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Research Article

PHYTOCHEMICAL SCREENING AND CYTOTOXIC ACTIVITIES OF *AEGLE MARMELLOS* (Linn.) LEAF

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Abstract:

Background: Medicinal plants are the mainstay of complementary and alternative medicine. The search for new natural remedies is still ongoing process against new and existence diseases. **Aim:** The objective of this study was to evaluate the phytochemical constituents and cytotoxic activity of methanolic extracts of *Aegle marmelos* (Linn.) (MEAM) leaves and its petroleum ether fraction (PEF), chloroform fraction (CLF), ethyl acetate fraction (EAF) & aqueous fraction (AQF). **Method:** A crude methanol extract and four fractions were prepared from the leaves of *Aegle marmelos* (AM). The preparations were assessed for phytochemical analysis by saponins, tannins, glycosides, steroids and alkaloids tests and cytotoxic activity by brine shrimp lethality assay. **Results:** Phytochemical screening of CME showed the presence of saponins, tannins, glycosides, steroids and alkaloids. Glycosides and steroids were found in the PEF. CLE showed the presence of only steroids and EAF showed the presence of only glycosides. AQF was highly enriched in saponins, tannins, glycosides and alkaloids. For cytotoxic activity among the extractives, the most potent activity was found in PEF and the ED_{50} value of PEF was 24.00 $\mu\text{g/ml}$. The ED_{50} values of other extractives such as CME, CLF, EAF and AQF were 40.00, 150.00, 184.63 and 240.02 $\mu\text{g/ml}$ respectively. The ED_{50} of vincristine sulphate was found to be 1.04 $\mu\text{g/ml}$. **Conclusion:** From the study it was found that AQF of AM leave enriched in phytoconstituents and PEF had significant cytotoxicity activity compared to other extracts.

Keywords: *Aegle marmelos*, Phytochemical Screening, Cytotoxic Activity, AQF, PEF.

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INTRODUCTION

Medicinal plants are richest alternative therapeutic tools for the prevention or treatment of many diseases. According to WHO (World Health Organization), 80% of the population of developing countries presently use plant based medicines for primary health care. The study of traditional human uses of plants (ethnobotany) is familiar as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in today medicine were derived from ethnobotanical sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant [1]. Many of the pharmaceuticals currently available in the market have a long history of use as herbal remedies, including aspirin, digitalis, quinine, and opium. Plants contains a number of biologically active compounds which are used for the treatment of large number of diseases. Alkaloids, flavonoids, steroids, glycosides, terpenes, tannins and phenolic compounds are biologically active ingredients. Phytochemical screening is preliminary most important step in the detection of biologically active compound. In addition to this today the enormous traditional knowledge of medicinal plants is playing an important role in the development of new pharmaceuticals.

Aegle marmelos (AM), commonly known as bael and belonging to the family Rutaceae, is a tree widely distributed throughout Bangladesh. It is a moderate-sized, slender and aromatic tree distributed throughout Southeast Asia as a naturalized species. The bright green leaves of AM are alternate and trifoliolate (rarely five-foliolate). The medicinal properties of AM are well described in Ayurveda, traditional Indian medicine. The leaf of this plant has a folkloric reputation for promoting intellect and enhancing memory. Traditionally, the plant has been used to treat fever, diabetes, diarrhoea, dysentery, abscesses and snake bites [2]. Phytochemical investigations of AM demonstrated several active elements including marmelosin, marmelide, luvangetin, auraptene, psoralen and tannin. The extract of the plant has been reported to possess important pharmacological effects including anti-diabetic, antihyperlipidaemic, contraceptive, anti-diarrhoeal, analgesic, antipyretic and anti-inflammatory, antimicrobial and anti-proliferative effects. Fresh leaf juice is used in asthmatic complaints and jaundice [3]. The Chinese used the leaves and young fruits to adulterate Opium.

The present study was conducted to estimate the phytochemical constituents and cytotoxic activities of petroleum ether fraction (PEF), chloroform fraction (CLF), ethyl acetate fraction (EAF) & aqueous fraction (AQF) of methanolic extract of *Aegle marmelos* (MEAM) Leaf.

MATERIALS AND METHODS

Chemicals and Eggs

Methanol, ferric chloride, chloroform, ammonia, sulphuric acid, sodium chloride, dimethyl sulfoxide (DMSO) was purchased from Active Fine Chemicals Ltd., Bangladesh. The eggs of the brine shrimp, *Artemia salina*, were collected from an aquarium shop Dhaka, Bangladesh.

Collection and Identification of Plant Materials

The leaves of AM collected from the city of Rajshahi, Bangladesh, and identified by an expert taxonomist. A voucher specimen was submitted to the herbarium of the Department of Botany, Rajshahi University.

Drying and Grinding of Plant Materials

The fresh fruit of the plants were first washed with water to remove adhering dirt. Then fruits were cut into small pieces, sun dried for 9 days and finally dried in an oven at temperature not more than 50 °C for better grinding. After drying, the entire portions were ground into coarse powder by a grinding machine and stored in an airtight container for further use.

Extraction and Fractionation of Plant Materials

Powdered sample having a weight of 250 g was taken in an amber colored reagent bottle and soaked in 500 ml of 95 % methanol at 25 °C. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper. Then the filtrate was concentrated with a rotary evaporator under reduced pressure at 50 °C temperature to give crude extracts. An aliquot of the concentrated methanol extract was fractionated as described previously, 35 and the resultant soluble fractions of petroleum ether (PEF), chloroform (CLF), ethyl acetate (EAF) and aqueous (AQF) were obtained for the experiment.

Phytochemical Screening

Phytochemical screening of different plant extractives were done according to qualitative phytochemical tests [4].

Test for Saponins

For this test to 2 ml of plant extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for Tannins

For this test to 1ml of plant extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Glycosides

For this test to 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for Steroids

For this test to 1ml of plant extract equal volume of chloroform was added and subjected with few drops of

concentrated sulphuric acid appearance of brown ring indicates the presence of steroids

Cytotoxic Activity**Brine Shrimp Lethality Assay**

The cytotoxic property of plant is determined by using brine shrimp lethality bioassay [5]. For this test eggs of Brine shrimp (*Artemia salina*) were added to a tank containing 3.8% NaCl and hatched for 48 h to produce mature shrimp called naupli. 4 mg of each of the samples were dissolved in DMSO to attain various concentrations such as 10, 20, 40, 80 and 120 µg/ml by using serial dilution technique. A vial containing 50 µl DMSO that was diluted to 5 ml was

considered as control group. 10 matured nauplii in 5 ml simulated sea water were applied to each of all experimental vials and also in the control vial. Numbers of the nauplii that lived after 24 h were counted in percentage by following equation:

$$\% \text{ Viability} = \frac{(\text{Total} - \text{Dead})}{\text{Total}} \times 100$$

The findings were presented graphically by plotting log of concentration versus percentage of mortality of nauplii from which ED₅₀ was determined by extrapolation and compared with the standard, vincristine sulphate.

Statistical Analysis

Results were expressed as mean. Microsoft Excel 2010 (Roselle, IL, USA) was used for the statistical and graphical evaluations.

RESULTS AND DISCUSSION**Phytochemical Screening**

The preliminary phytochemical screening of different extractives was done to ascertain the presence or absence of bioactive components. The results from the photochemical screening of CME showed the presence of saponins, tannins, glycosides, steroids and alkaloids. Glycosides and steroids were found in the PEF. CLE showed the presence of only steroids and EAF showed the presence of only glycosides. AQF was highly enriched in saponins, tannins, glycosides and alkaloids (Table 1).

Table 1: Phytochemical Test Results of Different Extractives of AM

Phytochemical Tests	CME	PEF	CLF	EAF	AQF
Saponins	+	–	–	–	+++
Tannins	+	–	–	–	+++
Glycosides	+	++	–	++	+++
Steroids	+++	++	+	–	–
Alkaloids	+	–	–	–	++

Here, CME = Crude methanol extract, PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, AQF = Aqueous fraction. + = Present in mild amount, ++ = Present in moderate amount, +++ = Present in large amount, – = Not present.

Brine Shrimp Lethality Assay

The effect of different concentrations of vincristine sulphate (standard) and extractives on nauplii viability is presented in Figure 1. The mortality was not observed in the negative control experiment. The ED₅₀ (effective dose of the extracts at which 50% nauplii are viable) of the test samples were calculated using the concentration versus % of nauplii viability

curve of the samples. Among the extractives, the most potent activity was found in PEF and the ED₅₀ value of PEF was 24.00 µg/ml. The ED₅₀ values of other extractives such as CME, CLF, EAF and AQF were 40.00, 150.00, 184.63 and 240.02 µg/ml respectively. The ED₅₀ of vincristine sulphate was found to be 1.04 µg/ml (Figure 2).

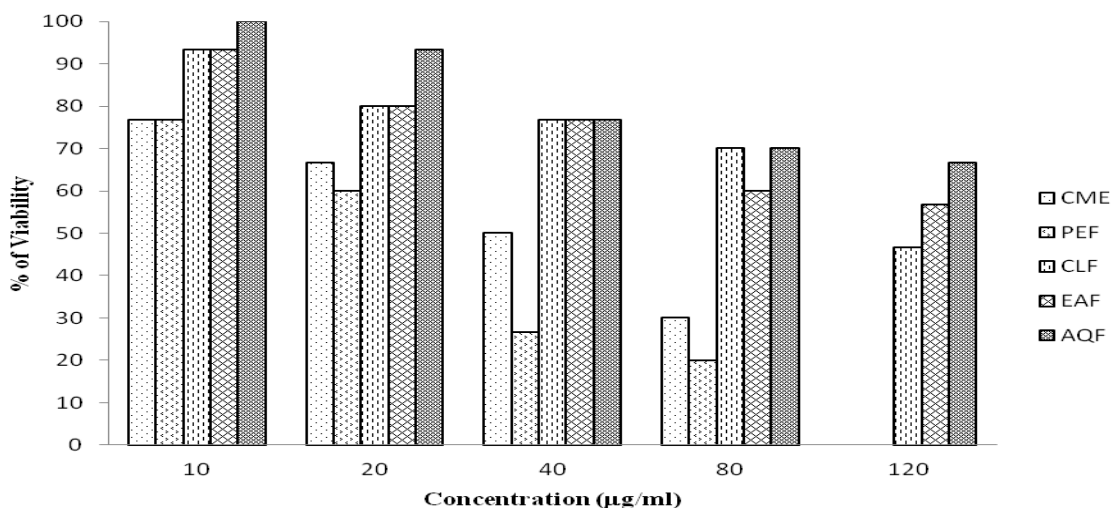


Fig. 1: Effects of CME and its Different Fractions on Various Concentrations on The Viability Of Brine Shrimp Nauplii After 24 Hrs Of Incubation. All Values Are The Mean Of Three Replicates. Here, CME = Crude methanol extract, PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, AQF = Aqueous fraction.

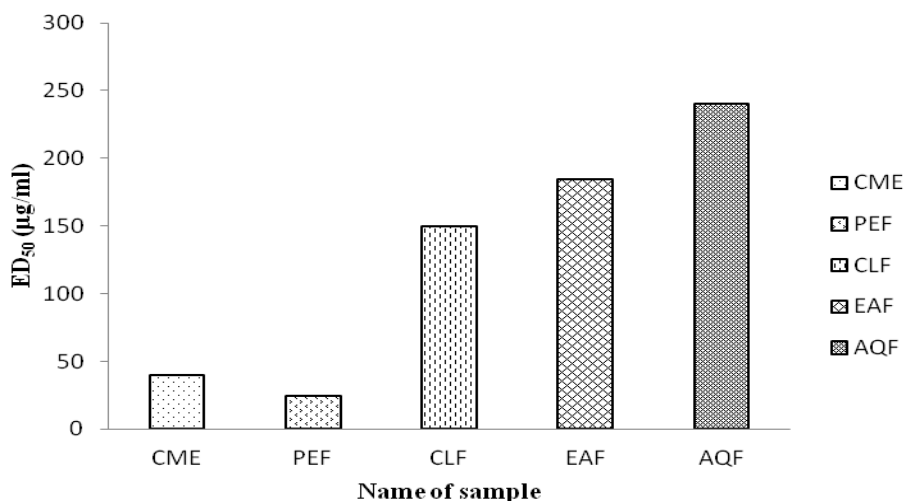


Fig. 2: ED₅₀ (µg/ml) values of CME and its Different Fractions on Brine Shrimp Viability. Here, VNS = Vincristine sulphate (Standard), CME = Crude methanol extract, PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, AQF = Aqueous fraction.

CONCLUSION

The results of this study showed that AQF of AM leaves may have role in biological activity due to presence of higher amount of saponins and tannins and PEF has significant cytotoxic activity.

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COMPETING INTERESTS

The authors proclaim that there is no competing interests exist about the content of this article.

AUTHORS' CONTRIBUTIONS

MA designed the study, wrote the protocol, managed the analyses of the study, carried out the tests, performed statistical and graphical evaluations and prepared the draft of the manuscript. The author read and approved the final manuscript.

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