



Phytochemical Screening of Methanolic Extract of *Aegle marmelos* and Ethanolic Extract of *Calendula officinalis*

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ABSTRACT

Medicinal plants are a major remedy for many ailments and have been used since ancient times. *Aegle marmelos* belongs to the *Rutaceae* family, known as bail, widely available in several places in India. Extracts of fruit, leaf showed antibacterial activity against all the used bacterial strains. *Calendula officinalis*, a member of the *Asteraceae* family, is an annual plant with yellow to orange flowers, mostly seen in the mediterranean region. Also known as pot marigold, it has been cultivated as a food and medicinal plant since the middle Ages. As a medicine, it has found applications in the treatment of inflammation and skin wounds. The aim of the present study was to screen the various phytochemicals from the ethanolic extracts of leaves of *Aegle marmelos* and methanolic extracts of leaves of *Calendula officinalis*. The extracts were subjected to qualitative phytochemical screening using standard procedures. Extracts of different leaves of *Aegle marmelos*, *Calendula Officinalis* were found to contain various secondary metabolites like Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids and Tannins. The phytochemicals generated data from the Extracts of different leaves of *Aegle marmelos*, *Calendula Officinalis* may be used as tools for quality control of drugs in the future, for the healing of a diversity of disease conditions.

Keywords: *Aegle marmelos*, *Rutaceae* family, *Calendula officinalis*, *Asteraceae* family, Pot marigold, Alkaloids.

INTRODUCTION

Aegle marmelos

Aegle marmelos belongs to the family *Rutaceae*. It is a slow growing medium to small size tree contains about 25 to 30 feet in height. The stem is soft, thick, a few spiny branches [1]. The subtropical fruit *Aegle marmelos* or commonly known as Bael. Though being native to northern India, widespread cultivation is found in other parts of India as well as in Pakistan, Thailand, Sri Lanka, Bangladesh and Burma. In different languages bael is named differently like Shivaphala, Bilva, in Sanskrit, Bel in Assamese Belo in Oriya, and Marathi, and Vilva marum in Tamil in India, Matoom in Thai, Bela in Spanish Be Li in Sinhalese [2]. *A marmelos* is grown in a temple as garden plant and their leaves are used to pray Lord Shiva [3]. Ripe fruit pulp of Bael are also used to prepare sweet drink which is

supposed to be effective against bacillary dysentery. The unripe fruits of Bael are reported to be useful in treating diarrhea, dysentery with spells of constipation, and stomach aches [4]. Aqueous and alcoholic extracts of *A. marmelos* leaves reported to have cardiotoxic effect. The major constituents of leaves extract were tannins, skimmianin, essential oil, sterol, triterpenoids, flavonoids and coumarins [5]. The crude extracts of *Aegle marmelos* reported widely to act as antidiabetic, anti-inflammatory and analgesic, antiulcer, antimicrobial, antihyperglycemic and antidyslipidemic, antiarrhoeal, oral hypoglycaemic, antifungal, gastric mucosal protective, antioxidant, anticancer, antiviral, radioprotective, cardioprotective, antiasthmatic, and antispermatic agent [6,7].

Calendula officinalis

Calendula officinalis commonly Known as Marigold; this annual

herb is from the Family Asteraceae, the Sunflower Family. It is made up of single, yellow to bright orange colored flowers that capture the strength and heat of the sun as it rises in the morning [8]. It is mostly seen in the Mediterranean region. Also known as pot marigold, it has been cultivated as a food and medicinal plant since the Middle Ages. As a drug, it has found applications in the treatment of inflammation and wounds of the skin [9]. Plant species have been reported to contain a wide range of chemical compounds, including carbohydrates, phenolic compounds, lipids, steroids, tocopherols, terpenoids, quinones. carotenoids have different health benefits. The major active ingredients of the plant include triterpene diol esters, saponins, and flavonoids including rutin and hyperoside [10,11]. Marigold bottle extracts have a variety of medicinal effects and are used as antiseptic, stimulant, diaphoretic and antispasmodic anti-pyretic agents. The flower fragments of the plant have antimicrobial effects on HIV. In-vitro, *Calendula officinalis* (CO) of the plant shows anti-cancer activity in a variety of tumor cells found in leukemias, fibrosarcomas, melanomas, breast, cervical, prostate, pancreas and lungs. It is also used internally for the treatment of gastritis, colitis and hemorrhoids [12]. Antibacterial activity can be found in methanolic extract whereas, antifungal activity can be found in both methanolic and ethanolic extract [13].

MATERIALS AND METHODS

Plant material

The leaves of *Aegle marmelos*, *Calendula officinalis* was Collected from the Herbal garden of Lucknow Model College of Pharmacy, Lucknow. Freshly Collected leaves of *Aegle marmelos*, *Calendula officinalis* was washed under running tap water; air dried, homogenized to fine powder, and stored in tightened light-protected containers.

Extract preparation

The leaves of *Aegle marmelos*, *Calendula officinalis* was collected, washed with water and shade dried at room temperature, then homogenized to fine powder and stored. Plant extract was prepared by the method known as Percolation. 25gm powder of both *Aegle marmelos* and *Calendula officinalis*, was taken in separate percolator. 50ml of Methanol was added in percolator containing powder of *Aegle marmelos*, 50ml of ethanol was added in a percolator containing powder of *calendula officinalis* after that both percolators were kept undisturbed for 24hrs. Repeat the step for three times. Each filtrate was collected in a separate container. The filtrate was allowed to evaporate at 60°C on water bath until completely dry extract was obtained. Extracts were stored in amber colored vial until used for experiment.

Phytochemical screening

Test of phenolic compound: Test of phenolic compound was done by lead acetate test. In this method few drops of 10% aqueous ferric chloride solution were added in 1ml sample of extract solution. Formation of blue or green colour indicated the presence of phenols (Figure 1).

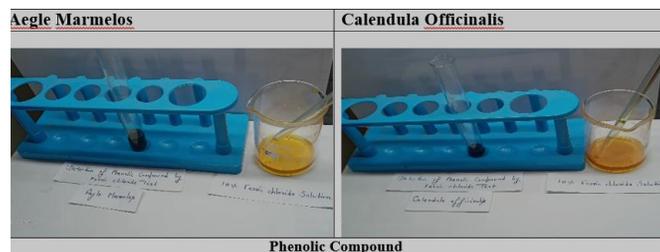


Figure 1: Phenolic compound test.

Test of Tanins: 0.25 g of extract was dissolved in 10 ml distilled water and filtered. 10% aqueous Ferric chloride solution was added in 1ml sample. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples (Figure 2).

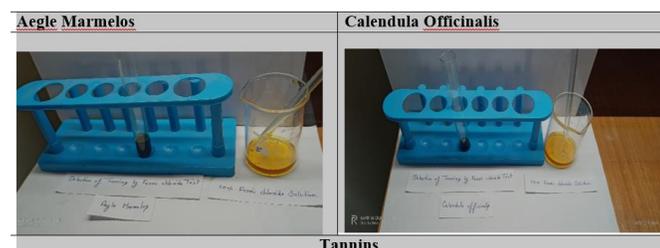


Figure 2: Tanin test.

Test of flavonoids: Flavonoid test was done by Lead Acetate test. In this test few drop of lead acetate sol was treated with 1ml of extract solution, Formation of yellow precipitate indicates the presence of flavonoids (Figure 3).

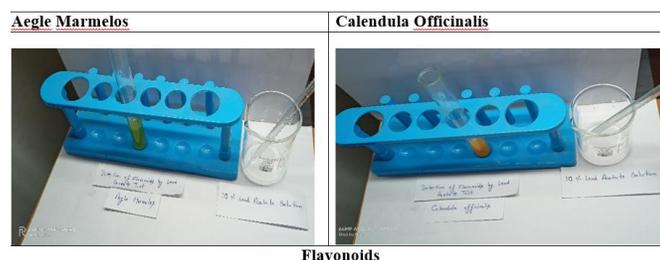


Figure 3: Flavonoid test.

Test of fixed oil and fat: Fixed oil and fat test was done by the help of spot test method. In this method small quantity of extract is placed between two filter paper, strains of oil on the filter paper shows the presence of oil/fat in the extract (Figure 4).

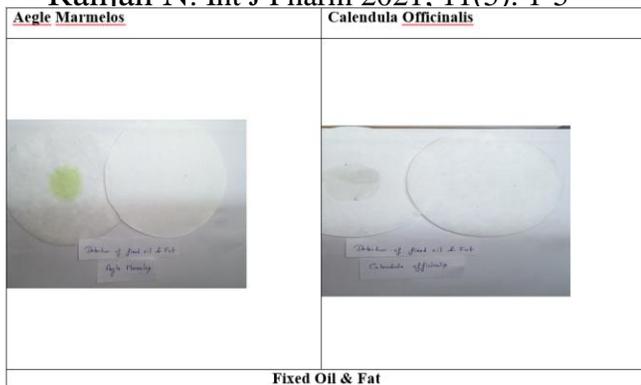


Figure 4: Test of fixed oil and fat.

Test of Saponins: Saponins test was done by the help of Froth test. In this method extract was diluted with distilled water up to 10 ml and shaken in a measuring cylinder for 15 min, formation of 1cm layer of foam indicate the presence of saponins (Figure 5).

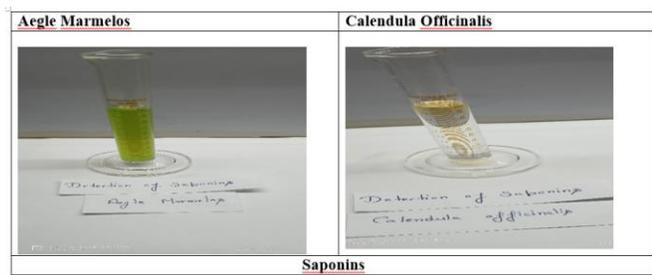


Figure 5: Saponin test.

Test of terpenoids: This test was performed by the method of Salkowski test, 5 ml of extract solvent was mixed in 2 ml of chloroform carefully add 3 ml concentrated (H₂SO₄) in it. A layer of the reddish brown colour was formed at the interface thus indicating a positive result for the presence of terpenoids (Figure 6).

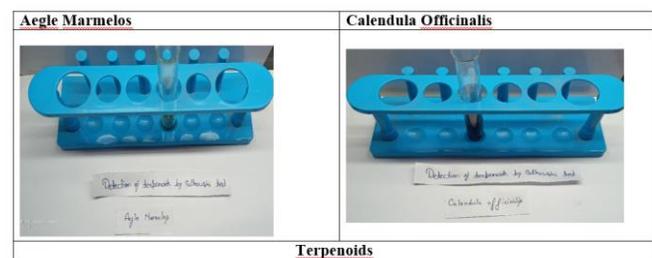


Figure 6: Terpenoid test.

Test of cardiac glycosides: This test was performed by the method of Keller killani test. 5 ml of extract solvent was taken and mixed with 2 ml of glacial acetic acid which contain one drop of ferric chloride (FeCl₃) solution, add 1 ml concentrated sulphuric acid. Brown ring was formed at the interface which indicated the presence of deoxy sugar of cardenolides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also

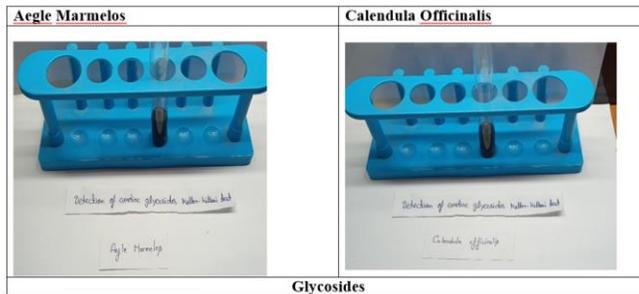


Figure 7: Glycoside test.

Test of reducing sugar: This test was performed by the method of Fehling's test. 2ml of extract was taken from each drug in separate test tube. Acidification was with dil. HCL so the sample was heated with fehling A and B solution. Formation of red precipitate indicate the presence of reducing sugar (Figure 8).

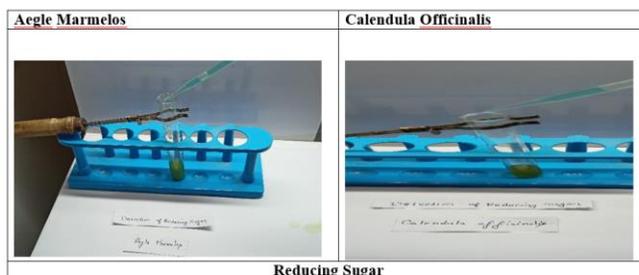


Figure 8: Reducing sugar test.

Test of protein and amino acids: This test was performed by the method of Millions test. Extract of each drug is taken in a separate test tube treat this extract with Millons reagent. After warming this sol a white precipitate is formed which is then converted in brick red colour, this indicates the presence of protein and amino acid in the extract (Figure 9).

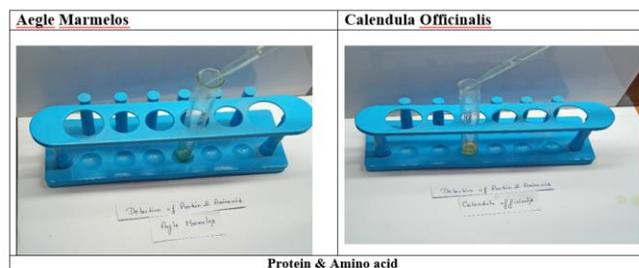


Figure 9: Protein and amino acid test.

Test of carboxylic acid: 1ml extracts was separately treated with a few ml of sodium bicarbonate solution. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acid (Figure 10).

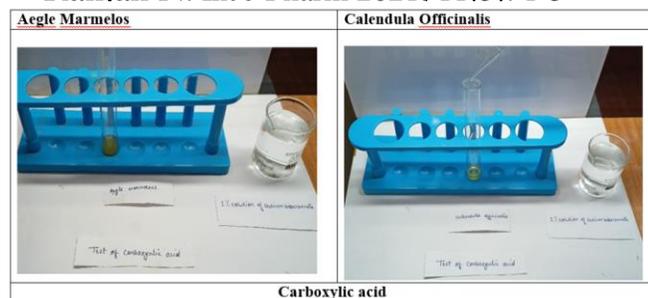


Figure 10: Carboxylic acid test.

RESULTS AND DISCUSSION

In the present investigation, preliminary phytochemical investigation of different Plant drug has been done in the different extracts, showed the presence of phytochemical constituents namely Phenolic compound, Tannins flavonoids, fixed oil and fat, Terpenoids in both extract and absence of Saponins, reducing sugar, protein and amino acids, Carboxylic Acid in both extract etc., (Table 1).

Name of Test	<i>Aegle marmelos</i>		<i>Calendula officinalis</i>
Phenolic Compound	+, +, +		+, +, +
Tannins	+, +, +		+, +, +
Flavonoids	+, -, -		+, +, +
Fixed oil and Fat	+, +, -		+, +, +
Saponins	-, -, -		-, -, +
Terpenoids	+, -, -		+, +, -
Glycosides	+, +, -		-, +, -
Reducing Sugar		-, -, -	-, -, -
Protein and Amino acid		-, -, -	-, -, -
Carboxylic acid		-, -, -	-, -, -

Table 1: The analysis of phytochemical screening of different leaves extract.

The initial phytochemical screening tests may be helpful in the screening of the bioactive compounds and eventually may help to detection and development of new drugs. Further, these tests make easy their qualitative separation and quantitative estimation of pharmacologically active chemical compounds [14]. The phytochemical screening in the present study has publicized the presence of phytochemical constituents namely Phenolic Compound,⁴

Tannins flavonoids, Fixed oil and Fat, Terpenoids in both leaves extract and absence of Saponins, reducing sugar, protein and amino acids, carboxylic acid in both leaves extract. These phytochemicals of medicinal plants have primarily reported for their medicinal value, which can be valuable for therapeutic index. saponins and glycosides proved as hypotensive and cardio depressant properties [15], which are helpful for the treatment of congestive heart failure and cardiac myopathy. Tannins have the potential of anti-hyperglycemic and anti-inflammatory activities. Moreover, the terpenoids have also been revealed to decrease blood sugar level in animal studies [16]. In addition, the steroids and terpenoids demonstrated the analgesic properties and central nervous system activities [15,16]. Hence the initial phytochemical studies are helpful in determining chemical constituents present in the plant material that may help for their quantitative assessment and also in locating the source of pharmacologically active chemical compound.

CONCLUSION

The results of phytochemical analysis showed the leaves extracts of *Aegle marmelos*, *Calendula officinalis* indicates their potential as a source of bioactive principles that may supply drugs for modern medicines. Further studies are therefore required to validate their antimicrobial, antihyperglycemic, anti-inflammatory, and anti-helminthic activities. In addition, isolation purification and characterization of the active principles are necessary to make the plant has novel interesting studies.

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