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Comparative study of phytochemical analysis and extractive value of *Aegle marmelos, Bauhinia racemosa* and *Psidium guajava*

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Abstract
The presence study conducted to study qualitative phytochemical and physiochemical extractive value of leaf of *Aegle marmelos, Bauhinia racemosa* and *Psidium gaujava*. It was evaluated using aqueous and methanol as a solvent to determine active components. Maceration method was adopted in extracting the phytochemical. This reveals aqueous and methanol leaf extracts of *Aegle marmelos* contain alkaloids, tannins, sterols, phenolic compounds, flavanoids wheres as aqueous and methanol leaf extracts of *Bauhinia racemosa* extracts present alkaloids, glycosides, tannins, sterols, phenolic compounds. Meanwhile leaf extracts of *Psidium gaujava* also contains Alkaloids, glycosides, tannins, saponins, sterols, phenolic compounds, saponins, Reducing sugar and Flavonoids.

Keywords: Salinity, PSB and characterization

Introduction
Plants have been used as medicines since thousands of years and are still being used in their natural and processed forms. Modern man as a result of his dependence on the quick results of allopathic medicines was neglecting the importance of plants as medicines for some years in the past. However, after growing awareness of unwanted side effects of the allopathic medicines has now understood the importance of medicinal plants for being devoid of side and adverse effects[1][4]. Therefore, while going through the varied literature on plants for their medicinal values gathered information on plants for their use against infectious and non-infectious diseases. It is reported that in India traditional healers are using 2500 plant species, out of which 100 species of plants serve as regular sources of medicine [11]. The medicinal systems such as Ayurveda, Unani and Siddha through systematic approach over course of many centuries have accumulated the vast information on medicinal plants.
Phytochemicals are the naturals bioactive compounds found in plants. These phytochemicals are primarily classified into two classes, i.e. primary and secondary constituents, according to their functions in plant metabolism. Primary constituents contain common sugars, amino acids, proteins and chlorophyll while secondary constituents comprises of alkaloids, terpenoids, steroids and flavonoids etc. [3]. Secondary metabolism is chemically & taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [17]. Plant chemicals are regards as secondary metabolites because the plants that manufacture them have little need for them. They are synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds etc i.e. any part of the plant body may contain active components [16]. The main purpose of the present study was preliminary phytochemicals qualitative phytochemicals analysis in the aqueous and methanolic extracts of *Aegle marmelos, Bauhinia racemose* and *Psidium guajava.*

Materials & methods
Selection and Collection of Plant Material
Whole fresh plant leaf of *Aegle marmelos, Bauhinia racemosa* and *Psidium gaujava* collected from campus of college of Veterinary and Animal Science, Udgir and suburban areas of Udgir city. The leaf of the plant was dried under shade and the powder of dried leaf was prepared by mechanical grinder. The dried powder was passed through the mesh sieve to obtained the fine powder.

Identification and authentication of plant

The plant *Aegle marmelos*, *Bauhinia racemosa* and *Psidium gaujava* was identified and authenticated by a botanist (Assistant professor, dept. of Botany, Shivaji Mahavidhyalaya, Udgir)

Method of extraction and Estimation of extractability percentage

The fine leaf powder prepared was stored in airtight glass bottles, which were kept in refrigerator. Fifty grams of the powder was taken in six separate 500 ml conical flasks. 200 ml of distilled water and methanol were respectively added to these six flasks. Flasks were Stoppard tightly and were kept in refrigerator for maceration. Maceration period lasted for 48 hrs. The flasks were shaken intermittently during the maceration period. After maceration, the contents in flask were filtered through the muslin cloth and the filtrate so obtained was once again filtered through Whatman filter paper. This filtrate was then transferred to sterilized evaporating bowl, which was then placed under fan for evaporation of the solvent so as to make the extract as much dry as possible. Color and physiochemical properties of extract are evaluated on the basis of its physical appearance. The percent extractability of each of these extracts was then determined as per the following formula suggested by Rosenthaler (1930) [14], while the consistency of each extracts was also noted.

$$\% \text{ Extractability} = \frac{\text{Weight of extract (gm)}}{\text{Powder used (gm)}} \times 100$$

Phytochemical study for qualitative analysis of certain active principles

The preliminary qualitative phytochemical analysis was done to detect presence or absence of various phytoconstituents in given leaf extract two best selected extracts per method described by Rosenthaler (1930) [14]and Raaman (2006) [12].

1. Test for Alkaloids

About 50 mg of extract was stirred with dilute hydrochloric acid and filtered. The filtrate was then subjected to following tests.

(A) Wagner's test: 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 ml distilled water and solution was further dissolved in water to make final volume 100 ml.

(B) Dragendroff's test: To a few ml of filtrate, 1 or 2ml of Dragendroff's reagent were added. A prominent yellow precipitate indicates positive test.

2. Test for glycosides

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

(A) Benedict's test: About 0.5 ml of filtrate was taken in a test tube and 0.5 ml 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.

(B) Fehling's test: About 1 ml of filtrate was taken in a test tube and added with 1ml of Fehling A and 1 ml 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.

(C) Folin Wu copper reagent test: A little amount of the extract was added to few drops of folin wu copper reagent, the development of red color gives positive reactions for glycoside

3. Test for detection of tannins

A little quantity of extract taken in a test tube was warmed and filtered. Tests were carried out with the filtrates using following reagent:

(A) Lead acetate test: Few drops of 5%nlead acetate solution were added to filtrate, formation of precipitate indicates the presence of tannins.

(B) Ferric chloride test: Few drops of ferric chloride were added to little amount of the filtrate, development of green color indicates presence of tannins

4. Test for saponins

The extract (50 mg) was taken in stoppered test tube and finally diluted upto 20ml 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 (100mg) was dissolved in 10ml of distilled water and filtered through Whatman filter paper no. 1 and filtrate was again used for following test.

(A) Xanthoprotein test: A little residue was taken in 2ml of water and to it 0.5 ml concentrated nitric acid was added. Appearance of white or yellow precipitate indicates presence of proteins.

(**B) Biuret test:** A few mg of residue were taken in water and 1 ml 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.

6. Test for phytosterols (Terpenoids)

Salkowski's test

A small amount of extract was taken in 2ml 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

Ferric chloride test

About 50 mg of extract were dissolved in 5ml 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.

8. Test for resins

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.

9. Reducing sugar

(A) Benedict's reagent test: The extract was added with Benedict's reagent in equal amount and mixture was heated for 2 minutes, appearance of brown to red color indicated presence of reducing sugar.

(B) Folin Wu copper reagent test: Few quantity of extract was added with few drops of Folin Wu copper reagent, the development of red color indicates presence o reducing sugar

10. Test for flavanoids

A small quantity of residue was dissolved in 5ml 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.

Result & Discussion

Physiochemical properties and percent extractability of plants extracts

The leaf powder of *Aegle marmelos*, *Bauhinia racemosa* and *Psidium gaujava* were subjected to aqueous and methanol solvent extraction by maceration process. The percent extractability, color and consistency of extracts of *Aegle marmelos, Bauhinia racemosa* and *Psidium gaujava* leaf were observed and recorded as shown in table 1. The color was brown for aqueous extract and bottle green for methanol extract of leaves of *Aegle marmelos*. Both the aqueous extract and methanol extract of leaves of *Aegle marmelos* were semisolid in consistency. While, the percent extractability of aqueous leaf extract of *Aegle marmelos* is observed to be 30% and that of the leaf methanol extract was found to be 8%. The color of aqueous and methanol extract of leaves of *Bauhinia racemosa* is slightly dark brown and dark green respectively. The consistency was found to be solid for both aqueous and methanol leaf extracts of *Bauhinia racemosa.* However, the percent extractability of aqueous extract and methanol leaf extracts of *Bauhinia racemosa* was observed to be different from each other and were found to be 2% and 15% respectively. The color observed for aqueous and methanol leaf extracts were dark brown and tan respectively and the consistency of aqueous and methanol leaf extracts of *Psidium gaujava* were found to be semi-solid and solid respectively. While, percent extractability of aqueous and methanol leaf extracts of *Psidium gaujava* is found to be 5% and 10% respectively.

Table 1: Percent extractability, color and consistency of aqueous and methanol extracts of *Aegle marmelos, Bauhinia racemosa* and *Psidium gaujava*

Sr. No.	Plants	Solvent	Extracts		
			Color	Consistency	Extractability [a] (%)
1	*Aegle marmelos*	Aqueous	Brown	Semi-solid	30
2		Methanol	Bottle Green	Semi-solid	8
3	*Bauhinia racemosa*	Aqueous	Slightly dark brown	Solid	2
4		Methanol	Dark Green	Solid	15
5	*Psidium gaujava*	Aqueous	Burgundy/Dark brown	Semi-Solid	5
6		Methanol	Tan	Solid	10

a: Mean of three observations

Qualitative phytochemical analysis

The qualitative test for detection of phytochemicals present in the aqueous extract and methanol extracts of *Aegle marmelos, Bauhinia racemosa* and *Psidium gaujava* are included in table 4. The qualitative test were carried out to find out the presence of active principles like alkaloids, glycosides, resins, terpenoids, reducing sugar, tannins, phenolics flavonoids and anthraqinones compounds. The aqueous leaf extract of *Aegle marmelos* was found to contain alkaloids, tannins, sterols, phenolic compounds, and flavanoids where as methanol leaf extract contains alkaloids, tannins, saponins, phenolic compounds and flavanoids. Sonu singh and Neeta singh (2016) [15] also observed the presence of alkaloids, tannins, steroids, flavonoids in the leaf extract of *aegle marmelos.* Presence of alkaloids, saponins, flavonoids and phenoilic compounds also reported by Hemalatha *et al.* (2013) [8] in aqueous leaf extracts of *A. marmelos.* Gupta *et al.* (2015) [7] carried out phytochemical analysis of *Aegle marmelos* and showed that the plant contains flavonoids, tannins, phenols, saponins, and terpenoids.

There is also observed that there is presence of alkaloids, glycosides, tannins, sterols, and phenolic compounds in aqueous leaf extracts of *Bauhinia racemosa* where as methanol leaf extracts tannins, saponins and phenolic compounds. Pawar and Nasreen (2016) [10] also observed that tannins, alkaloids, glycosides, sterols and saponins. The presence of alkaloids and tannins in leaf extracts of *Bauhinia racemosa* is reported by Ghumare *et al.* (2014) [6]. K.V. Bhaskar Rao (2010) [9] also reported presence of proteins, flavonoids, saponins, tannins and phenolic compound. The aqueous leaf extract of *Psidium guajava* was found to contain glycosides, tannins, phenolic compounds, saponins, resins, terpenoids and reducing sugars. However, the methanol leaf extracts of *Psidium gaujava* was found to bear glycosides, proteins, phenolic compounds, tannins, sterols and reducing sugars and flavonoids. Romasi *et al*. (2006) [13] also observed the presence of alkaloid, saponin, tannin, saponin, terpenoids and phenol in the aqueous leaf extract of *Psidium gaujava.* Vikrant Arya *et al.* (2012) [18] reported that *Psidium gaujava* contains of alkaloids, glycosides, tannins, saponins and sterols. Biswas *et al*. (2013) [2] carried out the phytochemical analysis of *Psidium gaujava* and showed that the plant contains glycosides, tannins, phenols, saponins, and terpenoids. Further on phytochemical analysis of both aqueous and methanol leaf extract of *Psidium gaujava* performed by Ekeleme *et.al* (2017) [18] also revealed the presence of tannin, alkaloids, saponins, glycosides and terpenoids.

Table 2: Qualitative phytochemical test of *Aegle marmelos, Bauhinia racemosa* and *Psidium gaujava* leaf extracts

S. No.	Phytochemical constituent	*Aegle marmelos*		*Bauhinia racemosa*		*Psidium gaujava*	
		Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
1	Alkaloid	+	+	+	-	+	+
2	Glycoside	-	-	+	-	+	+
3	Tannins	+	+	+	+	+	+
4	Saponin	-	+	-	+	+	-
5	Proteins	-	-	-	-	-	+
6	Sterols (Terpenoids)	+	-	+	-	+	+
7	Phenolic compound	+	+	+	+	+	+
8	Resins	-	-	-	-	+	-
9	Reducing sugar	-	-	+	-	+	+
10	Flavonoids	+	+	-	+	-	+

Note: "+" indicates presence "-" indicates absence

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