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PHYTOCHEMICAL ASSESSMENT AND PHYSICOCHEMICAL ANALYSIS OF AEGLE MARMELOS (L.) CORREA FRUIT FOUND IN KARACHI: A SYSTEMATIC EVALUATION OF SOLVENT-BASED EXTRACTS

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Abstract

Background: Aegle marmelos (L.), Rutaceae has been widely used in traditional medicines in South and Southeast Asian countries. Although it is documented for its ethnopharmacological relevance, comprehensive and standard documentation of its phytochemistry and physicochemical is still limited.

Objective: The aim of this study was to prepare and analyze the solvent-based extracts of powered fruit of A. marmelos by systematically determining the physicochemical parameters and conducting qualitative phytochemical screening in four different solvent systems; aqueous, ethanolic, ethyl acetate and chloroform.

Method: The fruits evaluated in this study were purchased from the local market of Karachi, powder was prepared and analyzed using sequential extraction technique with ethanol (90%), water, ethyl acetate and chloroform. The physicochemical parameters such as pH, conductance,

viscosity, total dissolved solids (TDS), density, loss on drying, extractive values, ash values were determined using standard pharmacognostic techniques. Qualitative phytochemical screening was carried out with the help of validated test for 17 classes of secondary metabolites.

Results: Organoleptic evaluation showed a bright yellowish-orange powder with bitter taste, latex like smell of moderately strong aroma and a gritty texture. The physicochemical analysis revealed that the pH of the sample was 4.62, the conductance value was 36.5 µS, the extractive value (alcohol soluble) was 71%, and the water soluble extractive value was 93%. Total ash was 3.92 ± 0.23%, with water-soluble ash (0.52 ± 0.12%) exceeding acid-insoluble ash (0.22 ± 0.14%). Loss on drying: 6.96%. Phytochemical screening indicated the difference in the distribution of bioactive constituents with aqueous and ethanolic extracts being rich in saponins, tannins, phenolic compounds, phytosterols, coumarins, steroids, glycosides, and fats oils, whereas the ethyl acetate and chloroform extracts mainly exhibited the presence of saponins, flavonoids, triterpenoids, and quinones. Only the ethanolic extract contained any alkaloids. All extracts were found to be free from cardiac glycosides, terpenoids and anthocyanins.

Conclusions: The A. marmelos fruit is a rich source of various pharmacologically active secondary metabolites and their distribution is observed to be polarity dependent. The high water soluble extractive value and its high phenolic content justify further quantitative and bioactivity oriented investigations, which support its traditional therapeutic uses.

1. INTRODUCTION

Aegle marmelos (L.) Correa is the common name of Bael, commonly referred to as Bengal Quince or Wood Apple and is part of the Rutaceae family. It is a medium-sized deciduous tree, native throughout the Indian subcontinent and is common in South and Southeast Asia [1,2] Entire part of this traditional plant have been used for the cure of diabetes ,diarrhea, dysentery, febrile and inflammatory diseases in various traditional Indian medicine systems such as Ayurvedic, Unani, and Siddha medicine [3,4]. The medicinal value of herbal remedies is closely related to their secondary metabolites such as flavonoids, alkaloids, tannins, terpenoids, saponins, phenolic compounds, and others [5 ,6] These medicinal components are typically found in many parts of plants and can be selectively extracted by solvents of varying polarity. Hence, the extraction of phytochemicals has to be done systematically using different solvent systems to obtain extensive phytochemical investigations.[7] Physicochemical characterization, such as loss on drying, ash values, extractive values, pH, viscosity and conductance, is one of the basic elements of the pharmacognostic standardization. These parameters are important quality markers which can be used to identify adulteration, determine purity and ensure the batch-to-batch reproducibility in the development of herbal drugs[8] . Although there is significant pharmacological interest in A. marmelos, majority of reported studies are not methodologically sound especially in terms of standard extraction procedure and systematic multi-solvent screening of phytochemicals . [9,10]

Hence the present work aimed to prepare aqueous, ethanolic, ethyl acetate and chloroform extracts of fruit powder of A. marmelos to study its physicochemical parameters and phytochemical content by following the standard methods.

2. MATERIALS AND METHODS

2.1.Plant Material Collection and Preparation

The fruits of A. marmelos were purchased locally from Karachi, Pakistan. The organoleptic characterization was used to confirm the botanical identity. The fruits were washed with distilled water twice to remove any excessive particulate matter and dirt. The fruits were then air dried at room temperature for 15 days. The dried fruit material was reduced mechanically into fine powder with the help of sieve size 120 μm to get uniform particle size. The powder formed was stored in a sealed air tight bottle, away from light and moisture, at room temperature until further testing was performed [11].

2.2 Preparation of Solvent Extracts

2.2.1 5 g accurately weighed Ethanolic Extract of powdered fruit soaked in 90% of 100 mL ethanol in a sealed conical flask.. The mixture was kept for continuous shaking for 6 hours, followed by an18-hour settling period**.** Following maceration, Whatman No. 1 was used to filter the mixture. The filtrate was collected for physicochemical and, phytochemical screening and determination of extractive value [12]. The aqueous, chloroform and ethylacetate extracts were prepared in the same manner. The aqueous extract was taken by macerating in chloroform water (100 mL) for 24 hours. All extracts were filtered and kept at 2-8°C under refrigeration until analyzed.

2.3 Organoleptic Characterization

Organoleptic properties of fruit powder were evaluated after standard pharmacopoeial methods of direct sensory examination of colour, odour, taste and texture. Each characteristic was assessed by three independent observers and recorded.

2.4 Physicochemical Parameter Determination

2.4.1 pH evaluation: The Ph measurement was done by calibrated instrument which was standardized with standard buffer solution of pH 4.0 and pH 9.0. Triplicate measurements were taken for pH of all extracts.

2.4.2 Conductance The conductivity of the extract solution was measured with a calibrated conductometer. These results were captured in microSiemens (µS).

2.4.3 Viscosity The kinematic viscosity was measured by an Ostwald capillary viscometer. 10 ml of each extract were placed in the viscometer and the efflux time was measured compared to that of distilled water. The absolute viscosity was determined by the formula: ηsample = ηwater × (t sample / t water) and the result was expressed in mPa·s.

2.4.4 Density A calibrated pycnometer was used to measure the density of each solvent extract. All measurements for mass and volume were made at ambient temperature and the density was expressed in kg/m3 units.

2.4.5 Total Dissolved Solids (TDS) The TDS of the aqueous extract was determined by the use of calibrated TDS meter and the results were reported in mg/L.

2.4.6 Alcohol-Soluble Extractive Value The fruit powder was stored for 24 hours in a closed flask with 90% ethanol (100ml) and constant shaking for the first 6 hours and undisturbed settling for the next 18 hours, after which the settled fruit powder was accurately weighed. Twenty-five ml of the filtered extract was taken to a watch glass which had been previously weighed and the extract was

dried on a water bath. Hot-air oven was used to dry the residue at 105°C until constant mass was achieved and then cooled in a desiccator. The air-dried drug was used as a reference to calculate the alcohol soluble extractive value as per the percentage [13].

2.4.7 Water-Soluble Extractive Value 5 g of sample was precisely weighed and placed in 100 mL of chloroform water in a closed flask and shaken for 6 hours several times and then left to macerate for an additional 18 hours. The 25 ml of the filtrate was dried at 105°C and extractive value was calculated as described above [13].

2.4.8 Total Ash Value Powdered drug 2.0g was accurately weighed, heated in a silica crucible at 450°C in a furnace until ash free from carbon was formed. The crucible was placed in a desiccator and then weighed. The percentage of air-dried drug as total ash values were noted [13].

2.4.9 Acid-Insoluble Ash The total ash was prepared with boiling 25 mL 5% (v/v) hydrochloric acid for five minutes. The residue was filtered using an ash free filter paper (Whatman No. 42) using hot distilled water till the filtrate was neutral, then it was dried and weighed after combustion. Acid-insoluble ash was determined per cent of air dried drug [13].

2.4.10 Water-Soluble Ash 25 mL of distilled water was used to boil the total ash for 5 minutes . The undissolved portion was filtered out, washed and burnt at 450°C for 15minutes. The water soluble ash was determined by subtracting water-insoluble ash from total ash (as percentage on air-dried drug) [13].

2.4.11 Loss on Drying (LOD) Fruit powder was spread thinly and evenly in a pre-weighed silica dish which was preweighed and dried for 5 hours at 105°C, before which 2 grams of the powder was weighed. The dish was placed in a desiccator then weighed, returned to the oven for 30 minutes, cooled again, and weighed again until constant weight was obtained. The percentage weight loss with respect to the initial sample weight was used to express LOD [13].

2.5 Qualitative Phytochemical Screening All four solvent extracts were analyzed by qualitative phytochemical analysis using the standard colorimetric, precipitation, and fluorescence tests for detection of alkaloids (Iodine test), saponins (froth test), flavonoids (NaOH and H_2SO_4 tests), sugars and carbohydrates (Fehling's test), reducing sugars (Fehling's test), steroids (Liebermann–Burchard test), terpenoids, triterpenoids (Salkowski's test), phenolic compounds ($FeCl_3$ test), glycosides (Legal's test), cardiac glycosides (Keller–Kiliani test), coumarins (NaOH test), tannins ($FeCl_3$ and NaOH tests), phlobatannins (HCl test), phytosterols (Salkowski's test and Hesse's test), quinones

(KOH and HCl tests), fats and oils (spot test) and anthocyanins (HCl/ammonia test) as described by Harborne (1998) and Trease and Evans (2002) [14,15].

2.6 Statistical Analysis Each physicochemical parameter was measured three times. Data are reported as mean ± SD.3.

3. RESULTS AND DISCUSSION

3.1 Organoleptic Characterization The organoleptic evaluation of A. marmelos fruit powder showed that there was an agreement among the evaluators regarding the sensory observations. The powder was described as bright yellowish-orange in colour, and had a latex-like and mildly aromatic smell, a distinctly bitter taste and a gritty texture. These properties have been reported earlier for the authenticated Bael preparations and are used as preliminary identification for crude drug. The findings are shown in the table mentioned below

Table 1. Organoleptic Properties of Aegle marmelos (L.) Fruit Powder

S. No.	Organoleptic Character	Observation
1	Colour	Bright yellowish-orange
2	Odour	Latex-like and mild aromatic
3	Taste	Bitter
4	Texture	Gritty

These observations match what has already been reported for authenticated Bael fruit powder. The yellowish-orange colour is most likely due to carotenoid pigments, the bitterness to alkaloidal constituents, and the faint latex-like smell to volatile terpenoids [2,6]. Simple as they are, sensory checks like these remain a useful first step for confirming the identity of a crude drug before any instrumental analysis is carried out.

3.2 Physicochemical Parameters

The fruit powder of A. marmelos and its extracts were subjected to physicochemical analysis to establish some important quality parameters. The aqueous extract had an acidic pH of 4.62, suggesting the presence of organic acids and acidic secondary metabolites. The water-soluble extractive value (93%) was significantly higher than the alcohol-soluble extractive value (71%) indicating that water-soluble constituents were predominant. The total ash (3.92 ± 0.23%) gives an indication of the total inorganic matter present in the drug. Water-soluble ash (0.52 ± 0.12%) confirms that the mass of alkali

salts and other water-soluble inorganic impurities is small, while low acid-insoluble ash (0.22 ± 0.14%) reveals that the content of siliceous impurities is small. The LOD of 6.96% is acceptable within the limits of the pharmacopoeia and is within acceptable limits of dryness for the storage of the product. The highest density was obtained with the chloroform extract (density of 1.27 kg/m³) which is similar to the density of pure chloroform, and the lowest density was obtained with the extract of ethanol (density of 0.75 kg/m³). Table 2 shows the complete results.

Table 2. Physicochemical Parameters of Aegle marmelos (L.) Fruit Powder and Extracts

S. No.	Parameter	Result
1	pH	4.62
2	Conductance	36.5 µS
3	Viscosity	0.914 mPa·s
4	Total Dissolved Solids (TDS)	22.81 mg/L
5	Density — Ethanolic Extract	0.75 kg/m³
6	Density — Aqueous Extract	0.90 kg/m³
7	Density — Ethyl Acetate Extract	0.78 kg/m³
8	Density — Chloroform Extract	1.27 kg/m³
9	Alcohol-Soluble Extractive Value (w/w)	71%
10	Water-Soluble Extractive Value (w/w)	93%
11	Total Ash Value	3.92 ± 0.23%
12	Acid-Insoluble Ash	0.22 ± 0.14%
13	Water-Soluble Ash	0.52 ± 0.12%
14	Loss on Drying (LOD)	6.96%

An aqueous pH of 4.62 points to the kind of organic acids already known to occur in A. marmelos, such as malic, citric and tartaric acid [7,9]. What is more interesting is that the water-soluble extractive

value (93%) came out well above the alcohol-soluble value (71%), suggesting that most of the fruit's bioactive load leans towards water solubility rather than alcohol solubility, a pattern also picked up in earlier work on this fruit's polysaccharides, tannins and glycosides [4,8]. The total ash (3.92 ± 0.23%) gives a general sense of the mineral content, and since the acid-insoluble ash stayed low at 0.22 ± 0.14%, well under the 0.3% mark usually taken as a sign of sand or soil contamination [13], the sample can reasonably be considered clean, with the washing step doing its job. The water-soluble ash (0.52 ± 0.12%) mostly reflects dissolved salts such as potassium, sodium and calcium. Finally, a loss on drying of 6.96% sits comfortably within pharmacopoeial limits, so the powder should be dry enough for storage without inviting microbial growth.

3.3 Qualitative Phytochemical Screening

The phytochemical profiles of the four A. marmelos fruit powder extracts have been found to be different from each other (Table 3). The aqueous extract showed multiple active metabolites, such as saponins, flavonoids, phytosterols, reducing sugars, phlobatannins, fats and oils, while ethanolic extract showed tannins, flavonoids, alkaloids, steroids, glycosides, coumarins, fats and oils, and phlobatannins. Ethyl acetate and chloroform extracts exhibited relatively low phytochemical profile, which mainly consisted of saponins, flavonoids, triterpenoids, and quinones, with reducing sugars also being found in the chloroform extract. The cardiac glycosides, terpenoids and anthocyanins were not detected after repeated testing in all four solvent systems. None of the extracts contained amino acids. Both the aqueous and ethanol extracts were positive for reducing sugars and carbohydrates, and negative for monosaccharides, pentose sugars and hexose sugars. The glycosides were found in both the aqueous and ethanolic extracts while cyanogenetic glycosides were not detected in either.

Table 3. Qualitative Phytochemical Screening of Aegle marmelos (L.) Fruit Extracts

(+ = Present; − = Absent)

Phytochemical Class	Aqueous Extract	Ethanolic Extract	Ethyl Acetate Extract	Chloroform Extract
Alkaloids	−	+	−	−
Flavonoids	+	−	+	+
Saponins	+	+	+	+

Phytochemical Class	Aqueous Extract	Ethanolic Extract	Ethyl Acetate Extract	Chloroform Extract
Sugars & Carbohydrates	+	+	−	−
Tannins	+	+	−	−
Cardiac Glycosides	−	−	−	−
Phenolic Compounds	+	+	−	−
Coumarins	+	+	−	−
Reducing Sugars	+	+	−	+
Phlobatannins	+	+	−	−
Terpenoids	−	−	−	−
Triterpenoids	−	−	+	+
Quinones	−	−	+	+
Phytosterols	+	+	−	−
Fats and Oils	+	+	−	−
Anthocyanins	−	−	−	−
Steroids	+	+	−	−

What stands out most in the phytochemical screening is how cleanly the results split along polarity lines. Alkaloids, for instance, showed up only in the ethanolic extract, which is not surprising given that most plant alkaloids are moderately polar to begin with. Flavonoids were a bit more puzzling: present in the aqueous, ethyl acetate and chloroform extracts, but missing from the ethanolic one. This

could simply come down to flavonoid-ethanol complexes forming and dropping out during filtration, rather than a genuine absence. Saponins, on the other hand, turned up in all four extracts, which fits with their amphiphilic nature, they are not fussy about polarity. Phenolics and tannins stuck to the polar extracts as expected, while triterpenoids and quinones showed up only in the ethyl acetate and chloroform fractions, in line with their lipophilic character [7]. Anthocyanins and terpenoids were not picked up in any extract; this may simply mean they were present only in trace amounts in the ripe fruit, broke down during drying, or were not really there in this particular batch of fruit to begin with. More encouragingly, the polar extracts in particular carried phytosterols, steroids, coumarins and phlobatannins, a combination worth paying attention to pharmacologically. Phytosterols are associated with cholesterol-lowering effects, coumarins with anti-inflammatory and anticoagulant activity, tannins and phlobatannins with antimicrobial, astringent action, and phenolics with antioxidant potential [1,3,5]. Put together, these findings offer a plausible chemical explanation for why A. marmelos has long been used against gastrointestinal complaints, diabetes and inflammation. The phytochemical picture that emerges here is broadly in line with earlier studies on the species [1–10], and the four-solvent approach used, backed by a fairly complete physicochemical dataset, adds a little more weight to the existing standardization data for this fruit.

4. CONCLUSION

The present study has shown that Aegle marmelos (L.) The Correa fruit is rich in a variety and polarity-dependent set of secondary metabolites of proven pharmacological relevance. The results of the physicochemical parameters, such as water-soluble extractive value is high, low acid-insoluble ash and acceptable moisture content, collectively validate the standard and purity of the plant material undertaken for the study. The differential phytoconstituent distribution among the various aqueous, ethanolic, ethyl acetate and chloroform extracts highlight the significance of choosing the appropriate extract solvent for research and formulation development. These results provide a solid pharmacogenetic basis for the quantitative phytochemical analysis, in vitro and in vivo bioactivity assessment and rational development of standardized A. marmelos based herbal therapeutics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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