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Physico-chemical and preliminary phytochemical screening of roots of some species of *Dashmoola*

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Abstract

Alkaloids, tannins, saponins, flavonoids, steroids, triterpenoids, proteins, fats, carbohydrates, phlobatannins and resin in the tree species of Dashmoola belongs to the different families were investigated and compared. The medicinal plants investigated are *Aegle marmelos*, *Stereospermum chelonoides*, *Gmelina arborea*. The samples are collected from eighteen months old plant. The plant extract is goes through the several qualitative analysis test to find out active constituents which are important in various ailments and drug development. The analysis of crude methanolic extract revealed the presence of flavonoids in all the extracts. The alkaloids are present in *A. marmelos*, *S. chelonoides*, while absent in *G. arborea*. The steroids, triterpenoids and saponins were not found in all the extracts. The significance of these plants in *Ayurvedic* medicines and the importance of chemical constituents were discussed with respect to the role in *Dashmoolarist*.

Keywords: dashmoola, medicinal plants, phytochemicals, flavonoids

Introduction

The Medicinal plants have been considered as important therapeutic aid for alleviating ailments of human being and these plants have been used for treatment of various ailments since immemorial time (Jain, 1991) [9]. Besides the rapid development of methods of organic synthesis in laboratory, medicinal plants continue to play a significant role in modern medicine due to their distinct biological and chemical properties.

In nature, a plant is able to synthesize complex molecules namely alkaloids, terpenoids, tannins, saponins, glycosides etc. collectively called secondary metabolites, (Harborne, 1973) [7] from simple one to through highly specific reaction mechanism, that they use for defense and communication.

It is difficult and expensive to duplicate such synthesis in laboratory. The compound synthesized by the plant play an important role as medicinal and pharmaceutical agents and not only as purified isolates and extractives but also led compound for synthesis optimization. Nature is still mankind's greatest chemist and many compound that remain undiscovered in plants are beyond the imagination. These secondary metabolites show potent biological activity against most of bacteria, viruses, ailments, endoparasites etc. It is rather difficult and expensive to prepare these phytochemicals in laboratories. Such compounds synthesized by plant naturally play an important role as medicinal and pharmaceutical agents. These phytochemicals are found in roots, stem, stem bark, leaves, fruits etc. (Debela, 2002) [4].

Medicinal plants are of great importance to the health of individuals and communities (Edeoga *et al.*, 2005) [6]. Many of these indigenous plant species are used as *Ayurvedic* medicine. *Dashmoola* (Ten roots) species comprises five tree species (Perennial / Brihat Panchamool) and five weed species (Seasonal / Laghu Panchamool) *viz.* *Aegle marmelos*, *Stereospermum chelonoides*, *Gmelina arborea*, *Premna obtusifolia*, *Oroxylum indicum*, *Tribulus terrestris*, *Solanum indicum*, *Solanum xanthocarpum*, *Desmodium gangeticum*, *Uraria picta*. The roots of these species are used in preparation of *Ayurvedic* drug namely Dashmoolarist (Herbal liquid), Dashmoola Kwath i.e. decoction, Dashmool Kadha, sweet preparation with jaggery, Dashmoola oil, Dashmoola Medicated Ghee, Dashmoola Haritaki (Combined with Hirda), These formulations are used in various disorders such as Pyrexia (different types of fever), acute and chronic asthma, all types of vata disorders etc. The drugs of Dashmoola are used in the treatment of various diseases of almost all the systems of body specifically works on the Respiratory, Gastro-intestinal and Central nervous system. Dashmoola are considered as immunomodulator, general restorative tonic.

The traditional medicinal uses of tree species are reviewed in Table 1. Despite extensive applications of these plant species in Ayurveda medicine, little information is available on

their phytochemical constituents. For preparation of *Dashmoolarist* the roots of *Dashmoola* plant species have huge demand in India as well as in the world market.

Considering all these facts the present investigation is designed to find out phytochemicals in the root samples of perennials/ *Brihat panchamoola* of eighteen months old plants.

Materials and Methods

Selection and authentication of plants

The three tree species of eighteen months old viz. *Aegle marmelos*, *Stereospermum chelonoides*, *Gmelina arborea*, were selected for present study on the basis of literature (Kapoor *et al.*, 1969)^[10] and indigenous traditional knowledge (Cohen *et al.*, 1991)^[3]. The plants are authenticated by Plant Introduction Officer and Asst. Prof. (Botany) Department of Agricultural Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri.

Collection of Plants

The selected plant materials were collected from the field of Medicinal and Aromatic Plants Project, Department of Agricultural Botany, MPKV, Rahuri. The plant material is washed with clean water and allow to shade dried for about 2-3 weeks. The dried materials were crushed to a coarse powder.

Preparation of Plant extract

The crude plant extract was prepared by Soxhlet extraction method (Kokate and Verma, 1971)^[12]. About 50 gm of powdered material were packed in to filter paper and run in soxhlet extractor. The extraction of phytoconstituents was carried out with methanol for about 6 cycles. The extract is filter and evaporate to get concentrated extract.

Physico-chemical analysis

All the samples were analyzed for their physico chemical parameters. The results are tabulated in table no. I

Table 1: Physico-chemical parameters of Roots of *Dashmoola* tree species (*Brihat panchmoola*)

S. No.	Test performed	<i>A. marmelos</i>	<i>S. chelonoids</i>	<i>G. arborea</i>	
1	Loss on drying @ 110 °C (%)	0.85	0.206	0.062	
2	Acid insoluble matter (%)	0.54	1.68	1.11	
3	Ash content (%)	6.24	9.05	5.45	
4	Water soluble extractive (%)	12.16	15.84	11.47	
5	Alcohol soluble Extractive (%)	9.24	22.0	21.18	
6	Thin Layer Chromatography	Solvent system	Toluene : Ethyl acetate : Formic acid		
		Rf Values (Retention factor)	0.38	0.3	0.48
			0.48	0.53	0.08
			0.53		
			0.63		
			0.69		
			0.78		
0.96					

Phytochemical analysis of crude extract

The crude extract were tested for the presence of active principles such as Alkaloids, tannins, saponins, flavonoids, steroids, triterpenoids, proteins, fats, carbohydrates etc. The following standard procedures were used. The results are tabulated in Table II.

Test for Alkaloids

Mayers test: 1.0 ml crude extract was mixed with 0.5 ml Mayers reagent (Potassium mercuric iodide solution) in test tube. The formation of cream colour precipitate indicate the presence of alkaloids.

Test for Tannins

For testing of tannins Ferric chloride test was followed as describe by Trease and Evens, 1983; Kokate, 1994. 1.0 ml crude extract was mixed with 0.5 ml ferric chloride in test tube. The appearance of blue green colour to the mixture, suggests the presence of tannins.

Test for Saponins

Froth test as suggested by Trease and Evens, 1983; Kokate, 1994; Sofowara, 1982 was followed for presence of saponins. 1.0ml crude extract was shaken vigorously with in 1.0 ml distilled water in a test tube. Formation of froth was taken as positive test for presence of saponins.

Test for Flavonoids

For detection of flavonoids, Alkaline reagent test prescribe by Trease and Evens, 1983; Ayoola *et al.*, 2008^[2] was used. 1.0 ml crude extract was mixed with few drops of sodium hydroxide solution. An intense yellow colour was form. Yellow colour turned to colourless on addition of few drops of diluted acid, marked the presence of flavonoids.

Test for steroids and Triterpenoids

Salkowski test was used for presence of steroids and triterpenoids. 1.0ml crude extract was mixed with chloroform and a few drops of conc. Sulphuric acid, shake well and allowed to stand for some time. Red colour at the lower layer indicated the presence of steroids and formation of yellow coloured layer indicates the presence of triterpenoids.

Test for proteins

Protein analysis was carried out by using Ninhydrin test. 1.0 ml crude extract was boiled with 0.2 % Ninhydrin solution (Indane 1, 2, 3, trione hydrate). A violet colouration indicates the presence of amino acids and proteins.

Test for Fats

Saponification test was carried out for the detection of fats. In a small quantity (about 1.0 ml) of crude extract few drops of 0.5 N alcoholic potassium hydroxide were added to which a drop of phenolphthalein was added separately and heated in a

water bath for 1 hr. The formation of soap indicates the presence of fixed oils and fats.

Test for carbohydrates

Fehlings test as suggested Kokate, 1994 was followed. Equal volume of Fehling A (copper sulphate in distilled water) and Fehling solution B (Potassium tartarate and sodium hydroxide in distilled water) reagents were mixed with few drops of crude extract and boiled, a brick red precipitate of cuprous oxide forms, indicate the presence of reducing sugar.

Test for Phlobatannins

Presence of Phlobatannins was estimated by using the procedure suggested by Edeoga *et al.*, 2005 [6]. About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Development of a red precipitate was taken as an evidence for the presence of Phlobatannins.

Test for Resin

5 ml of distilled water was added to extract and observe for turbidity.

Table 2: Chemical constituents of crude methanolic extract of Roots of *Dashmoola* tree species (*Brihat panchmoola*).

Sr. no.	Plant extract	Phytoconstituent											
		Alkaloids	Tannins	Saponins	Flavonoids	Steroids	Triterpenoids	Proteins	Fats	Carbohydrates	Phlobatannins	Resin	
1	<i>A. marmelos</i>	√	×	×	√	×	×	×	×	×	×	×	√
2	<i>S. chelonoides</i>	√	√	×	√	×	×	√	×	×	×	×	×
3	<i>G. arborea</i>	×	×	×	√	×	×	√	√	√	×	×	√

√: Presence of Phytoconstituent, ×: Absence of Phytoconstituent

Results

The physic-chemical parameters of the five perennial species are presented in Table 2. The results reveals that the *A. marmelos* recorded higher percentage loss (0.85%) while *G. arborea* recorded lower (0.062%) after drying @110°C. The acid insoluble matter was lower in *A. marmelos* (0.54%). *S. chelonoides* contain higher ash percentage (9.05%) while *G. arborea* was 3.62%. The water and alcohol soluble extractive was higher in *S. chelonoides* and lower in *A. marmelos*.

The thin layer chromatography of methanol extract is carried on silica plate. The solvent system used is Toluene: Ethyl acetate: Formic acid. The result indicates the presence of different chemical compounds in the methanolic extract with varying polarity which in turn gives different retention factor values. The retention factor (Rf values) are given in Table II.

The results of phytochemical characteristics of five tree species investigated and summarized in Table III, which reveals the presence of medicinally active constituents in the species under study. The analysis of crude methanolic extract of revealed the presence of flavonoids in all the extracts. The alkaloids are present in *A. marmelos*, *S. chelonoides*, *P. obtusifolia* and *O. indicum*. The steroids, triterpenoids and saponins are also absent in all extracts.

The *S. chelonoides* extract shows presence of alkaloids, tannins, flavonoids, proteins and carbohydrates. Besides the alkaloids the *O. indicum* extract shows presence of tannins, flavonoids, proteins, fats and carbohydrates.

Discussion

The results confirm the presence of constituent which are known to exhibit Medicinal as well as physiological activity (Sofowora, 1993) [15]. From all above analysis of methanolic extracts of roots of *Brihat panchamoola* shows the presence of different phytoconstituents showing different biological activities.

The alkaloids also shows analgesic (Antherden, 1969) [1] and anaesthetic property (Herourat *et al.*, 1988) [8]. The tannins shows antibacterial as well as anti-inflammatory activity (Duguid, 1989) [5]. Flavonoids enhance the effect of vitamin c and function as antioxidants. They are also known to be biologically active against liver toxins, tumours, viruses etc.

Flavonoids are found in almost all plant based foods and beverages. Flavonoids have antioxidant activity. Flavonoids are becoming very popular because they have many health effects. Some of the activities attributed to flavonoids include: anti-allergic, anti-cancer, antioxidant, anti-viral. The flavonoids quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. Epidermological studies have illustrated that heart diseases are inversely related to flavonoid intake. The flavonoids prevent the oxidation of low-density lipoprotein. The contribution of flavonoids to the total antioxidant activity of components in food can be very high because it can vary between 50 to 500 mg.

Saponins exhibit antifungal property. In agriculture studies particularly on the oat root, alfalfa and tomato have found that saponins can form antifungal compounds. Studies shows spraying saponins concentrated 0.1 to 0.2 % on leaves have reduced beetles, mites, aphids and various pests by significant amount.

Conclusion

The result reveals the presence of medicinally active constituents in the five tree species (*Brihat Panchmoolal*) of *Dashmoola*. The phytochemical compounds identified in this study have earlier been proved to be bioactive. The presence of some of these compounds have been confirmed by previous workers to have medicinal as well as physiological activity and therefore could be said to be responsible for the efficacy of the parts of the plants studied in treatments of different ailments. The plant extracts could therefore be seen as a potential source for (*Dashmoolalist*) important drug in *Ayurveda*. The continued traditional medicinal use of these plants is therefore encouraged while it is suggested that further work should be carried out to isolate, purify and possibly characterize the active constituents responsible for the activity of these plants. Also additional work should be embarked upon with a view to elucidate the possible mechanism of action of these extract

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