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Antifungal potentials of *Glycosmis pentaphylla* extractives

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ABSTRACT

The fresh flower, leaves, root, stem bark and stem wood of *Glycosmis pentaphylla* extracts ($CHCl_3$, CH_3OH, petroleum ether and acetone) were tested for their antifungal potentials against six pathogenic fungi *Fusarium vasinfectum, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Mucor* sp. and *Candida albicans* at concentrations of 200 µg/disc along with a standard Nystatin (50 µg/disc). The flower and leaf extracts showed activity against *F. vasinfectum, A. fumigatus, A.niger,C. albicans* and *Mucor*sp .incase of $CHCl_3$ extracts. The root, stem bark and stem wood extracts were responsive against *A. fumigatus, A.niger, C.albicans* and *Mucor* sp.The flower extracts (CH_3OH) was responsive against three pathogenic fungi *A. fumigatus, A .niger*and,*C. albicans*. For the leaf extract, *F. vasinfectum, A. fumigatus, A. niger, C. albicans* and *Mucor* sp. were responsive. The root extracts showed activity against *A. fumigatus, A. niger,C. albicans* and *Mucor* sp. In case of the stem wood extract *A. fumigatus, A. niger, A. flavus, C. albicans* and *Mucor sp.* were responsive. For the stem bark extract *A. fumigatus, A. niger, C. albicans* and *Mucor sp.* showed activity.The flower and leaf extracts (petroleum-ether) were responsive against *F. vasinfectum, A. niger,* and *C. albicans*. The root extracts showed activity against *F.vasinfectum A. niger, C. albicans* and *Mucor* sp. In case of the stem wood extract *A. fumigatus, A. niger*and *Mucor* sp. were responsive. For the stem bark extract *A. fumigatus, A. niger* and *C. albican s* were responsive. The flower extracts (acetone) were responsive against *F. vasinfectum,A.niger,C. albicans* and *Mucor* sp. The leaf extracts was responsive against *F. vasinfectum, C. albicans* and *Mucor* sp.The root extracts showed activity against *A. fumigatus, A.niger, C. albicans* and *Mucor sp.*The stem wood extracts showed activity against *A. fumigatus, A. flavus ,C. albicans* and *Mucor* sp. For the stem bark extract *A. fumigatus, A. niger, C. albicans* and *Mucor* sp. were responsive. According to the intensity of activity indices *G. pentaphylla* extracts ($CHCl_3$) could be arranged in a descending order of leaf > root> stem wood > stem bark > flower extract. For the Me OH extracts root > stem bark > leaf >stem wood > flower extract. In case of petroleum-ether extracts stem wood>stem bark>root>leaf>flower extract. For the acetone extracts stem wood > stem bark >leaf > root> flower extract

Introduction

Medicinal plants possess various medicinal properties and have been serving as the major sources of therapeutic agents for maintenance of human health. These medicinal plants are being used by the early human beings, as are done now, in a variety of forms, such as in the entire form and as powders, pastes,

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juices, infusions and decoctions. These various preparative forms of the medicinal plants may therefore conveniently and genuinely called the herbal medicine and this is why, the medicinal plants formed an integral part of the health management practices and constituted important items of medicines used in the treatment of diseases from the very early days of human civilization. *Glycosmis pentaphylla* (Retz.) DC belongs to the family Rutaceae which represents nearly 11 species. It is a shrub or small (1.5–5.0 m) tree widely distributed in India, Malaysia and Southern China to the Philippine Islands where it occurs in tropical forests at low altitudes (Wang *et al*., 2006)

Glycosmis pentaphylla (Retz) DC, Synonyms-*G. Arborea* (Roxb.) A. DC, *G. chylocarpa* Wight & Arn., *G. quinquefolia* Griff. Bengali/Vernacular names Ashshaora, Datmajan, Matmati, Kawatuti, Aidali, Fatik, Ban Jamir; Motkila (Comilla). Burma- Obok; Chinese-Ko muong; Hindi-Ban nimbu; Marathi- Kirmira; Malayalam- Malampanal, Panal; Sanskrit-Vanamimbuka; Tamil -Annam, Panal,Konji, Kattukkonji; Telugu- Golugu, Gonjipandu. Tribal Name-Hotiggira (Chakma); Si Ma Sere (Marma). English Name-Toothbrush Plant, Motar tree. The plant exist as a small erect shrub or tree, bearing large leaves 3-5 folio late; leaflets subopposite,7-15x2-5.5cm,elliptic-lance late, attenuate at base, entire to minutely crenellate-serrate on the margins, acute to round at apex, glandular on both sides, glabrous; rachis 6-10 cm long; petioles c. 2 mm long. Flowers in terminal or axillary, pisiform panicles. Sepals 5(4), 1-1.5 mm long, ovate-acute, ciliate on margins. Petals 5(4), white, 4-5 x 2-2.5 mm, obovate, surface gland-dotted. Stamens 8-10, longer and shorter filaments of longer ones. Ovary 2-2.5 mm across, ovoid, 5-celled; stigmaflat or obscurely lobed. Fruits: Unripe fruits yellowish white - pink, ripening reddish to darkvermilion. Ripe fruits globose - ellipsoid, 1-1.5cm across, with 2-3 seeds (occasionally only 1 seeded). Berry 1-1.2cmdiameter, ovoid,white turning pink,1-2-seeded., white, small berries pulpy, glandular, edible (Yoganarsimhan, 1996).

According to the history of potentiality *G. pentaphylla* is a widely reported medicinal plant in ethno botany. It has a beneficial effect on normalizing glucose level, lipid profile, liver marker enzymes, liver glycogen, and insulin. It is used in the maintenance of glucose homeostasis and thus used as a therapeutic agent in the management of diabetes mellitus. However, previous workers investigated this plant giving emphasis mostly on the chemical constituents and their medicinal profile but information on its various biological activities is still scanty. The ethnic people of India used the whole plant of *G. pentaphylla* for the treatment of cancer (Rekibul *et al.,* 2008).The plant reported to be a good source of antifungal agents. ie. amides from *Glycosmis mauritiana* (Greger *et al.*, 1994) and *G. pentaphylla* (Bandara *et al.*, 1990). Phytochemical analysis of this species were mainly focused on hydrophobic alkaloids, including those of the quinolone, quinazoline, acridone, carbazole types of components from leaves, root and stem bark (Bhattacharyya and Chowdhury, 1985; Muthukrishnan*et al.*, 1999). The plant is used for cough, rheumatism, anemia and jaundice (Mohammed *et al*., 2010; Gopi, 2000; Sastri, 1956).

Stems and roots of this plant are used for treatment of ulcer. Paste of leaves, with a bit of ginger, applied over the navel for worms and other bowel disorders. In parts of Asia the orange berry leaf is boiled down and used to reduce fever, liver complications and various intestinal parasites. The traditional healers in Gazipur district of Bangladesh utilize *G. pentaphylla* for the prevention of all forms of cancer (Ariful *et al*., 2010). Stem and fruits of *G. pentaphylla* is used by medicinal practitioners in Bangladesh for the treatment of rheumatoid arthritis (Mohammed *et al*., 2010). Roots were used in India against facial inflammation, rheumatism, jaundice and anemia (Oudhia *et al*., 2007). The root, stem and leaf of this plant are used in folklore medicine in Kerala and Tamil Nadu to cure fever, rheumatism etc (Balachandran *et al*., 2008).

Antifungal activities of this plant have been demonstrated by a number of recent workers Meera and Devi, 2009; Yasir *et al*., 2015; Abbas *et al*., 2011; whereas antibacterial properties of this plant were tested among others by Israt Jahan Bulbul and Nisrat Jahan, 2016; Isman, 2000; Reynolds, 1996; Muthukrishnan *et al*., 1999; Bershc and Vlietnick, 1991; Amran *et al*., 2011;Jeeshna *et al*., 2009 and Rahmatullah *et al*., 2010.

The present investigation was aimed at evaluating chloroform, methanol, petroleum ether and acetone extracts of some selected parts of *G. pentaphylla* against a number of pathogenic fungi using disc diffusion method.

Materials and Methods

The test fungi and their culture: Pure culture of six species of fungi viz., *F. vasinfectum*, *A. fumigatus, A. niger, C. albicans*, *Mucor* sp., and *P. notatum* were collected from the Department of Pharmacy, University of Rajshahi, and were maintained in the Molecular

Biology Laboratory, IBSc, RU. Potato dextrose agar (PDA) media, consisting of 20gmpotato, 2g dextrose,

1.5g agar and 100 ml distilled water, were used to perform the antifungal activity tests and for the maintenance of the sub cultures of the test organisms. The constituents of the medium was accurately weighed and dispersed in a conical flask with distilled water. It was heated in water bath to dissolve the ingredients until a transparent solution was obtained. The *pH* of the medium was adjusted to 5.6. The volume was adjusted by adding distilled water and sterilized in an autoclave at 121^{o} C and 15 lb. $inch^{-2}$ pressure for 15 min.

Preparation of plant materials for extraction: The fresh flower, leaves, root, stem bark and stem wood of *G. pentaphylla* were collected from the campus of the University of Rajshahi and different areas of Rajshahi division. All parts of this plant were cut into small pieces and dried at room temperature under shade keeping in wooden trays. The dried materials were powdered using grinder avoiding excess heat during grinding.

Chemical extraction of the collected materials: Chloroform, methanol, petroleum ether and acetone (Merck/Germany) were selected to extract five different parts of *G. pentaphylla* separately. The powdered materials were weighed and placed in separate conical flasks to add sufficient amount of solvents (500g × 1500ml × 3 times followed by filtration through What man filter paper at 24 h interval in the same collection flask) to yield the extracts. The output extracts were poured into glass vials and preserved in a refrigerator at 4ºC with proper labeling until used.

Tests for antifungal activity: The agar diffusion technique (Vander & Vlietinck, 1991) was employed while PDA medium was used for determining antifungal activity (Bauer *et al*., 1966) of the extracts where standard antibiotic discs of Nystatin (50µg/disc) were used for the comparison.

Results And Discussion

***G. pentaphylla* extracts in chloroform:** As shown in table 1 compared to inhibition zones of 20mm, 20mm, 22mm, 20mm and 21mmby the standard control Nystatin (50 µg/disc), the activity indices of the flower extracts were recorded 11mm, 12mm, 12mm,13mmand16mm respectively for

200 µg/disc application against *F. vasinfectum, A. fumigatus, A. niger, C. albicans* and *Mucor* sp. The leaf extract showed 11mm, 16mm, 12mm, 15mm and 20mm activity indices for 200 µg/disc application against*F. vasinfectum, A. fumigatus, A. niger, C. albicans* and *Mucor sp.*, while the inhibition zones for the standard were 20mm, 20mm, 22mm, 20mm and 21mmà.

The root extracts showed activity indices 17mm, 10mm, 12mm and 18mmfor 200 µg/disc application against *A. fumigatus, A. niger, C. albicans* and *Mucor sp.*, while the inhibition zones for the standard were 20mm, 22mm, 20mm and 21mm.

In case of the stem wood extract *A. fumigatus, A. niger, C. albicans* and *Mucor sp.*, showed activity indices 13mm,10mm,12mm and 16mmfor 200 µg/disc application, while the inhibition zones for the standard were 20mm, 22mm, 20mmand21mm.For the stem bark extract

A. fumigatus, A. niger, C. albicans and *Mucor sp.*, showed activity indices 15mm, 14mm,13mm and 16mm for 200µg/disc application, while the inhibition zones for the standard were 20mm, 22mm, 20mm and 21mm respectively and the results are shown in Table 1.

***G. pentaphylla* extracts in methanol:** The activity indices of the flower extracts were recorded 11mm, 10 mm and 12mmagainst three pathogenic fungal species *A. fumigatus, A. niger* and, *C. albicans G. pentaphylla* extracts in methanol whereas the standard Nystatin yielded 20mm,22mm and 20mm inhibition zones respectively. For the leaf extract *F. vasinfectum, A. fumigatus, A. niger, C. albicans* and *Mucor sp.*, showed activity indices 9mm,17mm, 10mm,12mm and 14mm for 200 µg/disc application, whereas the standard Nystatin had 20mm, 20mm, 22mm, 20mm and 22mm inhibition zones respectively. The root extracts showed activity indices 9mm,16mm, 8mm and 19mmfor 200 µg/disc application against *F.vasinfectum, A. fumigatus, A. niger* and *Mucor sp.*, while the inhibition zones for the standard were 20mm, 20mm, 22mm and 22mm. In case of the stem wood extract *A. fumigatus, A. niger, A. flavus, C. albicans* and *Mucor sp.*, showed activity indices 15mm,13mm,12mm,11mm and 13mmfor 200 µg/disc application, while the inhibition zones for the standard were 20mm, 22mm, 22mm, 20mm and 22mm. For the stem bark extract *A. fumigatus, A. niger, C. albicans* and *Mucor sp.*, showed activity indices 17mm, 10mm, 12mm and 14mm for 200 µg/disc application, while the inhibition zones for the standard were 20mm, 22mm, 20mm,and 22mm respectively and the results are shown in Table 2.

***pentaphylla* extracts in petroleum ether:** Compared to inhibition zones 20mm, 22mm and 20mmby the standard control Nystatin (50 µg/disc), the activity

indices of the flower extracts were recorded 10mm, 13mm and 11mm respectively for 200 µg/disc application against *F. vasinfectum*,

A. niger, and *C. albicans* . The leaf extract showed 12mm, 13mm and 11mm activity indicesfor200µg/disc application against

F. vasinfectum, *A. niger*, and *C. albicans* while the inhibition zones for the standard were 20mm, 22mm and 20mm.The root extracts showed activity indices 10mm, 13mm, 11mm and 12mmfor 200 µg/disc application against *F. vasinfectum A. niger,*

C. albicans and *Mucor sp.*, while the inhibition zones for the standard were 20mm, 22mm, 20mm and 22mm. In case of the stem wood extract *A. fumigatus, A. niger* and *Mucor sp.*, showed activity indices 10mm, 12mm and 14mmfor 200 µg/disc application, while the inhibition zones for the standard were 20mm, 22mm and 22mm. For the stem bark extract *A. fumigatus, A. niger* and *C. albicans* showed activity indices 10mm,13mm and 11mmfor 200 µg/disc application, while the inhibition zones for the standard were 20mm, 22mm and 20mm respectively and the results are shown in Table 3.

***G. pentaphylla* extracts in acetone:** The activity indices of the flower extracts were recorded13mm, 11mm,12mm and15mm against four pathogenic fungal species *F. vasinfectum, A. niger, C. albicans* and *Mucor sp.* whereas the standard Nystatin yielded 20mm, 22mm, 20mmand 22mm inhibition zones respectively. For the leaf extract *F. vasinfectum, C. albicans* and *Mucor sp.*, showed activity indices 14mm, 13mm and 16mm for 200 µg/disc application, whereas the standard Nystatin had 20mm, 20mm and 22mm inhibition zones respectively. The root extracts showed activity indices 13mm, 14mm, 12mm and 13mm for 200µg/disc application against *F. vasinfectum, A. niger, C. albicans* and *Mucor sp.*, while the inhibition zones for the standard were 20mm, 22mm, 20mm and 22mm.Incase of the stem wood extract*A. fumigatus, A. niger, A. flavus, C. albicans* and *Mucor sp.*, showed activity indices 11mm, 10mm, 13mm, and18mmfor 200 µg/disc application, while the inhibition zones for the standard were 20mm, 22mm, 20mm and 22mm.For the stem bark extract*A. fumigatus, A. niger, C. albicans* and *Mucor sp.*, showed activity indices 13mm, 11mm, 12mm and 15mm for 200 µg/disc application, while the inhibition zones for the standard were 20mm, 22mm, 20mm, and 22mm respectively and the results are shown in Table 4.

Table 1. Antifungal activity of the chloroform extracts of the different parts of *G. pentaphylla* in comparison with the standard antibiotics.

Test organisms	Activity index (diameter in mm) with the plant extracts					Standard
	Flower	Leaf	Root	Stem wood	Stem bark	
	200 µg/ disc	200 µg/ disc	200 µg/ disc	200 µg/ disc	200 µg/ disc	Nysatin 50µg/disc
F. vasinfectum	11	11	-	-	-	20
A. fumigatus	12	16	17	13	15	20
A. flavus	-	-	-	-	-	-
A. niger	12	12	10	10	14	22
C. albicans	13	15	12	12	13	20
Mucor sp.	16	20	18	16	16	21

Table 2. Antifungal activity of the methanol extracts of the different parts of *G. pentaphylla* in comparison with the standard antibiotics.

Test organisms	Activity index (diameter in mm) with the plant extracts					Standard
	Flower	Leaf	Root	Stem wood	Stem bark	
	200 µg/ disc	200 µg/ disc	200 µg/ disc	200 µg/ disc	200 µg/ disc	Nysatin 50µg/disc
F. asinfectum	-	09	09	-	-	20
A. fumigatus	11	17	16	15	17	20
A. flavus	-	-	-	13	-	22
A. niger	10	10	8	12	10	22
C.albicans	12	12	-	11	12	20
Mucor sp.	-	14	19	13	14	22

Table 3. Antifungal activity of the petroleum ether extracts of the different parts of *G. pentaphylla* in comparison with the standard antibiotics.

Test organisms	Activity index (diameter in mm) with the plant extracts					Standard
	Flower	Leaf	Root	Stem wood	Stem bark	
	200 µg/ disc	200 µg/ disc	200 µg/ disc	200 µg/ disc	200 µg/ disc	Nysatin 50µg/disc
F. asinfectum	10	12	10	-	-	20
A. fumigatus	-	-	-	10	10	20
A. flavus	-	-	-	-	-	-
A. niger	13	13	13	12	13	22
C.albicans	11	11	11	-	11	20

Mucor sp.	-	-	12	14	-	22

Table 4. Antifungal activity of the acetone extracts of the different parts of *G. pentaphylla* in comparison with the standard antibiotics.

Test organisms	Activity index (diameter in mm) with the plant extracts					Standard
	Flower	Leaf	Root	Stem wood	Stem bark	
	200 µg/ disc	200 µg/ disc	200 µg/ disc	200 µg/ disc	200 µg/ disc	Nysatin 50µg/disc
F. asinfectum	13	14	-	-	-	20
A. fumigatus	-	-	13	11	13	20
A. flavus	-	-	-	10	-	22
A. niger	11	-	14	0	11	22
C. albicans	12	13	12	13	12	20
Mucor sp.	15	16	13	18	15	22

Comparison of the chloroform, methanol, petroleum ether and acetone extracts:

The chloroform extracts of plant parts showed significant antifungal activity. The leaf was found to have maximum activity indices against *Mucor* sp. when tested by the disc diffusion method.

The present study clearly demonstrated that chloroform, methanol, petroleum ether and acetone extractives from various parts of *G. pentaphylla* have significant antifungal properties. The present data on the antifungal activity of the experimental plant are supported by a number of recent works. The present findings also fit well with those of Greger *et al*., 1992 in which they reported different activities against Sulfur containing cinnamides with antifungal activity from *Glycosmis cyanocarpa*. The present data on the antifungal activity of the experimental plant are in good agreement with Balakumar et *al*.2011in which they reported antifungal activity of Aegle marmelos (L.) Correa (Rutaceae) leaf extract on dermatophytes. The present findings also agree with those of Jeeshna *et al*. 2009 in which they explained antimicrobial property of the medicinal shrub, *Glycosmis pentaphylla*.

These results are also in agreement with the results of (Greger *et al.*, 1996) where they demonstrated antifungal and insecticidal activities have already been isolated from several *Glycosmis* species.

Conclusion

The present extractives represent novel leads and so future studies may be undertaken for the development of a pharmacologically acceptable antimicrobial agent or class of agents. Our findings suggest the possibility of using the extractives in the treatment of various fungal infections and of discovering new bioactive compounds which can also be used for prophylactic treatments. Especially the root wood and stem wood of the plant has strong potential for the isolation of antioxidant and antimicrobial agents for functional pharmaceutical uses.

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