

Full Length Research Paper

Phytochemical screening and antifungal activities of crude ethanol extracts of red-flowered silk cotton tree (*Bombax buonopozense*) and Calabash nutmeg (*Monodora myristica*) on *Candida albicans*

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The study was to determine the phytochemical compounds and antifungal activity of the extracts of the leaves, root, bark and seeds of Calabash nutmeg (*Monodora myristica*) and red-flowered silk cotton tree (*Bombax buonopozense*). The phytochemicals were extracted and analyzed using standard methods and the antifungal activity of the ethanolic extracts determined with clinical isolates of *Candida albicans* using the well diffusion method. The ethanolic extracts of *B. buonopozense* and *M. myristica* contained a mixture of compounds; saponins, tannins, general glycosides, steroids, alkaloids, flavonoids and triterpenoids. Saponins were present in all the plant extracts except the root of *B. buonopozense*. Extracts of *M. myristica* bark and seed contained more number of the different phytochemical compounds compared with the extracts of other plant parts. All the *B. buonopozense* extracts gave the same number of different phytochemical compounds although the type of compounds present varied from one part of the plant to another. All the plant extracts, except the bark of *B. buonopozense*, showed some level of significant activity against *C. albicans* ($p < 0.001$). Among these extracts, *M. myristica* seed and root parts were the most effective in inhibiting the growth of *C. albicans*. The antifungal activities of most of the plant extracts were also comparable to the standard Clotrimazole drug. These findings revealed the presence of major phytochemicals (saponins, tannins, alkaloids, steroids, general glycosides, flavonoids and triterpenoids) in the plants which could be responsible for the activities of the extracts on *C. albicans*.

Key words: Antimicrobial activity, crude ethanolic extract, antifungal activity, *Bombax buonopozense*, *Monodora myristica*

INTRODUCTION

Medicinal plants are major sources by which antimicrobial agents are obtained (Cowan, 1999) for the production of new drugs for human use (Panda et al., 2009). The

demand for more and more drugs from plant sources is continuously increasing (Panda et al., 2009) following resistance of some microorganisms to certain orthodox

drugs as well as their side effects. To meet this demand, scientists are increasingly becoming involved in screening of such plants with the aim of establishing their potential antimicrobial effects and identifying the bioactive compounds responsible for the antimicrobial properties (Aibinu et al., 2007; Ndukwe et al., 2005). With the ever increasing dependence of the world's greater population (80%) on folk medicines for the treatment of common infections and persistent diseases (Fajimi and Taiwo, 2000), such screening exercise is needed to ascertain the efficacy of the medicinal plants as well as their safety.

The use of plants in traditional medicine to treat diseases is attributed to the presence of antimicrobial compounds in them (Borchardt et al., 2008). These antimicrobial agents include different forms of alkaloids, sesquiterpenes, diterpenes, triterpenes saponins, triterpen aglycos, flavonoids, sterols, coumarin, quinines, monoterpenes, different proteins as well as lipids and tannins (Sofowora, 1993). The type and amount of phytochemical compounds present usually differ from one part of the plant to another part. This explains why in traditional medicine the same or different plant parts may be used in treating different diseases or same diseases (Addo-Fordjour et al., 2008). For instance, in Nigeria, roots, barks and leaves of *Newbouldia laevis* are used in the treatment of scrotal elephantiasis, dysentery, ringworm, syphilis, sore eyes and ear ache (Azoro, 2002).

Candidiasis is a mild, superficial fungal infection mainly caused by *Candida albicans*. Although *C. albicans* normally infects skin, mucosal surfaces (Kambizi and Afolayan, 2008) and even the gastrointestinal tract (GIT), it grows vigorously in the vagina, where pregnant women transmit the infection to their babies during birth (Lamb et al., 2000). It persists as one of the commonest and most troublesome sexually transmitted diseases in the world. To contain the disease, several drugs have been employed in the treatment of candidiasis over the years. However, recent reports of increasing resistance of *C. albicans* to drugs have raised a major concern worldwide (Goff et al., 1995; Nolte et al., 1997; Kieren et al., 1998). Consequently, the search for new antifungals especially from natural origins such as plants has become imperative. One way of achieving this is for scientists to depend on traditional knowledge of plants species used in treating candidiasis.

These plants can then be screened for their potential antifungal activities and where A recent study conducted by Addo-Fordjour et al. (2008) indicated that some herbalists in the Bomaa community of the Brong Ahafo Region, Ghana use possible the bioactive compounds responsible for their activities. *Bombax buonopozense* and *Monodora myristica* to treat candidiasis.

However, the study did not investigate the phytochemical basis and antifungal activities of the extracts of these plant species. The study was therefore, aimed at determining the phytochemical compounds and antifungal activity of the extracts of the leaves, root, bark and seeds of *M. myristica* and *B. buonopozense*.

MATERIALS AND METHODS

Plant collection

The seed, bark, root and leaves of *M. myristica* and *B. buonopozense* used for the investigation were collected from the Kwame Nkrumah University of Science and Technology (KNUST) Botanic garden, Kumasi, Ashanti Region. Ghana.

Test microorganisms

Clinical isolates for *C. albicans* were obtained from Microbiology laboratory of the Department of Pharmaceutics, KNUST (Kumasi, Ashanti Region. Ghana). The isolates were identified as *C. albicans* on the basis of germ tube production and microscopic observation of budding patterns from the organism. Prior to the identification, the isolates were preliminarily screened using 4% chloramphenicol agar, gram-staining techniques and pathogenicity testing. They were cultured and maintained on Sabouraud's Dextrose agar at 4°C.

Preparation of extracts

The plant materials of each plant part were air-dried and ground separately to powder with the help of an electric blender. The powder was sieved through a 1 mm mesh and stored in an airtight container for future use. 50 grams each of the powdered samples was extracted in 200 ml of ethanol using the Soxhlet extractor. The solvent was removed from the extract with the Büchi rotary evaporator and the residue dried to a constant weight in an electric oven at 50°C. The sample was further lyophilized to totally remove any possible leftover ethanol. The dry extracts were later re-dissolved in 1% dimethyl sulfoxide (Adeiza et al., 2009) to the final graded concentrations of 1, 0.5, 0.25, 0.125 g/ml.

Phytochemical screening

Phytochemicals present in the plants were analysed according to the standard procedures described by previous studies. Thus the presence of the following chemicals were determined in the plants: saponins (Nsiah and Opoku, 2005), tannins (Akinpelu et al 2008), general glycosides (Evans and Trease, 1989), steroids (Harbourne, 1973), alkaloids (Onakoya and Akinpelu, 2006), flavonoids (Sofowora, 1993) and triterpenoids (Evans and Trease, 1989).

Growth media preparation

Sabouraud dextrose agar powder (65 g) was suspended in 1 L distilled water. It was made to boil over a Bunsen flame to dissolve completely. 20 ml each of the mixture was poured into test tubes

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Table 1. Phytochemical compounds identified in the various parts of *B. buonopozense* and *M. myristica*.

Extract	Phytochemical compound						
	Saponins	Tannins	General glycosides	Steroids	Alkaloids	Flavonoids	Triterpenoids
<i>B. buonopozense</i> leaves	+	+	-	-	+	+	+
<i>B. buonopozense</i> bark	+	+	-	+	-	+	+
<i>B. buonopozense</i> root	-	+	+	+	-	+	+
<i>M. myristica</i> leaves	+	+	+	-	-	-	-
<i>M. myristica</i> bark	+	+	-	-	+	+	+
<i>M. myristica</i> seed	+	-	+	+	+	+	-
<i>M. myristica</i> root	+	-	-	+	-	+	-

+ = Present; - = Not detected.

and plugged with cotton wool. The test tubes were autoclaved at 121°C for 15 min.

Determination of antifungal activity

The 20 ml of stabilized Sabouraud agar was seeded with *C. albicans*, rolled in the palm to mix thoroughly, poured into sterile petri dishes and allowed to set. Using the agar well diffusion method (Parekh and Chanda, 2006), wells were created on the plates with a cork borer of 9 mm in diameter. The wells were filled with the different concentrations (0.125, 0.25, 0.5, 1.0 g/ml) of the plant extract to about three quarters full. The petri dishes were left on the working bench for 30 to 60 min for the extracts to diffuse into the agar. They were incubated for 3 days (36 h) at 37°C. After incubation, the plates were observed for the presence of a clear zone (zones of inhibition) around the wells. The diameters of these zones were measured as an indication of a positive antimicrobial activity of the extracts. Each experimental treatment was replicated thrice and the average of the three was calculated. The results were compared with a standard antifungal agent, clotrimazole (Sigma Chemicals, Steinheim-Germany) in concentrations of 0.0025, 0.0005, 0.001 and 0.002 g/ml. The vehicle (negative) control was 1% DMSO which was expected to show no inhibition against the microbes. All the plant extracts and the clotrimazole were dissolved in the 1% DMSO for their respective concentrations.

Statistical analysis

One way analysis of variance (ANOVA) was used to test for the significant differences between the diameters of inhibition zones obtained for the various extracts. The 11th Edition of the Genstat software (VSN International Ltd, Hemel Hempstead, UK) was used for the analysis at a significant level of 5%.

RESULTS

Extracted phytochemicals

The ethanolic extracts of *B. buonopozense* and *M. myristica* contained a mixture of compounds namely, saponins, tannins, general glycosides, steroids, alkaloids, flavonoids and triterpenoids (Table 1). Saponins and flavonoids were present in all the plant extracts, except that of *B. buonopozense* root and *M. myristica* leaves

respectively. Extracts of *M. myristica* bark and seed contained a wide range of phytochemical compounds compared with the other plant parts. In the case of *B. buonopozense*, all the extracts contained the same number of the major phytochemicals although the type of compounds differed from one part of the plant to another.

Antifungal activity

All the plant extracts used except the bark of *B. buonopozense* showed some level of activity against *C. albicans* (Table 2). The zone of inhibition ranged from 8 mm to 32.5 mm in the different plant extracts, whereas that of the standard drug ranged from 2.05 mm to 5.75 mm. The MIC of *B. buonopozense* extract also ranged from 0.059 g/ml to 1.873 g/ml, whereas that of *M. myristica* varied between 0.5 mg/ml to 5.5 g/ml as against the standard Clotrimazole drug (0.0027 g/ml), Table 2. The respective zones of inhibition differed significantly between the various treatments ($p < 0.001$) (Table 3). Extracts of *M. myristica* seed and root showed significantly greater antifungal activity than all the other treatments (Table 3). Generally, the inhibition zone diameter increased with increasing concentration. For example, in the extract of *M. myristica* root, the inhibition zone diameter was increased from 15 mm to 32.5 mm (with respective concentrations ranging from 0.125 to 1 g/ml). Thus, the bioactive extracts were dose-dependent in nature. The 1% DMSO serving as negative control revealed no antimicrobial activity.

DISCUSSION

Phytochemical analysis of the ethanolic extracts of the plant parts revealed the presence of saponins, tannins, steroids, triterpenoids, flavonoids, alkaloids and general glycosides. This result forms the scientific basis for the use of these plants by the herbal practitioners in the Boma community, since these secondary metabolites in plants have been shown to possess biological activity

Table 2. Antifungal activity of extracts of the various parts of *B. buonopozense* and *M. myristica* and clotrimazole at different concentrations (g/ml).

Extract	Diameter of inhibition zones (mm) at the various concentrations (g/ml)				Minimum inhibitory concentration (MIC)
	0.125	0.25	0.5	1	
<i>B. buonopozense</i> leaves	12.5±3.5	20.5±0.71	22.5±3.5	27.75±0.35	0.059
<i>B. buonopozense</i> bark	NA	NA	NA	NA	NA
<i>B. buonopozense</i> root	12.5±3.5	15±7.1	25±7.1	25±7.1	1.873
<i>M. myristica</i> leaves	8±2.8	10±0	12.5±3.5	17.5±3.5	5.297
<i>M. myristica</i> bark	9±1.4	10±0	12.5±10.6	15±7.1	0.732
<i>M. myristica</i> seed	22.5±3.5	25±7.1	30±14.1	32.5±10.6	0.145
<i>M. myristica</i> root	15±7.1	20±0	30±0	32.5±10.6	2.490
	0.00025	0.0005	0.001	0.002	
Clotrimazole*	2.05±0.2	2.65±0.2	5.5±0.07	5.75±1.1	0.0027

*Standard drug; NA = No activity.

Table 3. Comparison of the mean diameter of inhibition zone among the different plant extracts.

Extracts/standard drug	Mean diameter of zone of inhibition (mm)
<i>Bombax buonopozense</i> leaves	20.81 ^b
<i>Bombax buonopozense</i> root	19.38 ^b
<i>Monodora myristica</i> bark	11.63 ^c
<i>Monodora myristica</i> root	24.38 ^{a, b}
<i>Monodora myristica</i> seed	27.50 ^a
<i>Monodora myristica</i> leaves	12 ^c
Clotrimazole	NA

Means followed by different letters in the column are significantly different (that is, $p < 0.05$) whereas those followed by the same letters are not significantly different (that is, $p > 0.05$). NA = Not applicable.

(Soforowa, 1986). Generally, the composition of phytochemical compounds within the same plant species differed from one part to another. This explains why different parts of the same plant are sometimes used to treat several diseases (Addo-Fordjour et al., 2008).

The tannins mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes and cell envelope transport proteins (Ya et al., 1988). They also act by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes (Scalbert, 1991) in microbial cells. The activity of flavonoid is probably due to their ability to complex with extracellular and soluble proteins, as well as the disruption of microbial membranes (Tsuchiya et al., 1996). Regarding the alkaloids, they intercalate into cell wall and/or DNA of organisms thereby causing structural instability and genetic modifications. The saponins destabilize the cytoplasmic and plasma membranes of microbial organisms (Just et al., 1998).

The ethanolic extracts of the different parts of *B.*

buonopozense and *M. myristica* exhibited antifungal activities on *C. albicans*. The activities of the plant extracts are attributed to the presence of bioactive constituents outlined above (Borchardt et al., 2008). It is remarkable to note that the most important of the bioactive components namely alkaloids, tannins and flavonoids (Hill, 1952) were present in most of the plant parts. This situation could explain the fact that further purification of all the plant extracts (except the extract of *B. buonopozense* bark) could show significantly higher activities than even the standard drug as currently observed in this study. The lack of activity against *C. albicans* by the extract of *B. buonopozense* bark does not necessarily suggest the absence of bioactive constituents in the bark or its inability to cure the disease. Whereas the traditional healers in the Bomaa community used water as solvent and found the bark effective, ethanol was the solvent used in this study which did not produce any detectable activity. The performance of this extract therefore, seems to have been limited by the solvent

used in the extraction process. This was supported by the work of Parekh and Chanda (2006) who observed that the antimicrobial activity of plant extracts largely depends on the type of solvent used in the extraction process.

The antifungal activities produced differed significantly between the extracts of the different plant parts ($p < 0.001$). Even within the same plant species certain plant parts produced significantly more antifungal activity than others. For instance, the extracts of *M. myristica* root and seed produced significantly higher inhibition zone diameters than the leaf and bark extracts of the same plant. The differences in antimicrobial effects of the different plant parts (extracts) could be attributed to different phytochemical properties as well as differences in their concentrations within the plants. Among all the plant extracts, used *M. myristica* seed and root extracts proved most effective in inhibiting the growth of *C. albicans*. The phytochemical compounds identified in these plant parts might have occurred in higher concentrations resulting in relatively higher antimicrobial activities.

Compared with Clotrimazole, the findings indicated that the standard drug had a stronger bioactivity than the two plant extracts as per their MICs. However, further purification of the crude extracts could produce activities comparable to the standard antibiotics or even better as observed in most isolated active compounds from such sources. Generally, the antimicrobial activities of the *B. buonopozense* were higher than *M. myristica* as reported in other studies (Onakoya and Akinpelu, 2006). The results further indicated that the activities of most of the extracts were dose dependent, showing better activities at higher concentrations. The extracts of *M. myristica* seed and *B. buonopozense* leaf were more effective in inhibiting *C. albicans* at lower concentrations (0.125 and 0.25 g/ml) than all the other extracts. Additionally, the preliminary results described in this paper provided enough data for further purification and biological testings of different compounds present in the extracts.

Conclusion

The crude ethanolic extracts of the various parts (except the bark of *B. buonopozense*) of *B. buonopozense* and *M. myristica* produced significant antifungal activity against *C. albicans* which supports their use by some traditional healers in the Bomaa community to treat candidiasis. Though all the plant extracts showed high antifungal activity, the extracts of *M. myristica* seed and root were the most effective in inhibiting the growth of *C. albicans*. Phytochemical analysis of the extracts revealed saponins, tannins, alkaloids, steroids, general glycosides, flavonoids and triterpenoids which are probably responsible for the activities of the extracts on *C. albicans*.

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Conflict of Interests

The authors have not declared any conflict of interest.

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