Full Length Articles

THE PRELIMINARY PHYTOCHEMICAL SCREENING OF VARIOUS LEAF EXTRACTS OF PLANT *Limonia acidissima Linn*.

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ABSTRACT

The plant Limonia acidissima belongs to family Rutaceae. Various parts of this plant like fruit, seed, rind, bark, leaves and roots are being used to treat human and animal ailments. The fruits of the plant are most essential therapeutic portion containing bioactive molecules. This study is intended to reveal presence of some phytoconstituents from leaf extracts and review it thoroughly in order to validate its medicinal use. In the study it was found that aqueous and ethanolic extracts revealed maximum types of phytochemical classes as compared to acetone and petroleum ether extract which can be used for treatment with reference to its mentioned use. Many phytochemicals namely alkaloids, carbohydrates, glycosides, proteins, saponins, phenols, resins, etc. were detected which reflects its therapeutic value.

Key words: *Limonia acidissima*, leaf extract, phytochemical investigation

INTRODUCTION

The plants are being used in therapeutics across the globe since time immemorial. Even in current day situation man is looking for alternative therapies with plant as a source for newer phytomolecules. It is interesting to mention that significant population of the world still relies on plant medicines. India is home for millions of plant species with some famous for their medicinal properties and some still unexplored.

The fruits of the plant *Limonia acidissima* popularly called wood apple, or elephant apple are known as poor mens food. The fruit pulp of the plant possesses antidiabetic, wound healing, anticancer, astringent, antioxidant, hepatoprotective, antifungal, antispermatogenic activity, dyspepsia, stomachic activity (Vijayvargia and Vijayvargia, 2014, Pandey *et al*., 2014, Deshpande D J., 2006). Studies by some authors suggest fruit to bear promising antibacterial activity with dose dependent inhibition (Jayashree and Londonkar, 2014, Ponnuraj *et al*., 2015, Pandey *et al*., 2014). The leaves are mentioned to possess antibacterial activity, antilarvicidal, antioxidant, astringent, antifungal, diuretic activity, various types of gastropathy and cardiopathy (Vijayvargia and Vijayvargia, 2014, Prajapati *et al*., 2009, Deshpande D

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J., 2006, Naidu *et al.*, 2014, Attarde *et al.,* 2011). Many authors worked on antibacterial activity of leaves and results varied since some author given good remarks (Naidu *et al*., 2014, Panda *et al*., 2013), but some claims poor antbacterial activity (Patil *et. al.,* 2012, Thomas and Ponnammal, 2005). The plant has ethno-veterinary medicinal importance in Vidarbha region of Maharashtra, since tribals use leaves of tree against haemorrhagic septicaemia (Kulkarni *et al.*, 2014) in bovines. The leaves possess tannins and essential volatile oils (Vijayvargia and Vijayvargia, 2014, Prajapati *et al*., 2009, Nadkarni A. K., 1954). The active principles of the plants *Limonia acidissima* were known to be mostly residing in whole fruit and hence researcher were concentrated towards biological utility and pharmacological activity of only fruits. But, other parts of this plant including leaf remained untouched. Hence, taking into consideration its age old traditional medicinal use and ethnoveterinary use, the study on leaves is conducted to reveal certain phytochemicals.

MATERIALS AND METHODS

Materials – Distilled water, analytical grade solvents like ethanol, acetone and petroleum ether provided by Hi-Media, musclin cloth, Whatman filter paper number 1. The chemicals used for phytochemical analysis were all analytical grade.

Collection, identification and authentication of plant: The collection of plant material was done in the month of August. The branches possessing fresh green young to mature leaves were selected and submitted to Botany department of Rashtrasant Tukadoji Maharaj Nagpur University. The plant was identified as *Limonia acidissima* and herbarium sheet with appropriate information regarding identified plant species were filled up by an expert botanist and given authentication number **10056**. One herbarium sheet was deposited in the respective department for future reference.

(1) Preparation of extract - The extraction was done as per the methodologies employed by **Betoni *et al.*, (2006)** and **Mahomoodally** and **Dilmohamed (2016)** with very slight modifications. The extraction of leaves was done by cold exhaustive maceration method. The collected plant leaves were air dried under shed at normal room temperature. Dried leaves were subjected to grinding to a coarse powder form. Then 100 gm leaf powder was transferred into a stoppered round bottom long neck flask and added 400 ml of solvent. The plant powder was allowed to macerate for 24 hrs at room temperature with vigorous intermittent shaking of the flasks for 10 - 15 minutes after every 2 - 3 hrs to facilitate or accelerate the extraction process. The contents present in flask were taken out and filtered through muslin cloth with care that complete filtrate was obtained. The collected filtrate was again subjected for further filtration by Whatman filter paper no. 1. The final filtrate was collected in a petridish weighed previously. The marc remained after filtration in above process was again added with 400 ml solvent and allowed to macerate for next 24 hrs. Similar process was repeated for third time till complete decolorization of marc occurred and filtrates obtained through second and third maceration process were combinely

evaporated on hot water bath at 50ºc with previously obtained filtrate. Finally solvent free, semi solid extract was obtained.

The entire procedure was performed with four different solvents like water, ethanol, acetone and petroleum ether. The extracts thus obtained were stored in dessicator for further use. The percentage extractability has been calculated from above information for each individual extract.

(2) Qualitative Phytochemical analysis - The preliminary qualitative phytochemical analysis was done to detect presence or absence of various phytoconstituents in given leaf extracts of *Limonia acidissima* as per method described by **Rosenthaler (1930)** and **Raaman (2006).** The test revealing negative results were performed twice for confirmation.

1. Test for Alkaloids

Fifty milligrams of extract was stirred with dilute hydrochloric acid and filtered. The filtrate was then subjected to following tests. (A) Mayer's test

Few millilitres of filtrate were taken in a test tube and few drops of Mayer's reagent were added along side wall of test tube. Formation of white or creamy precipitate indicates positive test.

Mayer's reagent: Mercuric chloride (1.358 grams) was dissolved in 60 millilitres of water and potassium iodide (5 grams) was dissolved in 10 millilitres of water. These two solutions were mixed and volume was made up to 100 millilitres with water.

(B) Wagner's test (Rosenthaler, 1930)

To the little amount of filtrate, Wagner's reagent was added. Appearance of brown flocculent precipitate indicates the presence of alkaloids.

Wagner's reagent: Iodine 1.27 grams and 2 grams of potassium iodide were dissolved in 5 millilitres distilled water and solution was further dissolved in water to make final volume 100 millilitres.

(C) Hager's test

Few millilitres of filtrate were taken in a test tube and one or two millilitres of Hager's reagent were added. A prominent yellow color precipitate indicates positive test. Hager's reagent: A saturated aqueous solution of picric acid was made.

(D) Dragendroff's test

To a few millilitres of filtrate, 1 or 2 millilitres of Dragendroff's reagent were added. A prominent yellow precipitate indicates positive test.

Dragendroff's reagent: It was prepared by mixing solution A (17 grams of Bismuth subnitrate + 200 grams of tartaric acid + 800 millilitres of distilled water) and solution B (160 grams of potassium iodide + 400 millilitres of distilled water) in 1:1 proportion (V/V).

2. Test for carbohydrates

Extracts (100 milligrams each) were dissolved individually in 5 millilitres of water and filtered. The filtrates were then subjected to following test.

(A) Fehling's test: About 1 millilitre of filtrate was taken in a test tube and added with 1 millilitre of Fehling A and 1millilitre of Fehling B solution and mixed well by shaking. The test tube was heated on water bath for 2 minutes. Appearance of red precipitate indicates positive test.

Fehling - A solution: Copper sulphate (34.66 grams) was dissolved in distilled water and volume made upto 500 millilitres.

Fehling - B solution: Potassiun sodium tartarate (173 grams) and sodium hydroxide (50 grams) were dissolved in water and volume made upto 500 millilitres.

(B) Benedict's test : About 0.5 millilitre of filtrate was taken in a test tube and 0.5 millilitre of Benedict's reagent was added. The mixture was heated over boiling water bath for 2 minutes. A characteristic colored precipitate indicates test as positive.

Benedict's reagent: Sodium citrate (173 grams) and sodium carbonate (100 grams) were dissolved in 800 millilitres of distilled water and boiled to make it clear. Copper sulphate (17.3 grams) dissolved in 100 millilitres distilled water was added to it.

3. Test for glycosides

The extract (50 milligrams) was dissolved in concentrated hydrochloric acid for 2 hrs on water bath, this hydrolysate was filtrated and filtrate was used for following test.

Legal's test

About 50 milligrams of extract was taken in a test tube and small amount of pyridine was added to it and mixed well. After that sodium nitroprusside followed by 10% sodium hydroxide was added. Development of pink color indicates positive test.

4. Test for saponins

The extract (50 milligrams) was taken in stoppered test tube and finally diluted upto 20 millilitres by adding distilled water. The tube was shaken for 15 minutes and observed for formation of foam. A two centimeter foam layer indicates positive test.

5. Test for proteins and amino acids

The extract (100 milligrams) was dissolved in 10 millilitres of distilled water and filtered through Whatman filter paper no. 1 and filtrate was again used for following test. (A) Biuret test (Rosenthaler, 1930) A few milligrams of residue were taken in water and 1 millilitre of 1% solution of sodium hydroxide was added followed by a drop of 1% solution of copper sulphate. Violet pink color development indicates positive test for proteins.

(B) Ninhydrin test

Two millilitre of filtrate was taken in test tube and few drops of ninhydrin solution were added. A characteristic purple color indicates positive test for presence of amino acids.

Ninhydrin solution: About 10 milligrams of ninhydrin dissolved in 200 millilitres of acetone

(C) Xanthoprotein test (Rosenthaler, 1930) A little residue was taken in 2 millilitres of

water and to it 0.5 millilitre concentrated nitric acid was added. Appearance of white or yellow precipitate indicates presence of proteins.

6. Test for phytosterols

Salkowski's test (Rosenthaler, 1930. A small amount of extract was taken in 2 millilitres of chloroform and sulphuric acid was added alongside of test tube and test tube was shaken. Red color development in the chloroform layer and greenish yellow fluorescence in the lower layer indicates presence of sterols.

7. Test for phenolic compounds

(A) Ferric chloride test

About 50 milligrams of extract were dissolved in 5 millilitres of distilled water and transferred to test tube and to this 5% neutral ferric chloride solution was added. Development of dark green color indicates presence of phenolic compounds.

(B) Lead acetate test

About 50 milligrams extract was dissolved in distilled water and 3 millilitres of 10% lead acetate solution were added. A bulky white precipitate indicates presence of phenolic compounds.

8. Test for flavanoids (Rosenthaler, 1930)

A small quantity of residue was dissolved in 5 millilitres of ethanol (95%) and treated with a few drops of concentrated hydrochloric acid and 0.5 gram of magnesium metal turnings. Development of either pink or red color indicates presence of flavonoids.

9. Test for resins (Rosenthaler, 1930)

The alcoholic extract was dissolved in alcohol. To this, a few drops of water were added. The appearance of turbidity was considered as a positive test.

RESULTS

The fresh procured leaves with four different solvents i.e. distilled water, ethanol, acetone and petroleum ether were extracted. The percent extractability of *Limonia acidissima* leaves in water, ethanol, acetone and petroleum ether solvents were calculated as 24%, 14%, 2% and 1% respectively. The percent extractability, colour and consistency of individual extract in details are given in **Table 1**. The detailed phytochemical investigation of plant extracts were done to detect the presence or absence of various classes of phytoconstituents present in the extract. The maximum phytochemicals were detected from an aqueous and ethanolic extract. The phytoconstituents like alkaloids, carbohydrates and glycosides, proteins and amino acids, saponins, phenols, resins, etc. were detected. The results obtained in phytochemical analysis are presented in **Table 2**.

DISCUSSION

The percentage of extractability obtained through this extraction process is in accordance with the results obtained in the previous work done by various researchers. The highest percentage of extractability was obtained through aqueous extract i.e. 24%. However, percentage of extractability in water is not found in reviewed literature. The percentage of extractability with

ethanol is recorded as 14% in present study which is almost double as compared to the extractability (7.89%) mentioned by **Panda *et al.,* 2013**. The extractability with petroleum ether has been mentioned as 1% by **Panda *et al*., 2013** which is similar to our results. However, **Baneerjee *et al*., 2011** mentioned the total yield of 1.12 grams of petroleum ether extract; acetone extract 2.34 grams and alcohol extract 2.21 grams from 25 grams of leaves, which comes to 4.48, 9.36 and 8.84% respectively. As compared to this in present study petroleum ether and acetone extracts were less and alcoholic extract was yielded more (14%).

The aqueous extract showed presence of alkaloids, carbohydrates, proteins and amino acids, phenolic compounds and resins. As mentioned earlier phytochemicals in aqueous extract have not been mentioned in the literature it could not be compared with the present study. Ethanolic extract revealed glycoside and saponins in addition to those phytochemicals present in aqueous extract. The ethanolic extract revealed presence of phytoconstituents, which is in mere agreement with previous study by **Panda *et al.,* 2013** who detected all the phytochemicals in addition to phytosterols and flavanoids. **Singh and Vidyasagar, 2015** revealed the absence of steroids in leaf ethanolic extract which is in agreement with the present study. When the phytochemicals of the ethanolic extract obtained in this study compared with phytochemicals obtained in a study conducted by **Thilagavathi *et al.,* 2015**, it is observed that all the phytochemicals matched, except presence of saponin and absence of flavonoid and saponins in two studies. The acetone extract revealed alkaloids, phenols and resins, which were not found to be mentioned in reviewed literature. The petroleum ether extract in the present study revealed presence of alkaloids and resins. The results of phytochemical analysis with leaf petroleum ether extract seems almost match with previous results stated by **Attarde *et al*., 2011**, except for presence of steroids and absence of alkaloids. It is important to note that in the present study all the solvent extracts detected presence of alkaloids and resins. Many authors revealed presence of flavonoids and terpenoids/steroids in leaves, but not detected in this study from any of the tested extract. This variation could be due to the method of estimation of flavanoids which was followed in present study and alkaline reagent test followed by **Attarde *et al*., 2011**. However, resins were not detected by any author whichwere found in this study. However,these variations in presence of phytochemicals may be due to geographical and climatic conditions or habitat of the plant.

Hence, it can be said that water and ethanol are able to extract out most of phytochemicals and can employed for therapeutic purposes being repository of many phytoconstituents.

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Table No. 1. The colour, consistency and percent extractability of aqueous, ethanolic, acetone and petroleum ether extract of leaves of plant *Limonia acidissima.*

Sr. No	Type of extract	Quantity used	Colour	Consistency	Percentage of extractability
1	Aqueous	100 gm	Brown or coffee colour	Semisolid and pasty	24
2	Ethanolic (EtOH)	100 gm	Dark olive green	Semisolid and pasty	14
3	Acetone (Me_2CO)	100 gm	Dark green	Semisolid	2
4	Petroleum ether	100 gm	Sea weed green	Semisolid	1

Table No. 2. Phytochemical analysis of aqueous, ethanolic, acetone and petroleum ether leaf extracts of plant *Limonia acidissima.*

Sr. No	Active Principle	Test employed	Aqueous extract	Ethanolic extract	Acetone extract	Per. ether extract
1.	Alkaloids	Mayer's test	Absent	Absent	Absent	Absent
		Wagner's test	Present	Present	Present	Present
		Hager's test	Absent	Absent	Present	Present
		Dragendroff's test	Present	Present	Absent	Absent
2.	Carbohydrates	Fehling's test	Absent	Absent	Absent	Absent
		Benedict's test	Present	Absent	Absent	Absent
3.	Glycosides	Legal's test	Absent	Present	Absent	Absent
4.	Saponins	Foam test	Absent	Present	Absent	Absent
5.	Proteins and amino acids	Biuret test	Present	Absent	Absent	Absent
		Ninhydrin test	Present	Absent	Absent	Absent
		Xanthoprotein test	Absent	Present	Absent	Absent
6.	Phytosterols	Salkowski's test	Absent	Absent	Absent	Absent
7.	Phenolic compounds	Lead acetate test	Present	Present	Present	Absent
		Ferric chloride test	Present	Present	Absent	Absent
8.	Flavonoids	Test for flavonoids	Absent	Absent	Absent	Absent
9.	Resins	Test for resins	Present	Present	Present	Present