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Full Length Research Paper

Qualitative (phytochemical) analysis and antifungal activity of *Pentas decora* (De wild), a plant used traditionally to treat skin fungal infections in Western Uganda

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The ethanolic leaf extract of *Pentas decora,* a plant known in the local community as "Kabyakyasha" (in Runyankole Language), was evaluated for its antifungal activity against *Candida albicans, Epidermophyton floccosum, Microsporum canis* and *Trichophyton rubrum*, and qualitatively analyzed for its phytochemical composition. The disc diffusion method was employed to determine the antifungal activity. Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) were also determined using the tube dilution method. The *Pentas decora* ethanolic extract exhibited activity against *C. albicans* and *M. canis*, with MICs of 1000 and 1500 mg/ml respectively, while the corresponding values for the standard drug (clotrimazole) were 50 and 100 mg/ml respectively. The MFC values of the extract for *C. albicans* and *M. canis* were 2000 and 2500 mg/ml respectively while the corresponding MFC values for clotrimazole were 100 and 150 mg/ml respectively. However, both the extract and the standard drug had no activity against *E. fluccosum* and *T. rubrum*. The extract was also found to be rich in alkaloid and terpenoids. It can be concluded that the plant has some antifungal activity but there is need to do more tests and first ascertain its toxicity profile before it is declared sufficiently efficacious and safe for use by the community.

Key words: *Pentas decora,* anti fungal activity, skin fungal infections.

INTRODUCTION

Global priority is currently placed on combating malaria and HIV/AIDS. As the AIDS crisis leads to increasing number of countries to question their priorities in health expenditures, there is an evolving awareness that traditional medicine has a central role to play in combating new and re-emerging diseases with significant effect on human health. Among these are skin diseases (Burford et al., 2000). Recently, fungi have been recognized as important pathogens in Uganda; partly due to increasing debilitating rates that have been attributed to more widespread use of immunosuppressive therapies, indiscriminate use of broad-spectrum antimicrobial agents and immunosuppressive viral diseases like HIV/AIDS (Tasleem et al., 2011). In many poor and overcrowded areas, skin diseases, which are highly contagious, are widespread, with one-third or more of the population affected.

In developing countries, rapid urbanization increases overcrowding in slum areas, exacerbating the problem of skin diseases. Families often sacrifice part of their overstretched household budgets trying to treat the infections, only to see them reappear. Thus, controlling skin diseases through simple but effective public health measures is necessary and realistic for alleviating a common and solvable source of ill health (Hay et al., 2006).

Fungal infections are common nearly everywhere and can affect many parts of the body. For example, *Tinea pedis*, a fungal infection of the foot, is a treatable

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condition that causes cracking and swelling with itching between the toes. The most common fungal infection, *Tinea capitis*, affects the scalp, eyebrows, and eyelashes and sometimes causes massive hair loss. It is a contagious childhood disease that spreads extensively in schools. *T. capitis* is endemic in many developing countries and particularly in Sub-Saharan Africa, where the condition may affect more than 30% of children in primary schools. *Tinea imbricate* (Tokelau Ringworm) is an exotic and unusual infection that results in ornate circles of scaly plaque that can cover most of the body. This infection is seen in humid tropical regions, affecting as much as 30% of the population in some communities in the Western Pacific. Oral antifungal agents must be used to treat more severe infections, while topical treatments are given for lighter forms of infections (Hay et al., 2006).

Uganda has recently recorded an increase in fungal infections due to increased misuse of antibiotics and also as a result of the HIV/AIDS scourge (Global Health Governance, 2007). Furthermore, a person infected with human immuno-deficiency virus (HIV) is ten times more likely to develop fungal infections than an HIV-negative individual; consequently, the spread of HIV is accelerating the rise in fungal case rates. As a result, fungal infections remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents (Acha and Szyfres, 2003). Although mortality rates for fungal infections are lower than for many other conditions, the need for remedies should be met for several reasons: the illnesses can cause disfigurement, disability, or symptoms (such as an intractable itch) that reduce quality of life. In some cases, the initial condition can lead to a secondary infection or condition that can seriously jeopardize an individual's health. Skin diseases are so common and patients seek services for them in such large numbers that ignoring the diseases is not a viable option (Hay et al., 2006).

Skin diseases have received low priority until now, yet resolving the most common conditions is a realistic goal for primary health care systems. The burden of skin fungal diseases could be reduced substantially by focusing on a small group of conditions, particularly infections that make up the bulk of the community case load like fungal infections and pigment skin disorders. An effective search for new drugs, well trained health care team, and common prescribing practices could vastly improve community health and quality of life at low cost in countries where far too many people suffer from these diseases (Hay et al., 2006). Furthermore, many fungal infections in developing countries are contagious, with children being particularly more vulnerable. The cost to families of treating even trivial skin complaints is high and often ineffective and paying for the treatments may reduce a household's capacity to buy other essential items such as food (Hay et al., 2006). Due to affordability and availability problems, families have resorted to herbal treatment, and *P decora* is one of the plants used. The increasing failure and side effects of popularly used chemotherapeutics and appearance of multiple drug resistant strains among pathogenic microorganisms has led to a wide search of new compounds with antimicrobial activity. Therefore, development of novel drugs from traditional herbal medicines offers new prospects in modern healthcare (Shariff et al., 2006).

According to Hill (2001), traditional medicine (TM) occupies a central place among rural communities of developing countries for the provision of health care in the absence of an efficient primary health care system. However, the plant products they use have largely not been scientifically validated. The basis for use is usually the information handed down between generations. There is a danger that some of the materials might not be active or might be toxic. *Penta decora* var. *decora* (De Wild) belongs to the Rubiaceae family, *Pentas* genus, *decora* epithet, and varieties, *decora* (S.Moore), *triangularis* and *lasiocarpa* (Verdc.). *Penta decora* is also a synonym of *Dolichopentas decora* (S.Moore) (Kårehed and Bremer, 2007). It has glabrous leaves, less often sparsely pubescent or rarely hairy leaves. Inflorescence is made of 1 to 5 clusters each, with approximately 30 flowers with a corolla-tube of 3 to 12 cm in length. The capsule is obovoid-obtriangular and strongly ribbed. It has an altitude range of 1060 to 2400 m (http://www.eol.org/pages/29283).

Based on inflorescence colour, *P. decora* may be given various names including Penta 'Violet'(Violet Penta), Penta 'Red'(Red Penta), Penta 'White'(White Penta) and Penta 'Pink'(Pink Penta). Violet Penta has dark vibrant purple flowers; Red Penta has bright red flowers; White Penta has pure white flowers while Pink Penta has beautiful soft light pink flowers. All the plants have a well branched habit that keeps plants compact and with star-shaped flowers that are grouped together in a circled bunch atop dark green foliage [http://www.fowlersnursery.com/]. With regard to distribution *P. decora* and other members of the Rubiaceae family have wide distribution in African countries including Uganda, Kenya, Tanzania, Central African Republic, Democratic Republic of Congo, Ethiopia, Malawi and Nigeria, and there are various names given to the same species (Verdcourt, 1976). According to the review by Karou et al. (2011) various members of the Rubiaceae family are used widely in folk medicine. Table 1 shows the various uses of the members of the genus *Pentas* which have been reported by various researchers. Rubiaceae is a large family of 630 genera and about 13000 species globally, used both as ornamentals and in folk medicine to treat several diseases. The remedies are reported to be used in the the management of many diseases including abdominal irritation, abscesses, chancre, chicken pox, conjunctivitis, cough, cryptococcal meningitis, dermatitis, diarrhoea,

Table 1. Uses of *Pentas* spp in traditional medicine.

Scientific name	Local name	Medicinal indications	Region	Reference
Pentas brussei (K.Krause)	Mdob, Mudobe	Decoction of roots is taken as remedy for gonorrhoea, syphilis and dysentery	Kenya	Bukuru (2003)
Pentas decora (S.Moore)	-	Roots are pounded, mixed with ghee and rubbed on pimples	Kenya	Bukuru (2003)
Pentas decora var. *decora* (De Wild)	Kabyakyasha (Runyankole)	Leaf extract rubbed on ring worms	Uganda	Pers.com (2009)
Pentas hindsioides (K.Schum)	-	Pounded leaves are soaked in warm water for bathing against scabies	Kenya	Bukuru (2003)
Pentas lanceolata (Forssk) Deflers	Tigoch	Root and leaf used to treat lymphadenitis by topical and oral routes	Ethiopia	Giday et al. (2009)
Pentas lanceolata (Forssk).Defl. supsp. *quartiniana* (A.Rich), Verdc.	Mithaa	In case of snake bite, crushed fresh root homogenised in water, drunk or chewing any fresh parts of the whole plant or smoke bath of pounded sun dried leaf or soaking the whole body with crushed fresh leaf or chewing fresh root then soaking the bite area with the residue.	Ethiopia	Bekalo et al. (2009)
Pentas longiflora (Oliver)	Nekilango, Segimbe	Roots used as a cure for tape worms, itchy rashes and pimples; a decoction of the roots mixed with milk and taken as a cure for malaria.	Kenya	Bukuru (2003), Njoroge and Bussmann (2006b), Puyvelde et al. (1985)
	Muhuha	Powder of the roots mixed with butter and used as ointment to treat skin diseases such as scabies and pityriasis versicolor.	Rwanda	
Pentas micrantha (Backer)	-	Fresh roots chewed or boiled or pounded and soaked in water and the infusion drunk to treat cough.	Kenya	Bukuru (2003)

Table 1. Contd.

Pentas purpurea (Oliv)	-	Used by women to initiate menstruation; juice of the plant taken as a remedy for headache; decoction of roots mixed with sugar cane used for fever and rheumatic pain.	Tanzania	Bukuru (2003)
Pentas schimperiana subsp. *occidentalis* (Hookf.) Verdc.	Kamawong	Leaf concoction with bark of *Maesa lanceolata* taken orally to treat Hepatitis B liver infections	Cameroon	Focho et al. (2009)
Pentas schimperiana (A.Rich) Vertk.	Dibexxo	Fresh or dry root bark powder mixed with water and taken orally to treat epilepsy; root bark fine powder mixed with water and taken orally for mental illness.	Ethiopia	Mestin et al. (2009)
Pentas zanzibarica (Klotsch) Vetk	_	Extract from pounded leaves mixed with a little water and drunk as a drastic purgative; decoction of the root taken as remedy for gonorrhoea and syphilis or given to children as a tonic	Kenya	Bukuru (2003)
Pentas lanceolata (Forssk.) Deflers	tigoch	Lymphadenitis (charush); root, leaf applied topically or orally; boil (kursi): leaf/flower applied topically; meningitis (tikus): leaf/root applied orally or topically; Abdominal cramps (sheskan gazke): root applied orally; arthritis (pits): root/leaf applied orally, or topically;cow mastitis: root or leaf applied orally or topically.	Ethiopia	Giday et al. (2009)

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dysentery, eczema, fever, filariasis, gonorrhea, hepatitis B, itchy rashes, internal inflammation, leprous macular, malaria, measles, mycoses, pinworms, pubic lice, respiratory infection, ringworm, scabies, syphilis, tapeworm, trypanosomiasis, urinary tract infection, vomiting and wounds (Karou et al., 2011). In Western Uganda, *P. decora* is traditionally used in treatment of skin fungal infections. Fresh leaves of *P. decora* are squeezed with alcohol and applied locally on the affected part of the skin (Bakeewa, 2009). It is further reported that, based on leads provided by traditional healers, biological screening of many members of the Rubiaceae family has exhibited antimalarial, antimicrobial,

antihypertensive, antidiabetic, antioxidant and anti-inflammatory activities (Karou et al., 2011).

In terms of phytochemistry, biologically active compounds which include indole alkaloids, terpenoids and anthraquinones are reported to have been isolated from members of the Rubiaceae family including members of the genus *Pentas.* For example a phytochemical study of *Pentas longiflora* is reported by El-Hady et al. (2002) to have resulted in the isolation of compounds belonging to various chemical families including naphthoquinones, anthraquinones, coumarins and steroids. Similar investigation of *Pentas bussei* by the same authors resulted in the isolation and identification of highly oxygenated naphthohydroquinones and naphthohydroquinones of the benzochromene type. Other studies are reported to have isolated α-stigmasterol from the roots of *P. bussei* and *Pentas parvifolia* (Bukuru et al., 2002, 2003). Nevertheless, this list of isolated compounds is believed not to be conclusive (Karou et al., 2011).

It is further reported that a large number of Rubiaceae species that are used in TM have been tested *in vitro* for the antimalarial activities with a lot of success. This has been attributed partly to the fact that the main antimalarial drug, quinine, is of Rubiaceae origin, raising particular interest in members of the same family, as potential sources of novel compounds with antiplasmodial activity (Karou et al., 2011). However, from literature search made, there is virtually no study done yet to determine the antifungal efficacy, toxicity profile and phytochemical composition of *P. decora*. It is upon this background that this study was designed, to determine the efficacy of the plant on selected fungal strains and to determine, qualitatively, its phytochemical composition.

MATERIALS AND METHODS

Collection and identification of the plant

Leaves of the selected plant were collected from Kabungo village, Rwanja Parish, Kabira Sub-county, Ruhinda County in Buhenyi District, with the help of the informants, between April and May 2009. Only plants judged to be mature and disease-free were harvested in morning hours. The plant was identified by Mr. Rwaburindore Protase of Makerere University Herbarium in the Department of Botany. The plant specimen was assigned a voucher number AT/0001/52/KIU and kept in the Pharmacy laboratory of Kampala International University.

Preparation of plant material for extraction

This was done at the School of Pharmacy, Kampala International University, Western Campus, Bushenyi, Uganda.

Drying and pulverizing

The plant material was sorted and cleaned to improve on quality and the leaves were air-dried to constant weight in the shade at room temperature. The dried leaves were then ground into powder, sieved and packaged into clean containers until when needed. A sample of 140 g of the powdered leaves of *P. decora* was then extracted using ethanol.

Extraction

The powdered sample material was soaked, for 3 days in 800 ml of ethanol with occasional shaking. The extract was then filtered through a millipore filter into different conical flasks.

Separation of crude extracts from extracting solvents

The extract was concentrated to a minimum volume by using a distillation apparatus at a controlled temperature of 40°C. The concentrated extract was then allowed to evaporate to a constant weight in an incubator at 40°C. Too high temperatures were avoided to avoid possible inactivation of active ingredients. The yield was 12 g giving a percentage yield of 8.5%. The crude extract was then stored at 4°C awaiting screening.

Preparation of inoculum for drug sensitivity testing

Preserved strains were cultured on Saboraud dextrose agar, prior to antifungal susceptibility testing. The supernatant was adjusted to a turbidity of opacity equivalent to Mc Farland standard No.1. Then 1 ml of the suspension was diluted with 4 ml of sterile saline and used to inoculate each of the drug-containing media as well as the controls.

Test microorganisms

The microbial strains used were *Candida albicans, Epidermophyton floccosum, Microsporum canis* and *Trichophyton rubrum*; obtained from Microbiology Department, Mbarara University of Science and Technology.

Screening for antifungal activity

Sabouraud dextrose agar (SDA) is a suitable medium for the cultivating and differentiating fungi, yeast and mould. It is a peptone to which dextrose and agar are added with a pH of 5.6 and sterilized by autoclaving at 121°C for 15 min (Ghoshal et al., 2006; Sahm and Washington, 1990). Determination of the minimum inhibitory concentration (MIC) values followed guidelines by Prescott et al. (2002) and Manna et al. (2008) who stated that screening plant extracts against the test organisms normally shows that fungi vary widely in the degree of their susceptibility to antifungal agents. Determination of minimum fungicidal concentrations was based on findings by Banso and Adeyemo (2000) who suggested that some plant extracts have antifungal agents that are fungistatic at lower concentrations while becoming fungicidal at higher concentrations.

Preparation of the medium

This followed standard procedure for culture media preparation and also followed the manufacture's instructions. Thirty eight (38) grams of Saboraud dextrose agar medium was weighed and mixed with 1 L of sterile distilled water and the medium was then sterilized by autoclaving at 120°C for 20 min under 1 bar pressure. Under aseptic conditions, the medium was dispensed into 90 mm pre-

sterilized Petri-dishes to yield a uniform depth of 4 mm. The medium was allowed to cool at room temperature, undisturbed, until the culture medium hardened. It was then left in an incubator for incubation at 37°C for 24 h in an inverted position to test for sterility. Saboraud dextrose agar plates were then stored at 4 to 8°C and wrapped in plastic bags awaiting usage.

Preparation of dried discs for susceptibility assays

Whatman filter paper was used to make biodiscs (6 mm in diameter). The discs were sterilized by autoclaving. Then various concentrations (2500, 2000, 1500, 1000, 750 and 300 mg/ml) of plant extract, and for the standard drug clotrimazole, 150, 100, and 50 mg/ml were prepared. The discs were then placed onto sterile Petri dishes and 20 µl of the extract and standard drug dispensed in the middle of each disc, giving a minimum of 6 and 1 mg of extract and standard drug per disc respectively. They were then left to dry for 1 h.

Inoculation of plates for sensitivity determination

1 ml of the inoculum suspension was pipetted onto each of the SDA plates and distributed evenly over the surface of the medium by gently rocking the plate and excess fluid was pipetted off. Then, the plates were placed in an incubator for 10 min to dry before the discs were applied.

Application of discs and incubation of Petri dishes

Within 15 min after the inoculation of plates, discs were applied with flamed forceps in the center of the surface of one of each quadrant of the Petri dish, pressed down with slight pressure in order to ensure complete contact with the agar surface and left for aerobic incubation for 48 and 72 h in an inverted position for *C. albicans* and other dermatophytes, respectively. The diameter of the zone of inhibition in each case was then measured using a transparent millimeter scale. The experiments were done in triplicate and the mean of the results determined.

Determining MIC by the dilution method

Preparation of the culture medium

This also followed standard procedure: Twenty-five (25) grams of Saboraud dextrose broth was weighed and mixed with 1 L of sterile distilled water. Then the medium was sterilized by autoclaving at 120°C for 20 min under 1 bar pressure. After 1 h, the medium was poured in sterile screw-capped test tubes. 1 ml of inoculum containing approximately 10^5 colonies (based on Mc Farland standard) was added to each tube and then the medium was homogenized.

Determination of the minimum inhibitory concentration (MIC)

Various concentrations (2500, 2000, 1500, 1000, 750 and 300 mg/ml) of the extract were prepared. Then, 900 µl of the inoculated culture medium was dispensed in each screw-capped test tube and 100 µl of test extract was added. The preparation was then closed to prevent evaporation and then incubated at 37°C and examined for growth after 2 and 7 days for *C. albicans* and other microorganisms (*M. canis, E. floccosum,* and *T. rubrum*) respectively. MICs of the extract were defined as the lowest concentration of plant extract that visually showed no growth in comparison to that of growth control.

Determination of the minimum fungicidal concentration (MFC)

Ten (10)-fold dilutions of the culture medium in tubes that showed no visible fungal growth or turbidity in the minimum inhibitory concentration experiment were cultured into freshly prepared Saboraud dextrose agar plate to assay for the fungicidal effects of the extract. They were then incubated at 37°C for up to 2 and 7 days for *C. albicans* and other microorganisms (*M. canis, E. floccosum,* and *T. rubrum*) respectively. The minimum fungicidal concentration was regarded as the lowest concentration in the MIC test that did not yield any fungal growth on the solid medium used, after the ten-fold dilution.

Qualitative phytochemical analysis tests

Chemical tests were carried out on the ethanol extract using standard procedures to identify the constituents as described by Sofowara (1993), Evans (1989) and Harborne (1973).

Data analysis

Data from antifungal activity screening were analyzed using simple statistics from Microsoft Excel and recorded in appropriate tables as mean ± standard error of mean (SEM). Data from preliminary qualitative phytochemical analysis were recorded in a table as "+" for a positive test result and "–" for a negative test result.

Ethical considerations

This being an academic project, permission was granted by the Research and Ethics Committee of Kampala International University-Western Campus. Precautions were also taken where there was a possibility exposing the researchers to risks.

RESULTS

Phytochemical characteristics of *P. decora*

The phytochemical study carried out on ethanolic extract of *P. decora* showed that the leaves were rich in alkaloids and terpenoids.

Antifungal activity

The ethanolic extract of *P. decora* inhibited growth of *C. albicans* and *M. canis* but did not have an activity on *E. floccosum* and *T. rubrum.* The largest diameter of 4.8 ± 0.4 mm was obtained against *C. albicans*, while the lowest diameter of 3.7 ± 0.2 mm was obtained against *M. canis. E. floccosum* and *T. rubrum* appeared to be the most resistant to the effect of the leaf extract of *P. decora.* They were also resistant to the standard drug, clotrimazole.

The MIC of the extract against *C. albicans* was about 20 times that of the standard drug, clotrimazole while *M. canis* the MIC value for the extract was about 15 times

that of the standard drug. Relatively, higher MFC values were obtained compared to MICs for the ethanolic leaf extract of *P. decora.*

DISCUSSION

The burden of fungal infections and new strategies for new drugs

Skin diseases have received low priority until now, yet resolving the most common conditions is a realistic goal for primary health care systems. The burden of skin fungal diseases could be reduced substantially by focusing on small groups of conditions, particularly infections that make up the bulk of the community case load, such as fungal infections and pigment skin disorders. An effective search for new drugs, well trained health care team, and common prescribing practices could vastly improve community health and quality of life, at low cost, in countries where far too many people suffer from these diseases (Hay et al., 2006).

The results of this study, it is hoped, could help add on the database for medicinal plants used to treat fungal infections in Uganda. It was foreseen that if *P. decora* proved to have a significant activity with a reasonable minimum inhibitory concentration, it would significantly contribute to development of antifungal drugs as well as add information on *P. decora* research as argued by Premanathan et al. (2000). Today, natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action, utilizing the special feature of higher plants - their capacity to produce a large number of secondary metabolites (Castello et al., 2002). Recent studies have resulted in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases and knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agents as well as new sources of economic materials like oil and gums (Erturk et al., 2006; Mohanta et al., 2007).

Role of plant secondary metabolites in drug development

Finding healing powers in plants is an ancient idea. People on all continents have long applied poultices and imbibed infusion of hundreds, if not thousands, of indigenous plants, dating back to prehistory. There is evidence that Neanderthals living 60,000 years ago in the present-day Iraq used plants such as hollyhock which is still being used world wide (Ghoshal et al., 2006). Plants have an almost limitless ability to synthesize substances most of which are secondary metabolites, of which at least 12,000 have been isolated; a number estimated to be less than 10% of the total (Ghoshal et al., 2006).

Alkaloids which are heterocyclic nitrogen compounds were first medically useful as morphine isolated in 1805 from the opium poppy, *Papaver somniferum.* Now, alkaloids have been identified to have antifungal activity; for example piperin a compound of the alkaloid class isolated from *Piper nigrum*. The mechanism of action here is attributed to the alkaloid ability to intercalate with DNA (Ghoshal et al., 2006). There is, therefore, reason to believe that the activity of *P. decora* could be attributed to the presence of alkaloids as revealed by the preliminary qualitative phytochemical analysis (Table 2). Terpenoids and alkaloids have been proved to have antifungal activity (Ghoshal et al., 2006). Presence of alkaloids and terpenoids in the leaf extracts of *P. decora* as presented in Table 3 exhibit antifungal properties could serve as a basis for its traditional use as a medicinal plant. This agrees with what was reported by Ghoshal et al. (2006) that alkaloids, terpenoids and lactones are responsible for antifungal activity. Terpenoids are synthesized from acetic units, and as such they share their origins with fatty acids. They however differ from fatty acids in that they contain extensive branching and are cyclized and have been found to inhibit yeast growth (Chaurasia et al., 2004).

Antifungal tests on ethanolic extract of *P. decora* showed that the plant exhibit antifungal activity against C*. albicans* and *M. canis,* although it did not exhibit measurable activity against *E. canis* and *T. rubrum* (Table 4). However, this activity was achieved at a concentration which was 20 times that of the standard drug, which gives a ratio of 1:20. Considering the fact that we are comparing a crude extract with a pure drug, this is still reasonable. There is a possibility that if the active ingredient were to be isolated and purified, it might be found to be as effective as the standard drug or even more effective. Lack of activity for both the standard drug and the extract against *E. canis* and *T. rubrum* could have been due to drug resistance. This is probably because the pure cultures were isolated from patients who might have resistance to clotrimazole; a reason as to why this has to be verified in future research studies. However, in the event that the extract could prove to be active against clotrimazole-resistant fungi, this would be a good indication for potential of isolating a more effective antifungal agent from the plant.

The minimum inhibitory concentration values of the plant extract against the test organisms showed that fungi vary widely in the degree of their susceptibility (Table 5), which agrees with the report by Prescott et al. (2002) about the wide variation in susceptibility of fungi to antifungal drugs. However, there is need to carry more studies on this, using various extracts from the plant to be able to make accurate comparisons. When the broth culture of the extract and the test organisms used in the minimum inhibitory concentration test were sub cultured on a solid medium for the assessment of the MFC of the

Table 2. Qualitative phytochemical tests.

Test	Positive indicator	Class of compounds
1) About 0.5 g of the methanol extract was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% iron III chloride was added	A brownish green or blue-black coloration	Tannins
2) An aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid	Deposition of a red precipitate	Phlobatannins
3) To 400 ml of distilled water was added 50 g of powdered sample in a conical flask and boiled for 5 min. The mixture was filtered when still hot and 5 ml of sterile distilled water added to a test tube containing equal amounts of cooled filtrate. The test tube was stoppered and shaken vigorously for 30 s and then allowed to stand for 30 min	Formation of honey comb froth	Saponins
4) 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4.	A yellow coloration	Flavonoids
5) 2 ml of acetic anhydride was added to 0.5 g of the methanol extract with 2 ml H_2SO_4	The colour change from violet to blue or green	Steroids
6) 5 ml of the extract was mixed with 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) carefully added to form a layer (Salkowski test)	A reddish brown coloration on the interface	Terpenoids
7) To 5 ml of the extract, equal volume of Solkowski's reagent was added.,	A bluish-red solution that slowly changes to violet-red, with the fluorescence	Sterols
8) To 2 ml of the extract, 0.1 ml of 2 M sodium hydroxide was added, followed by a small quantity of 2 M hydrochloric acid and shaken	A white precipitate	Essential oils.
9) To 2 ml of the extract, 2 ml of Iron III chloride was added to the solution	A deep bluish-green solution	Phenols
10) To 1 ml of the extract, concentrated sulphuric acid was added followed by potassium dichromate crystals	An olive-green colour	Alkaloids

Table 3. Results of qualitative analysis of *P. decora*

Test	Results
Tannins	-
Phlobatanins	-
Saponins	-
Flavonoids	-
Steroids	-
Terpenoids	+
Phenols	-
Sterols	-
Essential Oils	-
Alkaloids	+

+ = Presence of constituent, - = Absence of constituent.

Table 4. Diameters of zones of inhibition (± SEM) for the standard drug and the ethanolic extract of *Pentas decora* against test microorganisms (excluding the diameter of the biodisc).

Test microorganism	Zone of inhibition (diameter) in millimeters (± SEM)	
	Standard drug (100 mg/ml)	*P. decora* (2000 mg/ml)
C. albicans	19.2 ± 0.5	4.8 ± 0.4
E. floccosum	-	-
M. canis	14.8 ± 0.3	3.7 ± 0.2
T. rubrum	-	-

- = No inhibition

Table 5. MICs (± SEM) of for *in vitro* activity of *Pentas decora* extract against selected fungi.

Plant extract and standard drug	MIC (mg/ml)			
	C. albicans	*E. fluccosum*	*M. canis*	*T. rubrum*
P. decora	1000 ± 3	-	1500 ± 2	-
Clotrimazole	50 ± 1	-	100 ± 1.5	-

- =, no *in vitro* activity at a concentration equal or less than 2500 mg/ml.

Table 6. MFCs (± SEM) for *in vitro* activity of *Pentas decora* extract against selected fungi.

Plant extract and standard drug	MFC (mg/ml)			
	C. albicans	*E. fluccosum*	*M. canis*	*T. rubrum*
P. decora	2000 ± 3	-	2500 ± 4	-
Clotrimazole	100 ± 2	-	150 ± 1.5	-

- =, no *in vitro* activity at a concentration equal or less than 2500 mg/ml.

extract, the results indicated that the minimum fungicidal concentration of the extract were obtained at a higher concentration than the MIC values (Table 6). This observation therefore suggests that the antifungal substance contained in the extract was fungistatic at lower concentrations while becoming fungicidal at higher concentrations of the extract. Similar observations have been reported by Banso and Adeyemo (2000).

Conclusion

The results of this study suggest a fairly good correlation between traditional therapeutic use and the *in vitro* antifungal activity. They show that the ethanolic extract of *P. decora* has antifungal effect on *M. canis* and *C. albicans.* The plant's ability to show antifungal activity may be attributed to the presence of alkaloids and terpenoids, which are known to be biologically active. The extract of the plant could therefore be useful in the treatment of fungal infections but there is need to first ascertain its toxicity profile.

RECOMMENDATIONS

Follow-up studies should concentrate on isolating and purifying the active compounds, carrying out toxicity tests and identifying the active compound (s) in order to establish its/their possible mechanism(s) of action.

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