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In vitro Antioxidant Activity of *Porana paniculata* and *Ipomoea quamoclit*-Two Ethnomedicinally Important Plants of *Convolvulaceae* Family

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Authors' contributions

This work was carried out in collaboration between all authors. Author ASK designed and performed the study, managed the literature searches, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JRR and VRMG supervised the work done, revised the methodology, managed the analyses of the study and approved the final draft of the manuscript. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: Present study deals with the *in vitro* antioxidant activity of *Porana paniculata* and *Ipomoea quamoclit* whole plants belongs to *Convolvulaceae* family.
Study Design: The present study was designed for *in vitro* evaluation of antioxidant activity of two ethnomedicinally important plants of *Convolvulaceae* family.
Place and Duration of Study: Division of Pharmacognosy, Raghavendra Institute of Pharmaceutical Education and Research, Krishnam Reddy Palli cross, Chiyyedu, Anantapuramu-515721, Andhra Pradesh, India, between June 2013 and July 2013.
Methodology: Plant materials were procured, shade dried and made into powder. The extraction

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was carried out by maceration using water and ethanol as solvent and then total flavonoid and total phenol content was determined by standard methods. *In vitro* antioxidant activity was performed by DPPH assay, superoxide anion scavenging activity assay, nitric oxide scavenging activity assay, hydrogen peroxide scavenging assay and metal chelating activity. Percentage inhibition of free radical formation and IC_{50} values were calculated.

Results: Total flavonoids were found to be 59.86 and 49.26 for HAPP and HAIQ mg/ml of standard compound quercetin and total phenols was found to be 33.34 and 39.32 mg/g of gallic acid. In DPPH method, the half inhibition concentration [IC_{50}] of HAPP, HAIQ and ascorbic acid were 71.68, 49.63 and 32.46 µg/ml respectively**.** In Superoxide anion scavenging activity assay, the half inhibition concentration [IC_{50}] of HAPP, HAIQ and standard compound BHA was found to be 54.61, 69.34 and 93.46 µg/ml respectively. In nitric oxide scavenging activity assay both the extracts HAPP and HAIQ moderately inhibited nitric oxide in dose dependent manner with the IC_{50} being 83.49 and 89.47 µg/ml and standard compound ascorbic acid was 78.93 µg/ml respectively**.** The IC_{50} of HAPP, HAIQ and gallic acid were found 25.65, 46.39 and 24.29 µg/ml respectively in hydrogen peroxide scavenging method and in metal chelating method, HAPP and HAIQ indicated that they have chelating activity with an IC_{50} of 49.27 and 65.41 µg/ml and alpha tocopherol was 32.48 µg/ml.

Conclusion: From the above findings it can be concluded that *Porana paniculata* and *Ipomoea quamoclit* extracts exhibited antioxidant activity.

Keywords: Porana paniculata; Ipomoea quamoclit; DPPH; superoxide; nitric oxide; hydrogen peroxide and metal chelation.

1. INTRODUCTION

Porana paniculata Roxb. having the synonym *Poranopsis paniculata* belongs to the family *Convolvulaceae* is an ever green creeper most abundantly available across India and well as different areas of Andhra Pradesh. Even though the plant is having medicinal uses both in ayurveda and folklore, its phytopharmacological nature was unrevealed. The plant possesses its significant use in the ayurveda and folklore medicine [1]. *Ipomoea quamoclit* Linn. also called as *Quamoclit pinnata* belongs to *Convolvulaceae* family is one of the most commonly seen plant in and around of the living area. In English it is called as Cypress Vine, Indian Pink and Cupid's Flower. The plant was claimed for many therapeutic uses in ayurveda and other countries medicines. Many groups of phytoconstituents were reported from *Ipomoea quamoclit* like alkaloids cyanogenetic and glycosides, quamoclins I-IV and jalapin. Pyrrolizidine alkaloids, and ergoline alkaloids and Anthocyanins were also reported from plant [2,3]. After conduction a thorough literature review, in the present study an attempt was made to reveal the total flavonoid and total phenol content and *in vitro* antioxidant activity of *Porana paniculata* and *Ipomoea quamoclit* in a systematic way.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

For the present study, *Porana paniculata* and *Ipomoea quamoclit* whole plants were collected from the forest area near to the Madanapalli of Chittoor district of Andhra Pradesh and the plant was botanically identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V. University, Tirupati, A.P., India and voucher specimens (RIPER/ASK/001 and RIPER/ASK/002) were preserved in Division of Pharmacognosy, RIPER, Anantapuramu, A.P., India for further reference.

2.2 Extraction

1000 g each of the powdered *Porana paniculata* and *Ipomoea quamoclit* were extracted by cold maceration method with ethanol: water (3:2) mixture as solvent. The maceration was continued for 72 hours after which, the contents were filtered and concentrated by rota evaporator. A resinous greenish extract was obtained for both the plants which were calculated for the yield, designated with HAPP for *Porana paniculata* extract and HAIQ for *Ipomoea quamoclit* extract and stored in desiccator till further study [4,5].

2.3 Total Flavonoids Determination

Aluminium chloride colorimetric method was used for flavonoids determination. Each plant extracts (0.5 ml of 1:10 gm/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg/ml in methanol [6].

2.4 Total Phenols Determination

Total phenols were determined by Folin Ciocalteu reagent. A dilute extract of each plant extract (0.5 ml of 1:10 g ml^{-1}) or Gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L^{-1} solutions of gallic acid in methanol: water (50:50 v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound [6].

2.5 Antioxidant Activity

2.5.1 DPPH assay

The scavenging ability of the natural antioxidants of the plant extract towards the stable free radical DPPH was measured in which 2 ml aliquot of DPPH methanol solution [25 µg/ml] was added to 0.5 ml HAPP and HAIQ solutions at different concentrations 20, 40, 60, 80 and 100µg/ml. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. L-Ascorbic acid was used as the standard [6,7]. The percentage inhibition was calculated by following formula:

$$\% \text{ DPPH scavenging activity} = 100 \times (A\text{c} - A\text{s})/A\text{c}$$

Where A_C = absorbance of the control and A_S = absorbance of reaction mixture [in the presence of sample or standard].

2.5.2 Superoxide anion scavenging activity assay

The scavenging activity of the *HAPP and HAIQ* towards superoxide anion radicals was measured here by a standard protocol. Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide [PMS-NADH] system through the reaction of PMS, NADH, and oxygen. It was assayed by the reduction of nitroblue tetrazolium [NBT]. In these experiments the superoxide anion was generated in 3 ml of Tris-HCl buffer [100 mM, pH 7.4] containing 0.75 ml of NBT [300 M] solution, 0.75 ml of NADH [936 M] solution and 0.3 ml of different concentrations of the HAPP and HAIQ like 20, 40, 60, 80 and 100 µg/ml. The reaction was initiated by adding 0.75 ml of PMS [120 M] to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. Butylated hydroxyanisole (BHA) was employed as standard [6,7]. The superoxide anion scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = 100 \times (A\text{c} - A\text{s})/A\text{c}$$

Where A_0 was the absorbance of the control [blank without extract] and A_1 was the absorbance in the presence of the extract or standard.

2.5.3 Nitric oxide scavenging activity assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10mM sodium nitroprusside in 0.5 ml phosphate buffer saline [pH 7.4] was mixed with 0.5 ml of HAPP and HAIQ at various concentrations like 20, 40, 60, 80 and 100 µg/ml and the mixture incubated at 25ºC for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent [33% in 20% glacial acetic acid] and incubated at room temperature for 5 min. finally, 1.0 ml naphthyl ethylene diamine dihydrochloride [0.1% w/v] was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm with a spectrophotometer. L-Ascorbic acid was used as the standard [7,8]. The nitric oxide radicals scavenging activity was calculated according to the following equations:

$$\% \text{ Inhibition} = 100 \times (Ac - As)/Ac$$

Where A_0 was the absorbance of the control [blank without extract] and A_1 was the absorbance in the presence of the sample or standard.

2.5.4 Hydrogen peroxide scavenging assay

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. HAPP and HAIQ (20-100 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide [7,8]. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds were calculated as follows:

$$\% \text{ Inhibition} = 100 \times (Ac - As)/Ac$$

Where A_0 was the absorbance of the control [blank without extract] and A_1 was the absorbance in the presence of the sample or standard.

2.5.5 Metal chelating activity

HAPP and HAIQ (20-100 µg/ml) was added to a solution of 2 mM FeCl2 (0.05 ml). The reaction was initiated by the addition of 5 mM Ferrozine (0.2 ml), the mixture was shaken vigorously and left standing at room temperature for 10 min. Alpha tocopherol was used as standard. Absorbance of the solution was then measured spectrophotometrically at 562 nm [8]. The percentage inhibition of ferrozine- Fe2+ complex formation was calculated as:

$$\% \text{ Inhibition} = 100 \times (Ac - As)/Ac$$

Where A_0 was the absorbance of the control [blank without extract] and A_1 was the absorbance in the presence of the sample or standard.

2.6 Calculation of 50% Inhibition Concentration (IC_{50})

A graph was plotted by taking the different concentrations of HAPP and HAIQ (20, 40, 60, 80 and 100 µg/ml) on X axis and the percentage inhibition on Y axis. From this, the IC50 values were calculated for all the methods.

3. RESULTS AND DISCUSSION

3.1Extraction

A thick green viscous matter about 28.9 gm was obtained from 1000 gm of plant material and the percentage was found to be 2.89% w/w for *Porana paniculata* (HAPP) and thick green viscous matter about 36.4 gm was obtained from 1000 gm of plant material and the percentage was found to be 3.64% w/w for *Ipomoea quamoclit* (HAIQ).

3.2 Total Flavonoids and Total Phenols Determination

Total flavonoids were found to be 59.86±0.54 and 49.26±0.53 for HAPP and HAIQ mg/ml of quercetin and total phenols were found to be 33.34±0.37 and 39.32±0.30 mg/g of gallic acid.

3.3 Antioxidant Activity

3.3.1 DPPH assay

DPPH radical scavenging activity of HAPP, HAIQ and standard ascorbic acid are presented in (Fig. 1). In this assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH free radical by a scavenger [A-H] causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. The half inhibition concentration [IC_{50}] of HAPP and HAIQ, ascorbic acid were 71.68, 49.63 and 32.46 µg/ml respectively (Table 1). The percentage inhibitions of HAPP and HAIQ along with the standard compound were calculated. Both the plant extracts exhibited a significant dose dependent inhibition of DPPH activity. The DPPH assay activity of HAPP was found to be near to standard compound ascorbic acid (Fig. 1).

3.3.2 Superoxide anion scavenging activity assay

Superoxide is biologically important since it can be decomposed to form stronger oxidative

species such as singlet oxygen and hydroxyl radicals, which are very harmful to the cellular components in a biological system. The superoxide anion radical scavenging activity of the HAPP and HAIQ was assayed by the PMS-NADH system. The superoxide scavenging activity of both HAPP and HAIQ was increased markedly with the increase of concentrations. The half inhibition concentration [IC_{50}] of HAPP, HAIQ and standard compound Butylated hydroxyanisole (BHA) was found to be 54.61, 69.34 and 93.46 µg/ml respectively (Table 1). The percentage inhibitions of HAPP and HAIQ along with the standard compound were calculated. These results suggested HAPP had notably near superoxide radical scavenging effects when compared to standard compound (Fig. 2).

3.3.3 Nitric oxide scavenging activity assay

Nitric oxide [NO] is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. Both the extracts that is HAPP and HAIQ moderately inhibited nitric oxide in dose dependent manner (Fig. 3) with the IC_{50} being 83.49 and 89.47 µg/ml and standard compound ascorbic acid was 78.93 µg/ml respectively (Table 1). The percentage inhibitions of HAPP and HAIQ along with the standard compound were calculated.

3.3.4 Hydrogen peroxide scavenging assay

Results showed that HAPP and HAIQ exhibited concentration dependent scavenging activities against hydroxyl radicals generated in reaction system. The IC_{50} of HAPP, HAIQ and gallic acid were found to be 25.65, 46.39 and 24.29 µg/ml respectively (Table 1). The percentage inhibitions of HAPP and HAIQ along with the standard compound were calculated. The potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxy substitution (Fig. 4).

3.3.5 Metal chelating activity

Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex [red colored] formation is interrupted and as a result, the red colour of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of colour reduction. The formation of the ferrozine– Fe2+ complex is interrupted in the presence of HAPP and HAIQ indicating that have chelating activity with an IC_{50} of 49.27, 65.41 µg/ml and alpha tocopherol was 32.48 µg/ml (Table 1). The percentage inhibitions of HAPP and HAIQ along with the standard compound were calculated (Fig. 5).

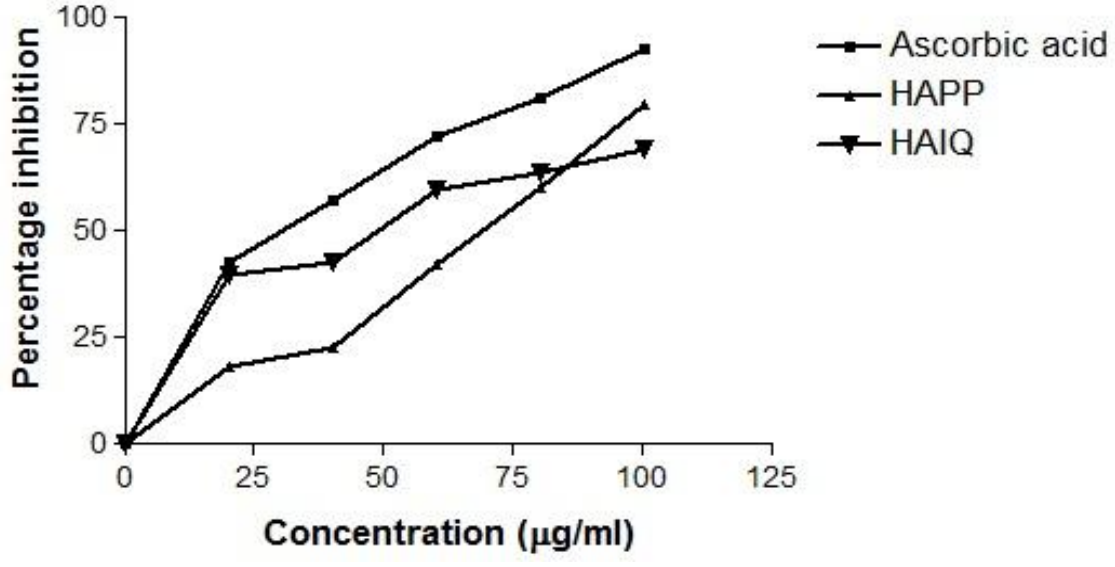

Fig. 1. Percentage inhibition in DPPH radical scavenging activity

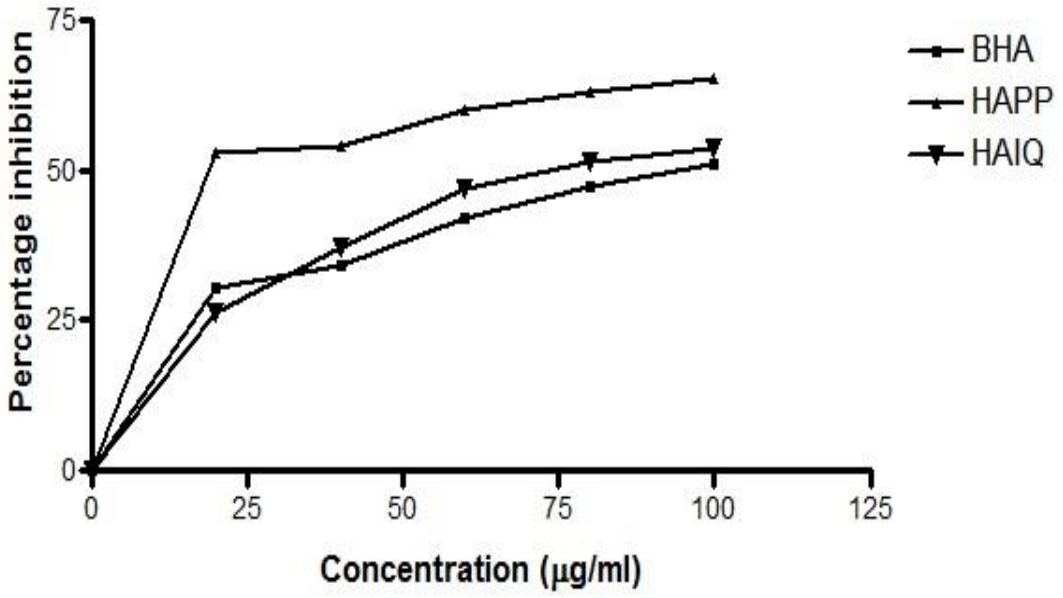

Fig. 2. Percentage inhibition in superoxide radical scavenging activity

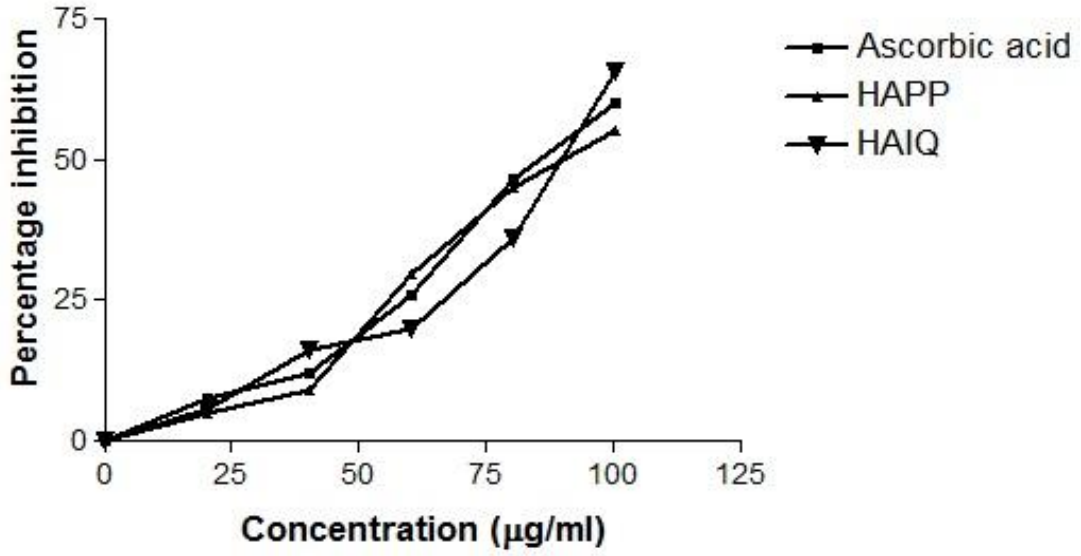

Fig. 3. Percentage inhibition in nitric oxide radical scavenging activity

Table 1. IC_{50} values of *Porana paniculata, Ipomoea quamoclit* and standard compounds

S. no	Method	IC_{50} values (µg/ml)		
		Standard	HAPP	HAIQ
01.	DPPH radical scavenging activity	32.46	71.68	49.63
02.	Superoxide radical scavenging activity	93.46	54.61	69.34
03.	Nitric oxide radical scavenging activity	78.93	83.49	89.47
04.	Hydrogen peroxide scavenging activity	24.29	25.65	46.39
05.	Metal chelating activity	32.48	49.27	65.41

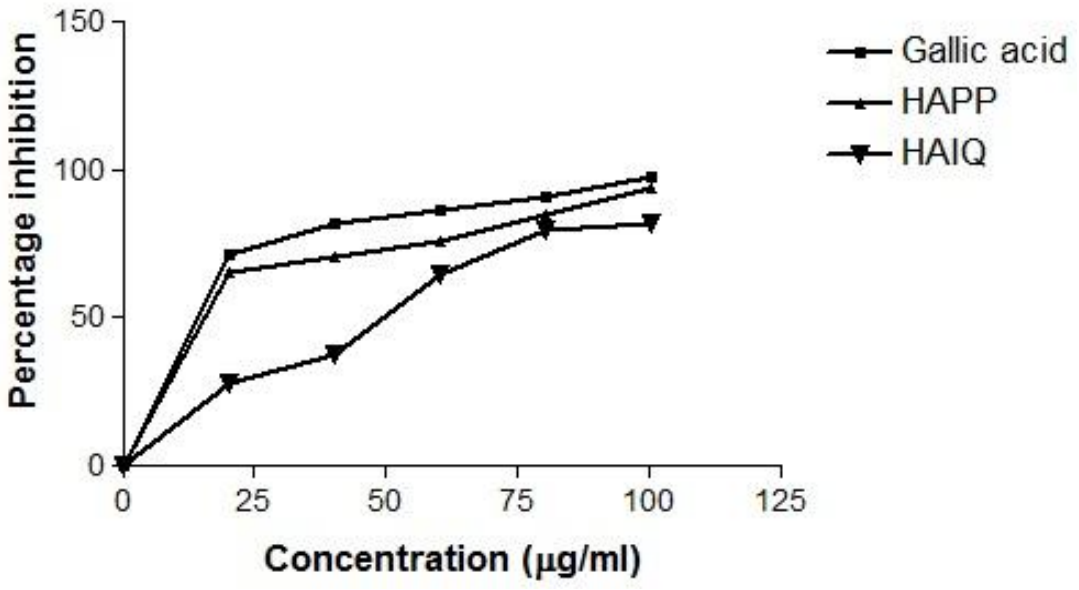

Fig. 4. Percentage inhibition in hydrogen peroxide scavenging activity

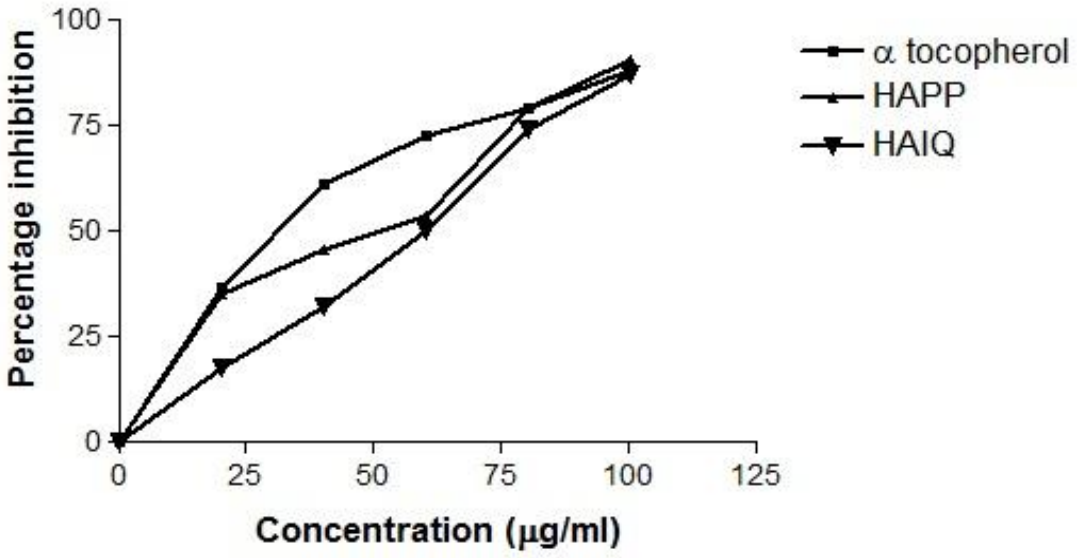

Fig. 5. Percentage inhibition in metal chelating activity

4. CONCLUSION

From the above results it can be concluded that the whole plant of *Porana paniculata* and *Ipomoea quamoclit* of *Convolvulaceae* family possesses significant antioxidant activity.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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