

Phytochemical Screening of Medicinal Plants *Aegle marmelos L.* and *Cardiospermum halicacabum L.* from Kolli Hills Tamil Nadu

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ABSTRACT

Plants are a local valuable with primary importance. This world is blessed with an abundance of medicinal plants. They are an alternative to antibiotics in non-served infections. They are also important in complementary and alternative medicine because they have developed the ability to produce various phytochemicals, which are used to restore health. Natural products derived from plants and animals provide a wealth of newer medicinal agents with clinical potential. The present study investigates the phytochemical analysis of *Aegle marmelos L.* and *Cardiospermum halicacabum L.* in different solvent extracts viz aqueous, chloroform, ethanol, and petroleum ether extracts. Alkaloids, Carbohydrates, Glycosides, Flavones, Flavanones, Gums, Mucilage, Oil, Fats, Protein, Amino acids, Saponins, Steroids, Tannin, Phenol, and Triterpenoids were the most abundant phytochemicals in both the aqueous and ethanolic extracts. Phytochemical analysis from plant extracts serves as a foundation for further phytochemical and pharmacological research, which could lead to the discovery of new clinically effective compounds.

Keywords: Phytochemical analysis, plant extracts, solvent extraction.

INTRODUCTION

Scientists all over the world are interested in the use of medicinal herbs in the treatment and prevention of diseases (Ansari et al., 2019; Vakayil et al., 2021; Kabeerdass et al., 2022; Vakayil et al., 2022). In its efforts to bring primary health care to the people, the World Health Organization confirms this. Plants have long been a rich source of pharmaceuticals (Kabeerdass et al., 2022), food additives, flavoring agents, colorants, binders, and lubricants. It is estimated that about 25% of all prescribed medicines today are derived from plants (Kabeerdass et al., 2021). Traditional medicine and medicinal plants are widely used as a normative basis for the maintenance of good health in most developing countries (Sankareswaran et al., 2021; Kabeerdass et al., 2022; Baburam et al., 2022; Sun et al., 2021; Vakayil et al., 2021; Vakayil et al., 2022). Furthermore, the extraction and development of several drugs and chemotherapeutics from medicinal plants, as well as traditional rural herbal remedies, has led to an increased reliance on their use in industrialized countries. Plant-

derived and natural compounds (Maghimaa and Palanisamy, 2016; Maghimaa and Palanisamy, 2019; Mathanmohun et al., 2021; Vakayil et al., 2021; Suriya Sabarinath et al., 2022; Sivakumar et al., 2021; Pandeewari et al., 2021) are gaining popularity around the world because they have potent, low-to-no toxic pharmacological compounds, are cost-effective, safer, and more dependable processes (Sivakumar et al., 2018; Vakayil et al., 2021; Pandeewari et al., 2021; Sivakumar et al., 2021; Kabeerdass et al., 2022; Suriya Sabarinath et al., 2022). Traditional medicines or traditional therapies, in which the whole or parts of plants are used as medicine, are used by 70–95 percent of the world's population.

Universal-level health issues bacterial Multidrug-resistant infections are coherent as leading pressures, and Multi-Drug Resistance bacteria cause many infections (Vakayil et al., 2020; Kabeerdass et al., 2021; Sharmila et al., 2022; Nair et al., 2022; Rakesh et al., 2022). This encouraged us towards our upcoming life with effective drugs. Pathogens causing infections are accountable for 15.5% of total MDR. Thereby, increasing mortality ultimately also leads to high healthcare costs (Vakayil et al., 2021; Kabeerdass et al., 2021). Therefore, drug resistance in microorganisms has become an unsolvable problem, and using existing drugs to treat infectious diseases is becoming less common. This situation has led to the discovery of drugs from a variety of sources, one of which is plant-based drugs. *Aegle marmelos*, a member of the Rutaceae family, is one such plant that contains pharmacologically active compounds in all parts of the plant. The global demand for traditional medicinal plant bi-products is on the rise these days. Medicinal plant procurement is mellow, cost-effective, and abundant in pharmacological active principles, and it is used as a home remedy by many. Human diseases are compared to modern medicine, which is tainted and has an excess of side effects. The need to assess the multi-infection targeted by antibiotic-resistant microorganisms has prompted scientists to look for new drugs with fewer side effects. Credential leaf extracts are used to treat intestinal parasitoids, syphilis, hemorrhoids, inguinal hernia, constipation, and diabetes. Many researchers have previously reported that compounds such as hydroxyl anthraquinones, glycosides, chrysophanic acid, and kampferin, which have a variety of biological functions, were isolated from *Aegle marmelos*. In the Indian subcontinent, medicinal plants have a high economic value. Nature has blessed us with an abundance of botanical diversity, with many different types of plants growing in different parts of the country. India is diverse in all three aspects of biodiversity: species, genetic diversity, and habitat diversity. *Cardiospermum halicacabum* is a climber in the *Sapindaceae* family that has been used as a medicinal plant since ancient times. *Sapinadaceae*, also known as the Litchi family or soap-berry family, is a large family that includes 158 genera and 2230 species. There are 24 genera and 72 species in the family in India. Typically found in the Himalayas of the North Eastern and North Western hemispheres. The family is mostly found in tropical and subtropical climates. *Cardiospermum halicacabum*, also known as the balloon plant or love in a puff, is a tropical and subtropical plant widely distributed. It grows as a weed along highways and rivers. *Cardiospermum* is derived from the Latin word *scardio*, which means heart, and *Sperma*, which means seed, and refers to the seed's white heart-shaped pattern. The name *halicacabum* comes from the Latin word *halicacabus*, which refers to a plant with inflated fruits. It's a small, delicate, smooth climber whose entire plant has

been used for centuries to treat a variety of ailments. The plant is a climber, with slender branches and pubescent or nearly glabrous pubescence. It climbs using tendrillar hooks. Leaflets are membranous and depressed, and the pyriform capsule is wrangled at an angle. Seeds are black and have a large white aril. The present study investigates the phytochemical analysis of *Aegle marmelos L.* and *Cardiospermum halicacabum L.* in different solvent extracts.

MATERIALS AND METHODS

Collection and processing of plant material

The plant material for *Aegle marmelos* and *Cardiospermum halicacabum* leaves were collected in Kolli hills, Namakkal, Tamil Nadu, India. The taxonomy of the plant was confirmed by St. Joseph's College in Trichy. The leaves are cleaned with tap water, shade dried and coarsely powdered. Fine leaf powder was used for the phytochemical analysis (Maghimaa and Alharbi, 2020).

Preparation of Leaf Extract

A 20g finely powder sample was placed in 250 ml Erlenmeyer flasks, each was then filled with 100 ml of solvents such as aqueous, chloroform, ethanol, and petroleum ether. Later the flasks were shaken in a rotary shaker for 6 hours before being left to macerate for 18 hours. Subsequently, the filtrate was filtered through Whatman no. 1 filter paper before being subjected to qualitative phytochemical analysis.

Phytochemical analysis

The concentrated extracts were subjected to phytochemical analysis to get a general idea of the constituents found in the crude extract. Grade techniques were used for the analysis of carbohydrates, proteins, alkaloids, flavonoids, tannins, phenols, terpenoids, triterpenoids, steroids, saponins, coumarin, quinine, anthraquinones, glycosides, starch, gum, and fixed oils present in the leaves of *Aegle marmelos* and *Cardiospermum halicacabum* (Vakayil et al., 2019).

Test for Protein

Biuret test

Two drops of 3% copper sulphate and a few drops of 10% sodium hydroxide were added to 2 ml of leaf extract. The presence of proteins was indicated by the formation of a violet or red color (Kancherla et al., 2019).

Test for Carbohydrate

Barfoed's test (Monosaccharide)

1 ml of Barfoed's reagent (a solution of cupric acetate and acetic acid) was added to 1 ml of leaf extract and heated in a boiling water bath for 3 minutes. The presence of carbohydrates was indicated by the formation of a red precipitate (Patil et al., 2021).

Molisch's Test

In Molisch's test, the leaf extract was supplemented with 5 ml of refined water, 2 drops of alcoholic α naphthol solution, and a dropper cautiously dispense conc H_2SO_4 alongside the test tube in a dropwise manner. Violet color formation at the combination of two liquids showed the subsistence of carbohydrates (Abirami and Maghimaa, 2019).

Test for Aminoacid**Ninhydrin test**

Three drops of 5% Ninhydrin solution were added to 1 ml of leaf extract and heated in a boiling water bath for 10 minutes. In a test tube, 3 ml leaf extract is supplemented with 3 drops of lead acetate solution (5%) then it was boiled in a water bath for 10 mins. The presence of amino acids was indicated by a distinctive purple color (Abirami and Maghimaa, 2019).

Test for Alkaloids**Wagner's Test**

To 1ml of the leaf extract, 1ml of Wagner's reagent (Iodine in potassium iodide solution) was added. The formation of a reddish-brown precipitate indicated the presence of alkaloids (Abirami et al., 2021).

Test for flavonoids**Ammoniumhydroxide Test**

The leaf extract was treated with ammonium hydroxide solution, which forms it a yellow fluorescence, indicating flavonoids.

A few droplets of watery HCL and a small quantity of magnesium were supplemented with 0.5 ml of leaf extract and simmered for a few minutes. The formation of the deep red color showed the existence of flavonoids (Abirami and Maghimaa, 2019).

Test for phenols**Lead acetate test**

1 ml of leaf extract was mixed with 3 ml of distilled water. To the mixture, 3 ml of 10% lead acetate solution was added. The formation of a bulky white precipitate indicated the presence of phenols (Dutta, 2015).

Test for tannin**Ferric chloride test**

A few drops of ferric chloride solution were added to 1 ml of leaf extract. If hydrolyzable tannins are present, a blue color appears, and if condensed tannins are present, a green color appears (Abirami and Maghimaa, 2019).

Test for glycosides**Borntrager's test**

The extract was chloroform-treated, and the chloroform layer was separated. Finally, a diluted Ammonia solution was added to the mix. The presence of glycosides was indicated by the formation of pink color (Patil et al., 2021).

Test for terpenoids

1 ml of the leaf extracts, chloroform (5 ml), and a few drops of conc. H₂SO₄ was added. The presence of terpenoids was indicated by the formation of a reddish-brown color at the interface (Dutta, 2015).

Test for saponin

Foam test

2 ml distilled water was added to 2 ml leaf extract and shaken for 15 minutes lengthwise in a graduated cylinder. Saponin was detected by the formation of a 1cm layer of foam (Patil et al., 2021).

Test for coumarin

Fill a test tube with a small amount of leaf extract and cover it with filter paper and treated it with 1 N NaOH solution. For several minutes, the covered test tube was placed in a water bath. After that, the paper was removed and exposed to UV light. The presence of coumarin was confirmed by green fluorescence in the paper (Amir et al., 2011).

Test for quinone

2 ml of conc. H₂SO₄ was added to 1 ml of leaf extract. The presence of quinone was indicated by the formation of a red color (María et al., 2018).

Test for anthroquinone

With a few drops of HCL, 0.5 mL of leaf extract was added. When the mixture was shaken, the presence of anthraquinone was indicated by the appearance of a pink, red, or violet color in the lower phase (Amir et al., 2011).

Test for fixed oils and fat

A small amount of the leaf extract is pressed between two filter papers. The presence of fixed oil and fats was indicated by an oil stain on the paper.

Test for gums and mucilages

5 ml of leaf extract was mixed with 5 ml of distilled water. With constant stirring, 25ml of absolute alcohol was added to this mixture. The presence of gums and mucilage was indicated by the presence of white or cloudy precipitate.

RESULTS AND DISCUSSION

This study determined the qualitative phytochemicals of *Aegle marmelos* leaf extracts using various solvents such as aqueous, chloroform, ethanolic, and petroleum ether extracts. Alkaloids, Carbohydrates, Glycosides, Flavones, Flavanones, Gums, Mucilage, Oil, Fats, Protein, Amino acids, Saponins, Steroids, Tannin, Phenol, and Triterpenoids were the most abundant phytochemicals in both the aqueous and ethanolic extracts (Table-1).

The presence of alkaloids, flavonoids, phenolics, steroids, and saponins were in petroleum ether, chloroform, and ethanolic extracts of *Cassia auriculata* L. (Manjush Ramesh et al., 2010). Similarly, using the Soxhlet extraction method, the leaves of *Aegle marmelos* were extracted in diverse solvents viz petroleum ether, chloroform, ethanolic, and aqueous. The crude extract was then tested for phytochemical studies, revealing phyto-constituents such as alkaloids, phenols, glycosides, flavonoids, tannins, gums, and mucilages. Microbes have developed multiple drug resistance as a result of the indiscriminate use of drugs commonly used to treat infectious diseases. Bacteria, unfortunately, have the genetic ability to transmit and acquire drug and chemical resistance (Nascimento et al., 2000). Phytochemical constituents such as tannins, flavonoids, alkaloids, and a variety of other aromatic compounds are secondary metabolites produced by plants that serve as defense mechanisms against microorganism predation.

This study reveals a qualitative phytochemical analysis of *Cardiospermum halicacabum* leaves are explored (Table 2). The subsistence of Alkaloids, Amino acids, Anthroquinone, Carbohydrates, Coumarin, Flavonoids, Glycosides, Gums, Mucilage, Oil, Fats, Phenol, Protein, Quinone, Saponins, Tannin, and Terpenoids in the aqueous extract of *Cardiospermum halicacabum*.

Carbohydrates, proteins, alkaloids, and saponins were found in the ethanolic extract of *Cardiospermum halicacabum* (Dinesh et al., 2014). Carbohydrates, alkaloids, phytosterol, saponins, tannins, and flavonoids were discovered in ethanol and chloroform extracts at the areal part of the plant. This study correlated with Arunkumaret al., 2013. Sugars, tannin, saponin, and sterols were found in the whole plant of *Cardiospermum halicacabum* aqueous extract. The ethanolic extract of *Cardiospermum halicacabum* leaves contains Carbohydrates, protein, lipids, phenol, saponin, flavonoids, steroids, and tannin (Senthilkumar and Vijayakumari, 2012).

Table 1 Phytochemical analysis of *Aegle marmelos* leaf with different solvent extract

Phytochemical compounds	Different solvent extract			
	Aqueous	Chloroform	Ethanol	Petroleum ether
Alkaloids	+	+	+	-
Carbohydrates & Glycosides	+	-	+	+
Flavones & flavanones	+	-	-	+
Gums & Mucilage	+	-	+	-
Oil & fats	-	-	-	+
Protein & Amino acids	+	-	+	-
Saponins	+	-	+	-
Steroids	+	+	+	+
Tannin & Phenol	+	+	+	-
Triterpenoids	-	-	+	-

Present (+), absent (-)

Table 2 Phytochemical analysis of *Cardiospermum halicacabum* leaf with different solvent extract

Phytochemical compounds	Different solvent			
	Aqueous	Chloroform	Ethanol	Petroleum ether
Alkaloids	+	+	-	+
Amino acids	-	-	+	-
Anthraquinone	-	-	-	-
Carbohydrates	+	+	+	-
Coumarin	+	-	-	+
Flavonoids	-	-	+	-
Glycosides	-	-	-	-

Gums & Mucilage	+	-	+	+
Oil & fats	-	-	-	+
Phenol	+	+	+	-
Protein	+	+	-	+
Quinone	-	-	-	-
Saponins	+	+	+	-
Tannin	+	+	+	-
Terpenoids	+	+	-	-

Present (+), absent (-)

CONCLUSION

The findings of this study are encouraging and can be used to standardize *Aegle marmelos* (L.) and *Cardiospermum halicacabum* (L.) formulations that contain these plants' leaves as a primary ingredient. Although this plant is a common one with a low risk of adulteration, the source of a herbal drug or its finished product should be 100% genuine plant material to ensure maximum efficacy. All of the aforementioned characteristics are diagnostic of *Aegle marmelos* and *Cardiospermum halicacabum* leaves, respectively. Preliminary screening tests may aid in the detection of bioactive principles, which could lead to drug development and discovery. Screening experiments like these serve as a foundation for further phytochemical and pharmacological research, which could lead to the discovery of new clinically effective compounds. As a result of this research, the medicinal value of *Aegle marmelos* and *Cardiospermum halicacabum* has been established.

Acknowledgment

The authors are thankful for the financial support from Tamilnadu State Council for Science and Technology (TNSCST), DOTE Campus, Chennai (S&T Project: TNSCST/STP-PRG/AR/2018-2019), and Department of Botany, Vellalar College for Women (Autonomous), Thindal, Erode- 638012. Tamil Nadu, India and the DST–FIST Centralized laboratory, Muthayammal College of Arts and Science, Rasipuram, Namakkal DT. Tamil Nadu, India for executing this work.

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