Original Article

Determination Of Phytoconstituents And Toxicity Profile In Methanolic Extract Of Aegle Marmelos

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Abstract

Aegle Marmelos is a traditional medicinal plant in india which belongs to Rutaceae family which possesses innumerable health benefits. The entire plant body including its leaves, stem, root, inflorescence and seed are proved to be significant medicinal value and hence it is one among the inevitable plant used in the preparation of various ayurvedic pharmacological products. The plant is a rich source of various components including eugenol, Vicenin- 2, linoleic acid, oleic acid, rosmarinic Calcium, Phosphorous and many more. its Ethanopharmacological properties such as Anti-diabetic, Anti- cancerous,Analgesic, Anti- inflammatory, Radiopreotective. In vivo toxicity study concluded that no mortality was find in toxicity study.

INTRODUCTION:

Traditional medicine, existed from ancient times, and is estimated by World HealthOrganization to be in use by up to 80% of the population in most of the developing countries mostly based on plants and plant materials. It is currently estimated that out of atotal of an approximately 420,000 plant species that exist in nature, over 248,000 species of higher plants have been identified, and of these 12,000 plants are known to have medicinal properties. However, less than 10% of all the plants have been investigated from a Phytochemical or pharmacological point of view. [1]

Traditional medicine is a main part of the cultural heritage of a society and it has develop in accordance with the life style and ancient practices of the society. During practical experiences of herbal remedies the therapeutic results of the various traditional medicinal systems around the world. Indian, Chinese, and Arabian traditional medicinal systems are highly developed. Traditional Indian medicinal systems have reached to various other countries such as Malaysia and America. According to a WHO report, around80% of the world's population primarily relies on traditional medicines. Aegle marmelos an important medicinal plant which is mostly find in western countries.[2]

Plant profile

Figure 1. Aegle marmelos

Taxonomy: [3--4]

Kingdom: Plantae

Phylum : Magnoliophyta

Class : Magnoliopsida

Subclass : Rosidae

Order : Sapindales

Family : Rutaceae

Genus: Aegle

Family : Rutaceae

Synonym / Vernacular names:
Plants species has been available all of the world, different different region identified through regional language i.e. Burmese
Burmese : Opesheet
English : Bael Fruit, Indian Bael, Holy Fruit, Golden Apple, Elephant Apple, Bengal Quince German : Belbaum, Schleimapfelbaum, Baelbaum)
Gujarati : Bili
Hindi : baelputri bela, sirphal
Indonesian : Maja batuh, maja)
Javanese : Khmer (bnau)
Malay : Bilak
Portuguese :Marmelos
Thai : Matum

Distribution:

Aegle marmelos is a medium slow-growing plant, sized tree, up to 10-15 m tall with short trunk. Its woof is thick, soft, shedding and spreading. Branches are prickly in some variety, the lower ones are baggy.[5]

Phytoconstituents

Leaves-

Mixture of phytosterolins and sitosterol, agroclanine, chanoclanine-I,II, lysergol, ergine,isoergine, erogometrine, lysergic acid, penniclanine

Seeds-

Ergoline alkaloids- agroclanine, chanoclanine-I,II, lysergol, ergine,isoergine, erogometrine, lysergic acid, penniclanine, racemic chacoclanine-II, elymoclarine, festuclarine, lysergene, isolysergol, molliclanine, setoclanine, isosetoclanine,ergometrinine, caffeic acid and isolysergic acid and few unknown alkaloids**.** [6]

Uses:

Leaves – Used in Diabetes and CNS depressant activities.

Root - Arthritis

PHARMACOLOGICAL ACTIVITY

- **Antioxidant Activity**
- **Anti Inflammatory**
- **Peptic Ulcer**
- **Wound Healing**

MATERIAL AND METHOD

Collection of plant

Leaves of Aegle marmelos of were collected from Herbal Garden of Rajasthan college of Agriculture Rajasthan, India, 313001.

Extraction of plant

The collected plant leaves was dried in the shade for about 10 days, powdered withmechanical grinder and stored in the airtight container for further study. The powdered plant material was extracted in Soxhlet apparatus assembly successively with solvents of increasing polarity, namely, Aqueous, petroleum ether, chloroform, ethanol, and methanol.For that, about 25 g of dried coarse powder was weighed, moistened with the respective solvents, packed in the Soxhlet extractor and extracted individually with 500 ml of each

solvent. After each extraction, the same dried marc was used for the subsequent extraction.Each extract was filtered, distilled off the solvent to obtain the dried extract. [7]

Morphological Studies:

After making powder drug evaluated morphological parameters like i.e. Its colour,odour, taste, size, shape and special features, like texture, touch, etc. Evaluation was carried based on the morphological and sensory profiles of whole drug. [8]

Physicochemical Constants: [9]

A. Ash values

Ash content of the crude drug is generally taken to be the residue remaining after incineration. It represents the inorganic salts naturally occurring in the drug and adhering to it, but may also include inorganic matter added for the purpose of adulteration.

Total ash is the residue remaining after incineration. Acid insoluble ash is the partof the total ash, which is insoluble in dilute hydrochloric acid. Water-soluble ash is the partof total ash, which is soluble in hot water.

Phytochemical Screening

Phytochemical broadcast of the extracts was conceded out according to the standard procedures [10-11] The Aqueous, petroleum ether, Ethanol, Chloroform and methanolic extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in them i.e. alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannins. For Detection of Phytochemical various solvent extract was used i.e. Aqueoue, Methanol, Petroleum ether,Ethyl Acetate etc.

A. Chemical test for alkaloids

Little quantity of dried extract with alcohol was shaken with dilute hydrochloric acid and filtered. The acidified filtrate was used to detect the presence of alkaloids by the following tests.

Mayer’s test

The acidified filtrate (2 ml) was treated with Mayer’s reagent (1 ml), shaken welland observed for the presence of creamy precipitate.

Wagner’s test

The acidified filtrate (2 ml) was treated with Wagner’s reagent (1 ml) and observedfor the presence of reddish-brown precipitate.

Hager’s test

The acidified filtrate (2 ml) was treated with Hager’s reagent (1 ml) and observed for the presence of yellow precipitate.

Dragendorff’s test

The acidified filtrate (2 ml) was treated with Dragendorff’s reagent (2 ml) andobserved for the presence of orange-red precipitate.

B. Chemical tests for glycosides

Little quantity of dried extract was hydrolyzed with dilute hydrochloric acid on a water bath for a few hours, and the hydrolysate obtained was used to detect the presence of glycosides by following tests.

Legal test

The hydrolysate (2 ml) was dissolved in pyridine (2 ml). Freshly prepared sodiumnitroprusside solution (2 ml) was added to it. Made the mixture alkaline with sodium hydroxide solution and observed for the formation of a pink color.

Baljet test

The hydrolysate (2 ml) was treated with sodium picrate solution (1 ml) and observed for the formation of a yellow to orange color.

Borntrager’s test

A little quantity of the residue obtained from the evaporation of hydrolysate was mixed with water and shaken with an equal volume of chloroform. The chloroform layer was separated and equal quantity of dilute ammonia solution was added to it and shaken well and observed for the formation of pink color in the ammoniacal layer.

Modified Borntrager’s test

A little quantity of the residue obtained from the evaporation of hydrolysate was treated with ferric chloride and dilute hydrochloric acid. Then, it was extracted with chloroform. The chloroform layer was separated, and an equal quantity of dilute ammoniasolution was added to it and shaken well and observed for the formation of pink color.

C. Chemical tests for phenolic compounds and tanninsFerric chloride test

A small quantity of the dried extract was mixed with water and treated with diluteferric chloride solution (5%) and observed for the presence of a blue color.

Gelatin test

The dried extract dissolved in the water was filtered. To the filtrate, a 2% solutionof gelatin containing 10% sodium chloride was added and observed for the presence of milky white precipitate.

Lead acetate test

The dried extract dissolved in the water was treated with a 10% lead acetate solutionand observed for the presence of bulky white precipitate.

Decolorization test

The dried extract dissolved in water was treated with dilute potassium permanganate solution and observed for the decolorization of potassium permanganate.

Chemical tests for flavanones and flavonoids

Aqueous sodium hydroxide solution was added to the little quantity of dried extractand observed for the yellow coloration of the solution.

Ammonia test

The filter paper wetted with a small quantity of an alcoholic solution of the driedextract was exposed to ammonia vapor and observed for the formation of yellow color.

Shinoda test

The dried extract mixed with alcohol was treated with magnesium or zinc and dilutehydrochloric acid and observed for the formation of orange-red or violet color.

D. Chemical tests for carbohydrates

A small quantity of ethanolic extract was mixed with water or alcohol and filtered.

The filtrate was subjected to the following tests to detect the presence of carbohydrates.

Molisch's test

The filtrate (2 ml) was treated with a few drops of Molisch's reagent and concentrated sulfuric acid (2 ml) was added through the side of the test tube without shaking and observed for the presence of violet ring at the junction of two solutions.

Fehling's test

The filtrate (1 ml) was treated with 1 ml each of Fehling's solution A and B andboiled in a water bath and observed for the formation of a reddish precipitate.

Benedict's test

The filtrate (2 ml) was treated with Benedict's reagent (2 ml). Then, the mixturewas heated in a boiling water bath and observed for the presence of reddish precipitate.

E. Chemical tests for proteins and amino acidsMillon's test

Little quantity of dried extract was treated with of Millon's reagent (2 ml) and observed for the formation of white precipitate, which on warming turn into a red coloredsolution.

Biuret test

Little quantity of dried extract was treated with a few drops of 2% copper sulfate solution. To this excess of potassium hydroxide solution was added and observed for the formation of violet colored solution.

Ninhydrin test

Little quantity of dried extract was treated with few drops of ninhydrin solution andheated on a water bath and observed for the presence of a violet color.

F. Chemical test for

terpenoidsSalkowski test

Little quantity of dried extract was dissolved in chloroform. An equal volume of concentrated sulfuric acid was added to it and observed for the appearance of red color inthe chloroform layer and greenish-yellow fluorescence in the acid layer.

G. Chemical tests for sterols

A little quantity of the alcoholic extract was refluxed with alcoholic potassium hydroxide solution until the saponification was observed. The mixture was diluted and extracted with solvent ether. The ethereal extract was evaporated, and the residue obtainedwas used in the tests for sterols.

Liebermann-Burchard test

The residue was taken with dry chloroform (1 ml), and then it was mixed with 2 mlof specially distilled acetic anhydride followed by a few drops of concentrated sulfuric acidthrough the sides of the test tube and observed for the formation of green color in the upperportion which changes to bluish violet.

Salkowski test

The residue was dissolved in chloroform, and an equal volume of concentrated sulfuric acid was added to it and observed for the red color in the lower layer.

Chemical tests for saponins

A small quantity of dried extract was diluted with distilled water (20 ml) in a graduated cylinder. The suspension was shaken for 15 min and observed for the formationof froth.

Hemolysis test

A drop of blood was placed in a slide and mixed with a small quantity of dried extract and observed for hemolysis.

H. Chemical tests for gum and mucilage

Absolute alcohol (25 ml) was added with an aqueous extract (10 ml) with constantstirring. Filtered and the precipitate formed was dried in air and examined for swelling properties.

Acute toxicity study

In a research study when a drug is administered to a biological system there will be some interactions may happen. In most case these are desired and useful, but many effects are not advantageous. Acute, subacute and chronic toxicity studies are performed by the manufacturers in the investigation of a new drug. Acute toxicity is involved in estimation of LD_{50} (It is the lethal dose (cuasing death) to 50% of tested group animals) [184]. Swiss Albino were fasted overnight, prior to dosing. The three dose levels are administered by the help of oral feeding needle over the prior of 24 hours. After the drug has been administered, food may be with held for a further 3-4 hours in rats. The purpose of sighting study is to allow selection of the appropriate starting dose for main study. The test substance is administered to a single animal in a sequential manner following from the fixed dose levels of 5, 50, 300 and 2000 mg/kg. The interval between dosing of each level is determined by the mortality/onset, duration and severity of toxic signs over the period of 24 hours, special attention given during the first 4 hours. Four hours after the drug administration, provide the food and water for 14 days and daily observed. [12]

RESULT

MORPHOLOGICAL STUDIES:

Powder leaf shows characteristics odour, with very smooth touch for leaf powder.

Table No. 1

Colour	Dark GREEN
Odour	Odorless
Taste	Pungent
Size	3.5-8cm long: 0.5-3 cm with
Shape	Ovate
Texture	Hairs on surface
Touch	Soft

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The Phytochemical analysis Aegle marmelos were revealed the presence of various chemical constituents such as alkaloids, saponins, glycosides, tannins, flavonoids,carbohydrate etc.

Table No. 2: Phytochemical Analysis

Phyto constituents	Chemical tests	Aqueous	Pet. ether	Ethanol	Chloroform	Methanol
Alkaloids	Dragendroffs	+	-	_	+	+
	Hager's test	+	-	_	+	+
	Mayer' test	+	-	_	+	+
	Wagner's test	+	+	+	+	+
Carbohydrate	Molish test	-	_	-	-	+
	Fehling's test	+	+	+	-	+
	Benedict's test	+	-	-	-	+
	Barfoed's test	-	-	-	-	-
Proteins	Biuret's test	+	+	+	-	+
	Milion's test	-	-	-	-	+
	Precipitation test	+	-	+	-	+
Amino tests	Ninhydrin test	+	-	-	-	+
	Xanthorproteic test	-	-	-	-	+
	Salkowski's test	+	+	+	+	+

	Shinoda test	+	-	+	-	+
	Brontrager's test	+	-	-	-	+
	Legal's					
	Zinc HCl test	+	-	-	-	+
	With 5% ferric chloride	+	-	-	-	+
	With KMnO4	+	+	+	+	+
	With lead acetate	+	-	-	-	+

The qualitative phytochemical analysis was performed using standard procedures.The phytochemical anlaysis of petroleum extracts showed the presence of saponins only while as the methanolic extracts showed the presence of alkaloids, terpenoids, flavonoids,carbohydrates, glycosides, tannins, saponnins and phenolics compounds.

Acute Toxicity & LD_{50} Value

The experimental mice was treated with different oral doses of extract shows appreciable changes in physical activity and signs of toxicity in table (3), while there are no signs of mortality up to (72) hr. post-treatment, indicating that the LD_{50} of the crude extracts in rats maximum doses not observed any mortality.

Table 3: LD_{50} dose determination and mortality rate of extract

Dose	**Observation** (Mortality or Moribund Status)
5 mg/kg b.w.	0/3
5 mg/kg b.w.	0/3
50 mg/kg b.w.	0/3
50 mg/kg b.w.	0/3
300 mg/kg b.w.	0/3
300 mg/kg b.w.	0/3
2000 mg/kg b.w.	0/3
2000 mg/kg b.w.	0/3

In this study, the lethal dose LD_{50} of extract administered to mice orally was up to 2000 mg/kg (body wt.), while there was no evident of death occurred among animals when given different doses of extract. The mice that received 05-2000 mg/kg dose of the extract survive and doesn't show any signs, the extract could be classified as practically non-toxic, since LD_{50} was found to lie between .05 gram and 02 gram/kg.

Consideration of wellbeing of every element intentional to be used within in body is very imperative. In present research test sample was given through oral route, hence acute oral toxicity assay of methanolic extract leaves was performed as per OECD 423 guidelines. The acute toxic group method set out in this Guideline is a stepwise process with the use of 3 animals of a single sex per step. Depending on the transience and/or the dilapidated status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods. For dermal toxicity study's methodology was followed that were according to OECD guideline It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no auxiliary testing is needed, dosing of three additional animals, with the same dose, dosing of three additional animals at the next higher or the next lower dose level. In present investigation it was observed that extract was not toxic up to the dose of 2000 mg/kg (Table 3). Hence, 2000 mg/kg was considered as Not Observed Adverse Effect Limit (NOAEL) for present set of experiment. Dose was selected accordingly. $1/10^{th}$ of NOAEL i.e. 200, 400 mg/kg were selected as dose further in vivo investigation.

Discussion

Phytochemical test analysis of this extracts has been carried out mainly contained flavonoids, alkaloids, phenols, tannins and saponins. Flavonoids and phenolic compoundshave multiple biological effects such as antioxidant activity. Flavonoids have also been reported to have anti-diabetics effect.

Plants have been used to treat many ailments since time immemorial. India has several plant species and thousands of them have claimed to possess medicinal properties.A number of plants have shown varying degree of hypoglycemic and anti hyperglycemic activity. The active principles present in medicinal plants have been reported to be capableof pancreatic β-cell regeneration, insulin secretion and reversal of insulin resistance.

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