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PHYTOCHEMICAL EVALUATION OF LEAF EXTRACTS OF *Aegle marmelos*

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ABSTRACT

The use of traditional medicines holds a great promise as an easily available source as effective medicinal agents to cure a wide range of ailments among the people particularly in tropical developing countries like India. The present study investigates the phytochemical analysis of the major bioactive constituents of *A. marmelos* leaf extracts. The extractive values of aqueous, acetone and chloroform extracts were found to be 7.5 %, 5.3 % and 6.4 % respectively. Qualitative phytochemical analysis of these three solvent extracts confirm the presence of Alkaloids, Saponins, Flavonoids and Phenolic compounds in all the three extracts; however these phytochemicals were more significant in aqueous extract. This indicates that the leaves can be useful for treating different diseases because the therapeutic activity of a plant is due to the presence of particular class of compounds and thus can serve as potential sources of useful drugs in future.

INTRODUCTION

The history of plant based health care goes back to antiquity and as old as human civilization. Plants have been primary source of medicines in the traditional healthcare systems around the globe, till recently and even currently in most of the developing countries. The approach to characterization and isolation of active ingredients from plants started in the late 19th century. Consequently chemical substances isolated are currently used as important drugs as such or as their derivative(s) today. Thus, there is considerable interest in the screening of plant and other natural product extracts in modern drug discovery programmes, since structurally novel chemotypes with potent and selective biological activity may be obtained (Cragg *et al*., 1997). A consideration of biological activity in addition to the isolation and structure elucidation stages in a phytochemical investigation may add a great deal to the overall scientific significance of the work. Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fibre to protect against diseases. They are non-nutritive compounds (secondary metabolites) that contribute to flavour colour (Johns, 1996; Craig, 1999; Agbafor and Nwachukwu, 2011). Globally, medicinal plants have been unique sources of medicines and constituted the most common human use of biodiversity (Hamilton, 2004; Hiremath and Taranath, 2010).

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Nearly 70% world population (mainly in the developing countries) rely entirely on such traditional medical therapies as their primary form of health care (Bewaji *et al*., 1985). The use of drugs derived from plants has been utilized as a source of many potent and powerful drugs for thousands of years all over the world (Lewis and Elvin-Lewis, 1977). Even in modern times, plant-based systems continue to play an essential role in health care and in the recent past increasing research evidence is getting accumulated, which clearly indicate the positive role of plant extracts for health care (Shabnam Javed *et al*., 2012). Since most plants have medicinal properties, it is of utmost importance that their efficacy and toxicity risks are evaluated (Olagunjua *et al*., 2009). The Knowledge of the chemical constituents of these plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers (Siddiqui *et al*., 2009). Therefore, the objective of the present research work was to perform the phytochemical analysis of three different extracts of fresh leaves of *Aegle marmelos*.

MATERIALS AND METHODS

Glassware and chemicals

Good quality glassware and chemicals were used for all tests. All the glass wares were of brand Borosil or Corning. They were washed with good detergent, rinsed in tap water and soaked in chromic acid clearing solution.

Clearing solution (Mahadevan and Sridhar, 1996)

Potassium dichromate - 60 g
Conc. $H_2 SO_4$ - 60 mL
Distilled water - 1 L

Potassium dichromate was dissolved in warm water, cooled and sulphuric acid was added slowly. It was mixed thoroughly and used for cleaning glassware. Then, they were rinsed thrice in tap water, finally rinsed in distilled water and dried in hot air oven. Dried glassware and media were sterilized in an autoclave for 15 min at 15 lb/sq inch pressure.

Chemicals

Analytical grade chemicals supplied by Loba, Hi-Media, S.D. Fine Chemicals, E. Merck, Qualigens and Sigma Chemicals (U.S.A) were used in this study.

Leaf collection and identification

The leaf specimens were collected in the month of August from Kumbakonam, Tamil Nudu, India and authenticated by Professor N. Raaman, Herbal Science Laboratory, centre for Advanced Studies in Botany, University of Madras, Chennai. After a thorough investigation leaves were checked for any pathological disorders and contamination of other plants and were washed with distilled water.

Preparation of extracts

The fresh leaves (300 grams) were grounded into paste and were extracted with water for 12 h at room temperature. This process was repeated successively with chloroform and acetone for 72 h at room temperature until the color of the extract becomes pale. The extracts obtained were filtered separately using Whatmann No. 1 filter paper. This was repeated for 2 to 3 times and similar extracts were pooled together and dried on water bath until the constant weight with dry mass was obtained for solvent extracts. The residual extracts were stored in refrigerator at $4^{\circ}C$ in small and sterile glass bottles. Percent extractive values were calculated by the following formula.

$$\text{Percent Extracts} = \frac{\text{Weight of dried extract}}{\text{Weight of leaf material}} \times 100$$

Preliminary phytochemical screening

The different qualitative chemical tests were performed for establishing the profile of the leaf extracts for its chemical composition. The following tests were performed to detect various phytoconstituents present in them.

Detection of alkaloids (Evans, 1997)

Solvent free extract (50 mg) was stirred with few mL of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows:

Mayer's test (Evans, 1997)

To a few mL of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate indicated the test as positive.

Mayer's Reagent

Mercuric chloride (1.358 g) was dissolved in 60 mL of water and potassium chloride (5.0 g) was dissolved in 10 mL of water. The two solutions were mixed and made up to 100 mL with water.

Wagner's test (Wagner, 1993)

To a few mL of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.

Wagner's reagent

Iodine (1.27 g) and potassium iodide (2 g) were dissolved in 5 mL of water and made up to 100 mL with distilled water.

Hager's test (Wagner *et al*., 1996)

To a few mL of the filtrate, 1 or 2 mL of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

Dragendorff's test (Waldi *et al*., 1965)

To a few mL of filtrate, 1 or 2 mL of Dragendorff's reagent was added. A prominent yellow precipitate indicated the test as positive.

Dragendorff's reagent

Stock solution

Bismuth carbonate (5.2 g) and sodium iodide (4 g) were boiled for a few min with 50 mL glacial acetic acid. After 12 h, the precipitated sodium acetate crystals were filtered off using a sintered glass funnel. Clear, red-brown filtrate, 40 mL was mixed with 160 mL of ethyl acetate and 1 mL of water and stored in amber-colored bottle.

Working solution

Ten mL of stock solution was mixed with 20 mL of acetic acid and made up to 100 mL with water.

Detection of saponins by foam test (Kokate, 1999)

The extract (50 mg) was diluted with distilled water and made up to 20 mL. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of saponins.

Detection of phytosterols (Finar, 1986)

Libermann-Burchard's test

The extract (50 mg) was dissolved in 2 mL of acetic anhydride. To this, one or two drops of concentrated H_2SO_4 were added slowly along the sides of test tube. An array of color changes showed the presence of phytosterols.

Detection of phenolic compounds

Ferric chloride test (Mace, 1963)

The extract (50 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution was

Table 1: Preliminary Phyto-Profile for *A. marmelos* leaf extracts

Different Solvents	Colour of extracts Value	Consistency	Yield (%age) Extractive
Water Extract	Brown	Sticky	7.5
Acetone Extract	Black	Dry	5.3
Chloroform Extract	Dark brown	Sticky	6.4

Table 2: Phytochemical screening of aqueous extract of *A. marmelos* leaves

S. No	Test	Test applied/Reagent used	Observation	Inference	Intensity
1	Alkaloids	Mayer's	No milky precipitate	Absent	-
		Wagner's	Reddish brown precipitate	Present	+
		Hager's	Yellow precipitate	Present	+
		Dragendroff's	Brick red precipitate	Present	+
4	Saponins	Foam test	Foam formation	Present	++
5	Phytosterols	Libermann-Burchard's	No color changes	Absent	-
7	Phenolic compounds	Ferric chloride	Dark green color	Present	+++
8	Flavonoids	Alkaline reagent	Yellow fluorescence	Present	++

Table 3: Phytochemical screening of acetone extract of *A. marmelos* leaves

S. No	Test	Test applied/Reagent used	Observation	Inference	Intensity
1	Alkaloids	Mayer's	No milky precipitate	Absent	-
		Wagner's	No reddish brown precipitate	Absent	+
		Hager's	No yellow precipitate	Absent	-
		Dragendroff's	No brick red precipitate	Absent	-
		Fehiling's	No red precipitate	Absent	-
4	Saponins	Foam test	Foam formation	Present	+
5	Phytosterols	Libermann-Burchard's	Color changes	Present	+
7	Phenolic compounds	Ferric chloride	Dark green color	Present	+
8	Flavonoids	Alkaline reagent	Yellow fluorescence	Present	+

Table 4: Phytochemical screening of chloroform extract of *A. marmelos* leaves

S. No	Test	Test applied/Reagent used	Observation	Inference	Intensity
1	Alkaloids	Mayer's	No milky precipitate	Absent	-
		Wagner's	Reddish brown precipitate	Present	++
		Hager's	No yellow precipitate	Absent	-
		Dragendroff's	Brick red precipitate	Present	+
4	Saponins	Foam test	Foam formation	Absent	+
5	Phytosterols	Libermann-Burchard's	Color changes	Present	++
7	Phenolic compounds	Ferric chloride	Dark green color	Present	+
8	Flavonoids	Alkaline reagent	No yellow fluorescence	Absent	+

added. A dark green colour indicated the presence of phenolic compounds.

Detection of flavanoids

Alkaline reagent test

An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavanoids.

RESULTS

The preliminary phyto–profiling for the leaves of *Aegle marmelos* were carried out. The extract values, colors and consistencies of the extracts were depicted in Table 1. Extractive values of aqueous, acetone and chloroform extracts of *A. marmelos* leaves were found to be 7.5 %, 5.3 % and 6.4 % respectively. Phytochemical screening of *A. marmelos* leaf extracts indicated the presence of different classes of secondary metabolites that are essential in herbal medicine. Among the phytochemicals obtained were alkaloids, saponins, flavonoids, phenolic compounds and Phytosterols. These phytochemicals were highly significant in aqueous extract (Table 2-4)

DISCUSSION

Phytomedicine represents one of the most important fields of traditional medicine all over the world and are of prime importance to the health of individuals and communities. The medicinal values of these economically important plant species is due to presence of some chemical substances which produce a definite physiological action on human body like alkaloids, tannins, flavonoids and saponin etc. (Edeoga et al., 2005; Khan *et al*., 2011). To promote the proper use of phytomedicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way (Subramanian and Suja, 2011). In the present study the quantitative analysis of *Aegle marmelos* leaf extracts was carried out in fresh leaf samples. Alkaloids, flavonoids, saponins, phytosterols and phenolic compounds were revealed to be present in *A. marmelos* leaf extracts. This shows high level of its possible medicinal values (Oloyed, 2005; Aja *et al*., 2010; John *et al*., 2011). Screening of plants for medicinal value has been carried out by number of workers with the help of preliminary phytochemical analysis (Dan *et al*., 1978; Ram, 2001; Mungole and Chaturvedi, 2011). A number of medicinal plants have been chemically investigated by several workers

(Battacharya *et al*., 1971; Kokate *et al*., 1998). The selection of plant part which yields maximum secondary metabolites is the prime or prerequisite step in this investigation. For this, different phytochemicals from *A. marmelos* leaf extracts were extracted by using water, acetone and chloroform solvents. Qualitative screening confirmed the presence of Alkaloids, Saponins, Flavonoids and Phenolic compounds in all the three extracts; however these phytochemicals were more significant in aqueous extract. The present of these four compounds support the use of the plant in folklore medications. Alkaloids are known to contain a lot of pharmacological properties.

They are mostly used as antidepressant (morphine), stimulants (caffine), anaesthetic (cocaine), antitumor (vinblastine) antimalaria (quinine), antibacterial (berberine) and amoebicide (emetine). (Bruneton, 1999; Heinrich *et al*., 2004*;* Gurib-Fakim, 2006). Saponins are glycosides possessed antimicrobial and inhibit Na^+ efflux, by blockage of the entrance of the Na^+ out of the cell, reducing congestive heart failure (Abou-Donia *et al*., 2008). These compounds are known to be immune booster and are said to demonstrate anti-inflammatory, homolytic, allelopathic, cholesterol lowering and anticancer properties. Flavonoids are known to have anti-inflammatory, anti-allergic, antiviral, antispasmodic and diuretic effect (Cowan, 1999). While Phenolic compounds have attracted a great attention in relation to their potential for beneficial effects on health. Over the last few years, several experimental studies have revealed biological and pharmacological properties of phenolics compounds, especially their anti-inflammatory activity (Castillo *et al*., 1989; Zhu *et al*., 1997), antiviral, and cytotoxic activity (Chhabra *et al*., 1984). It is a well documented fact that most medicinal plants are enriched with phenolic compounds and bioflavonoids that have excellent antioxidant properties (Shirwaikar *et al*., 2003; Mungole and Chaturvedi, 2011). Phenolics are active in curing kidney and stomach problems.

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