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Evaluation of antimicrobial and antioxidant activities of selected medicinal plants

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ABSTRACT
Medicinal plants are a rich source of therapeutic compounds that have tremendous uses in the pharmaceutical industries. Anisomeles malabarica (L.) R. Br and Andrographis echioides (L.) Nees have been used as traditional medicinal plants for various ailments. The two plants extracts was subjected to various phytochemical, pharmacological and microbiological studies. Antimicrobial activity was examined against four gram- positive, four gram-negative bacterial and two fungal strains. Methanolic leaves extracts were prepared and evaluated for free-radical scavenging capacity and antioxidant activity using DPPH, ABTS, FRAP and reducing power assay. The methanolic leaves extract of A. echioides (L.) Nees was more effective antioxidant activity compared to standard drugs. The extracts of A. malabarica (L.) R. Br and A. echioides (L.) Nees were the most effective extracts and showed a strong antimicrobial activity compared to standard drugs. This study demonstrated that A. malabarica (L.) R. Br and A. echioides (L.) Nees extracts exhibit more potential for antimicrobial and antioxidant activity which may be useful for nutritional and medicinal values.
Keywords: *A. malabarica (L.) R. Br, A. echioides (L.) Nees, antimicrobial, antioxidant.*

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INTRODUCTION

Medicinal plants have been traditionally used to treat human diseases for thousands of years because they have a vast and diverse assortment of organic compounds that can produce a definite physiological function on the human body. The most important of such compounds are alkaloids, tannins, flavonoids, terpenoids, saponins and phenolic compounds [1. Many compounds have been isolated from plants which could be used for the development of new drugs to inhibit the growth of bacterial and fungal pathogens and to quench ROS with possibly novel mechanisms of action and low toxicity to the host cell [2]. Reactive oxygen species (ROS)/free radicals have been implicated in the causation of more than 100 diseases including diabetes, inflammation, cancer, neurodegenerative disorders, atherosclerosis, liver cirrhosis, nephrotoxicity, etc., [3-5]. ROS are various forms of activated oxygen such as superoxide anion radicals (O_2^-) and hydroxyl radicals ($OH\cdot$), as well as non-free radicals species (H_2O_2) and singlet oxygen (O_2) [6]. Experimental, clinical and epidemiological studies have provided evidence in support of the role of ROS in the etiology of cancer [7]. All aerobic organisms including humans have antioxidant defense mechanisms that protect against oxidative damage. However, the natural antioxidant defense mechanisms can be insufficient and hence dietary intake of antioxidant components is important and recommended (8). Herbal plants considered good antioxidants since ancient times [9]. *A. malabarica (L.) R.Br* is a medicinal plant that belongs to *(Lamiaceae)* family, is distributed in southern tropical and tropical regions of Asia (10). Ethno botanically, the leaves of *A. malabarica(L.) R.Br* has been used in dyspepsia, intermittent fever and colic. In Indian traditional medicine, the infusion of the leaves are used for stomach ache, cough, cold, fever, epilepsy [11]. *A. malabarica(L.) R.Br* also known to possess antifertility, antispasmodic, anticancer, diuretic, antimicrobial and anticonvulsant activities [12]. *A. echioides (L.) Nees* plants are growing mostly in dry places, such as India, Sri Lanka and South Asian countries and exhibit diuretic [13], analgesic [14], anti-ulcer [15], hepatoprotective [16] and antioxidant [17,18] activities.Hence the present study has designed to evaluate the antimicrobial and antioxidant activity of methanolic leaves extract of *A. malabarica (L.) R.Br* and *A. echioides (L.) Nees* by using DPPH, ABTS, FRAP and reducing power assay methods.

MATERIAL AND METHODS

Collection of plant material

The fresh plants of *A. malabarica (L.) R.Br* and*A. echioides (L.) Nees*** was collected from Thanjavur District, Tamil Nadu, India and taxonomically identified by the Rapinat Herbarium and center for molecular systematic, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India.The collected plants were further surface sterilized and it was shaded and dried. Once completely dried, the plant leaves were ground using the electronic blender. Plant powder has been kept in a tight container until required.

Preparation of plant extract

100-g of both plant powder was subjected to extraction by using a Soxhlet extractor. The extraction was carried out in methanol. The extract was concentrated by a rotary vacuum evaporator and the left over solvent was evaporated to dryness using a water bath.

Determination of antioxidant activity by using an *in-vitro* method

DPPH radical scavenging ability assay

The percentage of antioxidant activity of extracts was assessed by DPPH free radical scavenging assay. The sample was reacted with the stable DPPH radical in methanol. 0.3 mM concentration of DPPH standard solution was prepared by dissolving 118.2 g of DPPH (1,1-diphenyl-2-picrylhydrazyl) in 1000 mL of methanol. The sample stock solution was made by dissolving 0.01g in 1 mL of respective solvents (100 mg/mL) and from that different concentration was prepared such as 10, 20, 30, 40 and 50 µg/mL. One milliliter of each sample solution was mixed with two milliliters of DPPH reagent and stored in a dark place and then allowed to react at room temperature for 30 minutes. When DPPH reacts with the antioxidant compounds present in the plant extracts, reduces the DPPH and changes its color from deep violet to light yellow. After 30 minutes, the absorbance was recorded at 517 nm in UV-Visible spectrophotometry and the percentage of radical scavenging activity i.e., antioxidant activity was calculated by following the standard formula. Control reading was read by adding one milliliter of solvent with two milliliters of DPPH reagent (19, 20).

$$\% \text{ of DPPH Scavenged} = \frac{\text{Ab of control} - \text{Ab of test}}{\text{Ab of control}} \times 100$$

Ab of control= Control Absorbance, Ab of test= Test solution Absorbance

The IC_{50} values were calculated by linear regression plots, where the abscissa represented the concentration of the tested sample and the ordinate the average percent of radical scavenging activity.

ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging assay

Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay. $ABTS^{\cdot+}$ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. $ABTS^{\cdot+}$ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µL of plant extracts to 3.995 mL of diluted $ABTS^{\cdot+}$ solution, the absorbance was measured at 30 minutes after the initial mixing. An appropriate solvent blank was run in each assay. All the measurement was carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula. Trolox was used as a standard substance (21).

$$\% \text{ of Scavenging Activity} = \frac{\text{Ab of control} - \text{Ab of test}}{\text{Ab of control}} \times 100$$

Ab of control= absorbance of ABTS radical + methanol, Ab of test= absorbance of ABTS radical + sample extract/standard.

The IC_{50} values were calculated by linear regression plots, where the abscissa represented the concentration of the tested sample and the ordinate the average percent of radical scavenging activity.

FRAP (Ferric Reducing/Antioxidant Power) ASSAY

The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2$. $3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3$. $6H_2O$ solution and then it was warmed at 37 ^{0}C. Different concentration of extracts (0.15 mL) was allowed to react with 2.80 mL of the FRAP solution for 30 minutes in the dark condition. Readings of the

colored product (ferrous tripyridyl triazine complex) was taken at 593 nm. Trolox was used as a standard substance (22).

Reducing power assay

Various concentrations of the extracts in corresponding solvents were mixed with phosphate buffer (2.5 mL) and potassium ferricyanide (2.5 mL). This mixture was kept at 50 ^{0}C in a water bath for 20 minutes. After cooling, 2.5 mL of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes whenever necessary. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared ferric chloride solution (0.5 mL). The absorbance was measured at 700 nm. Control was prepared similarly excluding samples. Various concentrations of ascorbic acid has used as standard. Increased absorbance of the reaction mixture indicates an increase in reducing power activity [23].

Determination of anti-microbial activity

The *in vitro* antimicrobial activity of the methanolic leaves extract of *A. malabarica (L.) R. Br* and *A. echioides (L.) Nees*is assessed by various assays towards pathogenic bacteria and fungi. The bacteria include both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Streptococcus epidermidis* and *E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi* andfungi *Aspergillus niger* and *Candida albicans.* Bacterial strain was maintained on Nutrient agar slants (Hi media) at 4 ^{0}C. The inhibitory action was observed in terms of the diameter of the inhibition zone formed around each disc caused by the disc diffusion of antimicrobial substances from the paper discs into the surrounding medium. The plant extracts have the maximum antimicrobial activity. The diameter of the inhibition zone for each extract against each microorganism was found to be less than the standard antibiotic drug (Gentamicin 10 µg/disc).

RESULTS

Free radical-scavenging ability by the DPPH assay

The DPPH is a stable radical with maximum absorption at 517 nm that can readily undergo scavenging by antioxidants [24]. It has been widely used to test the ability of compounds as free-radical scavengers hydrogen donors and to evaluate the anti-oxidative activity of plant extracts [25, 26]. Lower the IC_{50} value greater than its antioxidant activity. The anti-radical activity of the tested plant extracts were measured by the ability to scavenge DPPH free radicals and compared with standard in Fig-1. The methanolic leaves extract of *A. echioides (L.) Nees* showed significant antioxidant activity**.** The better antioxidant activity of the *A. echioides (L.) Nees* leaves extracts may be associated with higher phenolic compounds present in the leaves extracts.

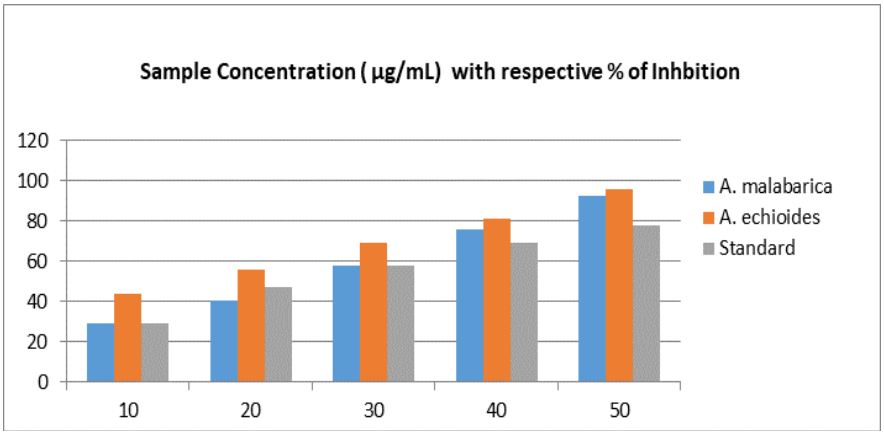

Fig-1: DPPH radical scavenging activity of the extracts

Free radical-scavenging ability by the ABTS assay

The working mechanism of the ABTS method for the evaluation of antioxidant activity is the same as that of the DPPH method but the ABTS method is more reliable than the DPPH method, due to the solubility of the ABTS reagent in both aqueous and organic solvents and rapid reaction with lipophilic as well as hydrophilic antioxidant species as compared to DPPH (27, 28). ABTS is a protonated radical that has a characteristic maximum at 734 nm, which decreases with the scavenging of proton radicals. The prominent scavenging activity is observed while increasing the concentration of the plant extract. The methanolic leaves extract **of** *A. malabarica (L.) R. Br* and *A. echioides (L.) Nees* show moderate ABTS radical scavenging activity compared to standard drug (Trolox) in Fig-2.

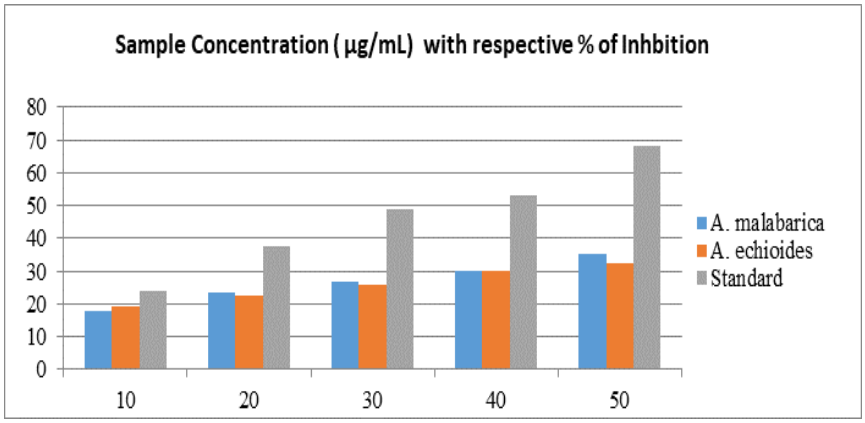

Fig-2: ABTS radical scavenging activity of the extracts

Free radical-scavenging ability by the FRAP assay

The FRAP assay assesses an antioxidant reducing capacity by converting a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) complex (29). Generally, the reducing abilities are related to the presence of compounds that exert their action by breaking the free radical chain by donating a hydrogen atom [30]. Therefore the antioxidant potential of different concentrations of ***A. malabarica (L.) R. Br and A. echioides (L.) Nees*** leaves exhibit moderate power reducing activity compared to the standard drug (Trolox) in Fig-3.

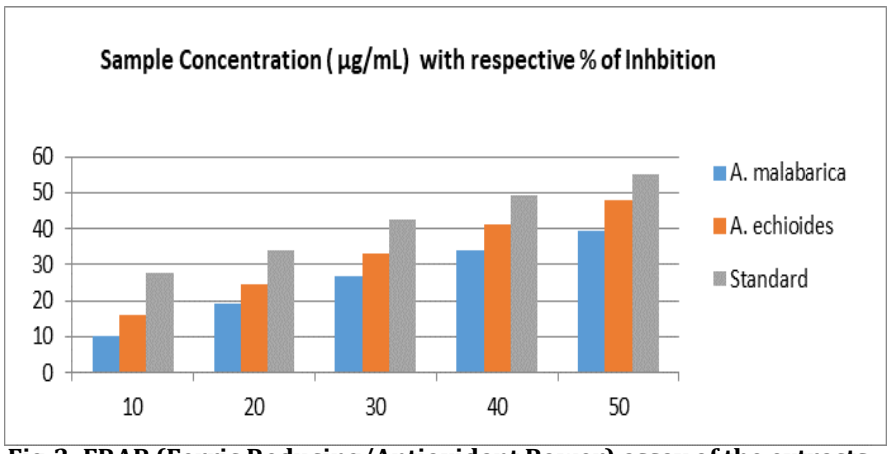

Fig-3: FRAP (Ferric Reducing/Antioxidant Power) assay of the extracts.

Free radical-scavenging ability by the Reducing power assay

The reducing power activity of A. malabarica (L.) R. Br and A. echioides (L.) Nees leaves extract exhibited better activity when increasing the concentrations of the plant extracts. Increasing absorbance at 700 nm indicated an increase in reductive ability. The tested plant extracts showed good reducing power activity that is compared with standard drug (Ascorbic acid) in Fig-4.

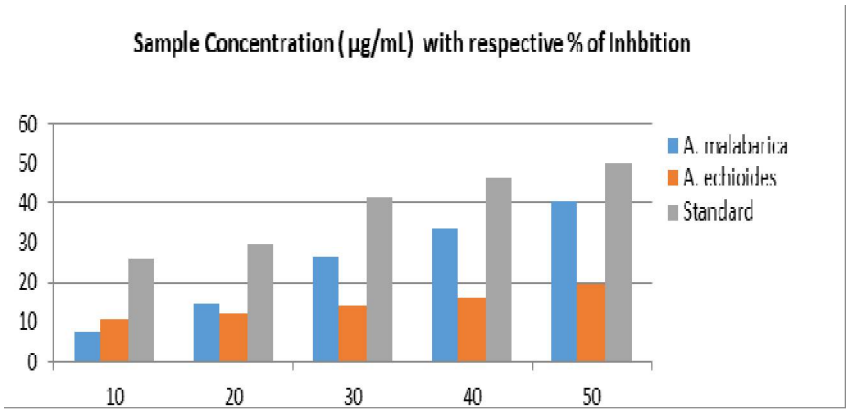

Fig-4: Reducing power activity of the extracts

IC_{50} value of different radical scavenging tests (DPPH, ABTS, FRAP and Reducing power)

In this study, the antioxidant activity of the plant extracts are evaluated using four different assays (DPPH, ABTS, FRAP and Reducing power). The obtained results summarized in Table-1 revealed that for the DPPH method, the lowest IC_{50} values corresponding to the most antioxidant substances are found for the plant extracts. Hence the higher activity shows the relation between abundant quantities of phenolic and flavonoids. For instance, *A. malabarica (L.) R. Br and A. echioides (L.) Nees* methanolic leaves extract plays a significant role in DPPH radical scavenging activity IC_{50} values of 24.53 µg/mL and 15.07 µg/mL.

Table-1: Percentage inhibitory concentration (IC_{50}) values of *A. malabarica (L.) R.Br* and *A. echioides (L.) Nees.*

S. No	Extract Name	IC_{50} Value µg/mL			
		DPPH	ABTS	FRAP	Reducing power
1	*A. malabarica (L.) R. Br*	24.53	85.34	64.16	49.07
2	*A.echioides (L.) Nees*	15.07	104.54	52.53	192.72
3	Standard	24.36	32.67	42.11	49.75

Antimicrobial activity

The present study showed that the two plants extract *(A. malabarica (L.) R. Br* and *A. echioides (L.) Nees*) are displayed antimicrobial activities against all the ten pathogens tested. Evaluation of antimicrobial activities of these plant extracts are presented in (Table-2, 3. Fig-5, 6). The tested plant extracts is potentially effective against gram-positive, gram-negative bacteria and fungi. When the two crude extracts are compared with standard antibiotic (Gentamicin).The plant extracts showed varying degree of antimicrobial potential due to different concentrations.

Table-2: Antimicrobial activity of *A. malabarica (L.) R. Br*

S. No	Name of organisms	Inhibition values in mm				
		25µL	50µL	75µL	100µL	Control
1	*Bacillus subtilis*	16	20	22	26	20
2	*Staphylococcus aureus*	14	16	19	22	20
3	*Enterococcus faecalis*	10	12	14	16	18
4	*Streptococcus epidermidis*	14	16	18	20	20
5	*Escherichia coli*	14	17	20	24	20
6	*Pseudomonas aeruginosa*	14	16	18	20	20
7	*Klebsiella pneumonia*	16	20	24	28	20
8	*Salmonella typhi*	14	16	19	21	18
9	*Candida albicans*	12	14	16	18	20
10	*Aspergillus niger*	16	18	20	22	20

Table-3: Antimicrobial activity of *A. echioides (L.) Nees*

S. No	Name of organisms	Inhibition values in mm				
		25 µL	50 µL	75 µL	100 µL	Control
1	*Bacillus subtilis*	20	23	26	30	20
2	*Staphylococcus aureus*	20	23	26	28	20
3	*Enterococcus faecalis*	20	24	26	29	18
4	*Streptococcus epidermidis*	18	22	24	26	20
5	*Escherichia coli*	20	24	26	28	20
6	*Pseudomonas aeruginosa*	20	24	27	30	20
7	*Klebsiella pneumonia*	20	22	24	27	18
8	*Salmonella typhi*	18	21	23	26	20
9	*Candida albicans*	20	24	26	29	20
10	*Aspergillus niger*	20	25	30	35	22

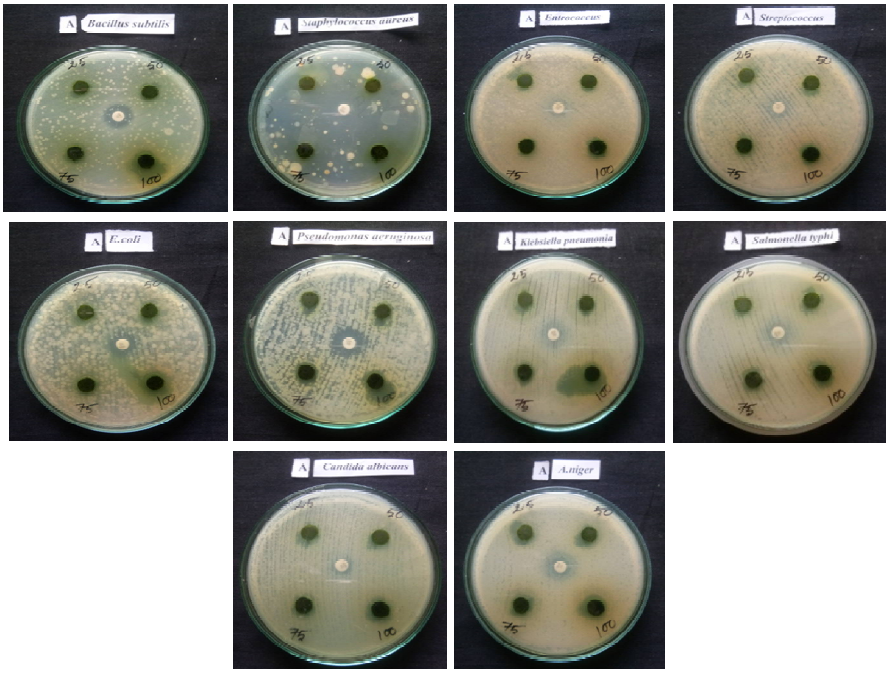

Fig-5: Antimicrobial activity of *A. malabarica (L.) R.Br* leaves extract

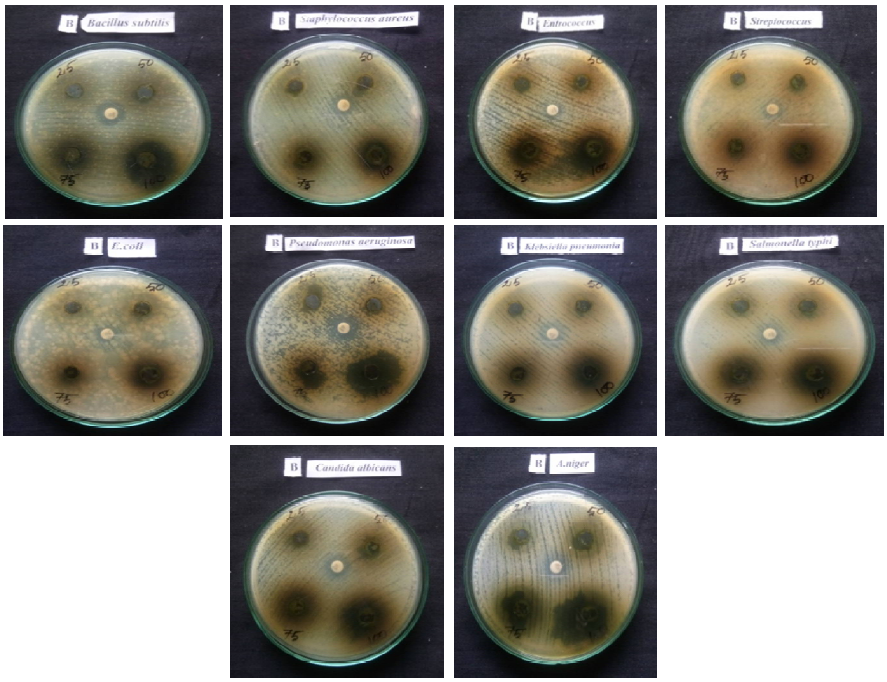

Fig-6: Antimicrobial activity of *A. echioides (L.) Nees* leaves extract

DISCUSSION

Antioxidant activity

In this study, methanolic extracts from two plants have been evaluated for their antioxidant activity. The antioxidant activity of the extracts is evaluated based on their ability to trap DPPH radicals. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts (31, 32). Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants are due to their scavenging activity and are useful for the management of diseases. DPPH is a stable free radical and can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Two plant extracts test inhibited the DPPH radical but, in different manners. This result proved that the extracts are capable of donating an electron that could react with DPPH radical. The variation are observed between the scavenging activities of the same extracts depends on the concentration of the plant extracts. The extract of *A. echioides (L.) Nees* have the lowest IC_{50} (15.07 µg/mL) followed by the *A. malabarica (L.) R. Br* extract (24.53 µg/mL) and the standard is (24.36 µg/mL), demonstrating that *A.*

echioides (L.) Nees has the greatest scavenging activity. Plant extracts are made of a mixture of several scavenging compounds which could act in a synergetic manner to enhance the antiradical activity. Moreover, the antiradical activity of the extracts to trap DPPH radicals depends on the availability and the ability of these extracts to give electrons (33). The scavenging activity of extracts of *A. malabarica (L.) R. Br* and *A. echioides (L.) Nees* against ABTS radicals in a concentration dependent manner. Comparable scavenging activity is observed between the extract and the standard drug. The IC_{50} values of the standard drug is 32.67 µg/mL, respectively while *A. malabarica (L.) R.Br* was85.34 µg/mL and *A. echioides (L.) Nees* is104.54 µg/mL. This observation indicates the strong antioxidant potential of the extracts which is confirmed with ABTS radicals (34, 35). The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and produces a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) (29). Generally, the reducing properties are linked with the presence of compounds that exert their action by breaking the free radical chain by donating a hydrogen atom (30). The absorbance of *A. malabarica (L.) R. Br* and *A. echioides (L.) Nees* increased, due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration as seen also in the reference antioxidant. Hence the plant extracts are able to donate electrons to free radicals stable in the actual biological systems. The methanolic leaves extract of *A. malabarica (L.) R. Br* and *A. echioides (L.) Nees* are a considerable antioxidant effect in ABTS assays. The reference antioxidant is (42.11 µg/mL) with higher scavenging activity at all the tested concentrations. Since the reducing power activity of the plant extract could serve as a significant indicator of the antioxidant potential. This property has assessed by measuring the ability of the extracts to transform Fe^{3+} to Fe^{2+} and to donate an electron. At 49.07 µg/mL, the extract of *A. malabarica (L.) R. Br* leaves showed the highest reducing activity and *A. echioides (L.) Nees* is lower than the standard. The ability of the extracts to reduce Fe^{3+} could be attributed either to the reducing agents such as phenol groups and the number and the position of the hydroxyl molecule on these groups (36). Plants polyphenols act as reducing agents and antioxidants by the hydrogen donating property of their hydroxyl group (37). Hence, these polyphenols may be responsible for the observed antioxidant in this study. The tested plant extracts showed higher antioxidant activity in the entire assay with the expectation of the DPPH assay having higher activity at all the tested concentrations.

Antimicrobial activity

In this present study, two plants extracts are investigated to evaluate their antimicrobial activity against four gram-positive (*B. subtilis, S. aureus, E. faecalis, S. epidermidis*) and four gram-negative (***E. coli, S. typhi***, ***P. aeruginosa,*** *K. pneumonia*) bacterial and two fungal (*C. albicans, A. niger)* strains using disc diffusion method. The results revealed that two plant extracts are potentially effective in suppressing microbial growth of bacteria and fungi with variable potency and the results are compared to standard drug (Gentamicin). Methanolic leaves extract of *A. malabarica (L.) R. Br* is found to be potentially effective against gram-positive (*B. subtilis, S. aureus)* gram-negative *(K. pneumonia, E. coli)* bacteriaand fungi (*A. niger).* Results showed thatcrude methanolic leaves extract of *A. echioides (L.) Nees* has potential against all tested gram-positive, gram-negative bacterial and fungi strains. These antimicrobial actions could be related to their chemical components in the crude extract (38). Therefore, the conspicuous antimicrobial activity exhibited by methanolic extract in the present study may be attributed to the presence of phenolic compounds.

CONCLUSION

The methanolic leaves extract of *A. malabarica (L.) R. Br* and *A. echioides (L.) Nees* contains natural active phytochemical components that possess significant antioxidant potential which is relatively equal to synthesized antioxidants. The results of this study clearly promise for the development of novel drug molecules. *A. malabarica (L.) R. Br* and *A. echioides (L.) Nees* methanolic leaves extract showed better inhibitory antimicrobial activity against all the tested pathogenic clinical bacterial and fungal strains. The antimicrobial activity results are also comparable to that of the antibiotic used as a standard reference drug. *In vitro* antimicrobial evaluation of secondary metabolite products of *A. malabarica (L.) R. Br* and *A. echioides (L.) Nees* forms a primary platform for further pharmacological investigation for the development of new potential antimicrobial compounds.

CONFLICT OF INTEREST

The authors declared no conflict of interest

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