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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *Anisomeles malabarica (L.) R.Br* LEAVES EXTRACTS

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ABSTRACT

Plants contain a variety of bioactive chemicals with tremendous uses in the pharma industry. Methanolic leaves extracts of *A. malabarica (L.) R. Br* was analyzed in order to find a new source of antimicrobial and antioxidant constituents. Four gram positive, four gram negative bacteria and two fungal strains were tested for antimicrobial activity. The antioxidant activity of methanolic extracts was evaluated using the DPPH, ABTS, FRAP, and reducing power assays. The antioxidant activities of the leaves extract showed that significantly inhibited all radicals. When compared to the standard, the methanolic leaves extracts of *A. malabarica (L.) R. Br* showed higher antioxidant activity. This research has found that *A. malabarica (L.) R. Br* extracts have higher antibacterial activity, which could be beneficial to nutritive and medicinal purposes.

Keywords: *A. malabarica (L.) R. Br,* antimicrobial, antioxidant, methanolic, leaves extracts.

INTRODUCTION

Medicinal plants have been utilized to cure disease for centuries because they contain a wide range of chemical constituents which can have a specific biological effect on

humans. Flavonoids, terpenoids, saponins alkaloids, tannins, and phenolic compounds are the most important of these phytochemicals **[1]**. A variety of these compounds were identified in plants, and they could be utilized to produce new medicines to prevent the growth of bacterial and fungal infections, as well as to extinguish ROS, using innovative modes of action and low toxicity to the cell membrane **[2]**. More than 100 illnesses have been related to reactive oxygen species (ROS)/free radicals, such diabetes, inflammation, cancer, neurological disorders, atherosclerosis, liver cirrhosis, nephrotoxicity, and more **[3-5]**. ROS as several forms of activated oxygen, including superoxide anion radicals (O_2^-) and hydroxyl radicals ($OH^\cdot$), as well as non-free radical 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, because the body's natural antioxidant defense mechanisms may be insufficient, dietary antioxidant supplementation is important and recommended **[8]**. Herbal plants considered as good antioxidant since ancient times **[9]**.

The therapeutic plant *A. malabarica (L.) R.Br* belonging to the (*Lamiaceae*) family and is found in Asia southern tropical and tropical areas **[10]**. The leaf of *A. malabarica (L.) R.Br* is used older to treat dyspepsia, intermittent fever, and colic. The decoction of the leaf can be used for stomach ache, cough, cold, fever, epilepsy **[11]** and halitosis, hysteria, amentia, anorexia, flatulence, intestinal worms, teething fever, intermittent fever, gout, swelling, and diarrhoea in Ayurvedic medicine **[12]**. Antifertility, antispasmodic, diuretic, and anticonvulsant properties have been reported for *A. malabarica (L.) R.Br* **[13]**. Hence the current study was designed to evaluate the antimicrobial and antioxidant activity of methanolic leaves extracts of *A. malabarica (L.) R. Br* by using DPPH, ABTS, FRAP and reducing power assay methods.

MATERIALS AND METHODS

Collection of plant material

The fresh plant of *A. malabarica (L.) R. Br* 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 plant was further surface sterilized and it was shaded

dried. Once completely dried, the plant leaves was grinded using the electronic blender. Plant powder was kept in a tight container until required.

Preparation of plant extracts

About 100-g plant powder was subjected to extraction by a Soxhlet extractor. The extraction was carried out in methanol. A rotary vacuum evaporator was used to concentrate the extract, and the remaining solvent was evaporated to dryness in a water bath.

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

DPPH radical scavenging ability assay

The percentage of antioxidant activity of extracts was assessed by DPPH free radical 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. 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 combined with two milliliters of DPPH reagent and kept in the dark for 30 minutes before reacting at room temperature. When DPPH combines with antioxidant chemicals found in plant extracts, the DPPH is reduced and the colour changes from deep violet to pale 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 standard formula. Control reading was readed by adding one milliliter of solvent with two milliliter of DPPH reagent **[14, 15]**.

% 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

Linear regression graphs were used to obtain the IC_{50} values, with the abscissa representing the concentration of the tested sample and the ordinate representing the average percent of radical scavenging activity.

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

The ABTS radical cation decolorization assay was used to determine the plant sample's free radical scavenging activity. The reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate

(1:1) produced the $ABTS^{\bullet+}$ cation radical, which was kept in the dark at room temperature for 12-16 hours before use. After diluting the $ABTS^{\bullet+}$ solution with methanol, an absorbance of 0.700 at 734 nm was obtained. The absorbance was measured 30 minutes after the addition of 5 liters of plant extracts to 3.995 liters of diluted $ABTS^{\bullet+}$ solution. In each test, a suitable solvent blank was used. All of the tests were repeated at least three times. The formula was used to compute the percent suppression of absorbance at 734 nm. As a standard drug, Trolox was employed **[16]**.

% 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. 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3$. $6H_2O$ solution were combined to make the fresh working solution, which was then warmed to 37 ^{0}C. For 30 minutes in the dark, different concentrations of extracts (0.15 mL) were allowed to react with 2.80 mL of the FRAP solution. At 593 nm, the colorful product (ferrous tripyridyl triazine complex) was measured. As a standard drug, Trolox was employed **[17]**.

Reducing power assay

Phosphate buffer (2.5 mL) and potassium ferricyanide were combined with various amounts of extracts in matching solvents (2.5 mL). This mixture was maintained in a water bath at 50 degrees Celsius for 20 minutes. When necessary, 2.5 mL of 10% trichloroacetic acid was added after cooling and centrifuged at 3000 rpm for 10 minutes. The solution was combined with distilled water (2.5 mL) and a freshly made ferric chloride solution in the upper layer (2.5 mL) (0.5 mL). At 700 nm, the absorbance was measured. The control was made in the same way as the samples, but without the samples. As a standard, various amounts of ascorbic acid were utilized. Increased absorbance of the reaction mixture indicates increase in reducing power **[18]**.

Determination of anti-microbial activity

The *in vitro* antimicrobial activity of the methanolic leaves extracts of *A. malabarica (L.) R. Br* 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* and fungi are *Aspergillus niger* and *Candida albicans*. Bacterial strains were kept at 4 degrees Celsius on Nutrient agar slants (Hi media). The diameter of the inhibition zone created around each disc as a result of disc diffusion of antimicrobial compounds from the paper discs into the surrounding medium is measured. The antibacterial activity of plant extracts is the highest. The diameter of each extract's inhibition zone against each microbe is shown to be less than that of a typical antibiotic medication. (Gentamicin 10 µg/disc).

RESULTS AND DISCUSSION

Free radical-scavenging ability by the DPPH assay

The DPPH is a stable radical with a maximum absorption at 517 nm that can readily undergo scavenging by antioxidant **[19]**. It has been frequently used to evaluate the anti-oxidative activity of plant extracts and to examine the ability of substances as free-radical scavengers or hydrogen donors **[20, 21]**. The interaction of antioxidants to produce oxidative free radicals is the principle of antioxidant activity. The role of DPPH method is that the antioxidants react with the stable free radical. During the free radical reaction, DPPH is converted into α, α-diphenyl-β-picrylhydrazine with colour change. The rate of colour change gradually decreases which indicates the scavenging potentials of the sample antioxidant. The scavenging abilities of methanolic extracts of *A. malabarica (L.) R. Br* was concentration dependent and expressed as IC_{50} values. Concentration of the sample necessary to decrease the initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was calculated. Therefore a lower IC_{50} value indicates a higher antioxidant activity. The inhibition range of *A. malabarica (L.) R. Br* antioxidant activity is 24.53 µg/mL at varied concentrations (10, 20, 30, 40, and 50 µg/mL), compared to the standard (Ascorbic acid) 24.36 µg/mL in Table-1 and illustrated in Fig-1,2. So the methanolic leaves extract of *A. malabarica (L.) R. Br* was showed good DPPH scavenging activity. Plant extracts are made up of a combination of various scavenging

chemicals that may work together to boost antiradical action. Furthermore, the antiradical activity of the extracts in trapping the DPPH radical is dependent on their availability and ability to donate electrons **[22]**. This extracts has a potential antioxidant which would enable them to play a beneficial role in terms of very significant preventive actions for human health.

Free radical-scavenging ability by the ABTS assay

$ABTS^{\bullet+}$ assay is an excellent tool to determine hydrogen donating antioxidants (scavenging aqueous phase radicals) and chain-breaking antioxidants (scavenging lipid peroxyl radicals). The suppression of $ABTS^{\bullet+}$ radicals in the methanolic leaves extracts of *A. malabarica (L.) R. Br* was concentration dependent. The inhibition range of *A. malabarica (L.) R. Br* antioxidant activity is 85.34 µg/mL at varied concentrations (10, 20, 30, 40, and 50 µg/mL), compared to the standard (Trolox) 32.67 µg/mL. The free radical scavenging activity of the leaves extracts found to possess better activity when the concentration of the leaves extracts increases. The ABTS radical scavenging capabilities of *A. malabarica (L.) R. Br* methanolic leaves extracts was lower activity than standard in **Table 2** and illustrated in **Figure 3, 4**.

Free radical-scavenging ability by the FRAP assay

Antioxidants can be defined as oxidant reductants and inactivators **[23]**. Previous research has also suggested that reducing power could be a useful predictor of possible antioxidant action. It has been suggested that anti-oxidative activity is linked to decreasing power. Therefore, the antioxidant potential of different concentrations of leaves extracts of *A. malabarica (L.) R. Br* was estimated for their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II). A comparable scavenging activity was observed between the extracts and the standard drug. The IC_{50} values of standard (Trolox) was 42.11 µg/mL, respectively will methanolic extracts of *A. malabarica (L.) R. Br* is 64.16 µg/mL. Standard record high inhibitory activities compared to the extracts of *A. malabarica (L.) R. Br* in **Table 3** and illustrated in **Figure 5, 6**.

Free radical-scavenging ability by the Reducing power assay

We measured the ability of the extracts to change Fe^{3+} to Fe^{2+} and donate an electron since the reducing power activity of the compounds could serve as a significant indicator of the antioxidant potential **[24]**. The reducing power activity of the

methanolic extracts increased with the concentration of the extracts. At the concentration higher than 30 µg/mL, a significant variation of the reducing property of the extracts was noted compared to the standard drug (Ascorbic acid) in **Table 4** and illustrated in **Figure 7, 8**. At 49.07 µg/mL, the extracts of *A. malabarica (L.) R. Br* showed the highest reducing capacity compared to standard (Ascorbic acid) 49.75 µg/mL. The methanolic leaves extracts of *A. malabarica (L.) R. Br* was demonstrated the higher reducing power property at 50 µg/mL. The capacity of the extracts to reduce Fe^{3+} could be related to reducing agents such phenol groups, as well as the number and position of the hydroxyl molecule on these groups **[25]**. Plant polyphenols use the hydrogen-donating property of their hydroxyl group to serve as reducing agents and antioxidants **[26]**. Hence, we could conclude that these polyphenols are responsible for the observed antioxidant in this study.

Table 5, below presence the IC_{50} (50% inhibitory concentration) of the methanolic leaves extracts tested. The results showed that the IC_{50} varied between 24.53 µg/mL and 49.07 µg/mL depending on the type of radicals. The different concentration of extracts tested for DPPH, ABTS and FRAPS radicals showed the better IC_{50} compared to the standard. The IC_{50} of the extracts of *A. malabarica (L.) R. Br* reducing power assay was lower (49.07 µg/mL) than the standard (49.75 µg/mL). The scavenging activity of *A. malabarica (L.) R. Br* extracts was comparable to standard drugs used in this study. This observation gives an indication of strong antioxidant potential of the extracts which is confirmed with DPPH, FRAP, reducing power, ABTS radicals. The findings suggest that the extracts could be used as free radical inhibitors or scavengers due to their proton-donating activity, or as a main antioxidant. The extracts were shown to have significant antioxidant activity and could be effective in the treatment of illnesses, according to the findings.

Antimicrobial activity

The results from the present study showed that the plant extracts of *A. malabarica (L.) R. Br* was displayed antimicrobial activities against all the ten human pathogens tested. Evaluation of antimicrobial activities of these plant extracts was recorded in **Table 6** and illustrated in **Figure 9**. The plant extracts showed promise against gram positive and gram negative bacteria as well as fungus. The plant extracts demonstrated various degrees of antibacterial potential when compared to a

standard antibiotic (gentamicin) due to different concentrations.

In the plant extracts 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 findings demonstrated that plant extracts had varying effectiveness in suppressing microbiological development of bacteria and fungi, and the results were compared to standard (Gentamicin). Methanolic leaves extracts of *A. malabarica (L.) R. Br* found to be potentially effective against gram positive (*B. subtilis, S. aureus)* gram negative *(K. pneumonia, E. coli)* bacteria and fungi (*A. niger).* The antimicrobial activity of the extracts of *A. malabarica (L.) R. Br* was estimate using standard conventional method. The presence of antimicrobial substances in this plant was demonstrated by the fact that varied concentrations of plant extracts inhibited the development of nearly all of the bacteria utilized in the assay. These antibacterial properties could be attributed to the crude extracts chemical components **[27]**. As a result, the presence of phenolic compounds in methanolic leaf extracts may be responsible for the high antibacterial activity observed in this investigation.

Table 1: Analysis of DPPH radical scavenging activity of leaves extracts of *A. malabarica (L.) R. Br*

S. No.	Extract Name	Sample Concentration (µg/mL) with respective % of Inhbition					IC_{50} Value µg/mL
		10	20	30	40	50	
1	*A. malabarica (L.) R. Br*	29.21±0.04	40.51±0.15	58.10±0.41	75.81±0.33	92.22±0.27	24.53
2	Ascorbic acid	29.08±1.23	47.50±1.50	58.17±1.98	69.01±2.05	78.14±2.97	24.36

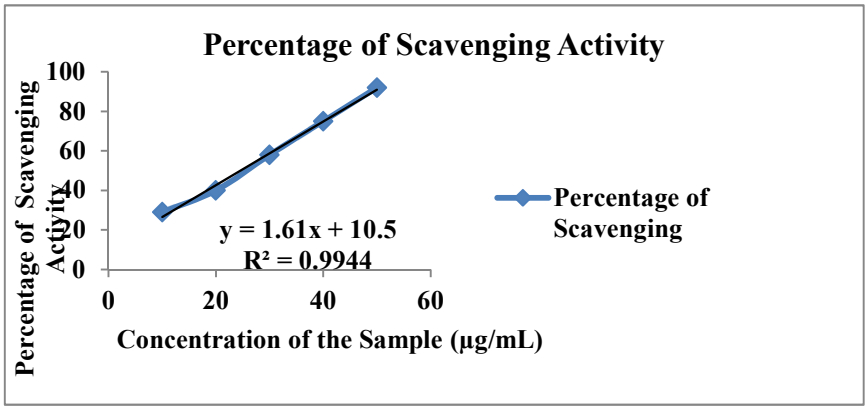

Figure 1: DPPH radical scavenging activity of the extracts of *A. malabarica (L.) R. Br*

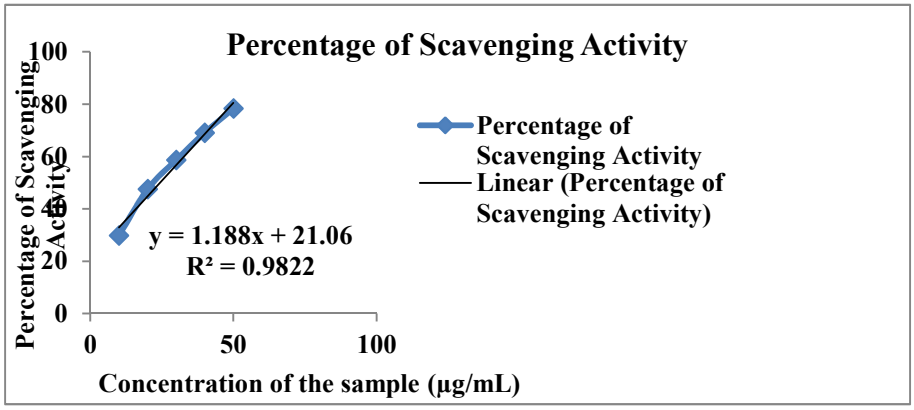

Figure 2: DPPH radical scavenging activity of standard (Ascorbic acid)

Table 2: Analysis of ABTS radical scavenging activity of leaves extracts of *A. malabarica (L.) R. Br*

S. No.	Extract Name	Sample Concentration (µg/mL) with respective % of Inhbition					IC_{50} Value µg/mL
		10	20	30	40	50	
1	*A. malabarica (L.) R. Br*	17.65±0.02	23.31±0.01	26.77±0.04	30.09±0.03	35.26±0.02	85.34
2	Trolox	23.8±0.43	37.5±0.50	48.7±0.90	53.1±1.05	68.4±1.07	32.67

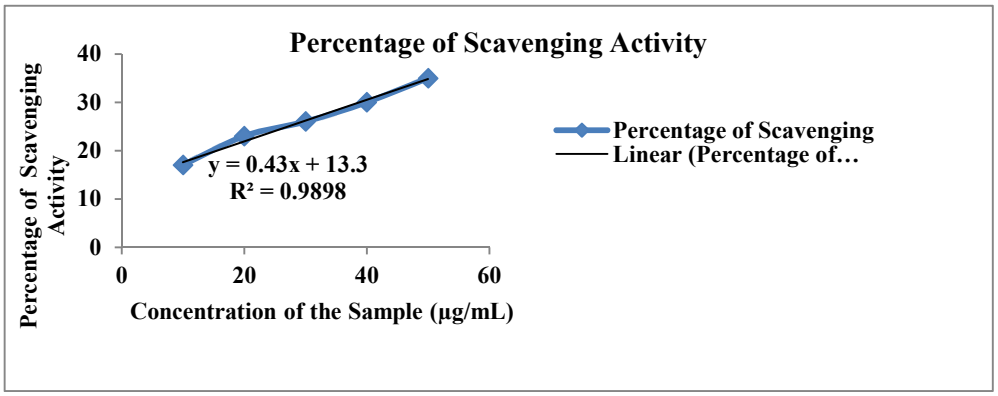

Figure 3: ABTS radical scavenging activity of the extracts of *A. malabarica (L.) R. Br*

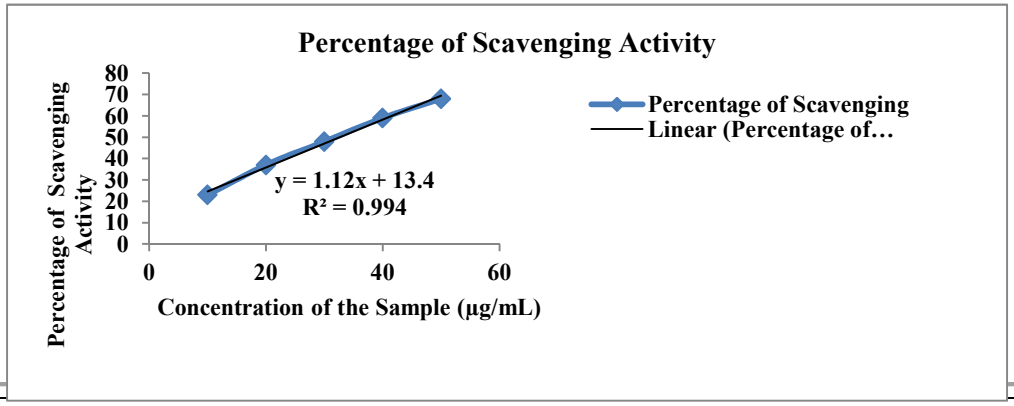

Figure 4: ABTS radical scavenging activity of standard (Trolox)

Table 3: Analysis of FRAP radical scavenging activity of leaves extracts of *A. malabarica (L.) R. Br*

S. No.	Extract Name	Sample Concentration (µg/mL) with respective % of Inhbition					IC_{50} Value µg/mL
		10	20	30	40	50	
1	*A. malabarica (L.) R. Br*	10.24±0.06	19.2±0.00	26.9±0.04	33.8±0.33	39.3±0.00	64.16
2	Trolox	27.60±0.01	34.01±0.11	42.72±0.04	49.09±0.13	55.06±0.12	42.11

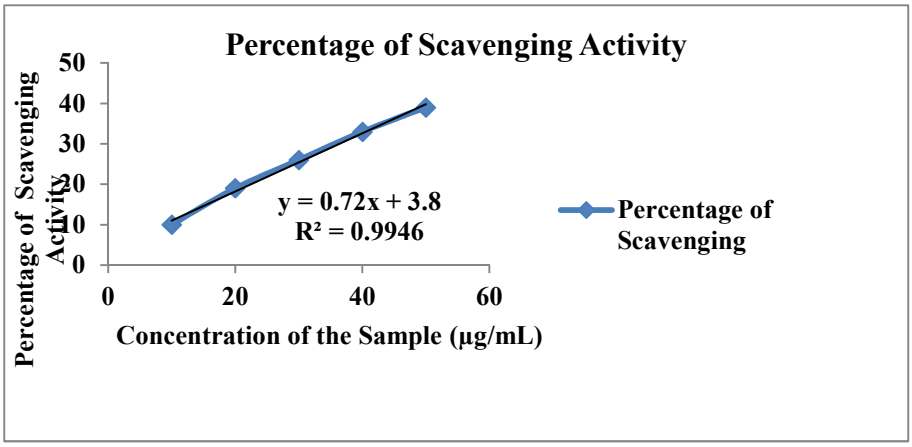

Figure 5: FRAP (Ferric Reducing/Antioxidant Power) assay of the extracts of *A. malabarica (L.) R. Br*

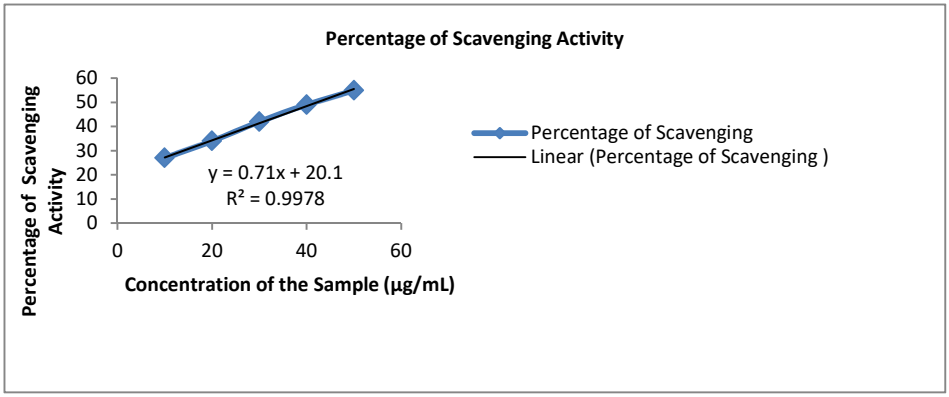

Figure 6: FRAP radical scavenging activity of standard (Trolox)

Table 4: Analysis of reducing power assay radical scavenging activity of leaves extracts of *A. malabarica (L.) R. Br*

S. No.	Extract Name	Sample Concentration (µg/mL) with respective % of Inhbition					IC_{50} Value µg/mL
		10	20	30	40	50	
1	*A. malabarica (L.) R. Br*	7.21±0.00	14.8±0.00	26.4±0.01	33.9±0.05	40.4±0.08	49.07
2	Ascorbic acid	25.80±0.03	30.11±0.10	41.02±0.14	46.18±0.03	50.02±0.01	49.75

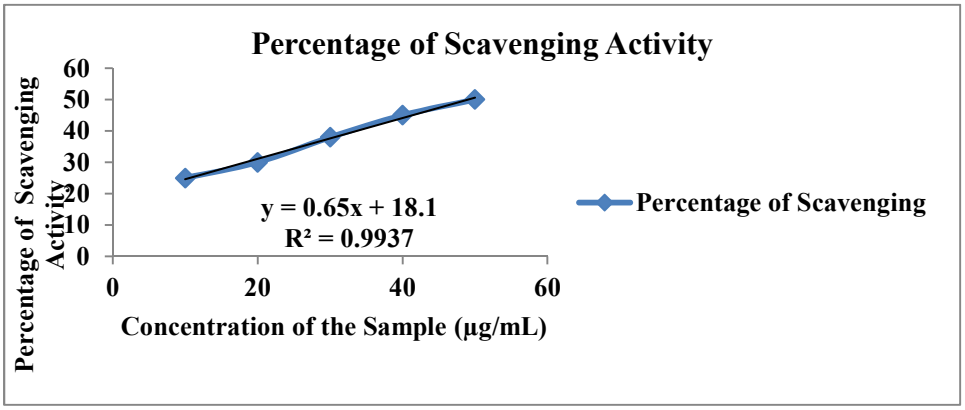

Figure 7: Reducing power activity of the extracts of *A. malabarica (L.) R. Br*

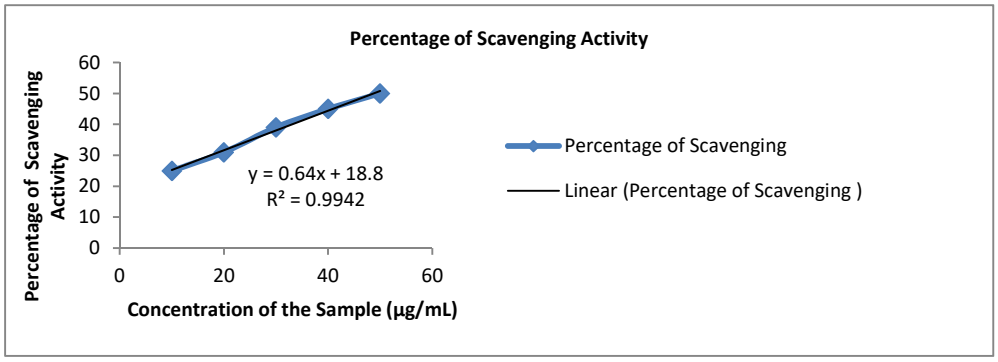

Figure 8: Reducing power activity of standard (Ascorbic acid)

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

Table 5: Fifty percentage inhibitory concentration (IC_{50}) values of extracts of *A. malabarica (L.) R.Br*

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	Standard	24.36	32.67	42.11	49.75

Table 6: Antimicrobial activity of *A. malabarica (L.) R. Br* leaves extracts

S. No	Name of organisms	Inhibition values in mm				
		25 µL	50 µL	75 µL	100 µL	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

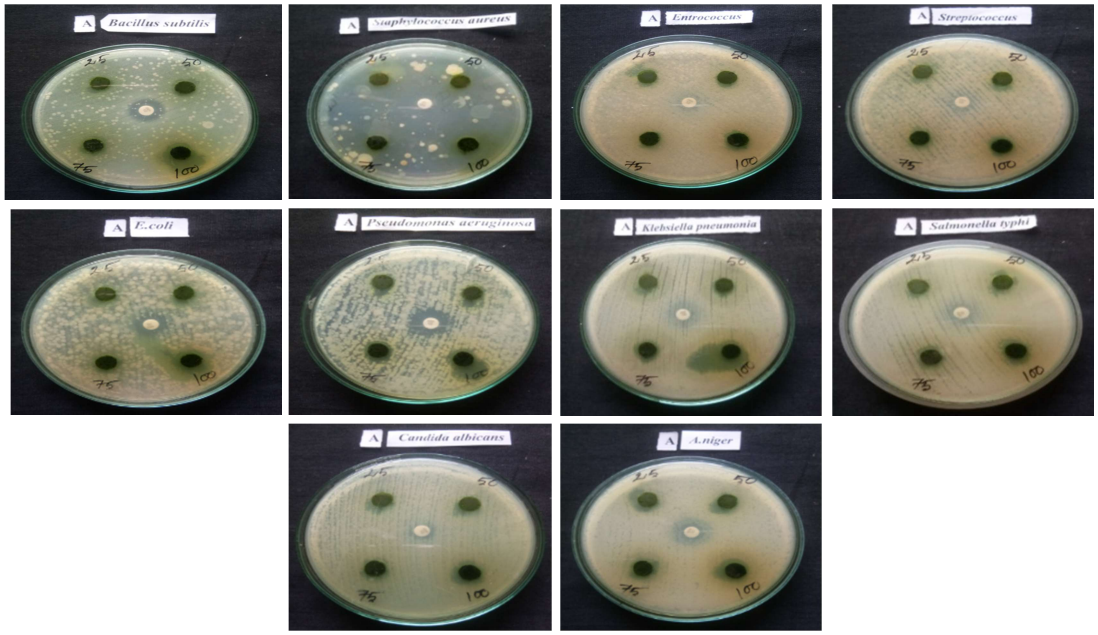

Figure 9: Antimicrobial activity of *A. malabarica (L.) R.Br* leaves extracts

CONCLUSION

The antimicrobial properties of methanolic leaf extract of *A. malabarica (L.) R. Br* is described in this work, which showed increased activity against the bacteria and fungi studied. The antioxidant activity of the methanolic leaf extract of *A. malabarica (L.) R. Br* is higher than that of the standard drug. These findings inspire further research into the plant chemical composition in order to separate and structurally characterize its active components.

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