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In vitro Antioxidant, Antimicrobial, Insecticidal and Cytotoxic Activities of the Medicinal Plants: *Allamanda cathartica* and *Mimusops elengi*

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAM designed the study, wrote the protocol and wrote the first and final draft of the manuscript. Authors MSA and FM performed the extraction, fractionation and biological studies. Authors MKEZ, RA, ABMHH and SZ revised the manuscript. Author DT performed the statistical analysis of the biological studies. All authors read and approved the final manuscript.

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ABSTRACT

A comparison of *in vitro* antioxidant, antimicrobial, insecticidal and cytotoxic activities of two medicinal plants, *Allamanda cathartica* and *Mimusops elengi* have been studied by spectrophotometric and disk diffusion methods in this research. It was observed that different leaf extract of *A. cathartica* exhibited less antioxidant activity in comparison to that of the extract of *M. elengi*. The highest antioxidant activity (4.588±0.001 μg/mL) was found in the dia-ion resin adsorbed fraction of *M. elengi* and the least activity (1.82±0.002 μg/mL) was found in the methanolic fraction of *A. cathartica*. From the results of antimicrobial activity test, it was observed that the petroleum ether fraction of *A. cathartica* showed the maximum inhibition with the diameter

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of 16–20 mm while the ethyl acetate fraction of *M. elengi* showed the least inhibition with the diameter of 10–16 mm. The insecticidal activity against the pathogenic bacteria *Tribolium castaneum* of *A. cathartica* was very pronounced with the LD_{50} values of 225.205 μg/cm^2 where as *M. elengi* showed the least and/or no insecticidal activity. All the extracts such as methanol, petroleum ether, chloroform, ethyl acetate and dia-ion-resin adsorbed fraction of both *A. cathartica* and *M. elengi* displayed higher level of toxicity towards brine shrimps lethality bioassay. Based on the results obtained, the *A. cathartica* and *M. elengi* leaf extracts could serve as the potential source of natural antioxidant, antimicrobial and anticancer agents. Also they could be used for the treatments of different oxidative disorders, infection diseases caused by the resistant microorganisms and cancer cells, respectively.

Keywords: Allamanda cathartica; Mimusops elengi; antioxidant; antimicrobial; insecticidal activity; cytotoxicity; microorganism; cancer cells.

1. INTRODUCTION

Intense pharmacological researches have been carried out on medicinal plants since the last few decades. This is because they are regarded as a potential source of new compounds of therapeutic values as well as leading compounds in the drug development strategies. The World Health Organization (WHO) estimated that about 80% of the population of the developing countries relies on traditional medicine for their primary healthcare [1]. Despite the great advances observed in modern medicine, plant kingdom is still making an important contribution to health care and there is need to screen bioactive compounds for further pharmacological studies [2]. It is well known that medicinal plants are rich sources of secondary metabolites with interesting biological activities. In general, these secondary metabolites are important sources with varieties of structural arrangements, biological activities and sources of antibiotics. Therefore, screening of the bioactive compounds from medicinal plants becomes very significant [3,4].

Allamanda cathartica, locally known in Bangladesh as “Malotilota”, is a tropical shrubs that belongs to the family *Apocynaceae*. Due to its rapid growth, pruning is often necessary, which can expose gardeners to the toxic sap that causes dermatitis symptoms of rash, blisters, and itch. Although incidence is much less and plant parts are toxic if ingested. All parts of *A. cathartica* contain toxic iridoid lactone, allamandin [5]. The bark, latex and the infusion of its leaves in small doses are cathartic. In Guyana, its latex is employed as a purgative and relieving colic. It has also been implicated in the treatment of malaria and jaundice [6]. On the other hand, *Mimusops elengi,* locally known as “Bakul” which belongs to the *Sapotaceae* family. The plant *M. elengi* has been frequently used for the treatment of chronic dysentery, antipyretics and also been used as analgesic [7,8].

Considering the importance and great pharmaceutical values, this study focused on the screened since not many studies on the isolation of their bioactive compounds had been carried out. In the earlier studies, heavy metals and minerals contents in *A. cathartica* and *M. elengi* were determined and also reported [9]. In this work, a comparative study of *in vitro* antioxidant, antimicrobial, insecticidal and cytotoxic activities were presented in the following sections.

2. MATERIALS AND METHODS

All the chemicals used in this study were of analytical grade. Methanol, acetone, petroleum ether, ethyl acetate were purchased from Merk, Germany and Fluka. Before use, all the solvents were further purified by distillation.

2.1 Plant Collection and Extraction Process

The leaves of *A. cathartica* and *M. elengi* were picked from the tree in the first period of the day from Talaimari region of the Rajshahi City Corporation area, Bangladesh during the period of May. The images and analyzed parts of the plants are shown in Fig. 1. The species of the plants were authenticated by one of the Professors at the Department of Botany, Rajshahi University. The collected raw leaves (7.35 Kg) were washed with distilled water and dried at ambient temperature (37–40°C) for the period of 15 days. After drying, the dried leaves (2.75 Kg) were milled separately in an electric grinder and kept in an airtight container at room temperature before extraction process. The ground powder (~ 2.70 Kg) of each *A. cathartica* and *M. elengi* were put into a polyvinyl drum which was previously loaded with methanol and

was kept overnight. Each extract from *A. cathartica* and *M. elengi* was filtered separately through a Whatman filter paper and was concentrated under reduced pressure at 45ºC using Buchi Rotavapor (R-200). Each obtained concentrated extract was designated as crude extract (~550 g). The crude methanol extract was then subjected into water for separating water soluble triturate. The water soluble triturate was passed through dia-ion resin column and consequently 100% methanol was flown through the resin. Finally, the deep violet portion was collected and was named as dia-ion resin adsorbed fraction. The residual water insoluble gummy part was subjected into petroleum ether, ethyl acetate, and chloroform to get the designed fractionates as petroleum ether, ethyl acetate and chloroform fraction, respectively.

2.2 Phytochemical Screening

Qualitative phytochemical screening of the different extractives of *A. cathartica* and *M. elengi* was carried out with a little modification according to the methods [10,11]. These tests are usually based on visual observation of color or precipitate formation after addition of specific reagents. A 0.5 g of the extractives was dissolved in distilled water and subjected to qualitative phytochemical tests for alkaloids, glycosides, tannins, steroids, and saponins. The results are shown in Table 1.

2.3 Determination of Total Phenolic and Flavonoid Content

Total phenolic and flavonoid contents were determined by modified Folin–Ciocalteu reagent and aluminum chloride colorimetric method, respectively [12,13]. A 2.5 mL of folin-ciocalteu reagent and 2.0 mL of Na_2CO_3 (7.5% w/v) solution was added to a 0.50 mL of *A. cathartica and M. elengi* extract (2.0 mg/mL). It was vortexed to make homogeneous mixture and then allowed to stand for 20 min. at ambient temperature. The absorbance was estimated at 760 nm with UV-vis spectrophotometer (Shimadzu UV-1609, Japan). A blank experiment was performed and a gallic acid standard curve with varying concentrations was constructed by plotting gallic acid concentration on the abscissa and absorbance on ordinates for quantification of total phenolic value. The phenolic value was obtained from the regression equation: $y=0.0088x + 0.005$ with $R^2=0.9999$ and expressed as mg/g gallic acid equivalent using the following equation.

$$C = (c \times V)/ M \quad (1)$$

where, C is concentration of total phenolic compounds in mg/g GAE, c is the concentration of gallic acid, V is the volume of extract, and M is weight of the extract.

Fig. 1. Images of the plant: (a) *Allamanda cathartica* (b) *Mimusops elengi*. Inside figure of the images are fresh, dried and ground leaves of the plants. The grounded leaves are used for the extraction process

In the case of flavonoid determination, the sample solution was made in a test tube where 0.5 mL of *A. cathartica* and *M. elengi* extracts were placed and then the following reagents were added sequentially. A 3.0 mL of methanol, 200 μL aluminum chloride (Sigma-Aldrich, USA,10%), 200 μL 1 M potassium acetate (Merck, Germany), and finally 5.6 mL distilled water. The test tube was then incubated at room temperature for 30 min. A blank experiment was performed in the same manner where distilled water was used instead of sample and aluminum chloride. The absorbance was taken at 420 nm. A standard curve with varying concentrations was constructed by plotting catechin concentration on the abscissa and absorbance on ordinates for quantification of total flavonoid values. The flavonoid value was obtained from the regression equation: $y=0.0052\ x + 0.0853$ with $R^2=0.9978$ and it is expressed as mg/g catechin equivalent using the equation (1). Detail experimental procedure has been reported according to [14].

2.4 Determination of Antioxidant Properties

The antioxidant capacities were determined by measuring total antioxidant, reducing power and DPPH radical scavenging activity test by the modified method described here [15–17]. A 1.0 mg of the pure compound with different concentrations was poured into a test tube. A 3.0 mL of reaction mixture containing 0.60 M sulphuric acid (Merck, Germany), 28.00 mM sodium phosphate (Sigma, USA) and 1% ammonium molybdate (Sigma, USA) were added into the test tube. The test tube was incubated at 95°C for 10 min. to complete the reaction. Then the absorbance of the solution was measured at 695nm by spectrophotometer (Shimadzu, Japan). The results were plotted as mean ±STD.

2.5 Determination of Reducing Power Capacity

The ferric reducing/antioxidant power capacity of *A. cathartica and M. elengi* extracts were determined by the method of [17]. The sample solution containing 5, 10, 20, 40, and 50μg/mL of separate extract was taken in a test tube and then 2.5mL phosphate buffer 0.2M (Sigma-Aldrich, USA), 2.5 mL potassium ferricyanide (Merck, Germany) were added. The reaction mixture was incubated at 50°C for 20 min to complete the reaction. Then 2.5 mL trichloroacetic acid (Merck, Germany) was added and the reaction mixture was centrifuged at 3000 rpm for 10 min, a 2.5 mL supernatant solution was withdrawn from the mixture and it was diluted with 2.5 mL of distilled water. Finally, 0.5 mL ferric chloride (Sigma, USA) solution was added in the supernatant solution. The absorbance of the solution was taken at 700 nm wavelength region, while the ascorbic acid (Sigma, USA) was used as standard.

2.6 DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) Radical Scavenging Assay

The DPPH (Sigma, USA) method was used to evaluate the free radical scavenging activity of the extracts *A. cathartica* and *M. elengi* [17]. A 1.0 mg of different concentrations, 3.0 mL of methanol (Sigma, USA) and DPPH were poured into a test tube. The test tube was incubated at 37°C for 30 min. in dark place to complete the reaction. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against a blank experiment. The blank solution contained all reagents except the plant extract. The percentage (%) inhibition activity was calculated from the following equation:

$$\% I = \{(A_o - A_1)/A_o\} \times 100 \qquad (2)$$

Where, A_o is the absorbance of the control and A_1 is the absorbance of the extract/standard. Then % inhibitions were plotted against concentration and from the graph the IC_{50} value was calculated. Here, (BHT) *tert*- butyl-1-hydroxytoluene was used as standard.

2.7 Antimicrobial and Insecticidal Activity Test

The antibacterial activity was determined by standard disc diffusion method by measuring the zone of inhibition and compared to that of the standard disc [18]. Four pathogenic bacteria such as: (i) *Bacillus subtilis* (ii) *Staphylococcus aurius* (iii) *Proteus volgaris* (iv) *Escherichia coli* have been used in determining the activity test. On the other hand, *Tribolium castaneum* and *Tribolium confusum* larvae were used for the insecticidal activity test. The mortality percentage was corrected by using the Abbott's formula [19]. The detail experimental procedures such as sterilization, preparation of fresh culture, preparation of sample and standard discs, preparation of the food medium, collection of eggs, determination of larval instars etc. have been reported by [14,20].

2.8 Cytotoxicity Bioassay

Brine shrimp lethality bioassay was used for the probable cytotoxic activity according to the methods [21,22]. The eggs of brine shrimp (*Artemia salina*) were collected from the aquarium shop of Kalabagan, Dhaka, Bangladesh and hatched in a small artificially partitioned tank with constant oxygen supply at temperature around 37°C. The artificial sea water containing 3.8% of sodium chloride was made by dissolving 38 g sodium chloride in 1000 mL distilled water. The pH of the brine water was at 8~9. In the partitioned tank, the eggs were hatched in the darkened side while the other part was under sunlight. With the help of light illumination, the larvae (nauplii) were attracted to one side of the tank and were easily collected from the non-hatched eggs. One day old matured nauplii were used for the experiment. The plant extracts dissolved in dimethyl sulfoxide (DMSO) were added into each vial to make the final concentration of 800, 400, 200, 100, 50 and 25 ppm. Each concentration was tested in triplicate. The controls were prepared in same manner in DMSO without the extracts. Typical 30 shrimp nauplii were used as negative control group. When the nauplii in the control showed a rapid mortality, then the test was considered to be invalid due to reasons other than the cytotoxicity of the test compounds. After 30 h treatment, the number of survivor was counted by magnifying glass and the percentage of death and LD_{50} were calculated by probit analysis [23]. The mortality percentage was corrected by using the Abbott's formula [19].

$$P_t = \frac{(P_o - P_c)}{(100 - P_c)} \times 100 \quad (3)$$

Where, P_t = corrected mortality%, P_o = observed mortality %, and P_c = control mortality %.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Contents

The phytochemical results from the extracts are presented in Table 1 which showed the presence of medicinally active secondary metabolites such as alkaloids, glycosides, tannins, steroids, saponins with the different fractionates of *A. catharita* and *M. elengi* extracts. All fractionates contained moderate amount of alkaloids while ethyl acetate fraction contained a large amount of the glycosides. These different groups of compound might be responsible for the antioxidant, antimicrobial, insecticidal and cytotoxic activities [24,25].

3.2 Total Phenolic and Flavonoid Content

Total phenolic and flavonoid contents of the different fractions of *A. cathartica* and *M. elengi* are shown in Fig. 2. It has been observed that the phenolic content was high in the extract of *M. elengi* in comparison to that of *A. cathartica* extract. Among the four fractions, the phenolic content was highest in the ethyl acetate fraction of *M. elengi* (36.55±0.01 mg/GAE) whereas a little amount of polyphenol was observed in the extract of *A. cathartica* (0.1239±0.001 mg/GAE). Also the highest content of phenolic component was found in the dia-ion-resin adsorbed fractions 34.78±0.01 mg/GAE of *M. elengi* while *A. cathartica* had 0.1653±0.001 mg/GAE. The phenolic content in the crude methanol, chloroform and petroleum ether fractions of *M. elengi* were found to be 22.17±0.02 mg/GAE, 11.34±0.02 mg/GAE, and 6.49±0.01 mg/GAE, respectively. The minimum values of phenolic content in the crude methanol, chloroform and petroleum ether fractions of the *A. cathartica* leaf extract were found to be 0.0899±0.001 mg/GAE, 0.1321± 0.001 mg/GAE, and 0.0726±0.002 mg/GAE, respectively.

Table 1. Qualitative phytochemical analyses results for different extractives of *Allamanda cathartica* and *Mimusops elengi*

Phytochemicals	*Allamanda cathartica*				*Mimusops elengi*			
	PE	ME	EA	CL	PE	ME	EA	CL
Alkaloids	+	+	+	+	+	+	+	+
Glycosides	-	-	+++	++	+	+	+	+
Tannins	-	-	+	+	-	-	+	+
Steroids	+	+	+	+	+	+	+	+
Saponins	+	+	++	-	+	+	+++	-

Key: "+" = mild amount, "++" = moderate amount, "+++" = large amount, " –" = not present.
PE=Petroleum ether, ME=Methanol, EA=Ethylacetate, CL=Chloroform

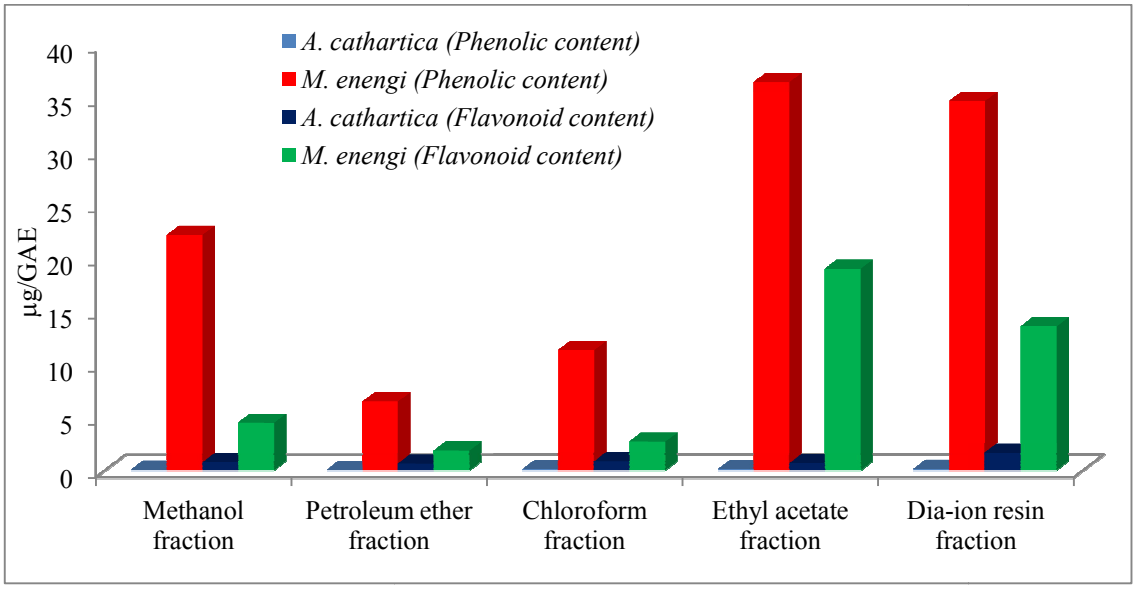

Fig. 2. Total phenolic and flavonoid contents of four different fractionates of the leaf extract of *Allamanda cathartica* and *Mimusops elengi*

Like phenolic component, the highest flavonoid content (18.97±0.11 mg/GAE) was also found in ethyl acetate fractionates among the four different extract of *M. elengi* leaf. The *A. cathartica* leaf extract contained the highest flavonoid in the dia-ion resin adsorbed fractionate (1.678±0.004 mg/GAE). However, this amount was low in comparison to that of the ethyl acetate frationante of *M. elengi*. The result of the flavonoid content in *M. elengi* extract followed the order of dia-ion resin adsorbed (13.55±0.13 mg/GAE)>crude methanol (4.48±0.05 mg)>chloroform (2.71±0.10 mg/GAE)>petroleum ether (1.86±0.13 mg/GAE) fractionates. On the other hand, the result of the flavonoid content in *A. cathartica* followed this trend acetone (0.8464±0.006 mg/GAE)>methanol (0.7521±0.006 mg/GAE)>and ethylacetate (0.6582±0.004 mg/GAE) fractionate.

3.3 Total Antioxidant Activity

Total antioxidant activities of the four different fractions of *A. cathartica* and *M. elengi* as well as the standard catechin are shown in Fig. 3 (a–b). The highest antioxidant activity was observed in the dia-ion resin adsorbed fraction (4.588±0.001) of *M. elengi* while the ethyl acetate fraction showed the highest (3.55±0.001**)** activity of *A. cathartica* in comparison to that of the standard catechin. The total antioxidant activity of different extractives of *M. elengi* and the standard exhibited the following order: Catechin>dia-ion resin (4.588±0.001)>ethyl acetate (4.521±0.002)>methanol (3.518±0.001)> chloroform (3.50±0.001)>petroleum ether (2.68±0.003). Total antioxidant activity of different extractives of *A. cathartica* and standard exhibited the following order: Catechin>petroleum ether (3.644±0.012)> chloroform (3.35±0.001)> ethyl acetate (3.31±0.001) >methanol (1.82±0.002).

3.4 Reducing Power Capacity

The iron reducing power capacity of the five different extractives such as petroleum ether, chloroform, ethyl acetate, and dia-ion resin adsorbed fraction of *A. cathartica* and *M. elengi* as well as the standard ascorbic acid are shown in Fig. 4(a–b). Among the four different extractives, the dia-ion resin adsorbed fraction of *M. elengi* showed the highest iron reducing capacity with the absorbance of 1.107+0.002 at 100 µg/mL concentration, followed by ethyl acetate 0.696±0.003, methanol 0.687±0.001, petroleum ether 0.72±0.001, and chloroform fraction 0.246±0.001 at 100 µg/mL concentration (Fig. 3a). The reducing power of the different extractives and standard exhibited by the following order: Ascorbic acid>dia-ion resin adsorbed>ethyl acetate> methanol>chloroform>petroleum ether.

Reducing power capacity of the different fractionates of *A. cathartica* was very weak as

compared to that of the standard ascorbic acid as well as the *M. elengi* fractionates (Fig. 3b). The activity was found in increasing trend with increasing concentration of the plant extracts. However, dia-ion resin fraction displayed the highest reducing power among the fractions. Different extractives and standard exhibited the following order of the reducing power capacities: Ascorbic acid>dia-ion resin adsorbed>petroleum ether>chloroform>ethyl acetate fractions.

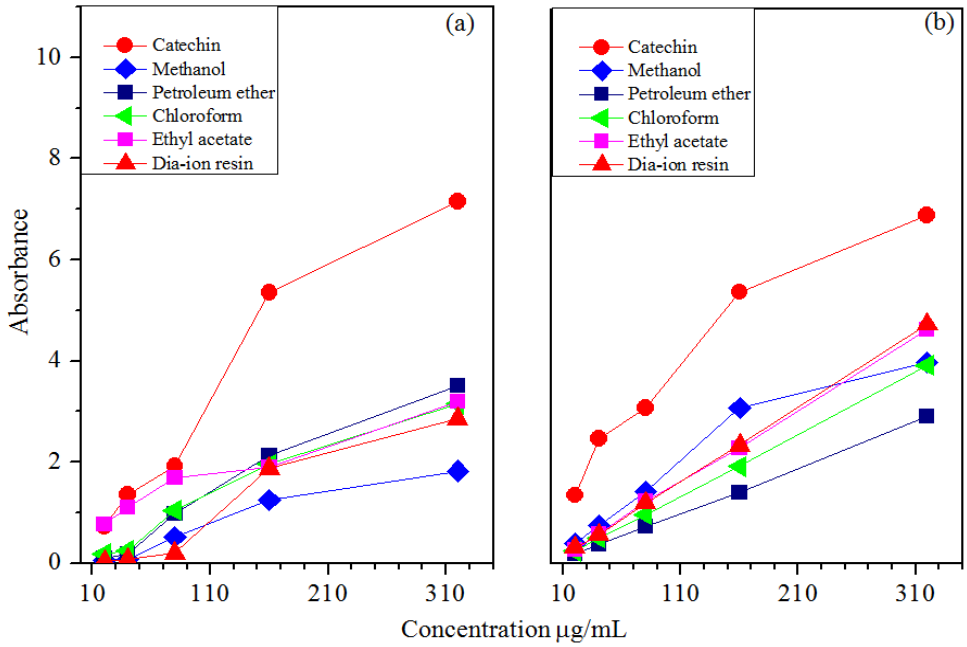

Fig. 3. Total antioxidant properties: (a) leaf extract of *Allamanda cathartica* (b) leaf extract of *Mimusops elengi*

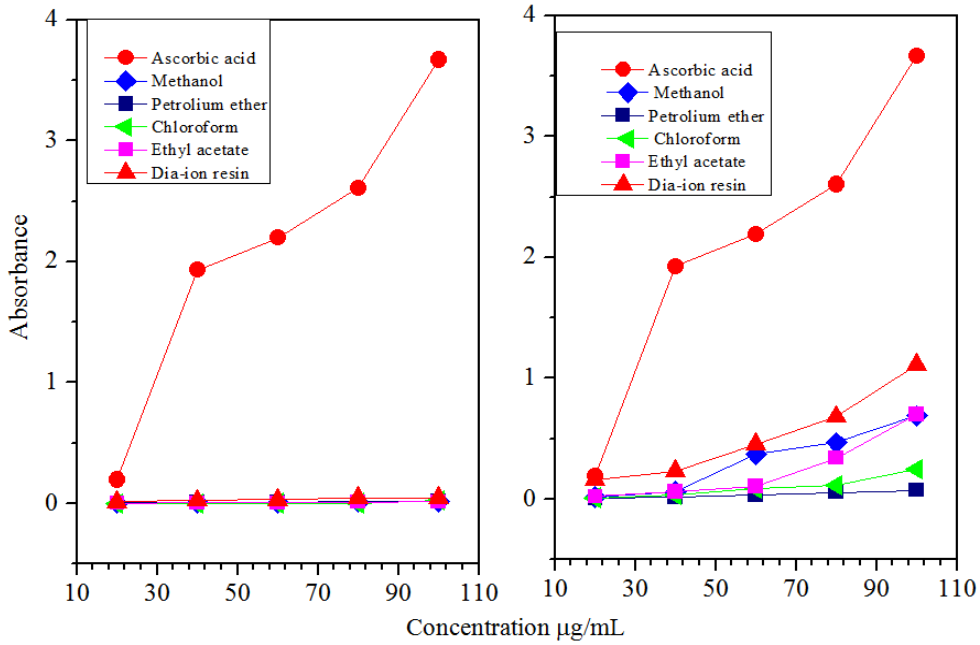

Fig. 4. Iron reducing power capacities: (a) leaf extract of *Allamanda cathartica* (b) leaf extract of *Mimusops elengi*

3.5 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of four different extractives of *A. cathartica* and *M. elengi* are shown in Fig. 5. It was observed that the DPPH radical scavenging activity is comparable to that of the standard butylated hydroxyl toluene (BHT). Among the four extractives of *M. elengi*, the highest DPPH radical scavenging activity was found in the dia-ion resin adsorbed fraction having the IC_{50} value of 31.80 μg/mL. On the other hand, the chloroform fraction showed the activity with the IC_{50} value of 32.21 μg/mL, followed by ethyl acetate with IC_{50} value 38.60 μg/mL, methanol with IC_{50} value 38.94 μg/mL and petroleum ether fraction having the value of IC_{50} value 45.00 μg/mL. It is clear from the result that all the extractives of *M. elengi* possess DPPH radical scavenging activity and the activity increases in the order of: BHT>dia-ion resin adsorbed>chloroform>ethyl acetate>crude methanol>petroleum ether. In *A. cathartica* the maximum radical scavenging activity was also shown by the dia-ion resin adsorbed fraction with the IC_{50} value of 55.76 μg/mL. The minimum scavenging activity was shown by the petroleum ether fraction with the IC_{50} value of 387.06 μg/mL. The DPPH radical scavenging activity of the extractives of *A. cathartica* has been arranged in the following descending order: BHT>dia-ion resin adsorbed>chloroform>ethyl acetate>crude methanol> petroleum ether fraction.

From the results, it has been observed that the dia-ion resin adsorbed fraction of both the *A. cathartica* and *M. elengi* showed the highest total phenolic content, total flavonoid content, total antioxidant activity, iron reducing power capacity and DPPH radical scavenging activity. Although, the properties are significantly different from each other of the two analyzed medicinal plants. From the observation, it is assumed that the dia-ion resin would be a good adsorbent for separating the polyphenols which are potent anti-oxidant and negatively correlated to IC_{50} of DPPH radical scavenging.

3.6 Antimicrobial Assay

The antimicrobial assay was performed on the four different fractionate of the *A. cathartica* and *M. elengi* extracts by using four pathogenic bacteria such as: (i) *Bacillus subtilis*, (ii) *Staphylococcus aurius*, (iii) *Proteus volgaris*, and (iv) *Escherichia coli*. The corresponding results are presented in Table 2 and Table 3, respectively. From the quantitative estimation of zone inhibitions of the different fractions showed various range of antibacterial activity against the bacteria. It has been observed that, the petroleum ether fraction of *A. cathartica* showed the maximum inhibition with diameter of 16–20 mm. On the other hand, ethyl acetate fraction of *M. elengi* showed the highest inhibition with the diameter of 10–16 mm. The other fractionates of *A. cathartica* showed the inhibition of 11–18 mm and the other fractionate of *M. elengi* showed the inhibition of 7–16 mm. This indicated that the higher quantities of antimicrobial components (phytochemicals) were present in the petroleum ether fraction of *A. cathartica*. The other fractions of *A. cathartica* also contained higher antimicrobial phytochemicals than that of the fractions of *M. elengi*. From the results we could recommend that the *A. cathartica* extracts are more antibacterial than that of the *M. elengi* extracts.

3.7 Insecticidal Assay

Mortality percentage of *Tribolium castaneum* adult due to the effect of different fractions of *A. cathartica* and their respective χ^2 values, regression equation, LD_{50}, and 95% confidence limit are shown in Table 4. The effects of different fractionates of *M. elengi* on *Tribolium castaneum* adult after 24, 48, and 72 hr treatment are shown in Table 5. It was seen from the table that all the fractions of *A. cathartica* are found to be toxic against *Tribolium castaneum.* Strong toxic effect was observed in the petroleum ether fraction with LD_{50} values of 684.376, 319.028 and 225.205 μg/cm^2 after 24, 48 and 72 hr, respectively. The LD_{50} values of chloroform fraction were 34289.35, 4308.567 and 804.082 μg/cm^2. The LD_{50} values of methanol fraction were 445092.1, 38709.1 and 9906.21 μg/cm^2. It was observed from Table 5 that no insect was killed by the treatment of the different fractionates of *M. elengi* extract. It could be reported that the *M. elengi* had very poor and/or no insecticidal activity.

3.8 Cytotoxicity Bioassay

The brine shrimp lethality assay has been used extensively in the primary screening of crude extracts as well as isolated compounds to evaluate cytotoxic, phototoxic, pesticidal, and many other activities towards brine shrimp that

could provide an indication of possible cytotoxic properties of the test material [26]. In this present study, the brine shrimp lethality test has been used to assess cytotoxic potential of the different fractionates of *A. cathartica* and *M. elengi* extracts. The lethality was determined on *Artemia salina* after 6~30 hr exposure. The LD_{50} values were calculated from the mortality percentage of brine shrimp and presented in Tables 6 and 7. The results were expressed as fractions which would not toxic when LD_{50}>1000 ppm, slightly toxic having the value of LD_{50} 500-1000 ppm, moderately toxic having the LD_{50} value of 100–500 ppm, and highly toxic having the LD_{50} <100 ppm [27].

The brine shrimp bioassay results (Table 6) of *A. cathartica* clearly demonstrated the toxic effects of the plant extracts which could be due to any of the secondary metabolites (alkaloid, flavonoid)

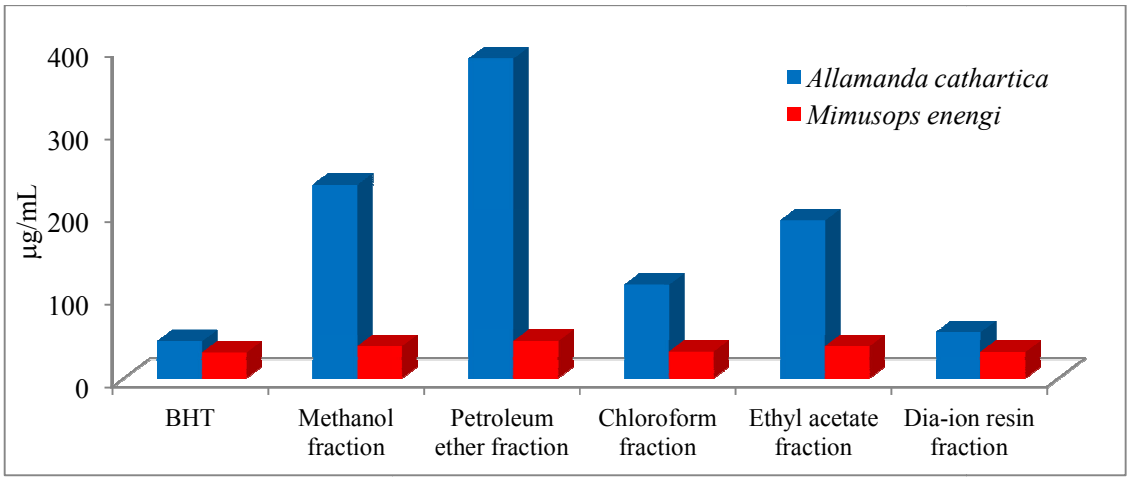

Fig. 5. DPPH radical scavenging activity of *Allamanda cathartica* and *Mimusops elengi* leaf extracts

Table 2. *In vitro* antibacterial activity of different fractionates of *Allamanda cathartica* leaf extract and standard streptomycin discs on gram positive and gram negative bacteria

Name of fractionate	Conc. (µg/µL)	Dose (µg/disc)	Inhibition zone (mm)			
			Bacillus subtilis	*Staphylococcus aureus*	*Esherichia coli*	*Proteus vulgaris*
Crude methanol	400	10	14	14	12	18
Petroleum ether	400	10	16	20	16	17
Chloroform	400	10	13	16	14	15
Ethyl acetate	400	10	12	12	16	17
Dia-ion resin	400	10	16	11	12	16
Streptomycin	400	10	24	25.5	23	28

Table 3. *In vitro* antibacterial activity of different fractionates of *Miusops elengi* leaf extract and standard streptomycin discs on gram positive and gram negative bacteria

Name of solvent fraction	Conc. (µg/µL)	Dose (µg/disc)	Inhibition zone (mm)			
			Bacillus subtilis	*Staphylococcus aureus*	*Esherichia coli*	*Proteus vulgaris*
Crude methanol	400	10	8	8	8	9
Petroleum ether	400	10	7	7	10	7
Chloroform	400	10	9	8	8	9
Ethyl acetate	400	10	10	16	12	10
Dia-ion resin	400	10	8	11	10	9
Streptomysin	400	10	26	29	23	23

present in the extract. Chloroform fraction was found to be highly effective (LD_{50}=47.86 μg/mL) whereas methanol and ethyl acetate fraction showed considerable action with their LD_{50} values less than 250 μg/mL. Petroleum ether fraction showed the toxicity with the LD_{50} value of 332.42 μg/mL. The inhibitory effect of the extract might be due to the toxic components present in the active fraction that possess ovicidal and larvicidal properties.

The cytotoxicity effect of *M. elengi* fraction was found to be very toxic (Table 7) in comparison to that of the *A. cathartica* fraction. The Ethyl acetate fraction was found be most active in having the LD_{50} value of 1.635 ppm. The next toxic fraction was the chloroform fraction which had the LD_{50} value of 9.182 ppm followed by petroleum ether with the LD_{50} value of the 64.159 ppm and the dia-ion resin adsorbed fraction having the LD_{50} value of 115.721 ppm.

Table 4. χ^2 values, regression equation, LD_{50} and 95% confidence limits of different fractions *Allamanda cathartica* leaf extract on *Tribolium castaneum* adult after 24, 48 and 72 h treatment

Fractionates	Treatment (h)	χ^2 for heterogeneity	Regression equation	LD_{50} (μg/cm^2)	95% confidence limits	
					Lower	Upper
Crude methanol	24	0.237	Y = 2.039 + 0.5241 X	445092.100	1.203	1.646E+11
	48	0.392	Y = 1.672 + 0.7253 X	38709.100	193.368	7748905
	72	1.624	Y = 1.509 + 0.8734 X	9906.210	634.466	154670.1
Petroleum ether	24	8.286	Y = -2.219 + 2.546 X	684.376	442.590	1058.249
	48	12.334	Y = -1.192 + 2.473 X	319.028	186.100	546.905
	72	7.925	Y = -3.496 + 3.611 X	225.205	158.376	320.235
Chloroform	24	0.339	Y = 2.414 + 0.5701 X	34289.350	122.854	9570309
	48	0.8184	Y = 2.097 + 0.7988 X	4308.567	628.894	29518.03
	72	0.538	Y = 1.632 + 1.159 X	804.082	492.280	1313.374

Table 5. Effects of different extractives of *Mimusops elengi* on *Tribolium castaneum* adult after 24, 48, and 72 h treatment

Fractionates	Treatment (h)	Number of insect killed				
		Dose-A	Dose-B	Dose-C	Dose-D	Control
Crude Methanol	24	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Petroleum ether	24	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Chloroform	24	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Dia-ion resin	24	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0

Table 6. Effects of different extractives of *Allamanda cathartica* leaf extract on *Artemia salina* nauplii after 30 h treatment

Extractives	χ^2 values for heterogeneity	Regression equation	LD_{50} (μg/cm^2)	95% confidence	
				Lower	Upper
Crude methanol	0.26	Y= 4.29 + 0.343X	111.61	32.38	384.69
Ethyl acetate	1.48	Y= 2.60 + 1.130X	131.14	92.23	186.47
Petroleum ether	1.04	Y= 3.27 + 0.682X	332.42	200.85	550.18
Chloroform	4.82	Y= 3.13 + 1.110 X	47.86	26.10	87.76

Table 7. Effects of different extractives of *Mimusops elengi* leaf extract on *Artemia salina* nauplii after 30 h treatment

Extractives	χ^2 values for heterogeneity	Regression equation	LD_{50} ($\mu g/cm^2$)	95% confidence	
				Lower	Upper
Ethyl acetate	2.196	Y= 5.345 + 0.193 X	1.635	3.627	7.369
Petroleum ether	0.217	Y= 2.427 + 1.422 X	64.159	43.902	93.765
Chloroform	5.011	Y= 3.895 + 1.148 X	9.182	3.585	23.51
Dia-ion resin	7.281	Y= 3.275 + 0.836 X	115.721	65.256	205.211

4. CONCLUSION

This study revealed the antioxidant, antimicrobial, insecticidal and cytotoxic activities on two medicinal plants *A. cathartica* and *M. elengi* using spectroscopic and disc diffusion methods. The results showed that all the five extractives of *A. cathartica* contained very few amounts of polyphenols and flavonoids. The extractives of *M. elengi*, on the other hand, contained a good amount of polyphenols and flavonoids. The microbial activity of *A. cathartica* was very active against pathogenic bacteria where as *M. elengi* showed the least microbial activity. From the insecticidal activity test, it was observed that the petroleum ether fraction of *A. cathartica* showed strong toxic effect against *Tribolium castaneum* for 72 hr exposure where as no toxic effect was observed in the case of *M. elengi* fraction. All the extracts of both *A. cathartica* and *M. elengi* displayed high level of toxicity towards brine shrimp lethality bioassay. On the basis of our obtained results, it might be suggested that both *A. cathartica* and *M. elengi* leaf extracts could be used as a potential source of natural antioxidant, antimicrobial and anticancer agents for the treatment of different oxidative disorder, infection diseases caused by the resistant microorganisms and cancer, respectively. Further research has been taken to isolate and characterize the individual bioactive compound under this program.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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