PHYTOCHEMICAL AND ANTIFUNGAL PROPERTIES OF ANARA (*Solanum incanum*) LEAF ON DERMATOPHYTES

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Abstract

Phytochemical and antifungal properties of anara leaf on dermatophytes were investigated. Anara leaves were purchased from Orie Obibi market, Obibiezena, Owerri-West, L.G.A, Imo State while samples from clippers were collected using swab sticks from barbing shops. Standard microbiological methods were adopted in the isolation of dermatophytes from the samples. Anara leaves were dried, ground, and extracted using ethanol, cold and hot water. Qualitative phytochemical screening was adopted in the determination of the phytochemical constituents of the leaf extracts. Agar well diffusion technique was adopted in the determination of the antifungal properties of the extracts. Tube dilution technique was adopted in the determination of the minimum inhibitory concentration of the extracts. Dermatophytes isolated from the clippers were *Trichophyton* and *Epidermophyton* species. The presence of saponins, tannins, phenols, alkaloids and anthraquinones were detected. Antifungal activities of the extracts showed zones of inhibition 12mm to 14m m with hot water extract, 16mm to 20mm with ethanol extract and none with cold water extract. Minimum inhibitory concentration was recorded at 250mg/ml and 500mg/ml with ethanol extract. Minimum fungicidal concentration was recorded at 500mg/ml with ethanol extract against *Epidermophyton* species. Cold and hot water extracts showed no fungicidal effect against the dermatophytes. The antifungal properties of the extract could be attributed to the phytochemical constituents of the leaf. The use of medicinal plants such as anara should be given attention for cheaper and easily available remedy to fungal infections in the society.

Key words: Antifungal, dermatophytes, phytochemical, extracts, infections, fungal.

INTRODUCTION

Anara leaf (*Solanum incanum*) is the most potent plants against pathogenic microorganisms. *Solanum incanum* (L) is one of the important traditional

medicinal plants belonging to *Solanacae* family. Antibacterial activity of *Solanum incanum* was studied (John Britto and Senthilkumar 2001; Pavitra *et al*., 2012) and presence of analysis of phytochemicals were also studied (Pavitra *et al*., 2012). Other solanum species, *Solaum torvum* (leaf, stem and roots) showed antibacterial and antifungal activity (Bari *et al*., 2010) and antibacterial activity of *Solanum surattense* whole plant extract (Patil Suhas *et al*., 2009) and leaf extract (Sheeba, 2010) were studied. Analysis, presence of phytochemicals and potent antibacterial activity of leaf, root and seed extracts were studied in *Solanum nigrum* (Sridhar *et al*., 2011).

Medicinal plants are used in both developing and developed countries as Source of drugs or as a source of herbal extracts for various therapeutic purposes. Use of plant derived natural compounds as part of herbal preparations and as alternative sources of medicine continues to play major role in the general wellness of the people all over the world. WHO estimates that 80% of the world population presently uses herbal medicine for some aspects of primary health care. This high co-dependence on herbal drugs has been facilitated by factors such as low cost of herbal drugs endearing them with the poor mass of underdeveloped and developing world; the 'green' movement in the first world that campaigns on the intrinsic safety and desirability of natural products and the individualistic philosophy of western society that encourages self-medication, with many people preferring to treat themselves with herbal remedies. In developing countries like Kenya, there is an increasing attempt to incorporate traditional medicine in health care systems (Verma and Singh, 2008). *Solanm incanum* is one of the important traditional medicinal plants. Many of the medicinal uses of *Solamum incanum* are based on its analgesic properties. Throughout tropical Africa a sore throat, angina, stomach-pain, colic, headache, painful menstruation and liver pain are treated with *Solanum incanum*. For these purposes, leaf, fruit and fruit decoctions are drank, fruits are chewed and sap swallowed, leaf sap is used for washing painful areas, and ash of burnt plants is mixed with fat and applied externally. Leaves are added to soup to improve the flavor. The large variation in toxicity makes it dangerous to transfer specific uses from one region to another, The fruit and seed are used in Africa and Asia to curdle milk and to make cheese. Also, the plant is employed in East and Southern Africa for the treatment of skin diseases, general infections, abdominal pains, fever, stomachache and indigestion. In addition, the fruit of *Solannum incanum* is used for the treatment of dandruff, skin diseases, sores and wounds in Tanzania (Habtamu *et al*., 2014). WHO in 2003 resolution recommended the inclusion of traditional healers in management of health.

This move was to help countries document traditional medicines and remedies and to ensure the safety and efficacies of these remedies is established. It's obvious th?t at least some plants contain compounds with pharmacological activity that can be harnessed as medicinal agents. Isolation of and experimentation with a single constituent provides information that can be adapted to more holistic understanding of the herbs action (Njoroge & Bussmann, 2006).

Solanum incanum belongs to the genus *Solanum* that contains aglycone, which is a steroidal alkaloid (containing nitrogen atom). Solanum incanum contains solanine which is a steroidal alkaloid whose pharmacological activity is against many bacterial organisms. Solanine is a bitter gluco-alkaloid first isolated from *Solanum nigram*, and it has also been isolated from other species such as *S. gigantium, S. incanum, S. tuberosum and S. aculaestrum,* this alkaloid is mainly concentrated in unripe fruits and in green potatoes and disappear in ripening process.

The rich content of antifungal substances in plants are being used biopesticide since up to the beginning of human civilization. Antifungal effects of plant and plant products emerge clearly every day. Antifungal substances which are obtained from plants have no side effect against environment thus, giving a significant advantage. Nowadays, a commercial pesticide used against plant diseases is found to cause damage to environment and human health. Because of that conducting a research of alternative control methods comes into prominence for minimizing used commercial pesticide. Research found that compounds in the structure of plants and essential oil were showed antifungal, antibacterial, insecticidal, nematicidal, herbicidal and antiviral activities, Bauer, (2007). Different parts of the plants are used as sedative, diuretic and digestive. They are also used in the treatment of coughs and colds (Yuanyuan *et al*., 2009). Leaves are used as haemostatic. Extract of the fruits and leaves of anara leaf (*Solanum incanumn*) are said to be useful in case of liver and spleen enlargemernt and in the treatment of cough. Paste of root is used to cure cracks in feet. The fume of burning seeds is inhaled for toothache. Due to the notable medicinal value of *S. torvum*, it was considered of interest to carry out a phytochemical and antimicrobial investigation of this species and the results leading to the antimicrobial screening are presented in this paper (Belboukhari *et al*., 2002).

MATERIALS AND METHODS

Sample Collection

Oral assent from the study participants was sort before collection of samples from the barbing tools used by the barbers. Sterile swab sticks moistened with sterile water was used to swab clippers used in barbing human hair. Fresh leaves of anara purchased from Orie Obibi market, Obibiezena, Owerri-West, L.G.A, Imo State. The leaves were dried, ground and kept for extraction.

Sterilization of Glasswares and Media

All the glasswares to be used in this study were sterilized using laboratory hot air oven at temperature of 160 ^{O}C for 2 hours and media used in this study were sterilized using the autoclave at a temperature of 121^{0} C at 15psi for 15 minutes. After the sterilization, the media were brought out together with the glassware and kept on a clean laboratory bench. The media were poured into the Petri dishes when cooled to 45 ^{O}C and allowed to solidify.

Extraction of active ingredients in the leaf powder

Twenty grams (20g) of ground leaf powder was subjected to extraction using soaking method in ethanol. Twenty grams (20g) each of the powder was poured into 95% ethanol in a beaker. The contents of the beaker were allowed to soak overnight. The content was filtered using muslin cloth and stored in a sterile container. This method was repeated using cold and hot water for cold and hot water extracts.

Isolation of fungi from barber's shop

The method described by Ebuara et al. (2020) was adopted in the isolation of fungi from the barber's shop. Swabs from sampled surfaces were inoculated in 10ml of peptone water by cutting the swabs aseptically into the peptone water, shaking and was allowed to stand for 20 minutes. 0.1 milliliter aliquot and was dropped onto the different media in the plates. A sterile bent glass rod was used to spread the aliquot evenly on the media Sabouraud dextrose agar. The plates were labeled accordingly. The inoculated plates were inverted and incubated in the incubator at a temperature of 37 ^{0}C for 24 hours except Sabouraud Dextrose agar plates were incubated at room temperature (28^{0}C) for three days.

Purification and preservation of isolates

After the various colony counts, fungal isolates was subcultured onto freshly prepared Sabouraud dextrose medium and was incubated for growth.

Identification of fungal isolates

The fungal isolates were identified by morphological characteristics on Sabouraud Dextrose Agar (SDA) and microscopic examination after lacto-phenol cotton blue staining technique. Each of the fungal isolates were separately collected with a sterile wooden stick or teasing needle and teased out on a drop of lacto-phenol cotton blue stain on a clean glass slide. The wet mount preparations were then viewed under the microscope for branched and unbranched hyphae (Yaradua et al., 2018).

Standardization of inocula

The fungal isolates were inoculated by transferring representative fungal isolates from fresh culture plate into sterile saline bottle. The mixture was shaken to achieve homogenous suspension. The homogenous suspension was adjusted to 0.5 McFarland's standard (**Idu and Igeleke, 2012**).

Antifungal susceptibility pattern of leaf extracts

The agar well diffusion techniques as described by **Idu and Igeleke (2012)** was adopted for this study to evaluate the antifungal susceptibility pattern of the leaf extracts. A sterile Pasteur pipette was used to drop 0.2 ml standardized inoculums equivalent to 0.5 McFarland's turbidity standards on the surface of already prepared and dry Mueller-Hinton agar. The inoculum was evenly spread using Hockey stick shaped glass rod. Two wells were carefully bored into each agar plate after standing for about 5 minutes with heat sterilized 6 mm diameter cork borer and labeled. The extracts were then poured into the wells and the plates were allowed to stand for about 30 minutes for proper diffusion of the solutions before being incubated at 28°C for 72 hours. Antifungal activity was evaluated by measuring the diameter of the zones of inhibition produced by the extracts against the test organisms in millimeters.

Determination of the minimum inhibitory concentration (MIC)

The method described by Berhe et al. (2018) was adopted in the determination of the ***minimum inhibitory concentration (MIC)*** of the leaf extracts. The minimum inhibitory concentration was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. Varying concentrations of the extracts (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml) was prepared. 0.1 ml of standardized test organisms were inoculated into the tubes containing the different concentrations of the extracts and controls were equally setup by using solvents and test organisms without extract. These were kept 28^0C for 3-5 days. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration.

Test for minimum fungicidal concentrations (MFC)

Tubes showing no visible growth from the MIC test was subcultured onto sterile nutrient agar plates and incubated at 28^0C for 72 hours. The lowest

concentration of the leaf extract yielding no growth was recorded as the minimum fungicidal concentrations as the case may be.

RESULTS AND DISCUSSION

RESULTS

Table 1 showed the cultural morphology and microscopic characteristics of the fungal isolates from the barber's shop. They were *Trichophyton* and *Epidermophyton* species.

Table 2 showed the phytochemical constituents of the leaf extracts. The presence of saponins, tannins, phenols, alkaloids and anthraquinones were detected.

Table 3 showed the antifungal properties of the leaf extracts against the dermatophytes. Zones of inhibition 12mm to 14m m with hot water extract, 16mm to 20mm with ethanol extract and none with cold water extract.

Table 4 showed the minimum inhibitory concentration of the leaf extracts against the dermatophytes. Minimum inhibitory concentration was recorded at 250mg/ml and 500mg/ml with ethanol extract.

Table 5 showed the minimum fungicidal concentrations of the leaf extracts against the dermatophytes. Minimum fungicidal concentration was recorded at 500mg/ml with ethanol extract against *Epidermophyton* species. Cold and hot water extracts showed no fungicidal effect against the dermatophytes.

Cultural morphology	Microscopy	Possible fungi
Pinkish, raised, fluffy, circular Colonies	Septate hyphae	*Trichophyton* species
Whitish, powdery flat colonies	Smooth, thin-walled hyphae	*Epidermophyton* species

Table 2: Phytochemical screening of the leaf extracts

Phytochemicals	Extracts/Results		
	Cold	Hot	Ethanol
Saponins	+	+	+
Tannins	+	+	+
Phenols	-	+	+
Alkaloids	+	+	+
Anthranoids	-	-	-
Anthraquinones	-	-	+
Phlobatannins	-	-	-
Cardiac glycosides	-	-	-

Key: + = Presence of phytochemicals - = Absence of Phytochemicals

Table 3: Antifungal susceptibility pattern of the leaf extracts

Fungal isolates	Extracts/Zones of inhibition (mm)		
	Cold	Hot	Ethanol
Trichophyton species	NI	12	16
Epidermophyton species	NI	14	20

Key: mm = Millimeter NI = No inhibition
CLSI guidelines = Clinical Laboratory Standard Institute

Table 4: Minimum inhibitory concentration of the leaf extracts

Fungal isolates	Extracts/Concentrations (mg/ml)		
	Cold	Hot	Ethanol
Trichophyton species	ND	ND	500
Epidermophyton species	ND	ND	250

Key: mg/ml = Milligram per milliliter ND = Not detected

Table 4.5: Minimum fungicidal concentration of the leaf extracts

Fungal isolates	Extracts/Concentrations (mg/ml)		
	Cold	Hot	Ethanol
Trichophyton species	ND	ND	ND
Epidermophyton species	ND	ND	500

Key: mg/ml = Milligram per milliliter ND = Not detected

DISCUSSION

Plants are known of their ability to maintain good health since antiquity. Nowadays, the interest in natural products as antimicrobial agents has greatly increased due to the emergence of multi-drug-resistant pathogens (Abdallah & Koko, 2017). This study investigated the phytochemical and antifungal properties of anara leaf on dermatophytes.

Table 1 showed the cultural morphology and microscopic characteristics of the fungal isolates from the barber's shop. They were *Trichophyton* and *Epidermophyton* species. Enemour et al. (2012) reported the isolation of ***Aspergillus*, *Trichophyton*, *Penicillum*, *Rhizopus* and *Mucor* species from combs, brush, clippers and apron used in barbershops.** Mbajiuka et al. (2014) reported the isolation of *Aspergillus, Mucor and Rhizopus species from* **hair dressing salons in Michael Okpara University of Agriculture, Umudike, Abia State.**

Table 2 showed the phytochemical constituents of the leaf extracts. The presence of saponins, tannins, phenols, alkaloids and anthraquinones were detected. Ibrahim et al. (2019) reported the presence flavonoids, steroids, tannins, alkaloids, phenols, saponins, and anthraquinones from *Solanum incanum* leaves (anara) bitter garden egg leaves.

Table 3 showed the antifungal properties of the leaf extracts against the dermatophytes. Zones of inhibition 12mm to 14m m with hot water extract, 16mm to 20mm with ethanol extract and none with cold water extract. Berhe et al. (2018) reported zones of inhibition ranging from 11mm to 16mm with ethanol extract of *Solanum incanum* against *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi,* and *Escherichia coli.*

Table 4 showed the minimum inhibitory concentration of the leaf extracts against the dermatophytes. Minimum inhibitory concentration was recorded at 250mg/ml and 500mg/ml with ethanol extract. Berhe et al. (2018) reported that *Solanum incanum* ethanol leaf extract showed minimum inhibitory concentration at 1.56mg/ml against *Escherichia coli* and *Staphylococcus aureus.*

Table 5 showed the minimum fungicidal concentrations of the leaf extracts against the dermatophytes. Minimum fungicidal concentration was recorded at 500mg/ml with ethanol extract against *Epidermophyton* species. Cold and hot water extracts showed no fungicidal effect against the dermatophytes.

The antifungal activities recorded with the extract could be attributed to the presence of important phytochemicals in the plant. Saponins are natural glycosides that act as hypo-glycemic, antifungal and serum cholesterol lowering agents in animals. They are essential elements in ensuring hormonal balance and synthesis of sex hormones. Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. Synergistic action of tannins, flavonoids, alkaloids and saponins are known to inhibit the growth of pathogens (Nwankwo et al*.,* 2014). Tannins exert antimicrobial activities by iron deprivation, hydrogen bounding or specific interactions with vital proteins such as enzymes in microbial cells. Herbs that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery.

CONCLUSION

This study showed that extracts from anara possessed antifungal properties against some fungal isolates from barber's shop. The ethanol plant extract showed fungicidal activity against *Epidermophyton* species which is one of the dermatophytes isolated from the clippers. This plant leaf could provide alternative medicine for the treatment of fungal infections in humans.

RECOMMENDATIONS

1. It is recommended that further work be done with the extracts with other fungal species to know the effect of the plant extracts against the organisms.
2. There should be toxicity study in order to justify the safety of using this plant extract in humans.

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