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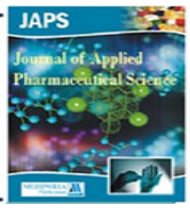

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In Vitro Antioxidant and Cytotoxicity Screening of Different Bark Extracts of *Aegle Marmelos* L

Kaiser Hamid, Farhana Diba, Kaniz Fatima Urmi, Muhammad Erfan Uddin, Fatema Tuz Zohera and Md. Razibul Habib

Kaiser Hamid, Farhana Diba, Md. Razibul Habib
Department of Pharmacy, East West University, Dhaka, Bangladesh.

Kaniz Fatima Urmi, Fatema Tuz Zohera
Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Muhammad Erfan Uddin
Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

For Correspondence
Md. Razibul Habib
Lecturer
Department of Pharmacy
East West University
Dhaka, Bangladesh.
Cell: +8801718423453

ABSTRACT

The present study was designed to investigate the antioxidant and cytotoxic activity of the methanol, ethyl acetate and n-hexane extracts of bark of *Aegle marmelos*. The antioxidant properties was assessed by using 1, 1-diphenyl-2- picrylhydrazyl radical (DPPH) and Nitric Oxide (NO) scavenging capacity. The methanolic, ethyl acetate and n-hexane bark extracts of *Aegle marmelos* showed potential DPPH free radical scavenging activity having IC_{50} values of 37.056 µg/ml, 43.379 µg/ml and 66.180 µg/ml respectively compared with the IC_{50} value of Ascorbic acid 33.447 µg/ml. The Ethyl acetate extract of *Aegle marmelos* displayed excellent antioxidant activity in nitric oxide (NO) scavenging capacity assay with IC_{50} value of 28.377 µg/ml compared with the standard ascorbic acid, IC_{50} value of 22.175 µg/ml. The cytotoxicity assay was performed by applying brine shrimp lethality bioassay method. Among all the fractions, n-hexane extract shown profound cytotoxic activity with LC_{50} value of 4.482 µg/ml that was followed by methanolic and ethyl acetate having LC_{50} values of 5.278 and 5.278 µg/ml respectively in comparison with the standard vincristine sulfate with LC_{50} value of 3.364 µg/ml. The findings of the present study suggest that the extracts from *Aegle marmelos bark* have potential cytotoxicity and antioxidant effects.

Keywords: *Aegle marmelos*, Cytotoxicity, DPPH, Antioxidant, Scavenging.

INTRODUCTION

Medicinal plants are rich sources of bioactive compounds and thus serve as an important raw material for drug production.They may constitute a valuable natural assets contribute a great deal to its health care systems. Free radicals contain one or more unpaired electrons, produced in normal or pathological cell metabolism. Reactive oxygen species (ROS) react easily with these free radicals to become radicals themselves. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals (O_2–) and hydroxyl radicals (OH•), as well as non-free radical species (H_2O_2) and the singled oxygen (O_2) (Gulcin *et al.*, 2003). In vivo, some of these ROS play an important role in cell metabolism including energy production, phagocytosis and intercellular signaling (Ottolenghi, 1959). However, these ROS produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects such as DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, aging and neuro-degenerative diseases (Gyamfi *et al.*, 2002; Osawa *et al.*, 1994; Noda *et al.*, 1997).

Fruits such as blueberry, cranberry and Sambucus nigra have been proven to be rich in flavonoids & other antioxidant properties that protect endothelial cells from oxidation, a key factor in the development of cardio-vascular diseases (Youdim *et al.,* 2002). Recent investigations have shown that the antioxidants with free-radical scavenging properties of plant origins could have great importance as therapeutic agents in aging process and free radical mediated diseases including neuro degeneration (Singh *et al.*, 1986; Singh *et al*., 1980). The use of antioxidants that scavenge ROS has been studied by evaluating its potential and therapeutic applications. The predictive value of in vitro cytotoxicity tests is based on the idea of 'basal' cytotoxicity – that toxic chemicals affect basic functions of cells which are common to all cells, and that the toxicity can be measured by assessing cellular damage. The development of in vitro cytotoxicity assays has been driven by the need to rapidly evaluate the potential toxicity of large numbers of compounds to limit animal experimentation whenever possible, and to carry out tests with small quantities of compound. *A. marmelos* belonging to the Family- Rutaceae, Subfamily- Aurantioideae, locally known as Bael, A small or medium-sized deciduous tree, armed with many axillary, straight, strong, long spines. Leaves 3-foliolate, rarely 5-foliolate. Flowers greenish-white, in short axillary panicles. It's fruit large, globose, with woody rind. Fruits are astringent, digestive, tonic, stomachic, laxative and is belived to be an invaluable remedy in obstinate cases of chronic diarrhoea and dysentery and in loss of appetite. Unripe fruit is used in diarrhoea, dysentery and ripe fruit for constipation. Dried slice of unripe fruit is regarded as astringent, digestive and stomachic, and is prescribed in diarrhoea and dysentery. The unripe fruit burnt in fire is taken in empty stomach for the treatment of chronic dysentery. Fresh leaves are astringent, digestive, laxative and febrifuge; useful in ophthalmia and inflammations; fresh juice is very useful in catarrh and feverishness; with black pepper it is given in anasarca and jaundice. Root bark is useful in hypochondriasis, melancholia and palpitation of heart. Seed extracts and crude alkaloids posseses moderate antifungal and antibacterial properties. Seed oil possesses antibacterial properties. Ethanol (50%) extract of the fruits and roots are hypoglycaemic and spasmogenic. Alkaloid present in leaves is efficacious against asthma. Leaf oil possesses broad spectrum antifungal properties

MATERIALS AND METHODS

Collection and identification of plant

The fresh bark part of the plant *Aegle marmelos* were collected from the area of Mymensing district during the month of February, 2011.The *Aegle marmelos w*as taxonomically identified by The National Herberium. The accession number of *Aegle marmelos* is 35486.

Plant material

The collected bark were washed thoroughly water, chopped, air dry for a week at 35-40^{0}C & pulverized in electric grinder. The powder obtained was successively extracted in methanol (55-60^{0}C). The extracts were the made to powder by using rotary evaporator under reduced pressure. Then we performed fractionation of crude extract & prepared methanol, ethyl acetate and n-hexane extracts of *Aegle marmelos* bark.

AMME = Methanol extract of *Aegle marmelos*
AMEA = Ethyl acetate extract of *Aegle marmelos*
AMNH = n-Hexane extract of *Aegle marmelos.*

Chemicals and drugs

DPPH (1, 1-diphenyl, 2-picrylhydrazyl), was obtained from sigma chemical co. USA. Ascorbic acid was obtained from SD Fine chem. Ltd., Biosar, India. Naphthyl ethylene diamine dihydrochloride was parched from Roch-light Ltd., Suffolk, England. Sodium nitroprusside was obtained from Ranbaxy Lab., Mohali, India.

DPPH radical scavenging activity

The free radical scavenging capacity of the extracts was determined using DPPH (Hasan *et al*, 2006; Alam *et al*, 2008). The methanol DPPH solution (0.004% w/v) was mixed with serial dilutions (0 to 100 µg) of extracts and after 10 min; the absorbance was read at 515 nm using a spectrophotometer. Ascorbic acid was used as a standard. The inhibition curve was plotted and IC_{50} values were calculated.

Nitric oxide scavenging assay

Nitric oxide radical scavenging was estimated on the basis of Griess Illosvoy reaction (Govindarajan *et al.*, 2003). In this investigation, Griess-Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/ v) instead of 1-napthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and *Aegle marmelos* extracts (5 to 200 µg/ml) or standard solution (ascorbic acid, 0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink colored chromophore formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions.

Cytotoxicity assay

Brine shrimp lethality bioassay was used for probable cytotoxic action (Meyer et al, Persoone, 1988) . The eggs of Brine shrimp (Artemia salina Leach) were collected and hatched in a tank at a temperature around 37°C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solutions of the sample were prepared by dissolving required amount of extracts in specific volume of pure dimethyl sulfoxide (DMSO) and 4 ml of seawater was given to each of the vials. Then specific volumes of sample were transferred from the stock solution to the vials to get final sample concentrations of 10, 25, 50, 250, 500 and

1000 µg/ml. In the control vials same volumes of DMSO (as in the sample vials) were taken. With the help of a Pasteur pipette 10 living nauplii were put to each of the vials. After 24h the vials were observed and the number of nauplii survived in each vial was counted. From this, the percentage of lethality of Brine Shrimp nauplii was calculated for each concentration of the extract.

RESULT AND DISCUSSION

DPPH radical scavenging activity

The DPPH radical scavenging activity of *A. marmelos* bark extracts shown in figure 1. Analyzing the figure we can see that by increasing the concentration of the extracts, activity were increased simultaneously whereas the IC_{50} values of methanolic and ethyl aetate extracts were found profound activity 37.056 µg/ml and 43.379 µg/ml respectively near to the standard ascorbic acid 33.447 µg/ml. On the other hand, the n-hexane extract of the plant given potential result 66.180 µg/ml.

Nitric oxide scavenging assay

The different extracts of the *A. marmelos* bark exhibited good result upon increasing the concentration of the plant extracts (Figure 2). The Ethyl acetate extract having an IC_{50} value of 28.377 µg/ml shown excellent result. On the other hand, n-hexane and methanolic extracts shown profound activity with an IC_{50} value of 45.853 µg/ml and 66.915 µg/ml respectively compared to 22.175 µg/ml that was the IC_{50} value for the reference ascorbic acid.

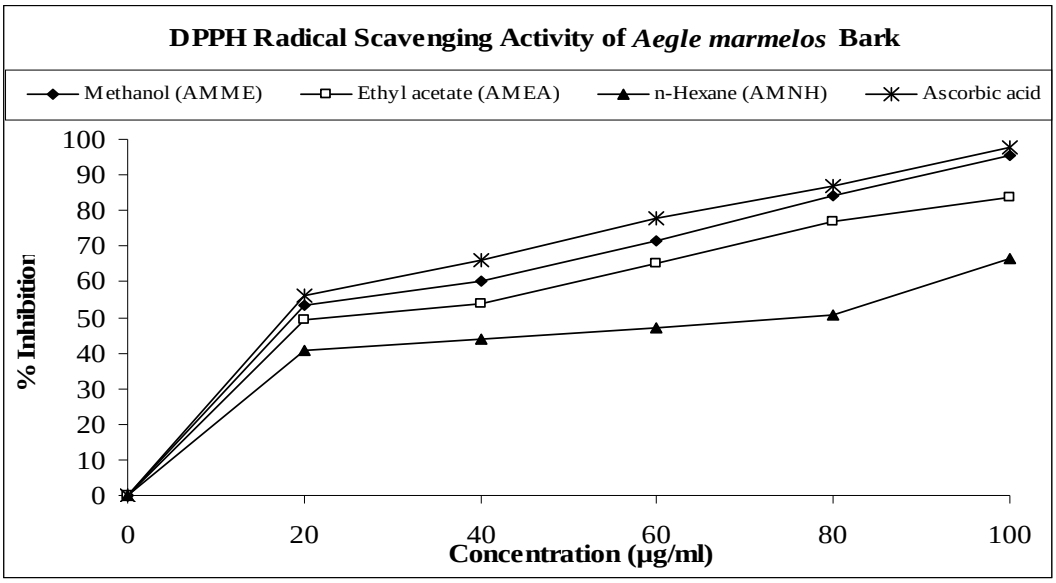

Fig. 1: DPPH scavenging activity of different extracts of bark of the *A. marmelos* and ascorbic acid.

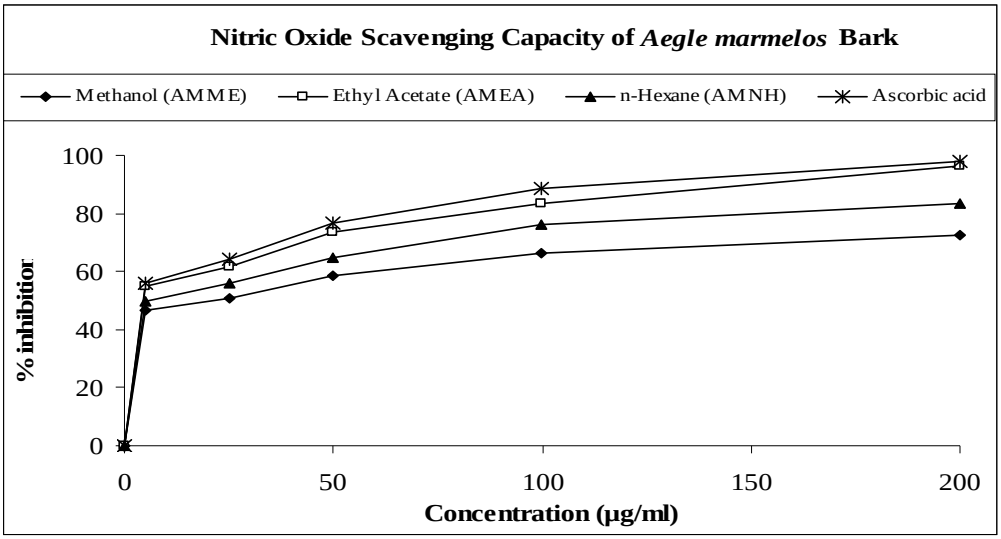

Fig. 2: NO scavenging activity of different extracts of bark of the *A. marmelos* and ascorbic acid.

Brine shrimp lethality bioassay

The LC_{50} values of different fractions of bark extracts of *Aegle marmelos* shown concentration dependent bioactivity (Table 1). The samples of methanolic, ethyl acetate and *n*-hexane extract of bark had the LC_{50} value of 5.278µg/ml, 5.278µg/ml and 4.482µg/ml respectively while vincristine sulfate had LC_{50} value of 3.364 µg/ml indicating the presence of potential bioactive substances. The n-hexane extract of bark showed highest cytotoxic activity having LC_{50} value of 4.482 µg/ml and the order of cytotoxicity is – AMNH > AMME = AMEA.

Table. 1: Cytotoxic activity of three different extracts of bark of the *Aegle marmelos.*

	Conc (µg/ml)	Log C	% mortality	LC_{50} (µg/ml)
AMME	10	1.000	30	5.278
	25	1.398	80	
	50	1.699	100	
	250	2.398	100	
	500	2.699	100	
	1000	3.000	100	
AMEA	10	1.000	30	5.278
	25	1.398	80	
	50	1.699	100	
	250	2.398	100	
	500	2.699	100	
	1000	3.000	100	
AMNH	10	1.000	20	4.482
	25	1.398	90	
	50	1.699	100	
	250	2.398	100	
	500	2.699	100	
	1000	3.000	100	

DISCUSSION

The relationship between diseases and free radicals have been proved by many studies. UV light, radiation, smoking, alcohol consumption tress and high cholesterol consumption can increase the process of cell oxidation (Kohen *et al*., 2002). This study aimed to establish a platform for in vitro evaluation of antioxidant capacity of herbal plants**.** The DPPH results indicate that the antioxidant properties of AMME shown more effective than AMEA and AMNH. In Nitric oxide scavenging assay, AMEA was more potent & effective than AMNH & AMME. So, it can be inferred that the antioxidant properties of *Aegle marmelos* different extracts is more potent & indicate the presence of potential bioactive substances that may help to prevent diseases (Burns *et al.*, 2000). In Brine shrimp lethality bioassay test, the extracts produced concentration dependent increment in percent mortality (table- 1). Here, AMNH showed very potent lethality whereas AMME & AMEA also showed very good lethality. Thus, the brine shrimp lethality bioassay is considered primary indications of cytotoxic action of *Aegle marmelos* different extracts is more potent & contain potential bioactive substances may have potential to tumor and cancer suppression.

CONCLUSION

The study indicate that the plant part contain potential bioactive compounds, which if properly and extensively studied, could provide many chemically interesting and biologically active drug candidates, including some with potential antioxidant, antitumor and antiproliferative properties. A thorough chemical study is required to isolate the molecules that are responsible for the activities as well pharmacological studies also to understand the mechanism of action.

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