was observed to be acidic whereas, aqueous extract of *Phyllanthus amarus* showed more acidity (3.58) compared to aqueous extract of *Senna alata* with pH of 5.42. Generally, the pH of *Phyllanthus amarus* was more acidic compared to that of *Senna alata* (Table 2).

Antimicrobial Activity of *Senna alata*: Water extract of *S. alata* was highly effective on *A. niger* with 27.2 mm zone of inhibition and on *A. flavus* with 20.1 mm zone of inhibition, while the least antifungal effectiveness of water extract of *S. alata* was recorded against *Candida albicans* with 14.1 mm zone of inhibition (Table 3). The best antibacterial water extract of *S. alata* was recorded against *Staphyloccus aureus* with 18.2 mm zone of inhibition while similar antibacterial effect of water extract of *S. alata* was observed against *Escherichia coli* and *Salmonella typhimurium* with 10.2 and 10.1 mm zone of inhibition respectively (Table 3). The antifungal effectiveness of ethanol extract of *S. alata* had the best zone if inhibition of 25.2 mm against *A. niger*, followed by *A. flavus* and *C. albicans* with 22.1 mm and 18.2 mm zone of inhibition respectively (Table 3).

Antimicrobial Activity of *Phyllanthus amarus*: Antimicrobial effectiveness of *P. amarus* was almost similar on *S. aureus* and *E. coli* with 20.1 mm zone of inhibition for ethanol extract and 15.1 mm zone of inhibition for water extract respectively (Table 3). *S. typhimurium* was inhibited by ethanol extract of *P. amarus* by 15.1 mm zone of inhibition and water extract of *P. amarus* by 20.1 mm zone of inhibition. Ethanol and water extract of *P. amarus* recorded 18.2 mm and 16.3 mm as zone of inhibition respectively against *A. niger*. The effect of *P. amarus* was also observed on *A. flavus* with 17.1 mm zone of inhibition for ethanol extract and 15.2 mm zone of inhibition for water extract (Table 3). Further observation showed that *C. albicans* was inhibited by ethanol extract (10.2 mm zone of inhibition) and water extract (16.2 mm zone of inhibition) of *P. amarus*.

Generally, ethanol and water extracts of *S. alata* significantly ($p<0.05$) inhibited *A. niger*, followed by *A. flavus*, *S. aureus*, *C. albican* while the least were *E.coli* and *S. typhimurium*. Similar observation showed that ethanol and water extracts of *P. amarus* had high significant ($p<0.05$) effect on *S. aureus*, followed by *A. niger* and *A. flavus*. Antibacterial effect of ethanol

extract of *P. amarus* on *E.coli* and *S. typhimurium* recorded significant (p <0.05) similarity (Table 3). However, both *S. alata* (ethanol and water extracts) and *P. amarus* (ethanol and water extracts) significantly (p <0.05) varied in their effectiveness with the exception of water extract of *S. alata* that showed significant (p <0.05) similarity between *E. coli* and *S. typhimurium*. This significant (p <0.05) similarity was also observed for ethanol extract of *P. amarus* on *E.coli* and *S. typhimurium* and for water extract of *P. amarus* on *A. niger* and *C. albicans*.

DISCUSSION

The leaf extracts of *Senna alata* and *Phyllanthus amarus* contain many biological active substances which have been proved as antimicrobial compound as shown in the work of Alade and Irobi [12] that tannins, steroids and phenol are among the antimicrobial substances in the phytochemical analysis of *Acalyphi weikeisiana* that can inhibit the growth of pathogenic fungi and bacteria. This was corroborated by Adekunle *et al*. [13] that the presence of tannins, saponins and steroids among other substances from the extracts of *Funtumia elastica* and *Mallotus oppositifolius* was likely to be responsible for the antifungal activity exhibited by these plants. The result obtained in this study therefore agreed with the findings of Barnabas and Nagarajan [14] and Onadapo and Owonubi [15].

These phytochemicals have been implicated in inhibiting cell wall formation in fungi leading to the death of the organisms [16]. Also, [17] reported a similar observation on the efficacy of *Moringa oleifera.* Their reports showed that the activities of these plants were due to the presence of tannins, saponin, flavonoids and anthraquiones in the plant extract. Similar phytochemical constituents (flavonoids and tannins) were also revealed in the plant (*Stachyterpheta indica*) against pathogenic bacteria as reported by Kumar *et al*. [18].

The phytochemical properties of both extracts revealed that *S. alata* have pH range of 5.42 and 6.0 for aqueous and ethanolic extracts respectively and pH ranges of 3.58 and 4.10 for aqueous and ethanolic extracts of *P. amarus.* Thus, the pH range of the two plants extracts is acidic and this could enhance their antimicrobial potency as curative measures for some disease.

Antimicrobial analysis of *P. amarus* was more effective against bacteria and *S.alata* against fungi. However, the ethanol extracts of both *S. alata* and *P. amarus* were more effective compared to water extract. The results showed that the test organisms were very sensitive to extracts from both plants. As reported by Oloke and Kolawole [19], the bioactive components of any medicinal plant may differ in their solubility depending on the solvents used for extraction. This may account for the differences observed in the effective ethanol and water extracts. Bacteria were sensitive to extracts of *P. amarus* as reported by Burkill [7] to treat bacterial infections such as otitis, abscesses and haemorrhoids. Fungi on the other hand were more sensitive to the extracts of *S. alata* as confirmed by Oliver-Bever [6] that *S. alata* can be used to cure fungi skin diseases caused by *Epidermophyton gloccosum* and *Trichophyton mentagrophte* in West Africa. This agreed with the suggestion of Maobe *et al*. [20] that application of this knowledge is likely to improve the use of traditional herbs if integrated with that of traditional healers.

Generally, the observed values for the antimicrobial plant extracts were moderately favorable against pathogenic bacteria and fungi. This then suggest that, they are promising antimicrobial agents which agreed with the suggestion of Takazawa *et al*. [21] that there is a need to employ broad range of solvents in the extraction of possible phytochemicals from medicinal plants. It is not common to have commercial antibiotics with antifungal and antibacterial effectiveness, this report open up the need to concentrate on the possibility of searching for active ingredients in these plants that can be deployed into world's chemotherapeutic usage.

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