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Research Article

PHYTOCHEMICAL SCREENING OF *AGLAIA ELAEAGNOIDEA* AND THEIR EFFICACY ON ANTIOXIDANT AND ANTIMICROBIAL GROWTH

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ABSTRACT

Phytochemicals are extensively found at different levels in many plants and serves as basic raw material in the manufacturing of medicine, nutrition, cosmetics, dyeing and other industries. The present study aimed to lighten the medicinal uses of the leaves and stem bark of *Aglaia elaeagnoide* plant in the treatment of different ailments such as astringent, antidiarrhoeal, antidysenteric, skin diseases, tumours in Indian medicine of Ayurveda. In the present work we investigated the phytochemical screening to find out new sources of natural antioxidant and antimicrobial activity source from the leaf and stem bark of *Aglaia elaeagnoidea* with different solvents such as chloroform, ethanol, methanol, petroleum ether and water. Phytochemical screening of all crude extracts of leaf and bark reveals the presence of alkaloids, steroids, phenols, flavonoids, tannins, coumarins, quinones, xanthoproteins, terpinoids, carbohydrates, fatty acids, leucoanthocyanins, saponins and emodins. *In vitro* antioxidant activity of ethanolic extracts of leaf and bark exhibited maximum phenolic compounds and scavenging activity. Phenolic compounds of leaf and bark exhibit positive correlation to antioxidant activity. All the crude extracts of leaf and bark showed low to moderate inhibition zone against *Staphylo coccus aureus*, *Bacillus subtilis, Escherichia coli*, *Vibrio vulnificus* and *Candida albicans.* The antioxidant and antimicrobial activity of different crude extracts of bark exhibited more efficacy compared to the leaf extracts. Thus, further development of new phytochemicals for the treatment of different disorders by using sustainable approach opens up possibilities in the usage of these as antioxidant and antimicrobial in various medicinal composition.

KEYWORDS: Phytochemicals screening, Crude extracts, Antioxidant, Antimicrobial, *Aglaia elaeagnoidea.*

INTRODUCTION

Phytochemicals of plants serve as basic raw material for many products including medicine, nutrition, cosmetics, dyeing and other industries. These provide potential new sources of drugs for antimicrobial, anticancer, anti-oxidant, anti-inflammatory, anti-diabetic, cardio disorders etc., with or without minimal side effects. Moreover, demand for isolation of phytochemicals are increasing across the globe because of availability, cost-effectiveness, non toxic and biodegradable nature[1]. The medicinal value of the plants lies in the bioactive compounds such as alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids etc., that are used to treat various diseases[2]. Many of these phytochemicals of medicinal plant exhibit antioxidant property and are able to remove oxygen from free radicals formed in cells and thus protect against oxidative damage and antimicrobial propertythat are able to kill microbes with minimizing many of the side effect[3-6]. Due to the potential therapeutic activity of phytochemicals recently, research has focused on medicinal plants for isolation of natural and low-cost antioxidant and antimicrobial compounds that can replace synthetic drugs[7]. A great number of plants worldwide showed a strong antioxidant activity such as *Asparagus racemosus*[8],*Terminalia chebula*[9], *Acacia arabica*[10], *Bidenspilosa*[11], *Polyalthiacerasoides (Roxb.) Bed* [12], *Teucriumpolium L.*[13]. Huge number of herbs possess a vast untapped source for antimicrobial activity that are *Betula utilis*[14], C*alotropis gigantean*[15], *Jatropha curcas* (Linn)[16], *Medicago sativa, Pimentadioica, Aloe* barbadensis[5], *Micromeria nervosa*[17], *Datura metel*[18].

Among medicinal plants, species of *Aglaia* genus has attracting for its secondary metabolites. This genus belongs to Meliaceae family and is widely distributed in coastal regions, tropical forests of Asia, Northern Australia and Pacific islands[19]. Baumann[20] reported that the leaf and twig extracts of *Aglaia ponapensis* possess cyclopenta (bc) benzopyran, ponapensin, and an aglaialactone, 5,6-desmethylenedioxy-5-methoxy-aglalactone, together with nine known compounds which showed potent NF-κB inhibitory action. The compounds were isolated from the bark of *Aglaia edulis* are aglaroxin A 1-O-acetate, 3′-methoxyaglaroxin A 1-O-acetate, 19,20-dehydroedulisone A, edulirin A, edulirin A 10-O-acetat, isoedulirin A, and edulirin B exhibited cytotoxic activity[21]. From A*. elliptifolia*, Cyclopenta(b)benzofurans compounds isolated and used in cancer chemotherapy, also showed potential activity to inhibit TNF-α or PMA-induced NF-κB activity in different mouse and human T-lymphocyte cell lines[22,23]. Aglaia species like *A. basiphylla* and *A. gracilis* exhibited potential insecticidal property against *Spodoptera littoralis* [24].

Aglaia elaeagnoidea (A. Juss) Benth is an evergreen tree, which is used as medicine for treatment of antipyretic, astringent, antidiarrhoeal, antidysenteric, anti-inflammatory, skin diseases and tumours[25]. It produces

antifeedant that act against insect pests like Helicoverpaarmigera and Spodoptralitura[26]. Henceforth, in this perspective, the aim of the study is screening of phytochemicals, total phenolic compounds, antioxidant and antimicrobial activity of different crude extracts of *A.* elaeagnoidea. The antioxidant and antimicrobial activity efficiency of all crude extracts were evaluated using DPPH scavenging and for the prevention and treatment of infectious diseases caused by microbial pathogens i.e., Gram-negative and Gram-positive bacteria and fungus.

MATERIALS AND METHODS

Materials

The solvents used in this present work are chloroform, ethanol, methanol, petroleum ether and that were purchased from Merck and Hi media. Dimethyl sulfoxide (DMSO) and DPPH (2, 2-diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich Chemicals. Other chemicals such as ascorbic acid, ferric chloride etc., were from Hi-media. *Aglaia elaeagnoidea* plant leaves and bark were collected from Puthupattu forest near Puducherry, India. The samples are shade dried for one week and powdered. The instruments used in the present experiment were Soxlet apparatus, rotary evaporator, laminar air flow and UV-Visible spectrophotometer, Pondicherry University.

Preparation of plant extraction

Aglaia elaeagnoidea plant leaves and stem bark powder were weighed (25g) and extracted with five solvents such as chloroform, ethanol, methanol and petroleum ether in soxhlet apparatus for 8 hrs. For aqueous extract 25g were taken in the 500 ml conical flask and boil it on magnetic stirrer for 30 min. After extraction, the extracts were filtered by using Whatman No.1 filter paper and all the extracts are subjected to rotary evaporator for complete evaporation of solvents. Then the resultant extracts were stored in a refrigerator at 4° C for further analysis.

Preliminary phytochemicals screening

The preliminary phytochemical analysis of the extracts is an important for further isolation of the compound. From the crude extracts of chloroform, ethanol, methanol, petroleum ether and water (500mg), stock solutions were prepared by using 50 ml of its own mother solvents. The presence of various secondary phyto-constituents were carried out using standard protocols[27,28].

Estimation of total phenolic compounds

To estimate total phenolics by Folin-Ciocallteu reagent method[29]. Gallic acid at different concentration by dissolving in 80% methanol (20, 40, 60, 80, 100 ppm) was taken to plot standard curve and the absorbance were recorded at 765 nm. 50 µl of leaves and bark extracts of *A. elaeagnoide* with different solvent samples were dissolve in 250 µl of Folin-Ciocallteu reagent (1:10 dilution) and add 500 µl of distilled water. All these samples are mixed well and allow to for 1 min at room temperature and then add 20% sodium carbonate (Na_2CO_3) at 1500 µl concentration. Shake the solutions vigorously and incubated in dark for 2 h. After incubation time, the absorbance were recorded at 765 nm. The obtained results were calculated in mg of gallic acid equivalent (mg GAE) per g of dry weight of the plant.

Evaluation of antioxidant activity

By using scavenging activity of free radicals of stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH), free radical scavenging of samples were estimated as described by Blois with modification[30]. The crude extracts like chloroform, ethanol, methanol, petroleum ether and aqueous of *Aglaia elaeagnoidea* plant leaves and bark at different concentrations were dissolved in methanol (25, 50, 100 and 200 ppm equivalent to 25, 50, 100 and 200 µg/ml, respectively) and from each, 3 ml is taken in the test tube. The DPPH (0.1 mM) was dissolved in methanol, 1ml was added to each test tube and all the test tubes are shaken vigorously and allowed to stand at 27° C in a dark place for 45 min. Ascorbic acid was used as a positive control. The control sample was prepared according to the same procedure without any extract and ascorbic acid. The absorbance of all the samples was measured by using Shimadzu double beam UV spectroscopy at a wavelength of 517 nm. The total radical scavenging activity of the tested crude extract samples was estimated as an inhibition percentage and was calculated by using formula,

Measurement of antioxidant activity (%)

$$\% \text{ of inhibition} = \frac{\text{Control} - \text{Extract}}{Control} x\ 100 \text{.....(1)}$$

Evaluation of antimicrobial activity

The antibacterial study was carried out using agar well diffusion method for all solvent extracts[31]. Each of these different extracts were dissolved in dimethyl sulphoxide (DMSO) at a concentration of 1mg/ml which served as the stock. From the stock solution prepared concentration (50 µg/ml) of all the extracts were tested for their antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* & *Bacillus subtilis*), Gram-negative bacteria (*Vibrio vulnificus* & *E.coli*) and a fungus, (*Candida albicans*) on Mueller Hinton Agar plates using well diffusion method. Briefly, 6 h old culture of all the microbes were swabbed over the surface of Mueller Hinton agar medium, after that 6 mm well was punched with the help of a cork-borer. To each well, 50 µl of plant extracts as an optimum concentration of 50 µg/ml was introduced and DMSO served as negative control. All the plates were incubated overnight at 37° C. Inhibition zone against tested bacteria and fungus were obtained by measuring the zone diameter (mm). Each method in this experiment was replicated three times.

Results and Discussion

Phytochemical screening

The preliminary biochemical screening of plants are useful to find the bioactive principles which lead to development of new drugs for antioxidant, antimicrobial, and anticancer[9,12,13,21]. The results of phytochemical screening of leaf and stem bark extracts were tabulated in Table.1 and Table.2 respectively. In our present study, preliminary phytochemical screening of leaf ethanolic and methanolic extracts showed the presence of alkaloids, steroids, phenols, flavonoids, coumarins, quinones, carbohydrates, fatty acids and saponins. Methanolic extraction of showed the presence of terpinoids. Aqueous

extract of leaf showed the presence of steroids, phenols, flavonoids, quinones, xanthoproteins, carbohydrates and saponins. The leaf extracts of chloroform and petroleum ether shows the presence of phenols, flavonoids, carbohydrates and fatty acids. While alkaloids are present shown in chloroform extracts respectively. On other hand *A. elaeagnoidea* bark ethanol and methanol extract showed the presence of alkaloids, phenols, flavonoids, tannins, coumarins, quinones, leucoanthocyanins carbohydrates and fatty acids.

Table 1: Phytochemical screening of *A. elaeagnoidea* leaf extracts

S.No.	Phytochemicals	Water	Ethanol	Methanol	Chloroform	Petroleum Ether
	Alkaloids	-	+	+	+	+
	Glycosides	-	-	-	-	-
	Steroids	+	-	-	-	+
	Phenols	+	+	+	+	+
	Flavonoids	+	+	+	+	+
	Tannins	+	+	+	-	-
	Coumarins	-	+	+	-	-
	Quinones	+	+	+	-	-
	Xanthoproteins	+	+	-	-	-
	Terpinoids	-	-	-	-	-
	Carbohydrates	+	+	+	-	-
	Phlobatanins	-	-	-	-	-
	Fatty acids	-	+	+	+	+
	Leucoanthocyanins	+	+	+	-	-
	Proteins	-	-	-	-	-
	Free amino acids	-	-	-	-	-
	Saponins	+	-	-	-	-
	Emodins	+	-	-	-	-

Table 2: Phytochemical screening of *A. elaeagnoidea* stem bark extracts

S.No.	Phytochemicals	Water	Ethanol	Methanol	Chloroform	Petroleum Ether
1.	Alkaloids	-	+	+	+	+
2.	Glycosides	-	-	-	-	-
3.	Steroids	+	-	-	-	+
4.	Phenols	+	+	+	+	+
5.	Flavonoids	+	+	+	+	+
6.	Tannins	+	+	+	-	-
7.	Coumarins	-	+	+	-	-
8.	Quinones	+	+	+	-	-
9.	Xanthoproteins	+	+	-	-	-
10.	Terpinoids	-	-	-	-	-
11.	Carbohydrates	+	+	+	-	-
12.	Phlobatanins	-	-	-	-	-
13.	Fatty acids	-	+	+	+	+
14.	Leucoanthocyanins	+	+	+	-	-
15.	Proteins	-	-	-	-	-
16.	Free amino acids	-	-	-	-	-
17.	Saponins	+	-	-	-	-
18.	Emodins	+	-	-	-	-

The presence of xanthoproteins were observed in ethanolic and aqueous extract. Aqueous extract showed the presence of steroids, phenols, flavonoids, tannins, quinones, carbohydrates, leucoanthocyanins, emodins and saponins. While, Chloroform and petroleum ether extracts shows the presence of alkaloids, phenols, flavonoids and fatty acids while, the steroids were present in the petrolleum extract. Bangajavalli and Ramasubramanian reported similar result in ethanol and water extracts of leaf and bark of *A.* elaeagnoidea.[32]

Total phenolic compounds

In plant kingdom, polyphenols are widely scattered in the plants. The total phenolic contents of leaves and bark extracts of *A. elaeagnoide* with different solvents were shown in Table.3. Generally, the leaf and bark of ethanolic extract of the plant had high total phenolic contents (49.51 & 51.72 mg GAE/g) followed by methanolic extract (48.72 &49.27 mg GAE/g), aqueous extract (42.28 & 46.71 mg GAE/g), chloroform (30.15 & 34.53 mg GAE/g) and petroleum ether (23.36 & 26.44 mg GAE/g). The total phenolic content ranged from 23.36 to 51.72 mg GAE/g. The phenolic compounds of plants possess various biological activities, that are related to their antioxidant activity[33]. Earlier reports also supporting the positive correlation between total phenolic compounds and antioxidant[34]. In this assay many other compounds of extract may interfere, that may react with the reagent and

gives only apparent estimation of total phenolic componunds[35].

Table 3: Total phenolic content of different extracts of A. *elaeagnoidea* leaf and stem bark

Solvents	mg of GAE/g of extract	
	Leaf Extract	Bark Extract
Water	42.28 ± 0.224	46.71 ± 0.242
Ethanol	49.51 ± 0.302	51.72 ± 0.407
Methanol	48.72 ± 0.245	49.27 ± 0.345
Chloroform	30.15 ± 0.716	34.53 ± 0.616
Petroleum	23.36 ± 0.556	26.44 ± 0.376

*Values are represented as the mean ± S.D. of three experiments

Antioxidant assay

The antioxidant assay of different crude extracts (chloroform, ethanol, methanol, petroleum ether and water) of leaves and bark samples at different concentrations (25, 50, 100, and 200 ppm) was tested through DPPH method and the results were shown in Fig. 1 and 2. Increase in percentage of inhibition with increasing concentration of crude extracts were observed from the results. Ethanolic and aqueous extract of leaf has maximum and minimum DPPH scavenging activity (65 ± 0.98% & 32 ± 0.71%). Stem bark extraction of Ethanolic and Petroleum ether showed maximum and minimum DPPH scavenging activity (70 ± 0.08% & 36 ± 0.60%). All the extracts of leaf and stem bark decolorized with DPPH, which is due phytochemicals reduction of DPPH radicals (DPPH*) equal to hydroxyl groups and prevents free radical formation. Acoording to Cai DPPH is nitrogen center free radical and odd electron which gives a strong absorption and its color changes from purple to yellow when in the presence of a radical scavenger and formed into reduced DPPH-H[34]. Several authors reported that the bioactivity of plants is attributed to phytochemical constituents, for instance flavonoids are a major group of phenolic compounds and tannins exhibit strong antioxidant[8,11]. Hence the presence of flavonoids phenols, tannins bioactive compounds in crude extracts of leaf and stem bark might have bind to free radicals (DPPH*) and then prevented oxidation chain reaction and exhibited as good antioxidant activity.

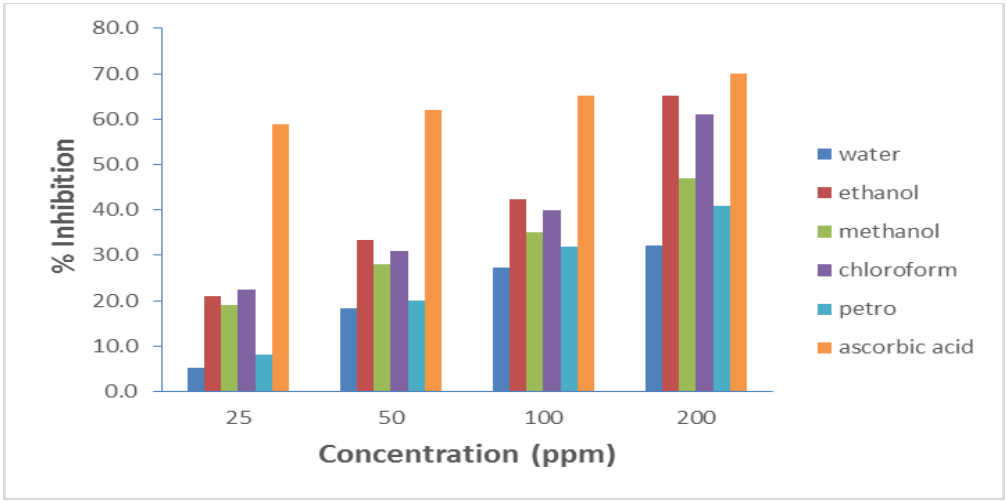

Fig. 1 Antioxidant activity of different crude extracts of *A. elaeagnoidea* Leaf

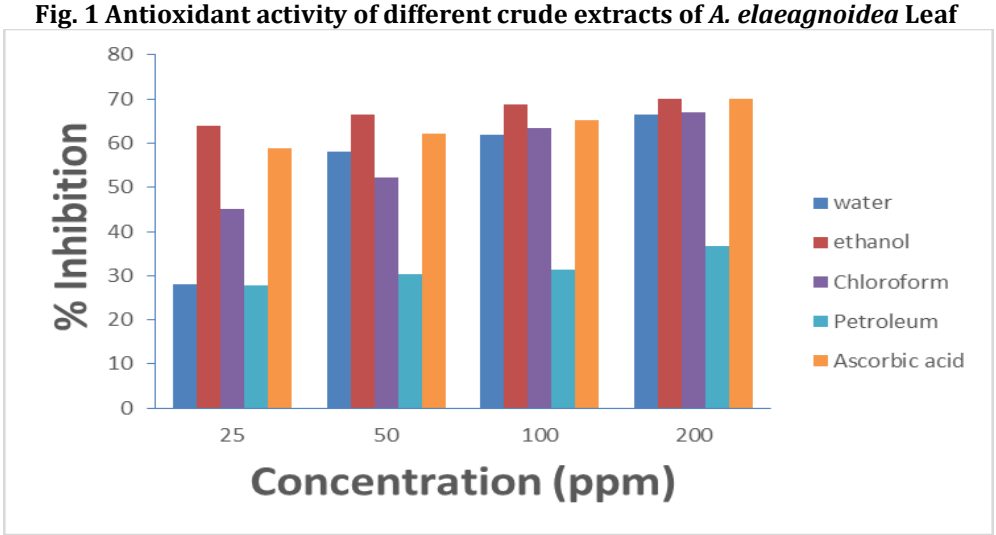

Fig.2 Antioxidant activity of different crude extracts of *A. elaeagnoidea* stem bark

Antimicrobial activity

In vitro antimicrobial activity of different extracts of leaves and stem bark was assayed by agar well diffusion method, the figures were presented in the Figure 3 and the results were tabulated in the Tables 3 and 4, respectively. *Invitro* antimicrobial assay was performed at 50 µg/ml as an optimum concentration. The maximum inhibition zone

of *E. coli* (10±0.22 and 13±0.19 mm) and *B.subtilis* (8±0.48 and 12±0.38 mm) exhibited in ethanolic extraction of both leaf and stem bark. The species *V. cholera* exhibited maximum inhibition growth in methanol and ethanol extraction leaf and bark (12±0.14 and 14±0.17) whereas, the chloroform and ethanolic extraction of leaf and stem bark inhibited maximum growth of *S aureus* (7±0.13 and 13±0.17 mm). Ethanolic extract of leaf and methsanolic extraction of bark have more effective against a fungus *C. albicans* (16±0.19 and 16±0.36 mm). Similar results for antimicrobial activity exhibited in other plants such as *Datura metel, Micromeria nervosa,* and *Jatropha curcas* [17,18]. Earlier reports on phytochemicals of plant extracts like flavonoids and tannins exhibit strong anti microbial activity[16]. Tannins may have potential cytotoxic effect and anti-neoplastic agent[36]. Saponins implicate as bioactive antibacterial and antifungal agents and steroids from plant possess antimicrobial, insecticidal, and cardio tonic activities, it was also used in cosmetics, nutrition, herbal medicine[37]. Both leaf and bark extracts A. *elaeagnoidea* showed low to moderate inhibition zone against all the bacteria and a fungus.

Table 4: Antimicrobial activity of different extracts of A. *elaeagnoidea* leaf.

Organisms	Diameter of inhibition zone (mm)				
	Aqueous	Ethanol	Methanol	Chloroform	Petroleum Ether
E.coli	8±0.59	10±0.22	9±0.55	8±0.49	8±0.29
B. subtilis	7±0.54	8±0.48	8±0.38	7±0.20	7±0.49
V. cholera	7±0.63	10±0.15	12±0.14	4±0.71	5±0.17
S. aureus	4±0.44	6±0.43	5±0.094	7±0.13	5±0.24
C. albicans	11±0.63	16±0.19	15±0.54	9±0.18	11±0.11

*Values are represented as the mean ± S.D. of three experiments

Table 5: Antimicrobial activity of different extracts of A. *elaeagnoidea* stem bark

Organisms	Diameter of inhibition zone (mm)				
	Aqueous	Ethanol	Methanol	Chloroform	Petroleum Ether
E.coli	13±0.07	13±0.19	12±0.084	7±0.12	6±o.42
B. subtilis	9±0.30	12±0.38	12±0.33	6±0.034	7±0.10
V. cholera	8±0.92	14±0.17	13±0.23	9±0.31	7±0.22
S. aureus	9±0.27	13±0.17	12±0.38	11±0.37	9±0.61
C. albicans	13±0.17	15±0.26	16±0.36	11±0.56	12±0.33

*Values are represented as the mean ± S.D. of three experiments

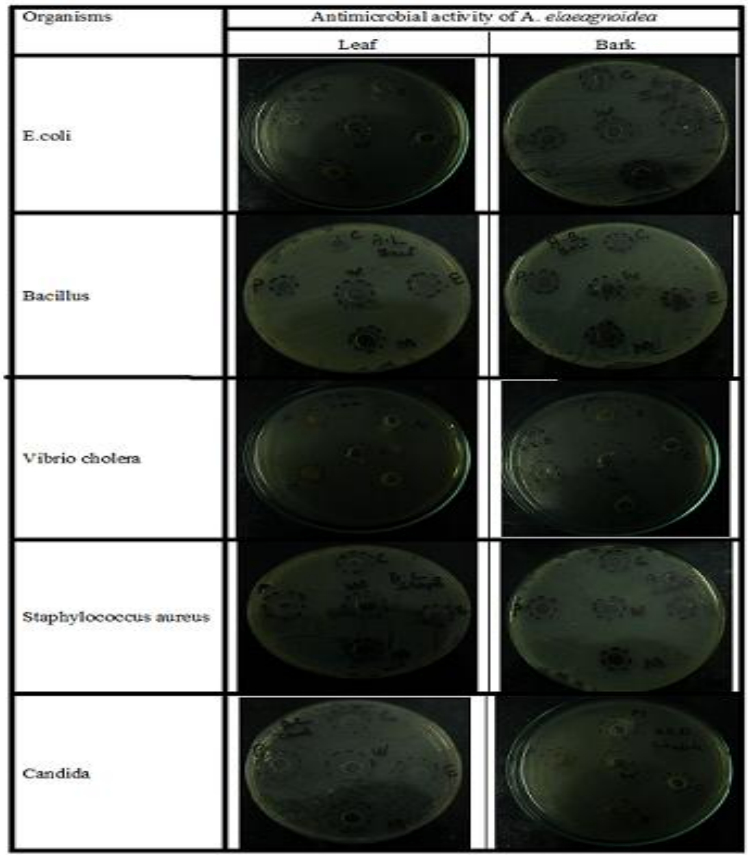

Fig. 3 Antimicrobial activity of different crude extracts of *A. elaeagnoidea* Leaf and bark

CONCLUSION

In summary, we have successfully demonstrated an important basic source of information for the use of the different crude extracts of leaf and bark of *A. elaeagnoidea*. secondary metabolites of the plant posses various potential phytochemicals. The different extracts of leaf and bark, positive correlation between total phenolic compounds and antioxidant, and efficacy on antioxidant and antimicrobial activity were evaluated. Further, it is clearly found that antioxidant and antimicrobial activity of different crude extracts of stem bark have more efficacy when compared to the leaf extracts.

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