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ORIGINAL ARTICLE

ANTIFUNGAL ACTIVITY OF *GOMPHRENA CELOSIOIDES* (SOFT KHAKI WEED) ON SELECTED FUNGAL ISOLATES

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ABSTRACT: Antifungal activities of extracts of *Gomphrena celosiodes* were investigated at different concentrations on *Aspergillus niger, Candida albicans* and *Trichophyton rubrum*. Extracts from the plants were used to challenge the fungi and the rate of the growth of fungal spores and hyphae was monitored. The phytochemical analysis of the extracts showed the presence of alkaloids, tannins, saponins, steroids, glycosides, terpenes and reducing sugars. However, the petroleum ether extract showed absence of saponins, reducing sugars, terpenes, alkaloids and tannins. The methanol extract of *G. celosiodes* had a fungicidal effect on the selected fungal isolates at a concentration of 2000µg/ml. The minimum inhibitory concentration (fungistatic) ranged from 2000 µg/ml to 1500 µg/ml. The results of this present investigation indicate that the study of primary metabolites and antifungal activity of this plant could be considered as a natural herbal source for treatment of some fungal diseases.

KEYWORDS: Antifungal, *Gomphrena celosiodes,* Fungal Isolates, Phytochemical analysis

INTRODUCTION

In Africa, approximately 80% of the population still relies on traditional healing practices and medicinal plants for their daily health care needs despite the immense technological advancement in orthodox medicine. Traditional medicine are the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental and social imbalance (Sofowora, 1982).

The consumption of a variety of local herbs and vegetables by man is believed to contribute significantly to the improvement of human health, in terms of prevention, and or cure of diseases because plants have long served as a source of therapy for different ailments (Tyler *et al.*, 1999).

Lewington (1990) reported that plants have always been the principal source of medicament, either in form of traditional preparations or as the pure active principle. Plants contain ions and phytochemicals that help boost individual health and cure diseases (Sofowora, 1984). Plants are a potential source of new drugs although their used as drugs are hindered by a lot of limitations like, lack of scientific proof of efficacy, absence of specific standard quality and dosage (Ghani, 1985). Although these limitations can be improved by research in which research programs to find out new natural and synthetic compounds with anti-microbial properties with minimum side effects have continued to engage scientists (Weitzman and Summerbell, 2005).

Multiple drug resistance is a common problem nowadays in the treatment of internal parasites and infectious diseases due to abuse and indiscriminate use of drugs. A number of pathogens are already developing resistance to available drugs (Davis, 1994; Service, 1995). The continued utilization of these drugs had resulted in hypersensitivity, immune-supression and allergic reactions (Ahmad *et al.*, 1998). Presently, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of these diseases but may often be adulterated with the attendant negative effects (Shariff, 2001). Most synthetic drugs are no longer the trend in most parts of the world and have given resurgence to the interest in traditional medicine or natural remedies that have little or no side effect (Olapade, 2002). Pathogens resistance to available drugs is alarming (Bhavnani and Ballow, 2000), therefore there is a need to search for new and effective therapeutic agents for the treatment of diseases caused by these organisms. Search for cure for these diseases from natural sources is growing because of degradable potential of herbal drugs

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apart from their efficacy (Dosumu *et al.*, 2010). *Gomphrena celosiodes* is a perennial or annual weed that belongs to the Kingdom; *Plantae,* Sub-Kingdom; *Tracheobionta* (Vascular Plants), Family; *Amaranthaceae* (Amaranth family), Genus; *Gomphrena* (globe amaranth), Species; *Celosiodes,* Common names include: *perpetua* (portugese; Brazil), *arrasa con todo* (Spanish) the weed grows in tufts or clumps. The stems are prostrate, procumbent and pilose (hairy).it grows to a height of about 6-12 inches (15-30cm) and a spacing of about 3-9 inches (7-15cm). The leaves are sessile, green, obovate to oblong shaped about 1.5-7.5 × 0.5-2.5cm. The flowers are white tinged with pink or red, globose to short- cylindric and 9-13mm in diameter. The roots are 1-10cm; and are fibrous. Propagation method is from the seeds, the seeds are well dispersed as they are very attractive to bees, butterflies and birds. It grows in full sunlight, partial shade or light shade. It blooms all year round and the foliage is evergreen. The plant grows abundantly in Africa with a few species occurring East and West of Africa, 120 species of the plant are found in Australia, Indo-Malaysia and tropical part of America and about 46 species are found in Brazil (Vieira *et al.*, 1994). It grows ubiquitously and can therefore be found growing on lawns, roadsides, sandy open areas, woodlands etc.

Gomphrena celosiodes growing in Nigeria

Ethno-botanical information of plant traditionally used for treating diseases is of particular importance to drug discovery, so collaborative work with traditional healers is paramount in this direction (Dosumu *et al.*, 2010). Therefore, this research is intended towards contributing to the ongoing effort to evolve new generation of plant based drugs with little or no limitations compared to the present trado-medical practices.

MATERIALS AND METHODS

2.1. Collection and Preparation of Plant Material

Fresh whole plant of *Gomphrena celosiodes* were collected from around the microbiology laboratory of the Federal University of Technology Minna, Niger state. The identity was confirmed by Dr I. C. J. Omalu of the department of Biological Science.

The whole plant of *Gomphrena celosiodes* was collected washed and air dried at room temperature for six (6) days after which it was ground with a sterile mortar and further blended into powder (micronization). This wwas to enhance the penetration of the extracting solvent to facilitate the release of the active components contained in the plant (Iyamabo, 1991) and also to reduce the surface area of the plant.

2.2. Preparation of Aqueous Extract

Fifty grams (50g) of the plant material was soaked in 250ml sterile distilled water in a conical flask and sealed with an aluminum foil to avoid contamination from the surrounding and allowed to stand for 72 hours (3 days), with frequent shaking and swirling (Onyeagba *et al.*, 2004).

The extract was then obtained by filtration using a muslin cloth and then further filtered using Whatman's filters paper No. 11.

The excess solvent was then evaporated using a rotary evaporator and the final extract was weighed, bottled and stored in a refrigerator till further use.

2.3. Preparation of Methanol Extract

The procedure for the preparation of the methanol extract is similar to that used for the aqueous extraction, instead of distilled water, 95% analytical grade methanol was used as the solvent for extraction.

2.4. Preparation of Petroleum-ether Extract

The procedure for the preparation of the petroleum-ether extract is similar to that used for the aqueous extraction, instead of distilled water, 95% analytical grade petroleum ether (petroleum spirit) was used as the solvent for extraction.

2.5. Concentration of Extracts

The aqueous, methanol and petroleum-ether extracts were evaporated to semi-solid using rotary evaporator. The extracts were then evaporated to dryness in an oven. Each dried extract was reconstituted into 2000µg/ml, 1500µg/ml, 1000µg/ml and 500µg/ml respectively. For the methanol and petroleum-

ether, 10% of the solvent was used to solubilize the extract before 90% of sterile distilled water was added to make up 100%. To each prepared 2000µg/ml, 1.0g of the extract was dissolved in 5ml of the diluents.

2.6. Screening of Test Organisms

The test organisms used for susceptibility includes *Trichophyton rubrum* isolated from children of the government orphanage F/layout, Minna, infected with dermatomycoses, *Aspergillus niger* and *Candida albicans* collected from the stock culture of the microbiology laboratory, Federal University of Technology Minna, Niger State. The organisms were then grown in Sabouraud's Dextrose Agar (SDA), and then confirmed by microscopic examination of the fungal colonies using Lacto-phenol cotton blue stain and then maintained on SDA slants prior to use.

2.7. Screening of the Extract for Antifungal Activity

The antifungal activity of the extract was determined using the radial growth method of Mann *et al*., (2008). Various concentrations of the extract were reconstituted in sterile distilled water and vortexes for homogeneity. 1.0ml of each concentration was introduced into McCartney bottles containing 19ml of each molten sterile Sabouraud's Dextrose Agar. The mixture was then thoroughly mixed and poured into pre-labeled sterile Petri-dishes to make a final concentration of 500, 1000, 1500, and 2000µg/ml respectively.

The plates were then kept for the medium to solidify and 4mm cork borer was used to bore wells on each plate. Pasteur pipette was then used to fill each labeled well with 2 drops of each plant extract and the plates were incubated at ambient temperature for 72 hours. The extent of radial growth was observed after incubation and interpreted as antifungal activity and recorded.

2.8. Standardization of the Fungal Inoculums

The modified method of the National Committee for Clinical Laboratory (NCCL, 1994) was used. Test organisms were grown for 5days on SDA at room temperature. Ten milliliters of sterile distilled water was added to each of the content in the slant bottles and followed by Tween 80, a universal solvent which facilitates the dispersal of the spores to produce a homogenous mixture. The slants were then shaken thoroughly to dislodge the spores. The mixture was transferred into a sterile test tube which was used as stock solution and serially diluted to obtain final spore suspension.

2.9. Determination of Minimum Inhibitory Concentration Using Test Tube Dilution Method

Varying concentrations of reconstituted extracts were evaluated for antifungal activity. One milliliter, 1.0ml of each concentration was added to 9.0ml of sterile Potato Dextrose Broth (PDB) in test tubes. 0.1ml of fungal suspension was then added into the test tubes and kept in the inoculating hood for 72 hours.

2.10. Phytochemical Screening of the Extracts

Preliminary phytochemical screening of extracts as described by Oyeleke and Manga (2008) was used to screen for the presence of secondary metabolites in the plant extracts.

RESULTS

Table 1: Phytochemical Constituents of Extracts of *Gomphrena celosiodes*

Metabolites	Extracts		
	Aqueous	Methanol	Petroleum ether
Alkaloids	+	+	-
Steroids	-	+	+
Saponins	+	+	-
Tannins	+	+	-
Glycosides	+	+	+
Reducing sugars	-	+	-
Terpenes	-	+	-
Phenols	-	+	+

Key: Positive (+): Present
Negative (-): Not present

Table 1 shows that the methanol extract of *Gomprena celosiodes* contains alkaloids, saponins, tannins, glycosides, terpenes, phenolic substances, steroids and reducing sugars. The Aqueous extract has only alkaloids, saponins, tannins and glycosides while the petroleum ether extract has the least constituents which include steroids, glycosides and phenols.

Table 2: Antifungal activity of aqueous extract of *G.celosiodes* in millimeters (mm)

Concentration (µg/ml)	Test organisms		
	A.niger	*C.albicans*	*T.rubrum*
2000	-	-	12±0.03
1500	-	-	10±0.01
1000	-	-	-
500	-	-	-

Key: (-) No Activity

Table 2 shows that the aqueous extract of *Gomprena celosiodes* had antifungal activity on only *Trichophyton rubrum*.

Table 3: Antifungal activity of methanol extract of *G. celosiodes* in millimeters (mm)

Concentration (µg/ml)	Test organisms		
	A.niger	*C.albicans*	*T.rubrum*
2000	30±0.03	28±0.01	26±0.02
1500	28±0.01	26±0.02	22±0.05
1000	28±0.03	22±0.04	20±0.01
500	22±0.01	20±0.01	22±0.03

Table 3 shows that the methanol extract of *Gomprena celosiodes* had antifungal activity on *Aspergillus niger*, *Candida albicans* and *Trichophyton rubrum* showing varying degrees of activity.

Table 4: Antifungal activity of petroleum ether extract of *G. celosiodes* in millimeter (mm)

Concentration (µg/ml)	Test organisms		
	A.niger	*C.albicans*	*T.rubrum*
2000	27±0.02	-	20±0.01
1500	24±0.03	-	18±0.04
1000	20±0.02	-	-
500	19±0.01	-	-

Key: (-) No Activity

Table 4 shows that the petroleum ether extract of *Gomprena celosiodes* had antifungal activity on *Aspergillus niger*, and *Trichophyton rubrum* but showed no activity on *Candida albicans*.

Table 5: Minimum Inhibitory Concentration of extracts of *G. celosiodes* (µg/ml)

Organisms	Extracts		
	Aqueous	methanol	petroleum ether
A.niger	-	500	1500
C.albicans	-	1000	-
T.rubrum	2000	500	1500

From table 5 the MIC of the extracts on the fungal pathogens shows 500µg/ml as the lowest for both *Aspergillus niger* and *Trichophyton rubrum* while *Candida albicans* was inhibited by 1000µg/ml. Only *Trichophyton rubrum* was susceptible to aqueous extract at the concentration of 2000µg/ml, others were resistant. Petroleum ether extract was active at 1500µg/ml against *Aspergillus niger* and *Trichophyton rubrum.*

DISCUSSION

Extraction of bioactive compound from medicinal plants permits the demonstration of physiological activity as well as facilitates pharmacological studies leading to the synthesis of more potent drugs with reduced toxicity (Ebana *et al*, 1991).

From this result, it shows that the higher the concentration of the extract the higher the potency. This hereby indicates that dilution or reduction in the concentration of the extract acts unfavorably in the antifungal activity of the extract due to either loss of some of the active components or availability of these components in trace amounts after dilution.

Early research work by Gerritsma-Van-der-Vijver and Botha (1986) on *Gomphrena celosiodes* extracts revealed the presence of saponins, steroids, non-reducing sugars, amino acids, phenols and flavonoids. The inhibitory effects of this medicinal plant may be due to the presence of these phytochemical components detected in the extract. Despite the complex and dynamic structure of the fungal cell wall, the extracts of *Gomphrena celosiodes* have been observed to posses phytochemical components that are capable of penetrating the fungal cell wall, this components may be acting singly or synergistically to inhibit the growth of the fungal isolates. The methanol extract of *Gomprena celosiodes* had antifungal activity on all the test organisms which can be related to the fact that the methanol extract of *Gomphrena celosiodes* was found to have extracted the highest amount of active components of the plant. It has been previously investigated by earlier researchers that have worked on *Gomphrena celosiodes* and other medicinal plants that the presence of these phytochemical components is probably the reason for the inhibitory effects of medicinal plants on microorganisms. The methanol extract of *Gomphrena celosiodes* was fungicidal at 2000µg/ml and fungistatic at a concentration range of 2000µg/ml to 1500µg/ml. This result here by concurs with an earlier investigation of the plant *Gomprena celosiodes* carried out by Dosumu *et al.,* (2010) on the Isolation of 3-(4-hydroxyphenyle) methylpropenoate and bioactivity of *Gomphrena celosiodes* extracts, which showed that the methanol extracts of *Gomprena celosiodes* possessed antifungal and antihelmintic activity.

CONCLUSION

This present study has shown that the extract of *Gomprena celosiodes* posses inhibitory effects on some fungal isolates. Therefore, *Gomprena celosiodes* seems to be a promising plant with respect to antifungal activities, it will however be interesting to lead further research into its mechanisms of action, isolation of active components and possibly elucidation of the structure of active components.

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