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PRELIMINARY PHYTOCHEMICAL SCREENING OF LIMONIAACIDISSIMA LINN.

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ABSTRACT :

The phytochemical screening of the medicinal plants are significant and have commercial interest in both research institutes and pharmaceutical companies for the development of the new drugs for treatment of various diseases. With the ever increasing interest of today's population towards natural products, LimoniaacidissimaL. emerged out to be one of the most eyes catching plant bearing multiple medicinal properties, belonging to family Rutaceae. Total of eight phytochemicals such as Alkaloid, Tannin, Flavonoids, Steroids, Saponins, Carbohydrate with Gum and Phenolic compound were tested in three different plant extracts showed presence and absence of their activity. For the screening of phytochemicals, Petroleum Ether (PE), Methanol (ME) and Aqueous (AQ) extracts were used.During qualitative test, different slandered methods were performed in order to check the activity of respective chemical compounds. The results revealed that, phytoconstituents such as Tannins, Sterols, Saponins, Carbohydrate, Gums and Phenolic compound were not observed in the Aqueous (AQ) extract of plant materials.In present study highest metabolites were present in Methanol extract; however it was less in the aqueous extract. The study supports the medicinal value of this plant and demonstrates that L. acidissimaplant may be used as nutraceuticals for disease prevention and health promoting benefits.

Keywords: *Limoniaacidissima,phytoconstituents, tannin, aqueous extract, medicinal properties*

INTRODUCTION

India is the birth place of renewed system of indigenous medicine such as Siddha, Ayurvedha and Unani. Traditional systems of medicines are prepared from a single plant or combinations of number of plants. The efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in a raw drug (Savithramma*et al.,* 2010; Vinoth*et al.,* 2011).

Limoniaacidissima (L.) of family Rutaceae (Citrus family) belongs to the monotypic genus *Limonia*, confined to India, Pakistan, Sri Lanka and Southeast Asia (Allen, 1967). It is also known as woodapple, elephant-apple, monkey fruit, curd fruit, Kath bel and Kaitha. This plant is given as a medicine for the treatment of various disorders (Khare, 2007).

L. acidissima is a deciduous, slow-growing, erect tree with a few upward-reaching branches bending outwards near the summit where they are subdivided into slender branch lets drooping at the tips. Its fruit is spherical in shape with 4.5- 12.5 cm diameter. The rind is grayish-white in color and 6-7 mm thick. It has woody and extremely hard outer shell (called as rind) which is very difficult to crack open. The leaves are pinnate, with 5-7 leaflets, each leaflet 23–34 mm long and 8–21 mm broad, with a citrus-scent when crushed. The fruit is a berry 4– 10 cm diameter, and may be sweet or sour. It has a very hard rind which can be difficult to crack open, and contains sticky brown pulp and small white seeds (Ghosh, 1982). The Pulp is whitish-brown, mealy, aromatic, resinous, sour or sweetish with many small white seeds embedded in it.

Syrups, drinks, jellies and jams can be prepared from its sticky pulp (Morton, 1987). The significant parts of the plant include its roots, fruits, bark and the leaves which are used for various therapeutic purposes (Manay, 2005). Traditionally the leaves have been used in the treatment of diarrhoea, wound healing, boils etc, which gives as an idea for its antimicrobial activity.

Considering wide utility of plants in variousfieldsespeciallyphytochemically, medicinally, economically significance was one of the aims to undertake present study; moreover very less reports on phyotochemical screening of *Limoniaacidissima* in general and region in particular were published in literature. Therefore present study with selected plant was conducted to screen secondary metabolites primarily.

Taxonomical Classification of plant

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliospida
Subclass	Rosidae
Order	Sapindales
Family	Rutaceae
Genus	*Limonia* L.
Species	*L. acidissima.*
Synonym	*Feronialimonia*(L.) Swingle

Material and Methods

Plant Materials

The plant material were collected from the different regions of Barshitakali and Washim (Maharashtra) area. Morphological character, identification and classification were confirmed with the help of different floras and expert persons. Morphology of various parts of plant were observed and mentioned. The characters from the literatures, several uses of plant parts and chemical metabolites present in them were also recorded.

Preparation of Extracts

The fruit, leaf and stem of *L. acidissima*were cut into smallest pieces, shade dried and powdered. The resultant powder was then subjected for successive extraction with methanol, petroleum ether, and water with Soxhlet apparatus. The extracts were then concentrated in vacuum under reduced pressure using rotary flash evaporator, dried in desiccators and stored in refrigerator at 4^{o}C till further use (Tanwer*et. al.,* 2010). The extractive values were deduced using following formula: Yield (%) = Dry weight of extract/Dry weight of plant powder×100.

Phytochemical Screening

The identification of the chemical constituents in plant extracts, standard procedures were carried out. For qualitative test, the following reagents and chemicals were used to check the presence of chemical constituents in crude extracts of plant material such as Mayer's reagent for Alkaloid, Ferric chloride for Tannin, Concentrated HCl for Flavonoids,Sulphuric acid for Steroids,Foam formation test for Saponins,Molish reagent and sulphuric acid for Carbohydrate with Gum and Folin-Ciocalteu reagent for Phenolic compound (Ghani, 2003; Trease and Evans, 1978). In each test 10% (w/v) solution of extract was taken unless otherwise mentioned in particular test. Components were identified by observing the characteristic color changes.

Determination of Alkaloids: Mayer's test: The solution of the extract (2 ml) and dilute hydrochloric acid (0.2 ml) were taken in a test tube. Then 0.1 ml of Mayer's reagent was added. The Precipitate of yellow color was formed that indicated the presence of alkaloids.

Determination of tannins: Ferric Chloride Test: The solution of the extract (5 ml) was taken in a test tube. Then 5%Ferric chloride solution (1 ml) was added. Greenish black precipitate was formed and indicated the presence of tannins.

Determination of Flavonoids: A few drops of concentrated hydrochloric acid was added in 1ml of respective plant solution extracts. Quick development of a red color indicated the presence of Flavonoids

Determination of Steroids: Sulphuric acid test: equal amount of solution of plant extracts and Sulphuric acid was mixed, immediateappearance of red color indicates the presence of steroid.

Determination of Saponins: 1 ml solution of the extracts was diluted with distilled water to 25 ml and shaken in a graduated cylinder for 20 minutes. Formation of foam confirmed the presence of saponins.

Determination of gums and Carbohydrate: The 2 ml solution of the extracts was taken in test tubes and then Molish reagent and sulphuric acid were added. As red violet ring was not observed at the junction of two liquids,therefore it was clear that gums and carbohydrate were not present in the extracts.

Determination of Phenolics: The total phenolic content in different extracts of *Limoniaacidissima*was estimated using standardized methods such as Folin-Ciocalteu reagent accordingly. The 20µl of the plant extracts (dissolved in the respective solvents) were taken in a test tube and made up to the volume of 1.0 ml using distilled water. Then 0.5 ml of freshly prepared Folin-ciocalteu phenol reagent (1:1 with water) and 2.5 ml of 20% sodium carbonate solution were added successively in each tube. Then the solution were mixed thoroughly and left in the dark room for 40 min for color development.

Results and Discussion

Phytochemical investigation provides the clue for further study of crude drug. The Petrolium ether, Methanol and Aqueous solvents were used in order to extract the plant metabolites that can be solubilized in these three different solvents. The Soxhlet extract method is efficient technique to get all that metabolites because of repeated extract of the material with the solvent system.The crude extracts were subjected for chemical group tests and identified various types of important chemical constituents. Results of different group tests are given in table 1.Total of eight phytochemicals tested in three different plant extracts showed presence and absence of their activity. Phytochemical screening showed that Alkaloids, Tannins, Sterols, Saponins and Phenolic compound were present in the Petroleum Ether (PE); Alkaloids, Tannins, Flavonoids, Sterols, Saponins and Phenolic compound were present in the Methanol (ME) however only Alkaloids and Flavonoids were present in the Aqueous (AQ) extracts showed extensively pharmacologic activity. Negative tests were detected for Flavonoids, Carbohydrate and Gums in Petroleum Ether (PE); Carbohydrate and Gums in Methanol (ME) and Tannins, Sterols, Saponins, Carbohydrate and Gums and Phenolic compound in Aqueous (AQ) extract (Table 1). Many phytochemicals were not dissolved in water might be a reasons for negative test in Aqueous extract of present study, on the other hand study confirmed that highest metabolites were present in Methanol extract (Table 1).

A primary metabolite is directly involved in the normal growth, development, and reproduction. Differentprimary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds such as antipsychotic drugs (Bray and Thorpe, 1954).

Flavonoids were found in the aqueous extracts of plant and are potent water soluble antioxidants (Borhade, 2012), the current results was obtained in accordance with the Flavonoids activity. The results of present investigation were additionally in accordance of flaxseeds (Tawheed and Monika, 2014).Moreover the presence of alkaloids, saponins, steroids, phenolic compounds and tannins was in similar with the findings by Sheeja*et al.,*(2005).

Table.1 Different Phytochemicals present in various extracts of *Limoniaacidissima*

SN	Metabolites Test	Petroleum Ether (PE)	Methanol (ME)	Aqueous (AQ)
1	Alkaloids	++	++	+
2	Tannins	+	+	--
3	Flavonoids	--	+	+
4	Sterols	++	++	--
5	Saponins	++	+	--
6	Carbohydrate	--	--	--
7	Gums	--	--	--
8	Phenolic compound	++	++	--

(+) indicates Present, (-) indicates absent.

Conclusions

The preliminary phytochemical analysis of *Limoniaacidissima* L. had revealed the presence of various phytoconstituents such as Saponin, Tannin, Steroid, Protein, Carbohydrate, Alkaloids, Flavanoids. The report may be useful in the isolation and characterization of active phytoconstituents for bioactivity and have great importance as therapeutic agents (Amin *et al.,* 2017). Moreover "Kaitha" was analyzed for nutritional, phytochemical, antioxidant and antibacterial activity for use as functional foods and nutraceutical and flavoring agents to provide health significance. These results were additionally support beneficial health claims. Hence, there is wide scope for future research and further pharmacological investigation on *L. acidissima*. If some of the phytochemical from wood apple will be proved for its traditional uses, the cultivation of the plant will have great profitable business for the farmers as well even in the road side and barren land.

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