

Studies on Qualitative and Quantitative Estimation of Primary and Secondary Metabolites in Various Solvents Extracts of *Aegle marmelos*

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Abstract: The present study was an attempt to analyze the qualitative and quantitative analysis of phytochemical in different solvents extracts of *A. marmelos*. Primary metabolites like total soluble carbohydrates, proteins and secondary metabolites such as flavonoids, total phenols, and tannins were estimated using standard protocols. The qualitative and quantitative analysis is very essential for identifying and quantifying the compounds present in the medicinal plants. The results obtained from the present study provides evidence that various solvent extracts of *A. marmelos* leaves contains different types of primary and secondary metabolites which are having highly medicinal values and this justifies that the use of plant species as traditional medicine for treatment of various diseases. The finding of this study suggests that this plant leaves could be a potential source of natural medicine that could have great importance as therapeutic agents in preventing various diseases. The results are very much encouraging but scientific validation is necessary before being put in to practice. The present study suggest for future researcher on this plant that further Isolation, purification, characterization, structural elucidation and evaluation of a specific biological activity and preclinical and clinical studies of Bioactive compounds of this plant extracts were also necessary to emerge as medicine in the market.

Keywords: *Aegle marmelos*, Leaves, Secondary Metabolites, Phytochemical Screening, Medicinal Plant

1. Introduction

Traditional medication is that the accumulation of data, skills and practices supported the theories, believes and experiences associated with completely different cultures that square measure meant to keep up health additionally on stop, diagnose, improve or treat physical and mental sicknesses. Healthful plants have bioactive compounds that square measure used for action of assorted human diseases and additionally play a very important role in healing. Phytochemicals have 2 classes i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins, sugar and amino acids. Secondary constituents contain several compounds like terpenoids and alkaloids.

Phytochemicals square measure present within the healthful plants leaves, vegetables, fruits, bark and roots that have process and defend from numerous diseases [1]. in step with the global Health Organization ancient seasoning medicines square measure outlined as present, plant-derived substances with lowest or no industrial process that are used anciently to treat unhealthiness, at intervals native or regional healing practices. Seasoning medication is additionally known as phytotherapy or phytomedicine. The extent to that it will be used is terribly broad, and also the healthful properties will be achieved by employing a plant's seeds, berries, roots, leaves, bark, or flowers. Herbalism encompasses a long tradition of use outside of standard medication. It's changing into a lot of main stream as enhancements in analysis and

internal control in conjunction with advances in clinical analysis show the price of seasoning medication in the treating and preventing unwellness [2]. The India is endowed with a rich wealth of medicinal plants diversity and Natural products of medicinal plants offer vast resource of novel medicinal agents with potential in clinical ahead. Extraction, purification methods and solvent selections are important for the draw of the phytochemicals because Phytoconstituents are not dissolving in all solvents and those compounds quality and quantity rates are also different in different solvents. For that which solvent obtain maximum phytochemicals and there quality and quantity is also most important. The use of plants for medicinal purposes dates back to antiquity [3] and has been very important in the health care delivery of every nation at one or other stage. The natural resources how so ever large are bound to diminish hence effective strategy is needed for sustainable utilization. Cultivation of medicinal and aromatic plants is constrained due to lack of suitable technology, which led to low yield and poor quality. Consequently, medicinal herbs are predominantly harvested in sufficient quantities from the wild in an unregulated manner [4]. The present study was an attempt to analyze the qualitative and quantitative estimation of phytochemical in different solvents extracts of *A. marmelos*.

2. Materials and Methods

2.1. Plant Material

The leaves of *A. marmelos* were collected around Gulbarga University campus in June 2014 and plant was identified by a taxonomist and a voucher sample was deposited in the Herbarium of Medicinal Plants of the Department of Botany Gulbarga University Kalburgi, Karnataka. The leaves of the specimen were washed with tap water followed by 70% alcohol, & shade dried.



Taxonomy	
Kingdom	: Plantae
Order	: Sapindales
Family	: Rutaceae
Sub family	: Aurantioideae
Genus	: <i>Aegle</i>
Species	: <i>A. marmelos</i>

Figure 1. Showing Aerial part with taxonomical classification of *A. marmelos*.

2.2. Chemicals

Methanol, ethanol, ethyl acetate, Petroleum ether, Diethyl ether, Sulphuric acid, Chloroform, Hydrochloric acid, Potassium hydroxide, Hexane, Silica Gel 60-120 mesh, Tween 80 Phosphate buffer saline, FCR Reagent, all the chemical, solvents and reagents used were analytical grade obtained from Hi-media.

2.3. Preparation of Extracts

The extraction procedure used for the isolation of crude

drug from plants has been practiced since from long time. The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on type of substance that is being isolated. Plant material is shade dried before extraction. It is essential that drying operation is carried out under controlled condition to avoid chemical changes. It is essential for plant taken to be free from diseases i.e. not affected by any bacterial or fungal infections. Normally the crude extract is taken by cold extraction with the help of non-polar to polar solvents.

About 100 g of the shade dried powder leaves were taken separately and dissolved in 500ml of distilled water and magnetically stirred in a separate container for overnight at room temperature in the case of aqueous solution. Similarly 100gm of powdered leaves were dissolved in 500ml of different solvents such as Chloroform, Petroleum ether and ethanol. These extract were then collected by evaporation in the plates and stored in refrigerator for further use.

2.4. Qualitative Estimation of Primary and Secondary Metabolites

The extracts so obtained after each successive cold extraction were qualitatively estimated for the presence of various primary and secondary phytochemicals.

2.4.1. Qualitative Tests for Carbohydrates

Substance with general formula of $C_x(H_2O)_y$ is called as carbohydrate because they contain hydrogen and oxygen in the same proportion as in water. Molish's test is most comely used for carbohydrates in this test to the Extract; Molishch's reagent is added and shakes carefully. Add 2 mL of conc. H_2SO_4 along the walls of the test tube and allow it to stand for 2 min and observe the formation of reddish violet color at the junction of two liquids.

2.4.2. Qualitative Test for PROTEINS and Amino Acids

Proteins are complex nitrogenous compounds which occur in plants and animals. Proteins on hydrolysis with strong acids or by enzymes yield a mixture of amino acids. Ninhydrin test is most commonly used test for proteins in this test to the Extract; Ninhydrin reagent is added and observes the formation of blue color.

2.4.3. Qualitative Test for Tannins

The tannin compounds are widely distributed in many species of medicinal plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. Ferric Chloride test is most commonly used for tannins in this test, to the Extract added 1% Ferric chloride solution and observed the formation of the Blue green or brownish green color.

2.4.4. Qualitative Test for Flavonoids

Two different methods were used to determine the presence of flavonoids. The Shinoda test is most commonly used for flavonoids and in this test, to the extract; 5 mL of dilute ammonia solution was added to a portion of total flavonoid extracts followed by addition of concentrated

H₂SO₄. The yellow color is formed after adding H₂SO₄ and is disappeared on standing. Few drops of aluminum solution added and again gained the yellow color [5, 6].

2.4.5. Qualitative Test for Phenols

Phenols are the aromatic compounds in which OH (hydroxyl) group is directly attached to benzene ring depending on the attachment of OH Group to benzene ring the phenols are classified. E.g.: Cresols, Catechol, etc., Ellagic acid test is most comely used for phenolic test in this test to the extract 3 drops of 5% NaNH₂ solution is added and observe the formation of Muddy/Niger brown precipitate.

2.5. Quantitative Estimation of Primary and Secondary Metabolites

2.5.1. Estimation of Carbohydrates by Anthrone Method

Take 0.5 ml of plant extracts in three different test tubes and add distilled water to make the volume up to 1 mL then add 1 mL of anthrone reagent to all the test tubes. Keep for 10 min in boiling water bath. Observe the color formation, cool it and absorbance was measured at 640 nm in UV spectrophotometer. The amount of carbohydrates was calculated using standard graph of glucose [7].

2.5.2. Estimation of Reducing Sugar by Dintro Salicylic Acid Method

One ml of extract (petroleum ether, chloroform and ethanol) was pipetted out in to the test tube and 3 mL of Dintro Salicylic acid reagent is added to it. The mixture was heated for 5 min, in boiling water bath. 1 mL of 40% Rochelle salt was added to it, when it is still warm the content were cooled under running tap water and absorbance was measured at 540 nm in UV spectrophotometer. The amount of reducing sugars was calculated using standard graph of maltose.

2.5.3. Estimation of Amino acid by Ninhydrine Method

Take 0.5 mL of plant extracts in three different test tubes add 1ml of ninhydrine reagent and final volume was made up to 2 mL with distilled water. The test tubes with contents were heated in boiling water bath for 20 min., incubate at room temperature for 15 min and read the intensity of purple against reagent blank in a UV spectrophotometer at 570 nm. The amount of amino acid in the sample was determined with the help of standard curve [8].

2.5.4. Estimation of Protein by Lowry's Method

Take 0.5 mL of plant extracts in three different test tubes. Add 0.9 mL of distilled water to make the final volume up to 1 mL then 5 mL of Alkaline copper reagent was added and incubated for 10 min at room temperature. After incubation 0.5 mL of FCR reagent was added and mixed thoroughly and kept in dark for 30min. the blue color was developed, absorbance was measured at 660 nm in UV spectrophotometer. The standard graph was used to calculate the amount of protein present in the sample [9].

2.5.5. Estimation of Tannins by Folin Denis Method (FDR)

Take one ml of plant extracts in three different test tubes add 5 mL of FDR reagent and 10 mL of sodium carbonate solution to it and dilute with distilled water and shake well. The blue color intensity was measured at 700 nm after 30 min. A standard graph was drawn by plotting concentration verses absorbance of the standard tannic acid and the amount of tannins present in the sample was calculated.

2.5.6. Estimation of Phenols by Lowry's Method (1951)

Take 1 mL of plant extracts, add 0.5 mL of distilled water and add 0.5 mL of FCR reagent. After 3 min add 2 mL of 20% sodium carbonate solution to each tube and make up the volume to 3 mL with distilled water. Mix thoroughly places the tubes in boiling water bath for 15 min and cool. Measure the absorbance at 650 nm against reagent blank in UV spectrophotometer. The amount of phenols was calculated with the help of catechol standard curve.

2.5.7. Estimation of Flavonoid

Determination of Total Flavonoid Content by UV-Spectrophotometric Method, Firstly, 2 mL of the sample solution was accurately removed in a volumetric flask (10 mL) by adding 0.6 mL of NaNO₂ (5%) solution, shaking up, and then standing for 6 min. Secondly, 0.5 mL of the Al (NO₃)₃ (10%) solution was added to the volumetric flask, shaken, and left to stand for 6 min. Finally, 3.0 mL of the NaOH (4.3%) solution was added to the volumetric flask, followed by addition of water to the scale, shaken, and left to stand for 15 min before determination. Using the sample solution without coloration as reference solution and 500 nm as determination wavelength, the coloration method was used to determine the content of flavonoids in the sample by ultraviolet-visible detector [10].

3. Results

3.1. Qualitative Estimation of Primary and Secondary Metabolites

3.1.1. Qualitative Test for Flavonoids

Petroleum ether extracts, chloroform extract and ethanol extracts of *A. marmelos* have responded positively to Shinoda tests, indicating the presence of flavonoids. The yellow coloration is observed after adding H₂SO₄ and it disappeared on standing. Few drops of 1% aluminum solution were added to a portion of flavonoid extract and again yellow coloration was Observed, this indicates the presence of flavonoids.

3.1.2. Qualitative Test for Carbohydrates

The petroleum ether, chloroform and ethanol extracts of the plant *A. marmelos* were showed positive to Molisch test confirming the presence of carbohydrates by the Formation of reddish violet color at the junction of two liquids.

3.1.3. Qualitative Test for Proteins

All the 3 extracts of plant have shown positive result for Ninhydrin test by the formation of blue color and indicate the

presence of proteins

3.1.4. Qualitative Test for Phenols

The formation of Muddy/Niger brown precipitate indicates the presence of phenols and phenolic compounds in the tested 3 various extracts of *A. marmelos*.

3.1.5. Qualitative Test for Tannins

The formation of the blue green or brownish green color indicating the presence of tannins in the various extracts of the *A. marmelos*.

Table 1. Qualitative Estimation of Primary and Secondary metabolites of *A. marmelos*.

Plant extract	Flavonoids	Carbohydrates	Proteins	Phenols	Tannins
PE	+	+	+	+	+
CE	+	+	+	+	+
EE	+	+	+	+	+

PE: Petroleum ether extract; CE: Chloroform extract; EE: Ethanol extract; +: Presence

3.2. Quantitative Estimation of Primary and Secondary Metabolites

3.2.1. Estimation of Total Carbohydrates by Anthrone Method

The Leaf part of *A. marmelos* extract of petroleum ether, chloroform and ethanol consist of 110 µg, 150 µg and 160 µg of carbohydrates respectively. Among the Carbohydrates, Monosaccharide's and Oligosaccharides are found widely in the plant glycosides bounded to Flavonoids and Isoprenoids.

3.2.2. Estimation of Reducing Sugars by Dinitro Salicylic Acid Method

A. marmelos plant leaf part extract of petroleum ether, chloroform and ethanol consist of 400 µg, 510 µg and 390 µg of reducing sugars respectively. In these, the oxygen of

carbonyl group is not attached to any other structure. These reducing sugars readily react with metals reducing into insoluble form.

3.2.3. Estimation of Amino Acid by Ninhydrine Method

The leaf part of *A. marmelos* plant extract of petroleum ether, chloroform and ethanol consist of 10 µg, 4 µg and 18 µg of amino acids respectively. Among various amino acids present in the plants, Tryptophan is the most important as a precursor for the synthesis of different class of secondary metabolites.

3.2.4. Estimation of Protein by Lowry's Method

The leaf part of *A. marmelos* extract of petroleum ether, chloroform and ethanol consist of 40 µg, 20 µg and 300 µg respectively. The basic regulatory principle in the expression of secondary product formation is the synthesis of Secondary metabolic enzymes. In higher plants the enzymatic activity of secondary metabolism appears before or during the phase of secondary product synthesis and the expression depends on the denovo pathway.

3.2.5. Estimation of Total Phenols by FCR Method

The leaf part of *A. marmelos* extract of petroleum ether, chloroform and ethanol consist of 580 µg, 490 µg and 540 µg of total phenols respectively. The presence of phenols in all plants tissue is the characteristics feature. It is made up of aromatic ring with hydroxyle substituent derived from phenylealanine of Shikamic acid pathway. Many Phenols with a dihydroxy grouping possess the chelate metals that may be present biologically in plant system.

3.2.6. Estimation of Total Tannins by FDR Method

The leaf part of *A. marmelos* extract of petroleum ether, chloroform and ethanol consist of 84 µg, 40 µg and 68 µg of total tannins respectively. The tannins generally been shown to have an antagonistic effect on garlic acid.

Table 2. Quantitative Estimation of Primary and Secondary metabolites of *A. marmelos*.

Plant extract	Total Carbohydrates (µg/mL)	Reducing sugars (µg/mL)	Amino acids (µg/mL)	Proteins (µg/mL)	Phenols (µg/mL)	Tannins (µg/mL)
PE	110	400	10	40	580	84
CE	150	510	4	20	490	40
EE	160	390	18	300	540	68

PE: Petroleum ether extract; CE: Chloroform extract; EE: Ethanol extract

4. Discussion

Medicinal plants have played an important role in maintaining human health and improving the quality of human life since many years. The use of natural products for therapeutic purposes is in practice from ancient times. The world health organization has estimated that 80% of the earth inhabitants relied on traditional medicine for their primary health care needs. Herbs have been used as food and medicine from many countries to cure the diseases. The traditional medicines are used to cure all kind of diseases especially they were believed to possess

anti-tumor or immune stimulating properties. Phytochemicals analysis of plants extracts indicates the presence of primary and secondary metabolites such as protein, carbohydrates, phenols, glycosides, flavonoids, and tannins which possess medicinal values. The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in the plant, such as alkaloids, flavonoids, steroids, tannins and phenolic terpenes, volatile oils which are synthesized and deposited in specific parts or in all parts of the plant. The plant secondary products may exert their action by resembling endogenous metabolites, ligands,

hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential sites.

Therefore, random screening of plants for bioactive compounds is as important as, the screening of ethno botanically targeted species. Phytochemical screening of *Cassia auriculata* L extract has revealed the presence of alkaloids, flavonoids, Phenolic, steroids and saponins in petroleum ether, chloroform and ethanol extracts as reported [11]. In present study, the Leaf part of *A. marmelos* has been subjected to extract crude drug in 3 different solvents such as petroleum ether, chloroform and ethanol by cold extraction method, the crude drug so obtained was tested for phytochemical studies which reveals the presence of phyto-constituents such as phenols, glycosides, flavonoids, tannins and quantitative estimation indicates that the presence of carbohydrates, proteins, amino acids, reducing sugars, total phenols and tannins etc. in all the above 3 different organic solvent extracts. There are some other several secondary metabolites present in plant extracts.

The quantity of primary and secondary metabolites present in the plant sample is also important for their biological activity is also been estimated. The studies on *Murrayakoenigii* indicates the presence of alkaloids, proteins and amino acids, phenolic, saponins, flavonoids, terpinoids present in petroleum ether, chloroform extract and ethanol extract [12].

Similar studies also reveals that phytochemical analysis of *Andrographis alata* indicates the presence of saponins, flavonoids, triterpenoids, glycosides, gums and mucilages, tannins, phenolic compounds and steroids, in petroleum ether, chloroform and ethanol extracts [13].

5. Conclusion

The results obtained in the present study indicates that the *A. marmelos* leaves have the potential to act as a source of useful drugs because of presence of various phytochemical such as carbohydrate, protein, phenols, flavonoids and tannin. The results are very much encouraging but further scientific validation is necessary before being put into practice.

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