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Phytochemical Screening of Lawn-Forming Moss *Hyophila involuta* in Kuvempu University Campus, Shivamogga District, Karnataka

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A B S T R A C T

Hyophila involuta is a lawn-forming moss belongs to family Pottiaceae of Bryophyta. Specimen was collected from Kuvempu University Campus, Shivamogga, Karnataka during 2011-2012. Soxhlet extraction method was used to obtain crude extracts by using the successive solvents like petroleum ether, chloroform, acetone, ethanol and water. The highest yield was obtained in the chloroform extract followed by water, petroleum ether, acetone and ethanol. Preliminary phytochemical analysis of extracts revealed presence of alkaloids, saponins, tannins, phenols, glycosides and flavonoids. It may helpful to further studies and investigations in the field of phytochemistry, pharmacological and drug industry.

Keywords: *Hyophila involuta*, moss, secondary metabolites, phytochemistry, crude extract

A R T I C L E I N F O

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1. Introduction

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (Fatima et al., 2006). Secondary metabolites are chemicals produced by plants; and their function in growth, photosynthesis, reproduction

and other primary processes are not known yet. Secondary chemicals are important in plant use by widely used especially in Asia (Bodeker, 2000).

Bryophytes are very interesting, diverse and distinct group in botany. Approximately 25,000 species of bryophytes are exists in the world. One of the important feature that helped bryophytes to survive and maintain their place in today's flora is their content of biologically active compounds (Bodade et al., 2008). Few molecular level works are investigated some chemical compounds, which are generally these plants are contain high amounts of terpenoids, phenolics, glycosides, fatty acids and some rare aromatic compounds are present (Savaroglu et al., 2011). These bryophytes are having some of the important medicinal value. These are used in pharmaceutical products (Banerjee, 1974; Asakawa, 1981; Ohta et al., 1997), horticulture (Perin, 1962; Adderley, 1964), household purposes and also ecologically an important as good indicators of environmental conditions. Mosses generally grow in humid and moist condition and their relative resistance to microbial attack indicates their ability to produce some active inducible antimicrobials (Asakawa, 2001; Bodade et al., 2008). Though their antimicrobial potentiality and medicalscenarios have not been explored comprehensively till now, the study of literature indicates that in bryophytes flavonoids, biflavonoids, isoflavonoidstannins, phenolic compounds the active components providing resistance against microorganisms and also acting as natural antioxidants (Hahn et al., 1995; Polterait, 1997; Basile et al., 1999; Chobot et al., 2012). *Hyophila involuta* is a lawn-forming moss with upright shoots. On the shoot tip, the leaves form a rosulate group, which makes the shoot tips look like a little star. This moss is most widely spread in the tropical region. It grows on limestone rocks, moist house foundations (so called "cement moss"), walls on the shore and temporarily flooded rocks.

2. Materials and Methods

Collection and identification of plant: *Hyophila involuta* (Hook.) A. Jaeger. was collected from Kuvempu University campus, Shivamogga, Karnataka during 2011-2012 and it was identified by using standard manuals (Chopra, 1975; Ganguli 1985; Nair et al., 2005). Herbarium of the specimen was deposited in Department of Botany, Kuvempu University, Shankaraghatta, Shivamogga.

Preliminary screening of secondary metabolites

The collected plant material was washed 4-5 times in running water for removal of other plant parts and soil debris. After the completion of washing, this material was spread on paper in room for shade drying. After 8 days, the plant material was fully dried and that is kept in polythene cover. This dried material was powdered by using mixer grinder. Then this powdered material was packed in zip-lock cover and stored for further work. The powdered plant material was extracted successively with petroleum ether, chloroform, acetone, ethanol and water in a soxhlet extractor for 24 hours (40 cycles). Then these extracts were dried under room temperature. The yield of each extracts was recorded and all the extracts were preserved in cool and dry place. The freshly prepared extracts were subjected to standard phytochemical analysis to ensure the presence of following phytoconstituents such as alkaloids, steroids, saponins, tannins, phenols, glycosides, terpenoids and flavonoids by standard procedures (Horborne, 1993; Iyengar, 1995; Siddqui and Ali, 1997). The tests were based on the visual observation of color change or formation of a precipitate after the addition of specific reagents.

Qualitative Phytochemical analysis

Test for alkaloids

Mayer's Test (Potassium Mercuric Iodide)

Mayer's reagent was added to the acidic test solution (small quantity of extract + 2 ml of respective solvent). Formation of yellow coloured precipitate indicates the presence of alkaloids.

Wagner's test (Solution of iodine in potassium iodide)

Wagner's reagent was added to the acidic test solution (small quantity of extract+2ml of respective solvent) brown coloured precipitate was formed indicates the presence of alkaloids.

Test for flavonoids

Flavonoid test: To the plant extract, magnesium turnings was added with few drops of concentrated H_2SO_4 through sides of test tubes magenta colour was formed indicates the presence of flavonoids.

Lead acetate test: Extract with treated with few drops of 10% lead acetate solution, formation of yellow colour precipitate indicates the presence of flavonoids.

Test for glycosides To the extract few drops of glacial acetic acid solution was added and boil for a minute and cooled. Then two drops of ferric chloride was added. Then concentrated acid was added slowly two layers are formed. Formation of reddish brown ring at the junction indicates the presence of glycosides.

Test for saponins

Foam test: Plant extracts of 5 ml was taken to which a drop of sodium bicarbonate was added and shaken well. Formation of honeycomb like froth indicates the presence of saponins.

Test for steroids

Salkowaski test To the test solution (small quantity of extracts +2ml of respective solvent) 2 ml of concentrated sulphuric acid were added, shaken well for sometimes and allowed to stand, the lower layer colour was turns with red indicating the presence of steroids.

Test for tannins

Ferric chloride test

Test solution (small quality of extractions + 2ml of respective solvent) taken in a test tube, few drops of 5% ferric chloride solution was added, appearance of greenish grey precipitated indicates the presence of tannins.

Test for terpenoids

Salkowaski test: Few drops of sulphuric acid concentration was added to the test solution (small quantity of extract + 2ml of respective solvent), shaken well and allowed to stand, the lower layer colour turns yellow indication the presence of terpenoids.

Liebermann-Burchord test: The test solution (small quantity of extract + 2ml of respective solvent) was treated

with acetic anhydride mixed well and concentrated sulphuric acid was added along the sides of test tube. Deep red colour formed indicates the presence of triterpenoids.

3. Results and Discussion

In the present study, Soxhlet extraction method was used to obtain crude extracts by using the successive solvents like petroleum ether, chloroform, acetone, ethanol and water. The yield of plant extract were shown in Table 1. The result of the plant extract shows that the highest yield was obtained in the chloroform extract followed by water, petroleum ether, acetone and ethanol. The data of preliminary screening of phytochemical analysis of secondary metabolites of all the five extracts of *Hyophila involuta* in Table 2. Preliminary phytochemical analysis of extracts of *Hyophila involuta* revealed presence of alkaloids, saponins, tannins, phenols, glycosides and flavonoids. The pet ether extract showed positive for alkaloids, steroids, tannins, glycosides, saponins, terpenoids, flavonoids and shows negative for phenols. The chloroform extracts shows the presence of only few in alkaloids, tannins and terpenoids, the remaining secondary metabolites were absent. In acetone extract shows the positive result on alkaloids, tannin, glycosides and saponin remaining secondary metabolites were absent (Table 2). In ethanolic extract, it shows the positive result in alkaloids, steroids, tannins and flavonoids. However, during the analysis of methanol extract reveals the absence of all secondary metabolites. While comparing pet ether extract shows the maximum secondary metabolites and minimum or nil in water extract (Table 2). Our results corroborates with the results of Tanwar et al. (2012) and Nasreen et al. (2010), they also reported the presence of above phytochemicals in *Tinospora cordifolia*. The different pharmacological actions of *T. cordifolia* like other medicinal plants can be attributed to the presence of array of secondary metabolites in it, alkaloids, flavonoids, phenols, steroids, saponins, glycosides etc. (Singh et al., 2003). Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori et al., 1994). The availability of specific phytochemicals in plant gives it specific medicinal properties. Therefore, presence of above phytochemicals in *H. involuta* can is correlated with its medicinal potential. Similar reports on phytochemical composition of various medicinal plants were made earlier by many workers (Chopra et al., 1956; Del-Rio et al.,1997; Koche et al., 2010). However, it is very essential to isolate the bioactive fractions from these major groups so that it can be used further in designing specific drugs.

Table 1: Total yield of the extracts obtained in organic solvents of *Hyophila involuta*
(100 g plant powder in 500 ml of solvent)

Organic solvents	Yield in gram	Yield in Percentage
Petroleum ether	1.6	1.6
Chloroform	3.2	3.2
Acetone	1.5	1.5
Ethanol	1.2	1.2
Water	2.36	2.36

Table 2: Phytochemical analysis of secondary metabolites of *Hyophila involuta*

Secondary metabolites	Extracts				
	Pet ether	Chloroform	Acetone	Ethanol	Water
Alkaloids	+	+	+	+	–
Steroids	+	–	–	+	–
Tannins	+	+	+	+	–
Phenols	–	–	–	–	–
Glycosides	+	–	+	–	–
Saponins	+	–	+	–	–
Terpenoids	+	+	–	–	–
Flavonoids	+	–	–	+	–

Note: +- Presence, – - Absence

4. Conclusion

The phytochemical analysis of the medicinally important bryophytes are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. The previous phytochemical analysis and present study showed nearly the similar results due to the presence of the phytochemical constituents. Preliminary phytochemical analysis of extracts of moss *Hyophila involuta* revealed that presence of alkaloids, saponins, tannins, phenols, glycosides and flavonoids. It may helpful to further studies and investigations in the field of phytochemistry, pharmacological and drug industry.

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