



Antioxidant and Antimicrobial Potentials of *Acanthus Illicifolius* and *Excoeceria Agallocha* Mangrove Plant Raw Leaf Extracts

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ABSTRACT:

In this study biological activities of whole raw leaf extract of a mangrove plant named *Acanthus illicifolius* and *Excoeceria agallocha* were investigated. The extraction was performed by using a soxhlet apparatus. Antimicrobial activity and antioxidant activity were determined in the raw leaf extracts of selected plants. Based on the research results *Excoeceria agallocha* raw leaf (EARL) extract exhibited higher antibacterial activity against gram (-)ve bacterial species like *Pseudomonas aeruginosa* (ZOI 6mm) and *Streptococcus pyogenes* (ZOI 5.7mm) and antifungal activity with *Candida albicans* (ZOI 6.5mm). In addition EARL extract also showed higher antioxidant DPPH activity (IC_{50} -36.05 μ g mL⁻¹) and less ABTS activity (IC_{50} -100.34 μ g mL⁻¹) compared to the extracts of *A. illicifolius* with DPPH and ABTS activities (IC_{50} -72.05 μ g mL⁻¹) and (100.346 μ g mL⁻¹) respectively. *A. illicifolius* showed higher antibacterial activity against gram (-)ve bacteria *Streptococcus pyogenes* and also exhibited high antifungal activity. According to current research observations, the *E. agallocha* leaf extract has an excellent antioxidant potentials for application as a bioactive compounds in pharmaceuticals. Moreover, the *A. illicifolius* extract exhibited significant antibacterial and antifungal activities. This experimental results confirmed the efficiency of the extracts as natural antimicrobials and suggested the possibility of employing them in the utilization of drugs for the treatment of infectious diseases.

1. Introduction

The species of *A. illicifolius* and *E. agallocha* belong to the *Acanthaceae* and *Euphorbiaceae* families known as herb holly Mangrove and shrub blind eye Mangrove. Medicinal plants are used in the traditional treatments of various diseases on an empirical formula [1-5]. The plants have been extensively used in traditional medicine and have great pharmaceutical significance and exhibit Antimicrobial, Antiviral, and insecticidal activities [6-9]. Mangrove plants are found to contain biological activities like antibacterial antifungal and antiviral compounds [10]. *A. illicifolius* is a medicinal plant used against paralysis, asthma, and snakebite, antioxidant, analgesic, anti-inflammatory, and ulcers [11,12]. *A. illicifolius* leaf extract revealed that potent antioxidant, antimicrobial against gram(+) ve and

gram(-) ve bacteria and enriched with nutritional content [13]. *A. illicifolius* soluble extract of petroleum ether and chloroform exhibit potent analgesic and antioxidant activities [14]. The ethanoic stem bark extract of *A. illicifolius* exhibited moderate antibacterial activity against *Bacillus subtilis* and *Bacillus megaterium* [16]. The leaf extract of *A. illicifolius* has potent antioxidant activity in making anti-acne cream [17]. Several research studies revealed that the leaf part of the *E. agallocha* plant is used in the treatment of eye diseases and in heart problems [18]. In general, the secondary metabolites of plants can exhibit biological characteristics as they are antimicrobial [19-20] and antioxidant [21].

Madhurima bhaski et al., analyzed the dry leaf extracts of *A. illicifolius* and *E. agallocha* collected from



Sundarbans Kolkata of the west Bengal region by using the solvents viz., hexane, ethyl acetate, acetone, methanol, and evaluated the microbial activity against phytopathogens and fungi [22]. The studies of Ch. Govinda Swamy et al., revealed the antimicrobial activity of dry leaf extract of *A.ilicifolius* with solvents ethanol, methanol, chloroform, acetone, and aqueous *Streptococcus epidermis* *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *T.rubrum*, *L.plataraum*, and *Candida albicans* with the ZOI with Gentamycin and Clotrimazole as standards [23]. Hasan et al., analyzed the antibacterial and antioxidant activities of mangrove ethyl acetate extract in *Avicennia Marina* leaves which have potent antioxidant activity [24]. Fungal endophytes separated from *Ocimum bascillium* exhibit potent antioxidant and antibacterial activities [25]. Mangrove plants with endophytic microorganisms are depicted as having a vital role in pharmacological applications [26]. Antibacterial activities of crude extract of roots of mangroves of *Rhizophora apiculata* against other strains of bacteria to determine their spectrum of activities [27]. The ethanoic stem extract of *A.ilicifolius* exhibited good antibacterial activity against human pathogens [28]. Mangrove plants that pretended to have the highest IC₅₀ value were found in leaves of methanol extract [29].

A.ilicifolius as holly mangrove or sea holly is a species of herb or shrub that belongs to the family *Acanthaceae*, native to Southeast Asia and Australia. It has shallow top roots and develops stilt roots. In good medicinal plants, the endophytic bacteria separated from *A.ilicifolius* exhibit more antibacterial activity against human pathogens[30].

E.agallocha a mangrove species blind eye mangrove, milky mangrove, and river poison tree belongs to the family *Euphorbiaceae*. It grows in brackish water or saline water, it is widely west in India, Australia, and Western Australia. It is observed in backwater areas of the ocean and low salinity areas. It grows as a tree and latex is poisonous causing skin irritation and temporary blindness. The leaf extract of *E.agallocha* has good antibacterial activity against *Salmonella typhi* and no fungal activity against *C.parapsilosis*[31].

soxhlet extraction is the rapid and innovative technique for extraction of maximum amount of secondary metabolites which is identified as a green sample

preparation technique due to its high efficiency and eco-friendly technique. The Objective of this study was to evaluate the antioxidant and antimicrobial activities of the raw leaf extracts of *A.ilicifolius* and *E.agallocha* which are grown in the Coringa.



Acanthus.iclifolius

Figure 1.0The selected plant species are represented in the Figure-1.0

Materials & Methods

Materials

All the chemicals used were of analytical grade. DPPH(1,1-diphenyl-2-picrylhydrazyl), Ascorbic acid, Methanol, ABTS(2,2'-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid, Potassium persulphate, and all standard compounds are procured from Sigma-Aldrich. (India)

Plant material and chemicals

The *Acanthus ilicifolius* raw leaf extract(AIRL) and *Excoeceria agallocha* raw leaf extract (EARL) picked by hand from Coringa region (16-30 17-00, N and 82-14 to 82-23 E longitudes) in May 2020, quickly moved to a laboratory. After cleaning, the leaves were made into a paste with the help of a mortar or pestle. The raw leaf extract was filtered and stored in vials at 4°C in cold conditions and further analysis.

Extraction

The raw leaves were collected from (*Acanthus ilicifolius*) and (*Excoeceria agallocha*) and are made into paste with the help of a grinder and obtained extract is mixed with water and methanol is used as solvents. The 0.1gm/mL of leaf extracts are obtained and transferred to petriplates for determination of the Zone of inhibition exhibited by the bacteria.



Anti-microbial activity

The sensitivity of different pathogenic microorganisms in the extract was evaluated according to the agar dilution method. The bacterial species included two Gram -ve negative bacteria (*Salmonella enterica* (MTCC1253), *Pseudomonas aeruginosa* (MTCC 2453) and two Gram-positive bacteria (*Streptococcus pyogenes* (MTCC 442), *Bacillus cereus* (MTCC 1305), and fungal strain (*Candida albicans*) (MTCC183) were chosen based on their clinical and pharmacological importance. The bacterial strains and fungal strains obtained from the Department of Microbiology, Osmania University, Hyderabad, India were used for evaluating antibacterial activity. The bacterial stock cultures were incubated for 24 hrs at 37°C on nutrient agar. The bacteria were grown on Mueller-Hinton agar plates at 37°C. The stock cultures were maintained at 4°C for the growth of fungi potato dextrose agar was used. filter paper Whatman No.1 discs of 5mm diameter were autoclaved by keeping them in a clean and dry petri plate. The discs were soaked in compound solutions for 5 hrs and were taken as test material. After 5 hrs the discs were dried under shade. The concentrations of compound solutions per disc accounted for 0.1 gm/mL and were carefully transferred to spread on cultured Petri plates. The discs immersed in ethanol were employed as the control positive and streptomycin as the control negative. For assessment of the antibacterial activity, LB agar medium was used and the medium was sterilized at 121°C for 30 min. The agar plates were prepared by pouring about 10 ml of the medium into 10cm Petri dishes under aseptic condition and left undisturbed for 2hrs to solidify the medium. 1ml of inoculum containing a suspension of *Streptococcus pyogenes*, *Bacillus cereus*, *Salmonella enterica*, *Pseudomonas aeruginosa* and *Candida albicans* was poured on to the plates separately containing solidified agar media.

The prepared sterile filter paper discs were impregnated with the compound solutions and shaken thoroughly and these test plates were incubated for a period of 48 hrs in BOD at 37°C for the development of inhibitory zones and the average of 2 independent readings for each organism in different compound solutions were recorded in (Table 1). The diameter of the inhibition zones were measured after 1 day at 37°C for bacteria and recorded with the aid of a plastic ruler.

DPPH assay

DPPH assay was used to determine the antiradical scavenging activity of plant extracts (AIRL and EARL) was determined on the basis of the radical scavenging effect on the DPPH free radical. Samples were prepared by suspending 1ml of different concentrations of extracts mixed with 3ml of DPPH solution (0.004% in methanol) at various concentrations. The tubes were incubated in the dark for 30 min at room temperature and the optical density was measured at 517 nm using a UV-Vis spectrophotometer. The absorbance of the DPPH control (extract replaced by methanol) was also noted. Ascorbic acid was used as the reference standard. The following equation was employed to calculate radical scavenging activity. (Elmastas et al., 2006)

$$\% \text{ DPPH} = (A_b - A_s) / A_b \times 100$$

Where A_b is the absorbance of control and A_s is the absorbance of the sample, respectively.

ABTS assay

The ABTS assay was used to determine the antiradical activity of the AIRL and EARL extract with slight modification (Li et al., 2011). Samples were prepared by suspending DPPH solution 1ml of different concentrations of plant extracts (5-100 $\mu\text{g mL}^{-1}$) were added to 4ml of ABTS solution in labeled tubes and the tubes were incubated for 30 min followed by measuring the absorbance at 730 nm. Ascorbic acid was used as the reference standard. The ABTS radical was generated by mixing 7mM ABTS stock solution with 2.45 mM Potassium Persulfate and the mixture was left in the dark for 12–16 h at room temperature. The resulting solution was diluted with distilled water and subjected for absorbance in the region between 0.70 at 730 nm. The following equation was employed to calculate radical scavenging activity.

$$(\text{ABTS}\%) = (A_b - A_s) / A_b \times 100$$

Where A_b is the absorbance of the control and A_s is the absorbance of the sample solution respectively.

Results

Antimicrobial activity of the extract

The ZOI assessment results of the two species of the AIRL and EARL extracts are presented in Table 1. As a negative control, streptomycin did not show any



bactericidal activity or inhibitory effects on both gram(+)ve and gram(-ve) bacteria. The EARL extract showed slightly higher ZOI of (3.7mm) with *Salmonella enterica* compared to the ZOI of (3.6 mm) with the AIRL extract. The AIRL and EARL extracts with the concentration of 100 mg/mL exhibited equal values of ZOI (3mm) and (3mm) against the bacterial species gram (+)ve *Bacillus cereus* with streptomycin as standard. The AIRL extract exhibited a higher Zone of inhibition of (6.5mm) with *Streptococcus pyogenes* and a lesser zone of inhibition of (5.7 mm) in EARL extract. The gram (-)ve bacteria *Pseudomonas aeruginosa* exhibited a prominent zone of inhibition (6.0 mm) in EARL and less ZOI (4.0 mm) in AIRL. The antifungal activity was further tested for AIRL And EARL extracts of plant species by taking streptomycin as control. The results summarized in Table 1 revealed that the zone of inhibition with *Candida albicans* was found to be more in EARL at (6.5 mm) compared to raw leaf extract of AIRL at (6.0 mm).

The Studies of V. Anil et al., on the antibacterial activity of *A. ilicifolius* dry leaf ethanoic extract showed the Zone of Inhibition as (18 mm) by *Pseudomonas aeruginosa* [32]. Ramasubbuyran et al., stated that the ZOI showed by *E. agallocha* leaf extract against *Pseudomonas aeruginosa* is (15.00±0.50) mm maceration taking as DMSO and fluconazole as standards [33]. Raghavanpillai Sabu et al., carried out studies on against *streptococcus aureus* on dried leaf extract of *E. agallocha* extracted from the ethyl acetate solvent in the range (23.5±1.3)mm [36]. The studies carried out on antifungal activity on dried leaf extract of *A. ilicifolius* extracted from the solvents aqueous, ethanol and methanol [34], aqueous [35]. Raghavanpillai Sabu et al., carried out on studies antifungal activity against *Candida albicans* in the range 10.3±0.6mm on dried leaf extract of *E. agallocha* extracted from the ethyl acetate solvent [36]. But the present research study results revealed that the ZOI of the extract of EARL found against *Candida albicans* in ethanol solvent was 6.5 mm.

The descending order of ZOI in AIRL

Streptococcus pyogenes > *Pseudomonas aeruginosa* > *Salmonella enterica* > *Bacillus cereus*.

The descending order of ZOI in EARL

Pseudomonas aeruginosa > *Streptococcus pyogenes* > *Salmonella enterica* > *Bacillus cereus*

The photographs of the microbial species are shown in figures from 6(A)-6(E).

Figure 1.1 Zone of Inhibition (ZOI) of different bacterial strains and fungal strains from AIRL and EARL Figure(1.1A-1E). The ZOI is presented in the photograph in Figure 6(A) with C streptomycin being as control (7.5 mm). The ZOI of raw leaf extracts are *A. ilicifolius* (3.6 mm), and *E. agallocha* (3.7mm). The ZOI is posted in the photograph in Figure 6(B) with C streptomycin being as control (9.5 mm). The ZOI of raw leaf extracts are *A. ilicifolius* 6.5mm, *E. agallocha* (5.7 mm). The ZOI is posted in the photograph in Figure 6(C) with C streptomycin being as control (6.0 mm). The ZOI of raw leaf extracts are AIRL(3mm), and EARL(3mm). The ZOI is posted in the photograph Figure 6(D) with the ZOI of plant leaf C streptomycin being as control (8.0 mm). The ZOI of extracts are *A. ilicifolius* (4 mm), *E. agallocha* (6 mm). The ZOI is presented in the figure 6(E) with C being as control (13mm). The ZOI of raw extracts are *A. ilicifolius* (6 mm); and *E. agallocha* (6.5mm).

Antioxidant activity

DPPH Assay

The antioxidant activity of AIRL and EARL is determined by the DPPH Assay. The results of the antioxidant activity of AIRL and EARL extracts are compared at various concentrations of 50,100,150,200,400 µg mL⁻¹ by the DPPH method taking Ascorbic acid as standard. The results showed a clear trend between the concentration and antioxidant activity of AIRL and EARL extracts (Table 2). IC₅₀ is a suitable parameter for determining the antioxidant activity of different extracts. It stated that high IC₅₀ leads to low antioxidant activity. The AIRL extract had higher IC₅₀ at (129.05) µg mL⁻¹ compared to the EARL extract at (36.05) µg mL⁻¹ and Ascorbic acid standard (106.8) µg mL⁻¹ respectively. The above results revealed that EARL extract exhibited higher antioxidant DPPH activity than AIRL extract. The values are summarized in Table 2.



Correlation of DPPH activity vs concentration of extracts

The R^2 value AIRL was found to be 0.6597 indicating more than 65% of inhibition is affected by the concentration of the ingredients. The regression curve of raw leaf extract of EARL by DPPH method. The R^2 value was found to be 0.8105 indicating more than 81% inhibition is affected by the concentration of the ingredients.

The 50% inhibition of DPPH activity by different plant materials was determined by analysis. The EARL has the highest IC_{50} value (105.47) $\mu\text{g mL}^{-1}$ values represented in plot 3(B) whereas extract-1 showed the lowest IC_{50} value of (74.31) $\mu\text{g mL}^{-1}$ of AIRL observed in plot 3(A) when compared with Ascorbic acid as a standard showing IC_{50} value is 172.59 $\mu\text{g mL}^{-1}$ are observed in Figure 3(C).

ABTS assay

The ABTS antioxidant activity of extracts of AIRL and EARL are analyzed by taking the concentration at 25,50,75,100,125,250 ppm respectively by taking Ascorbic acid as standard. The extract of AIRL at 25 ppm concentration has exhibited antioxidant activity at 19.54 $\mu\text{g mL}^{-1}$. At 25 ppm concentration of EARL showed the antioxidant activity as 19.39 $\mu\text{g mL}^{-1}$. At 50 ppm concentration of EARL observed the antioxidant activity was found to be 33.58 $\mu\text{g mL}^{-1}$ and raw leaf extract of AIRL at 50 ppm concentration showed radical activity as 32.57 $\mu\text{g mL}^{-1}$. The antioxidant activity was found to be 54.86 $\mu\text{g mL}^{-1}$ and 58.39 $\mu\text{g mL}^{-1}$ higher in raw leaf extract of AIRL at 75 ppm and 100 ppm concentrations respectively. In the case of raw leaf extract of EARL the antioxidant activity is found to be 54.12 $\mu\text{g mL}^{-1}$ at 75 ppm and 56.69 $\mu\text{g mL}^{-1}$ at 100 ppm concentrations. The % scavenging activity was found to be 65.19 $\mu\text{g mL}^{-1}$ in raw leaf extract of AIRL at 125 ppm concentration and at the same concentration, the % scavenging activity of raw leaf extract of EARL was observed as 65.2 $\mu\text{g mL}^{-1}$. The % ABTS Activity was found to be 76.18 $\mu\text{g mL}^{-1}$ and 76.6 $\mu\text{g mL}^{-1}$ is nearly equal in raw leaf extract of AIRL and EARL at 250 ppm concentration respectively. The IC_{50} value of ABTS activity by different plant species was determined graphically and presented in figures from 4(A)-4(F) respectively.

Correlation of ABTS activity with concentration of extracts

The regression curve of raw leaf extract of AIRL has been carried out by ABTS method. The R^2 value was found to be 0.7532. The IC_{50} value 99.95 indicated the moderate antioxidant activity of the Raw leaf extract of AIRL and values are represented in plot 5(A). The regression curve of EARL extract by ABTS method. The R^2 value was found to be 0.7732 indicating more than 77% inhibition is affected by the concentration of the ingredients. The IC_{50} value 100.35 $\mu\text{g mL}^{-1}$ indicated the higher antioxidant activity of the EARL extracts and values are represented in plot 5(B). The regression curve of standard Ascorbic acid by ABTS method. The R^2 value was found to be 0.7227 indicating more than 72% inhibition is affected by the concentration of the ingredients. The IC_{50} value 154.88 $\mu\text{g mL}^{-1}$ indicated the antioxidant activity of the extract of Ascorbic acid and values are represented in plot 6(C).

The antioxidant activity of the dry leaves extract of *A. ilicifolius* collected from the Tamilnadu state of India and employed the DPPH method and found the concentration as $68.65 \pm 0.5 \mu\text{g mL}^{-1}$ and with vitamin C as standard the concentration was resulted as $76.25 \pm 0.33 \mu\text{g mL}^{-1}$.

Conclusions

As expected, our experiments demonstrated that EARL extract exhibited a sufficient good antibacterial and antifungal activity compared to the AIRL extract. The extract of EARL also exhibited higher DPPH and ABTS activity than extracts of AIRL with Ascorbic acid as a reference standard. The research results revealed that the EARL and AIRL raw leaf extracts exhibited good antibacterial, antifungal, and antioxidant activity. The research study findings will be useful to pharmaceutical and biological researchers in new drug discovery and designs and in formulating the required new drug molecules.

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Ethical Consideration

The research work followed all the research ethics.

Conflict of interest

All the authors have declared they don't have a conflict of interest.

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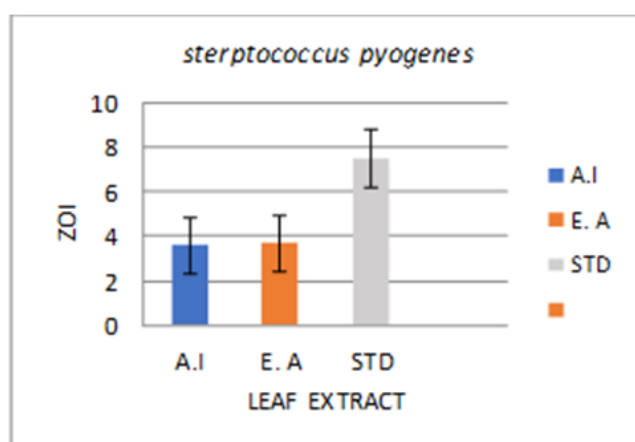
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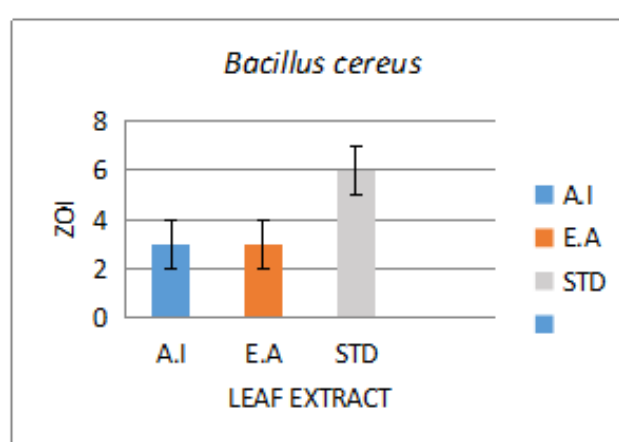
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Bacterial strains	ZOI (mm)	ZOI (mm)	Streptomycin control(mg mL ⁻¹)
	AIRL extract	EARL extract	
<i>Bacillus cereus</i> MTCC 1305	3 ^{ab}	3	6
<i>Staphylococcus pyogens</i> MTCC 442	6.5	5.7*	9.5
<i>Salomonella enterica</i> MTCC 1253	3.6	3.7	7.5
<i>Psuedomonas aeruginosa</i> MTCC 2453	4	6	8
<i>Candida albicans</i> MTCC 183	6	6.5	13

Table 1. Antibacterial and Antifungal activity of AIRL and EARL extracts



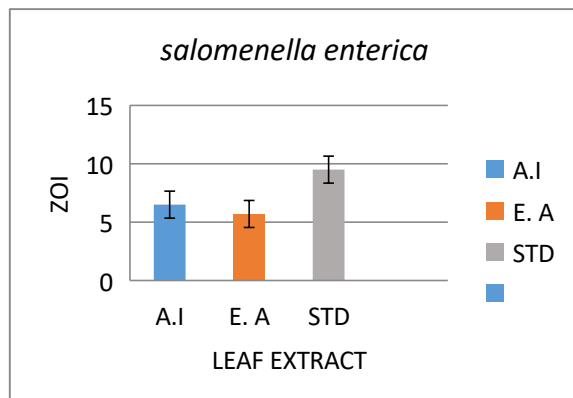
1(a)



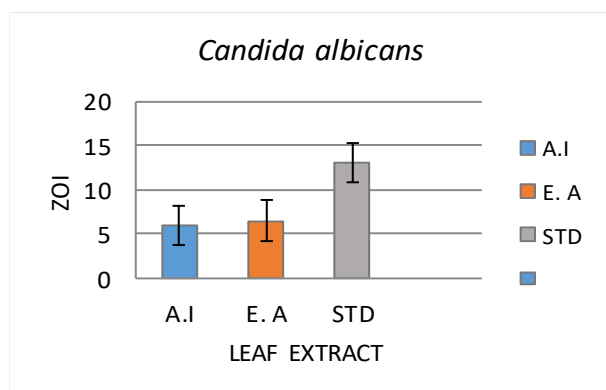
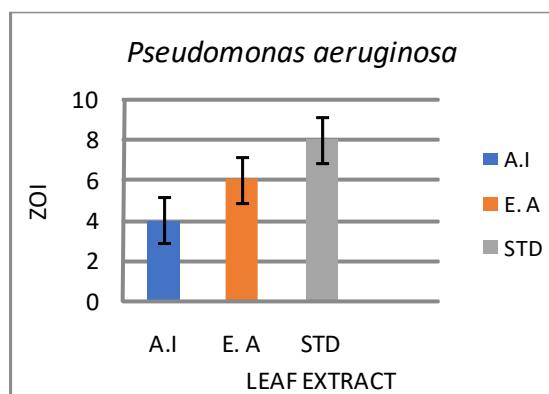
1(b)



1(c)



1(d)



1(e)

Species	50			100		150	200	400	IC ₅₀ (µg mL ⁻¹)	linear regression Y=MX+C	R ²
	ppm	ppm	ppm	ppm	ppm						
AIRL	30±5.5 ^{ab}	50±4.3	60±5.2	65±5.1	70±4.2	129.05	Y=0.0959X+37.74	0.6712			
EARL	50±5.1	60±5.6*	68±5.2**	72±4.9	78±5.7	36.527	Y=0.0959X+37.74	0.7732			
Ascorbic acid (std)	55±5.6	68±4.4	72±5.2	78±5.5	85±5.1	106.8	Y=0.0755X+58.014	0.8168			

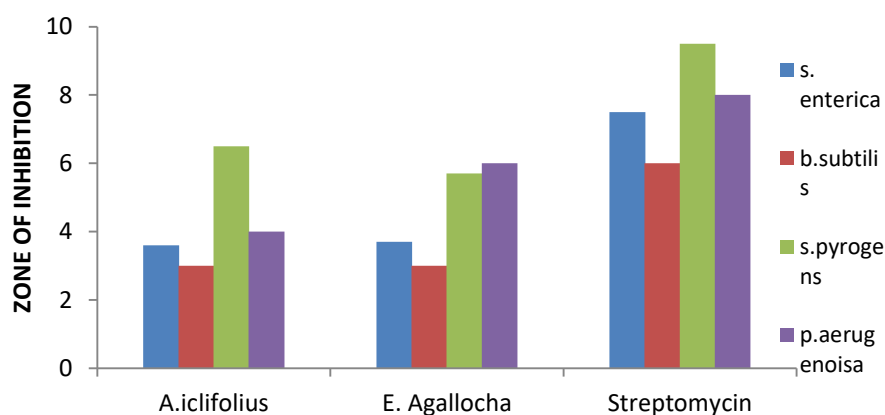
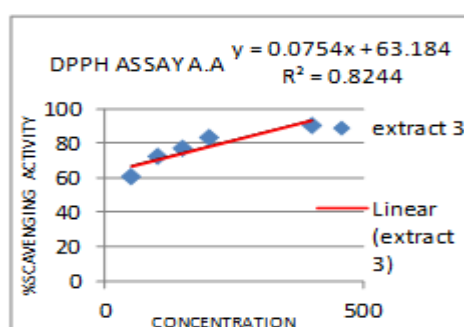
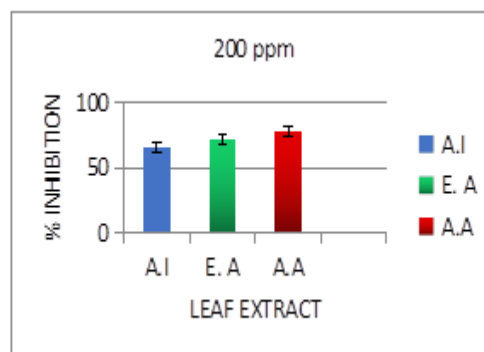


Figure 1.0. Comparison between the *antibacterial* activity exhibited by plant species AIRL, EARL, and streptomycin.

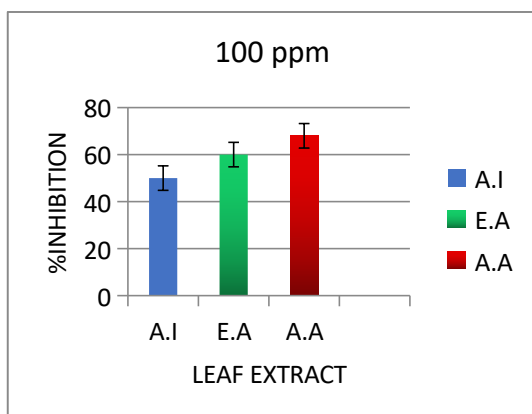
Table 2. DPPH activity at different concentrations and IC₅₀ and linear regression analysis



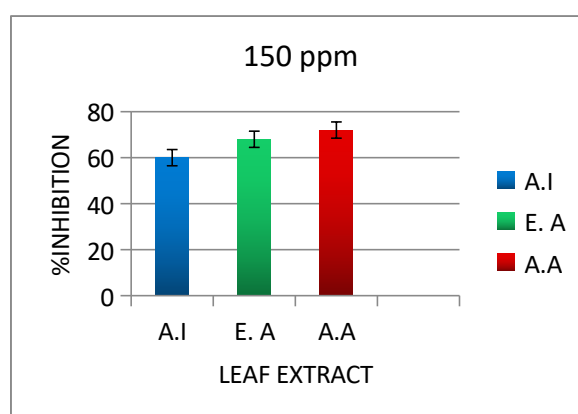
3(a)



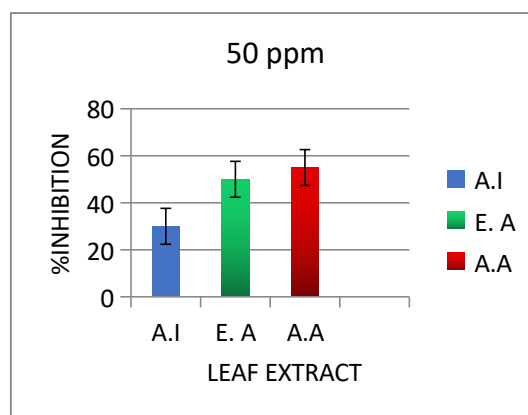
3(b)



3(c)



3(d)



3(e)

Figure 2. Antioxidant Activity (DPPH Method) of the AIRL and EARL at different concentrations from Figure 2A-2E with A-400 PPM, B-200 PPM, C-100 PPM, D-150 PPM, E-50 PPM

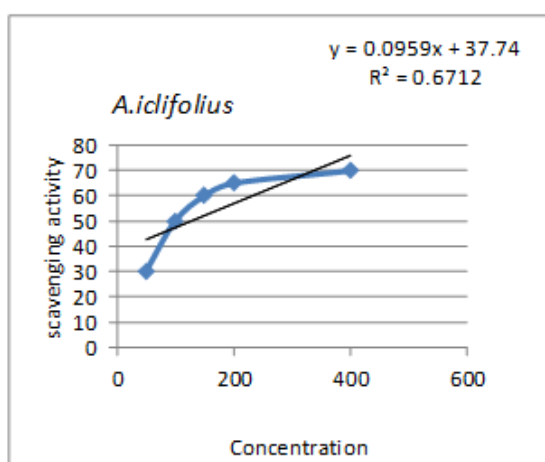


Figure -3(a)

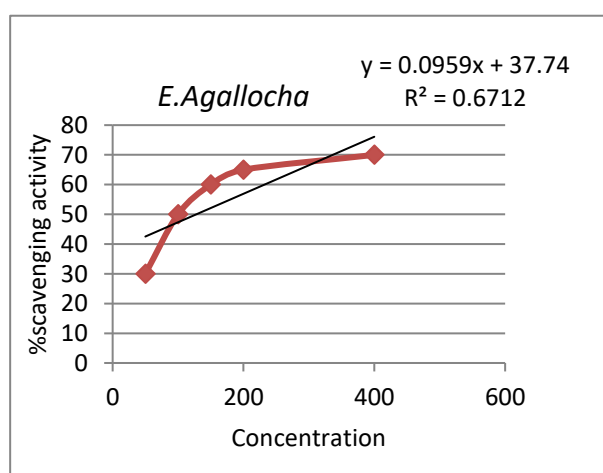


Figure -3(b)

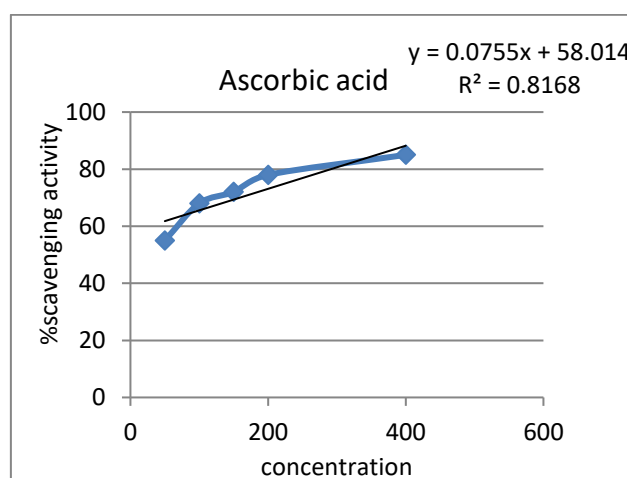


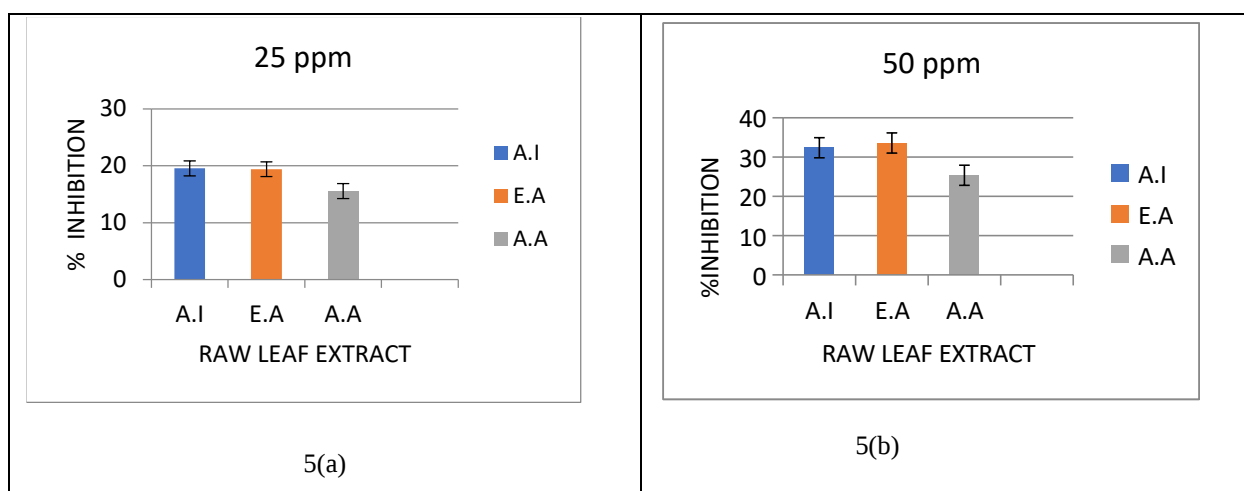
Figure-3(c)



Figure 3. The linear correlation between %scavenging activity and concentration is presented in figures from 3A to 3C (A- ATRL B- EARL C- Ascorbic acid)

Table 3. ABTS activity at different concentrations and IC₅₀ and linear regression analysis

Plant species	% ABTS Activity (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulphonicacid]) radical scavenging activity						IC ₅₀ mg/ml	Linear regression equation	R ²
	Concentration mg/mL ppm	25 ppm	50 ppm	75 ppm	100 ppm	125 ppm	250 ppm		
<i>Acanthus iclifolius</i>		19.54	32.37	54.86	58.39	65.19	76.18	99.956	Y=0.2305X+27.075 0.7532
<i>Excoceria agallocha</i>		19.39	33.58	54.12	56.69	65.2	76.5	100.346	Y=0.2312X+26.828 0.7732
<i>Ascorbic acid (std)</i>		15.56	25.36	45.18	48.58	50.58	60.45	154.88	Y=0.1808X+22.12 0.7227



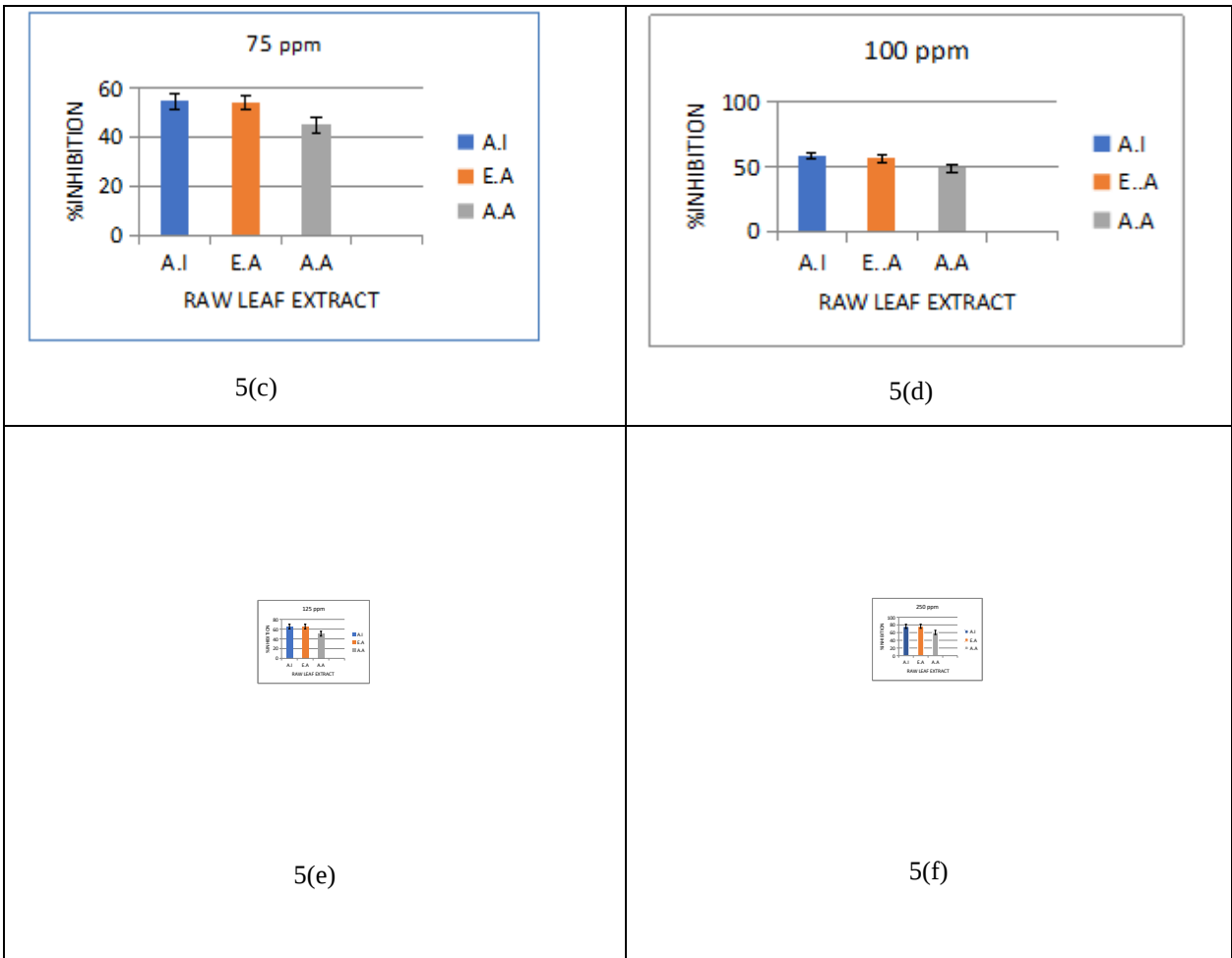
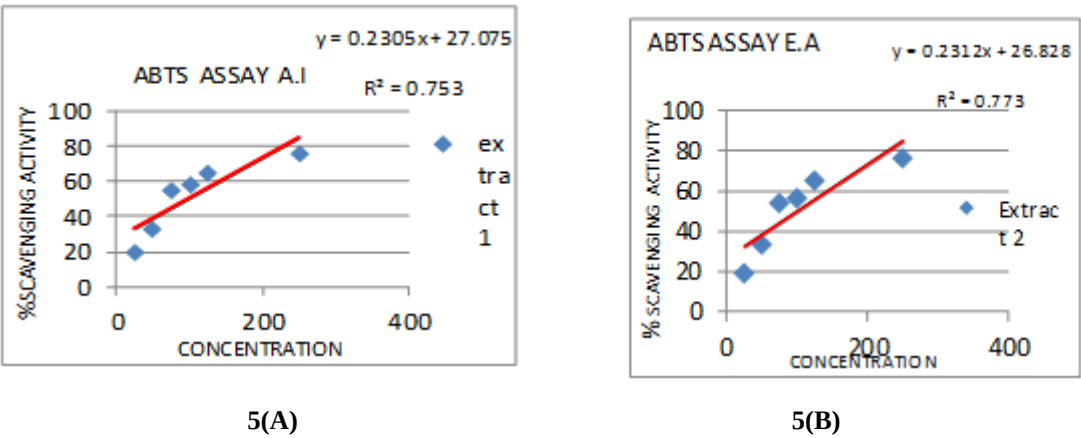
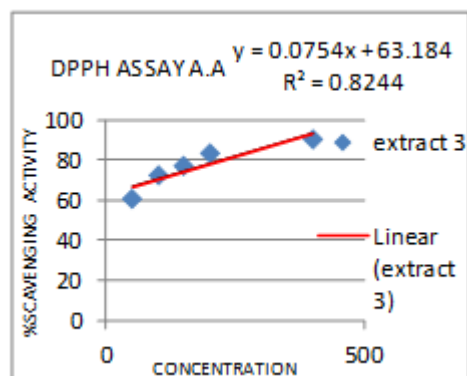


Figure 4 Antioxidant Activity by ABTS Method of the selected plant species at different concentrations from Figure (4A-4F) A-25PPM, B-50PPM, C-75PPM, D-100PPM, E-125PPM, F-250PPM





5(C)

Figure 5. The linear correlation between %ABTS scavenging activity and concentration is given in Figure (5A-5C) A- AIRL-B- EARLC- Ascorbic acid.

The antioxidant activity of the dry leaves extract of *A.ilicifolius* collected from the Tamilnadu state of India and employed the DPPH method and found the concentration as $68.65 \pm 0.5 \mu\text{g mL}^{-1}$ and with vitamin C as standard the concentration was resulted as $76.25 \pm 0.33 \mu\text{g mL}^{-1}$.

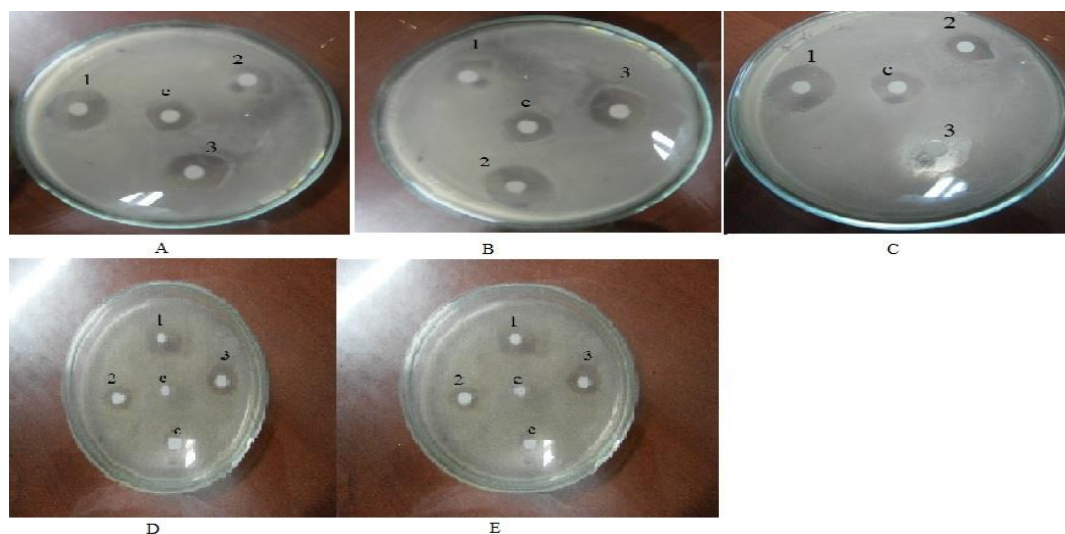


Figure 6. The photographs showing antimicrobial activity from Figure 6A-6E A *Salmonella enterica* B *Streptococcus Pyrogenes* C *Bacillus cereus* D *Pseudomonas.aeruginosa* E *Candida albicans*