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Original Article

ANTIOXIDANT ACTIVITES AND PHYTOCHEMICAL ANALYSIS OF METHANOL EXTRACT OF LEAVES OF *HYGROPHILA AURICULATA (*SCHUMACH) HEINE

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ABSTRACT

Objective: The aim of the present investigation was to evaluate the phytochemical constituents, and antioxidant properties of the methanol extract of leaves of *Hygrophila auriculata*.

Methods: The phytochemicals in the methanol extract of leaves of *H. auriculata* were determined qualitatively and quantitatively using standard methods. The antioxidant activities were carried out by DPPH free radical scavenging assay, $ABTS^{\cdot+}$radical cation scavenging assay $OH^{\bullet}$ radical scavenging assay, $NO^{\bullet}$radical scavenging assay, Fe^{2+}chelation assay, Fe^{3+}reducing power assay and phosphomolybdenum reduction assay methods.

Results: The methanol extract of leaves of *H. auriculata* revealed good radical scavenging activities and reducing power activities which were found to increase with the increasing concentration of the extract. The presence of phenols and flavonoids in the methanol extract of leaves of *H. auriculata* were 342.64 mg/g and 124.4 mg/g, respectively.

Conclusion: The present study revealed that the methanol extract of leaves of *H. auriculata* showed significant antioxidant activities.

Keywords: *H. auriculata*, Phenols, Flavonoids, DPPH, $ABTS^{\cdot+}$, $OH^{\bullet}$, $NO^{\bullet}$, Reducing power.

INTRODUCTION

The plant *Hygrophila auriculata* (Schumach.) Heine, which belongs to Acanthaceae, has been traditionally used for the treatment of inflammation, pain, urinary infection, edema, gout and as a diuretic. It is described in ayurvedic literature as Ikshura, Ikshugandha, and Kokilasha having eyes like the Kokila or Indian Cuckoo [1]. The plants are widely distributed throughout India, Nepal, Sri Lanka, Malaysia and Burma. Leaves are tonic, aphrodisiac and hypnotic; useful in diarrhoea, dysentery, thirst, urinary calculi, urinary discharges, inflammations, biliousness, anaemia, constipation, anuria and cough; also applied for gleet, lumbago and pains in the joints. Seeds are cooling, tonic, aphrodisiac, sedative to gravid uterus and constipating; given for gonorrhoea and spermatorrhoea. Decoction of the root is used as a diuretic in dropsy. The plant is used in cancer and tubercular fistula [2].

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals used in the study were of analytical grade and procured from Merck India Pvt. Ltd.

Plant material

Leaves of *Hygrophila auriculata* were collected from Maduravoyal at Chennai, India. The plant (fig. 1) was authenticated by Prof. Dr. N. Raaman, Director, Centre for Advanced Studies in Botany, University of Madras, Guindy, Chennai, India. The plant is a sub shrub, usually growing in marshy places along with water courses. The stem is reddish brown and the shoot has 8 leaves and six thorns at each node (fig. 1). The leaves occur in whorls, the outer pair of leaves is larger, lanceolate, scalerous, margins are minutely dentate, subsessile, and the thorns are strong, straight or curved. Flowers occur in axillary whorls, bract and bracteoles are leafy. The calyx is four-lobed, and the lobes are unequal. Corolla with 5 petals, gamopetalous, unequally 2-lipped, middle lobe of the lower lip with a yellow palate; corolla purple coloured. Stamens four, in two pairs, filaments unequal; anthers divergent; ovary two celled; four ovules in each cell. The fruit is in the form of dehiscent capsules [3].

Fig. 1: Habitat of *Hygrophila auriculata*

Preparation of the extract

The collected leaves were shade dried for 15 d. The dried leaves were powdered mechanically and stored in air tight container for further analysis. The powdered leaves of *H. auriculata* were extracted with methanol and concentrated at room temperature. The extract obtained was stored in a refrigerator for further analysis.

Qualitative phytochemical screening

Chemical tests for screening and identification of bioactive chemical constituents present in the methanol extract of leaves of *H. auriculata* were carried out using the standard procedures [4-6].

Quantitative phytochemical estimation

Estimation of total phenol by Folin-Ciocalteu reagent method

Folin-Ciocalteau method was used to determine the total phenolic compounds [7] with slight modifications. Methanol extract (0.05 ml) of leaves of *H. auriculata* was mixed with 0.5 ml of Folin Ciocalteu reagent (1:10 diluted with distilled water). After 5 min, 1 ml of aqueous Na_2CO_3 (20%) was added. The mixture was then allowed to stand for 30 min incubation in dark. The quantification of phenolic

compounds was performed spectrophotometrically by measuring the absorbance in UV-VIS spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

Estimation of total flavonoids by $AlCl_3$ method

Aluminium chloride colorimetric method was used to determine the total flavonoids [8] with slight modifications. Methanol extract (0.1 ml) of leaves of *H. auriculata* was mixed with 0.5 ml of 10 % aluminium chloride, 0.5 ml of 1 M potassium acetate and 0.5 ml of distilled water. It was incubated at room temperature for 30 min. The absorbance of the reaction mixture was measured by spectrophotometer at 415 nm. The total flavonoid content was expressed in terms of quercetin equivalent, which is a common reference standard.

***In vitro* antioxidant activity**

DPPH radical scavenging assay

The antioxidant activity of methanol extract of leaves of *H. auriculata* was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical with slight modifications [9, 10]. One ml of 0.1 mM DPPH solution in methanol was mixed with 1 ml of various concentrations (10-70 µg/ml) of methanol extract of leaves of *H. auriculata*. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used as the reference standard. Mixer of 1 ml methanol and 1 ml DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis spectrophotometer at 517 nm. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of DPPH radical inhibition} = \frac{\text{Control-Sample}}{\text{Control}} \times 100$$

ABTS$^{\bullet}$ radical cation scavenging assay

The antioxidant capacity was estimated in terms of the ABTS$^{\bullet}$ radical cation scavenging activity following the procedure described by Delgado-Andrade *et al.* [11]. Briefly, ABTS was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 h before use. The ABTS solution (stable for 2 d) was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance at 730 nm of 0.70±0.02. After the addition of 10 µl of the aqueous extract to 4 ml of diluted ABTS$^{\bullet}$ solution, the absorbance was measured at 30 min. The ABTS $^{\bullet}$ radical-scavenging activity of the samples was expressed as

$$\% \text{ of ABTS}^{\bullet+} \text{ radical inhibition} = \frac{\text{Control-Sample}}{\text{Control}} \times 100$$

Hydroxyl radical (OH$^{\cdot}$) scavenging assay

Various concentrations (2-14 µg/ml) of methanol extract (1 ml) of leaves of *H. auriculata* were added with 1.0 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1.0 ml of dimethyl sulphoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90 °C for 15 min in a water bath. After incubation, the reaction was terminated by the addition of 1.0 ml of ice-cold TCA (17.5% w/v). An amount of 0.5 ml of Nash reagent (75.0 g of ammonium acetate, 3.0 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and made up to 1 L with distilled water) was added and left at room temperature for 15 min [12]. The reaction mixture without sample was used as control. The intensity of the colour formed was measured spectroscopically at 412 nm. Ascorbic acid was used as the reference standard. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of OH}^{\cdot} \text{ radical inhibition} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

Nitric oxide radical scavenging assay

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent [13]. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, 1 ml of sodium nitroprusside (10 mM), in phosphate-buffered saline was mixed with different concentrations (2-14 µg/ml) of methanol extract (1 ml) of leaves of *H. auriculata* and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Griess reagent [1% sulphanilamide, 2% *o*-phosphoric acid and 0.1% of N-(1-naphthyl) ethylenediamine dihydrochloride] were added. Ascorbic acid was used as positive control. The absorbance of the chromophore formed was read at 546 nm. The percentage of NO radical scavenging activity was calculated using the following formula:

$$\% \text{ of NO}^{\cdot} \text{ radical inhibition} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

Fe $^{2+}$chelation assay

The chelation of ferrous ions by methanol extract (1 ml) of leaves of *H. auriculata* was estimated by method of Dinis *et al.* [14]. Briefly, 50 µl of 2 mM $FeCl_2$ was added to 1 ml of different concentrations of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml). The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. Na_2EDTA was used as positive control. The percentage inhibition of ferrozine–Fe^{2+}complex formation was calculated as

$$\% \text{ of Fe}^{2+} \text{ chelation activity} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

Ferric (Fe $^{3+}$) reducing power assay

The reducing power of methanol extract of leaves of *H. auriculata* was determined by slightly modified method of Ravisankar *et al.* [15]. One ml of each plant extract of different concentrations (10-70 µg/ml) was mixed with phosphate buffer (1 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 ml, 1%). The mixtures were then incubated at 50 °C for 20 min. One ml of trichloroacetic acid (10%) was added to each mixture, which were then centrifuged for 10 min at 1036 x g. The upper layers of the solutions (1 ml) were mixed separately with distilled water (1 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbances were measured at 700 nm using a spectrophotometer. Ascorbic acid was used as the standard reference.

Phosphomolybdenum reduction assay

The antioxidant capacity of the methanol extract of leaves of *H. auriculata* was assessed as described by Prieto *et al.* [16]. The methanol extract of leaves of *H. auriculata* in dilution from 10 to 70 µg/ml was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in a water bath at 90 ^{o}C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as standard reference. Increased absorbance of the reaction mixture indicates the increase in phosphomolybdenum reduction.

RESULTS AND DISCUSSION

Phytochemical screening

The methanol extract of leaves of *H. auriculata* was taken for various qualitative phytochemical tests and it showed the presence of various phytoconstituents such as terpenoids, alkaloids, flavonoids, phenolic compounds, steroids, glycosides and saponins as depicted in table 1.

Phytochemical estimations

The total phenolic content (estimated by Folin-Ciocalteu method) in the methanol extract of leaves of *H. auriculata* was 342.64 mg/g and the total flavonoid content (estimated by $AlCl_3$ method) was 124.4 mg/g (table 2). Free radicals are produced during normal cellular function in the body; these molecules are missing in an electron, giving them an electric charge. To neutralize this charge, free radicals try to withdraw an electron from, or donate an electron to a

neighbouring molecule. The newly created free radical, in turn, looks out for another molecule and withdraws or donates an electron, setting off a chain reaction that can damage hundred of molecules. Antioxidants such as phenolic compounds and flavonoids halt this chain reaction. These antioxidants are themselves free radical, donating electrons to stabilize and neutralize the dangerous free radicals. The phenolic compounds and flavonoids present in the methanol extract of leaves of *H. auriculata* work against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage [17].

Table 1: Qualitative phytochemical screening of methanol extract of leaves of *H. auriculata*

S. No.	Phytoconstituents	Chemical reagents	Results
1.	Terpenoids	$CHCl_3$+Conc. H_2SO_4	+
2.	Alkaloids	Dragendroff's reagent	+
3.	Flavonoids	NaOH solution	+
4.	Phenols	$FeCl_3$ solution	+
5.	Steroids	Acetic anhydride+conc. H_2SO_4	+
6.	Glycosides	5% NaOH+Fehling's solution	+
8.	Saponins	Foam test	+

+: Present

Table 2: Quantitative phytochemical estimation of phenols and flavonoids of methanol extract of leaves of *Hygrophila auriculata*

S. No.	Contents	Amount mg/g
1.	Total Phenol	342.64
2.	Total Flavonoid	124.4

DPPH radical scavenging assay

DPPH is a stable free radical, used as a substrate to evaluate antioxidant activity. DPPH assay is based on the measurement of the scavenging ability of antioxidants present in the methanol extract of leaves of *H. auriculata* towards the DPPH free radical. The method is based on the reduction of purple colored methanol solution of DPPH radical in the presence of methanol extract of leaves of *H. auriculata*, which is having hydrogen donating antioxidants, by the formation of yellow colored non radical form of DPPH. Lower absorbance indicates higher DPPH free radical scavenging activity [18]. The methanol extract of leaves of *H. auriculata* was able to reduce purple colored 1,1-diphenyl-2-picryl hydrazyl (DPPH) to yellow colored 1,1-diphenyl-2-picryl hydrazine. The maximum DPPH radical scavenging activity was 85.71% at 60 µg/ml [table 5]. The IC_{50} of methanol extract of leaves of *H. auriculata* was 31.88 µg/ml while that of the standard ascorbic acid was 10.73 µg/ml [table 6 and 7].

$ABTS^{\bullet+}$ radical cation scavenging assay

In the $ABTS^{\bullet+}$ radical cation scavenging assay, a blue chromophore of $ABTS^{\bullet+}$ is produced by the reaction between ABTS and potassium persulfate and in the presence of methanol extract of leaves of *H. auriculata,* preformed cation radical gets reduced and the remaining radical cation concentration after reaction with antioxidant compound was then quantified [19]. The maximum ABTS+• radical cation scavenging activity was 62.30% at 12 µg/ml concentration [Table 8]. This demonstrates its high antioxidant activity with IC_{50} of 9.06 µg/ml concentration. It was compared with standard ascorbic acid with the IC_{50} of 9.68 µg/ml concentration [table 6 and 7].

Table 5: DPPH radical scavenging activity of methanol extract of leaves of *H. auriculata*

S. No.	Concentration(µg/ml)	% of inhibition
1.	10	12.60±0.88
2.	20	25.96±1.82
3.	30	47.05±3.29
4.	40	61.34±4.29
5.	50	83.19±5.82
6.	60	85.71±5.99

Table 6: DPPH, $OH^{\cdot}$ and $NO^{\cdot}$ radical scavenging activities of standard (Ascorbic acid)

S. No.	Concentration (µg/ml)	DPPH	$ABTS^{+}$	$OH^{\cdot}$	$NO^{\cdot}$
1.	2	03.34±0.23	25.00±1.75	35.17±3.02	37.14±2.59
2.	4	13.88±0.27	32.25±2.25	52.42±2.51	40.00±2.81
3	6	21.59±1.51	37.9±2.65	69.35±1.07	48.57±3.39
4.	8	37.25±2.60	43.54±3.04	89.14±6.23	54.28±3.79
5.	10	70.62±4.94	51.61±3.61	92.24±6.45	60.00±4.23
6.	12	70.95±4.96	52.41±3.66	97.67±6.83	62.85±4.39

Table 7: IC_{50} values (µg/ml) for methanol extract of leaves of *H. auriculata* and standard

S. No.	Assay	Methanol extract	Standard (Ascorbic acid)
1.	DPPH	31.88	10.73
2.	$ABTS^{\bullet+}$	09.06	09.68
3.	$OH^{\bullet}$	06.23	03.81
4.	$NO^{\bullet}$	10.38	06.17
5.	Fe $^{2+}$chelation	08.39	05.82

Table 8: ABTS.+radical cation scavenging activity of methanol extract of leaves of *H. auriculata*

S. No.	Concentration(µg/ml)	% of inhibition
1.	2	2.70±0.18
2.	4	21.28±1.48
3.	6	39.54±2.76
4.	8	44.12±3.08
5.	10	59.60±4.17
6.	12	62.30±4.36

Hydroxyl radical (OH·) scavenging assay

An extremely reactive hydroxyl free radical has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [20]. OH·has the capacity to react with nucleotides in DNA and cause strand breakage, which contributes to mutagenesis, cytotoxicity and carcinogenesis. Hydroxyl radicals are the major active oxygen causing lipid peroxidation in enormous biological damage. The highly reactive hydroxyl radical can cause oxidative damage to proteins, DNA and lipids [21] by abstracting hydrogen atoms from unsaturated fatty acids. Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA. The hydroxyl radicals formed by the oxidation process and react with dimethyl sulfoxide (DMSO) to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent.

The maximum OH·radical scavenging activity was 92.50% at 12 µg/ml concentration [table 9]. The IC_{50} of methanol extract of leaves of *H. auriculata* was 6.23 µg/ml concentrations whereas it was 3.81 µg/ml concentrations for the standard ascorbic acid [table 6 and 7].

Table 9: Hydroxyl radical scavenging activity of methanol extract of leaves of *H. auriculata*

S. No.	Concentration(µg/ml)	% of inhibition
1.	2	16.96±1.18
2.	4	27.65±1.93
3.	6	48.13±3.36
4.	8	81.90±5.73
5.	10	85.50±5.98
6.	12	92.50±6.47

Nitric oxide is implicated in diseases such as cancer and inflammation [22]. It also mediates smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated cytotoxicity [23]. Sodium nitroprusside spontaneously generates nitric oxide at physiological pH, in aqueous solutions. The nitric oxide generated is converted into nitric and nitrous acids on contact with dissolved oxygen and water. The liberated nitrous acid was estimated using a modified Griess-Illosvoy method. Nitrous acid reacts with Griess reagent to form a purple azo dye. In presence of antioxidants, the amount of nitrous acid will decrease and the degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. The maximum NO· radical scavenging activity was showed 51.90% at 12 µg/ml concentration [table 10]. The methanol extract of leaves of *H. auriculata* showed significant nitric oxide radical scavenging activity with the IC_{50} of 10.38 µg/ml concentration, whereas it was 6.17 µg/ml concentrations for the standard ascorbic acid [table 6 and 7].

Table 10: Nitric oxide radical scavenging activity of methanol extract of leaves of *H. auriculata*

S. No.	Concentration(µg/ml)	% of inhibition
1.	2	13.33±0.93
2.	4	25.65±1.79
3.	6	36.43±2.55
4.	8	46.51±3.25
5.	10	48.16±3.37
6.	12	51.90±3.63

Ferrozine can quantitatively form complexes with Fe^{2+}ion. However, in the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction, therefore, allows the estimation of the chelating activity in the presence of coexisting chelator. The transition metal ion, Fe^{2+} possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals [24]. The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. The active methanol extract of leaves of *H. auriculata* interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. The maximum Fe^{2+}chelating activity was 61.25% at 12 µg/ml concentration [table 11]. IC_{50} of Fe^{2+}chelating activity was 8.39 µg/ml which was compared with the standard EDTA of 5.82 µg/ml concentration [table 12 and 7].

Table 11: Fe^{2+}chelating activity of methanol extract of leaves of *H. auriculata*

S. No.	Concentration (µg/ml)	Chelation (%)
1.	2	23.21±1.62
2.	4	32.67±2.29
3.	6	39.64±2.77
4.	8	47.67±3.34
5.	10	59.64±4.17
6.	12	61.25±4.29

Table 12: Fe $^{2+}$chelating activity of standard (EDTA)

S. No.	Concentration (µg/ml)	Chelation (%)
1.	1	03.75±0.08
2.	2	09.43±0.01
3.	3	21.33±0.04
4.	4	27.45±0.02
5.	5	31.16±0.05
6.	6	51.54±0.02

The reducing properties are generally associated with the presence of reductones, such as flavonoids and phenolic compounds, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [25]. Reductones are also reported to react directly with peroxides and also with certain precursors of peroxides, thus preventing peroxide formation [26]. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of methanol extract of leaves of *H. auriculata*. Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex to ferrous form. Fe^{3+} reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action, and can be strongly correlated with other antioxidant properties [27]. By measuring the formation of Pearl's Prussian blue at 700 nm, it is possible to determine the concentration of ferrous ions. Increase in absorbance of the reaction mixture indicated increase in reducing power of the extract which is shown in fig. 5. The methanol extract of leaves of *H. auriculata* showed good ferric reducing power (0.429) at 60 µg/ml concentration [table 13], which was compared with the standard (0.289) ascorbic acid [table 14].

Table 13: Ferric (Fe^{3+}) reducing power and phosphomolybdenum reduction of methanol extract of leaves of *H. auriculat.*

S. No.	Concentration (µg/ml)	Fe^{3+}reducing power	Phosphomolybdenum reduction
1.	10	0.388±0.027	0.007±0.000
2.	20	0.407±0.028	0.008±0.001
3.	30	0.412±0.029	0.015±0.001
4.	40	0.417±0.029	0.025±0.002
5.	50	0.423±0.030	0.035±0.002
6.	60	0.429±0.030	0.046±0.003

Table 14: Ferric (Fe^{3+}) reducing power and phosphomolybdenum reduction of standard (Ascorbic acid)

S. No.	Concentration (µg/ml)	Fe^{3+}reducing power	Phosphomolybdenum reduction
1	2	0.154±0.01	0.015±0.01
2	4	0.189±0.01	0.093±0.00
3	6	0.209±0.01	0.172±0.01
4	8	0.214±0.01	0.205±0.01
5	10	0.247±0.01	0.317±0.02
6	12	0.289±0.02	0.359±0.02

Phosphomolybdenum assay revealed the reduction of MO (VI) to MO (V) by the the methanol extract of leaves of *H. auriculata* and formation of a MO (V) complex at acidic pH. Increase in absorbance of the reaction mixture indicates increase in reducing power [28]. The significant increase in absorbance of extract was found to be 0.046 [table 13] whereas, the standard ascorbic acid was 0.359 [table 14]. The result obtained was confirmed by the high potency of the methanol extract of leaves of *H. auriculata* towards the reduction of transition metal ions. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants.

CONCLUSION

The results of antioxidant activities of methanol extract of leaves of *H. auriculata* showed the potential source of natural antioxidants and could significantly inhibit free radicals by dose-dependent concentrations. The differentiation in the antioxidant activity may be ascribed to their different group of phenolic and flavonoids compounds. The higher antioxidant activity is due to the higher phenolic content of methanol extract of leaves of *H. auriculata*. Based on the results obtained, it can be concluded that the plant contains essential phytochemical constituents and possess active antioxidant property.

CONFLICT OF INTERESTS

Declared None

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