Studies on Phytochemical Analysis of *Aegle marmelos* (Indian Bael)

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Abstract: -

The present work deals with the phytochemical screening of A. marmelos (L.). Common name of A. marmelos is Belpatra. Every part of Aegle marmelos plant such as its fruits, stem, bark, and leaves possess medicinal property and is used for treating various eye and skin infections. This plant-derived secondary metabolite components like flavonoids, quinine, terpenoid, etc. and conduct certain biological functions that enhance therapeutic activities such as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties. The present study was carried on aqueous and methanolic extracts of Aegle marmelos to investigate the presence of medicinally important phytochemicals in the leaves of different varieties/accessions. The extract revealed the presence of various phytochemicals such as tannins, flavonoids, alkaloids, terpenoids, cardiac glycosides, and reducing sugars, proteins and amino acids in all the varieties of A. marmelos and phlobatannins were absent.

Key words: - *Aegle marmelos, Rutaceae, Extract, Soxhlet apparatus, Phytochemical, Methanol, Hexane,*

Introduction: -

India is widely known as the botanical garden of the world since it is the largest producer of medicinal herbs. Many Medicinal plants possesses therapeutic value and can also be used in drug development. 80% of the population of developing countries depend on traditional medicines, mostly natural plant products, for their primary health care needs as estimated by WHO. Because of the growing recognition of natural products, the demand for medicinal plants has been increasing all over the world. They have minimal toxicity, pharmacologically active, and provide an easy remedy for many human ailments as compared to the synthetic drugs which are a subject of adulteration and side effects. The ability to synthesize compounds from medicinal plants in terms of secondary metabolite owning antimicrobial potential makes plants an invaluable source of pharmaceutical and therapeutic products.

Out of many valuable medicinal plants *Aegle marmelos* (Bael) is well known in Indian traditional medicinal science due to multipurpose use for various purposes. *Aegle marmelos* belongs to genus *Aegle* and family Rutaceae. This plant is commonly known as bael in Hindi and Golden apple in English and found in sub-Himalayan regions, Northern India, Indo-China, Burma and Thailand. To bridge the gap between traditional knowledge and modern scientific knowledge, different parts of this plant were assessed for aphrodisiac, antidiarrheal, antidysentery, antioxidant, anti-inflammatory, antipyretic and many more diseases.

Plants consist of various kinds of chemical constituents known as phytoconstituents. Phytoconstituents serve the plants by contributing some secondary functions like; helps in plant growth, safe guarding the plants by activating defense mechanism, imparting colour, odor, and flavour to the plants. Also, the phytochemical investigations showed the presence of secondary metabolites in different extracts of *A. marmelos* leaves. The Bael fruit pulp as well as leaf extract contains many functional and bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, terpenoids, and other antioxidants which may protect us against chronic diseases. The biological evaluations such as antioxidant, anti-inflammatory, cardiotonic, hypoglycaemic, anti-dyslipidaemia, anti-cancer effects were also done with the leaves collected from different places.

Every part of *Aegle marmelos* plant such as its fruits, stem, bark, and leaves possess medicinal property and is used for treating various eye and skin infections. Leaf is considered to be one of the highest accumulatory parts of the plant containing bioactive

compounds which are synthesized as secondary metabolites. These plant-derived secondary metabolite components like flavonoids, quinine, terpenoid, etc conduct certain biological functions that enhance therapeutic activities such as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties. This plant is contemplated as rich sources of ingredients that can be used in the synthesis and production of drugs. The present study was, therefore, aimed at evaluating the and antibacterial activity of *Aegle marmelos* aqueous and methanolic leaf extracts.

Materials and Methods: -

1. **Collection and Identification of Plant Material:** The fresh leaves of Aegle marmelos from 18 varieties/accessions were collected from the Sugaon village in dist. Latur. Pathological disorders and contamination of plants were checked after washing with distilled water.
2. **Laboratory Equipment's:**

a. **Glass wares, apparatus instruments:** Micropipettes, Scalpels, PH meter, Autoclave, Incubators, Laminar air flow, Whatman Filter Paper, Microwave oven, Soxhlet Extractor.

b. **Chemicals required:** Methanol, Chloroform, Hydrochloric acid, ethanol, acetone.

3. **Extract Preparation:** The leaves of the plant were properly washed in tap water and rinsed in distilled water. The leaves were shade air-dried for 4-6 days. The dried leaves of plant were crushed using pestle mortar to obtain a powdered form which was stored in airtight glass containers at 4°C until used. Extraction for the phytochemical screening was performed by Soxhlet extractor to obtain the extract. 28gm of leaf powder and another 38gm of powder of powdered sample was soaked in methanol and hexane (260mL and 500 mL) separately as a solvent and extraction was continued for 36 hours and 48 hours respectively in Soxhlet extractor. The extracts were then filtered and concentrated to a final volume of 100ml and subjected to phytochemical analysis.
4. **Phytochemical Analysis:** Qualitative phytochemical analyses of extracts were performed to examine the presence of bioactive compounds by using following standard methods. Phytochemical screening of Aegle marmelos extracts by Junaid R Shaikh and M K Patil (2020).

➢ **Test for proteins:**

- **Ninhydrin test:** 2ml of Crude extracts boiled with 2 ml of 0.2 % solution of Ninhydrin, appearance of violet colour signifying the presence of amino acids and proteins.

➢ **Test for carbohydrates:**

- **Fehling's test:** Equal volume of Fehling A and Fehling B reagents were mixed together and added to extracts and gently boiled. A brick red precipitate appeared at the bottom of the test tube specified the presence of reducing sugars.
- **Benedict's test:** Extract mixed with 2 ml of Benedict's reagent and boiled, a reddish-brown precipitate formed which showed the presence of the carbohydrates.
- **Iodine test:** Extract were mixed with 2 ml of iodine solutions respectively. Dark blue or purple coloration designated the presence of the carbohydrates in leaf extract.

➢ **Test for phenols:** Extract were mixed with 2 ml of 2 % solution of FeCl3 respectively. A blue-green or black coloration indicated the presence of phenols and tannins in extract.

➢ **Test for tannins:** Extract were mixed with 1 drop of 2 % solution of FeCl3 and 0.3ml of distilled water respectively. A blue-green coloration indicated the presence of phenols and tannins in extract

➢ **Test for flavonoids:**

- **Shinoda test:** Extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.
- **Alkaline reagent test:** Extract were mixed with 2 ml of 2 % solution of NaOH respectively. Appearance of intense yellow colour which turned into colourless on addition of few drops of dil. acid which

indicated the presence of flavonoid compound.

➢ **Test for glycosides:**

- **Liebermann's test:** Extracts were mixed with 2 ml of chloroform and 2 ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.
- **Salkowski's test:** Extract were mixed with 2 ml of chloroform. Then 2 ml of concentrated H2SO4 was added carefully and shaken gently. A reddish-brown colour specified the presence of steroidal ring, i.e., glycone portion of the glycoside.
- **Keller-Kilani test:** Extract mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2 % solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycoside

➢ **Test for steroids:** Extract mixed with 2 ml of chloroform and conc. H_2SO_4 and was added sidewise in dropwise manner. A red colour ring produced in the lower chloroform layer indicated the presence of steroids.

Or

Another test also can be performed by mixing extract with 2 ml of chloroform. Then 2 ml of conc. H2SO4 and acetic acid were poured into the mixture. The development of a greenish colour indicated the presence of steroids.

➢ **Test for terpenoids:** Extracts were dissolved in 2 ml of chloroform and evaporated to dryness. In that mixture, 2 ml of concentrated H_2SO_4 was added and warmly heated for about 2 minutes. A greyish colour indicated the presence of terpenoids.

➢ **Test for alkaloids:** Extract was mixed with 2 ml of 1 % HCL and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

➢ **Test for phlobatannins:** About 2 ml of aqueous extracts were added to 2 ml of 1 % HCl and the mixture was boiled. Deposition of a red precipitate was taken as evidence for the presence of Phlobatannins.

Table 1: Phytochemical screening of *Aegle marmelos*

Sr. No.	Test	Procedure	Observation
1.	Ninhydrin test (detection of proteins and amino acids)	0.1 ml filtrate + 1 drop of ninhydrin solution (10mg Ninhydrin) + acetone.	Purple colour
2.	Fehling's test (detection of carbohydrates)	Fehling's And B reagents +2 ml extract +A&B solutions pick up+ Boiling	Red colour
3.	Benedict's Test (detection of reducing sugars)	0.3 ml filtrate + 0.3 ml Benedict's reagent + boil for 2 min.	Green/Yellow/Red colour
4.	Iodine test (detection of carbohydrates)	2 ml extract+ 2 ml iodine solutions+ presence of carbohydrates	Dark blue or purple colour
5.	Test for phenols (detection of ferric chloride test)	0.2 ml extract+2drop of ferric chloride 2%+indicated phenol	Dark green or blue + black colour
6.	Test for Tannins	0.1 ml filtrate+0.3 ml D/W+1drop of ferric chloride	Blue/green colour
7.	Shinoda's test (Detection of flavonoids)	0.1 ml alcoholic filtrate + Mg ribbon + 2 drops of conc. HCl.	A pink to cream colour
8.	Alkaline reagent test (detection of flavonoid test)	2 ml extract +2%NaOH +few drops diluted acid	Yellow colour

9.	Liebermann's test (Detection of glycosides)	2ml chloroform+2ml of acetic acid+concH2SO4	Violet/ green/blue colour
10.	Salkowski's test	0.1ml filtrate +,2 drops of conc. H2SO4	Red colour
11.	Keller-Killani test	0.2 ml filtrate + 0.3 ml glacial acetic acid + 1 drop of 5 % ferric chloride + conc. sulphuric acid (along the side of test tube)	Brown colour
12.	Test for Steroids	2 ml chloroform + conc.H2SO4 added sidewise.	Red colour
13.	Test for terpenoids	2 ml of chloroform & evaporated to dryness + 2 ml of conc.H2SO4 heated 2 min.	Greyish colour
14.	Test for alkaloid	0.1 ml filtrate + 2ml of1% HCL	Red colour
15.	Test for phlobatannins	2ml aqueous extract+ 2ml of 1%Hcl	Red precipitate colour

Result and Discussion: -

3.1. Phytochemical Profiling: The present study was carried on aqueous and methanolic extracts of *Aegle marmelos* to investigate the presence of medicinally important phytochemicals in the leaves of different varieties/accessions. The extract revealed the presence of various phytochemicals such as tannins, flavonoids, alkaloids, terpenoids, cardiac glycosides, and reducing sugars, proteins and amino acids in all the varieties and phlobatannins were absent (Table 2). The presence of different phytochemicals in methanolic extract and hexane extract of a single unidentified variety of *Aegle marmelos* have been reported; however, our study report to the best of our knowledge on qualitative and quantitative comparative analysis of various varieties/accessions available in India.

Sr. No.	Solvent used for extraction	Test Name	Observation	Inference
1.	**METHANOL**	Ninhydrin test	Purple colour	Present
2.		Fehling's test	Red colour	Absent
3.		Benedict's Test	Green / yellow/red colour	Present
4.		Iodine test	Dark blue or purple colour	Present
5.		Test for phenols	Dark green or blue + black colour	Present
6.		Test for Tannins	Blue/green colour	Present
7.		Shinoda's test	Pink to cream colour	Present
8.		Alkaline reagent test	Yellow colour	Present
9.		Liebermann's test	Violet/green/blue colour	Absent
10.		Salkowski's test	Red colour	Present
11.		Keller-Killani test	Brown colour	Present
12.		Test for Steroids	Red colour	Present
13.		Test for terpenoids	Greyish colour	Present
14.		Test for alkaloid	Red colour	Present
15.		Test for phlobatannins	No Red Precipitate colour	Absent

Sr. No.	Solvent used for extraction	Test Name	Observation	Inference
		Ninhydrin test	Purple colour	Present
		Fehling's test	Red colour	Absent
1.		Benedict's Test	Green / yellow/red colour	Present

2.	HEXANE	Iodine test	Dark blue or purple colour	Absent
3.		Test for phenols	Dark green or blue + black colour	Present
4.		Test for Tannins	Blue/green colour	Present
5.		Shinoda's test	Pink to cream colour	Present
6.		Alkaline reagent test	Yellow colour	Absent
7.		Liebermann's test	Violet/green/blue colour	Present
8.		Salkowski's test	Red colour	Present
9.		Keller-Killani test	Brown colour	Absent
10.		Test for Steroids	Red colour	Present
11.		Test for terpenoids	Greyish colour	Present
12.		Test for alkaloid	Red colour	Present
13.		Test for phlobatannins	No Red Precipitate colour	Absent

Conclusion: -

This study contributes to the present knowledge of the presence of different active phytochemical compounds of *Aegle marmelos* plants possessing different groups such as alkaloids, carbohydrates, flavonoids, phenols, saponins, steroids, and tannins. Their isolation is solvent dependent. *Aegle marmelos* contains different classes of secondary metabolites and these metabolites are further used as herbal and ayurvedic medicines. For the chemical characterization the selection of solvent should be done carefully because there are some solvents in which the phytochemical test shows positive result and same extract shows negative result in other solvent.

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