IN VITRO ANTIOXIDANT ACTIVITY OF FLOWERING TOPS OF *IPOMOEA PALMATA*

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Summary

The flowering tops of the *Ipomoea palmata* (morning glory) family:- convolvulaceae, a vigorous fast growing perennial twig climber commonly found widely throughout the Saurashtra region and it is popular as ornamental plant. Flowering tops were taken into the present study. The primary phytochemical study and in vitro anti oxidant study was performed on methanolic extract flowering tops. The Methanolic extract flowering tops of *Ipomoea palmata* were prepared and evaluated for its primary phytochemical analysis for total phenolic content and in vitro anti oxidant activity study by DPPH free radical scavenging activity, super oxide free radical activity and nitric oxide scavenging activity. Results indicate that Methanolic extract flowering tops of *Ipomoea palmata* have noticeable amount of total phenols which could be responsible for the anti oxidant activity of Methanolic extract flowering tops of *Ipomoea palmata* remains unclear and could be further investigated by detailed phytochemical investigation.

Key-words: Flowering tops of *Ipomoea palmata*, *in-vitro* anti-oxidant activity, Morning Glory

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Introduction

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Exogenous chemical and endogenous metabolic processes in the human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing bio molecules, resulting in cell death or tissue damage. Oxidative damage plays a significantly pathological role in human disease. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, ageing, inflammatory response syndrome, respiratory diseases, liver diseases, cancer and AIDS.[1-3] Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite.[4, 5] The antioxidants can neutralize the ill effects of free radicals by scavenging or chain breaking (like vitamin A, C, β-carotene, etc.) or some other mechanism of action. These antioxidants must be constantly replenished since they are 'used up' in the process of neutralizing free radicals. [6]

Flavanoids are natural product and can be regarded as C6 –C3- C6 in which each C6 moiety is a benzene ring. The variation in the state of oxidation of connecting C3 moiety determines the properties and class of each such compounds. Flavanoid compounds and the related coumarins usually occur in plants as glycosides in which one or more phenolic hydroxyl group are combined with sugar residues. The hydroxyl group are nearly always found in position 5 and 7 in ring A, whole ring B commonly carries hydroxyl or alkoxyl group at the 4' position or at both 3' and 4' positions. Glycosides of flavanoids compounds may bear the sugar on any of the available hydroxyl groups. Flavanoids anthocyanins, abs sugar free pigments anthocyanidins have shown to posses various biological properties related to antioxidant mechanism.

Many of the natural antioxidants, hence, especially flavanoids, exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, anti allergic, antithrombotic, and vasodilatory actions. Antioxidant activity is a fundamental property important for life. Many of the biological functions, such as antimutagenicity, anticarcinogenicity, and anti aging, among others, originate from this property. One of the flavanoid containing plant is *Ipomoea palmata* Forssk. (Family: Convolvulaceae) which is vigorous fast-growing perennial twining climber from tropical America, growing to the top of

the tree canopy and forming a dense blanket of foliage over all vegetation. It is also found widely in Saurashtra region. This plant is known as blue morning glory, ocean blue morning-glory, morning glory vine and it is widely grown as ornamental decorative plants. The flowers are a striking purple-blue. The flowers open in the morning and fade to magenta in the evening. The bloom time is spring/fall. The colourful flowering tops contain flavanoid glycosides so we have assessed flowering tops of morning glory for its in-vitro antioxidant activities.

Materials And Methods

Plant Material and preparation of extract

Ipomoea palmata flowering tops were collected and identified by comparing it with specimen in the month of February and March, when it was of pink colour in the spring fall. The flowerings tops were dried at control temperature not exceed 50° C and made to a fine powder (particle size ~0.25mm). The powders of flowering tops were extracted with methanol in the soxhlet apparatus. The solvent was then evaporated under reduced pressure at 50° C and dried in vacuum dryer. Then dry powder of methanolic extract of *Ipomoea palmata* – MEIP obtained was then used for the preliminary phytochemical screening, assessment of total phenolic content and antioxidant activity through various *in vitro* assays.

Chemicals

L-Ascorbic acid, Gallic acid, curcumin, 2,2-diphenyl-1-picrylhydrazyl (DPPH),Ethylene diamine tetra acetic acid (EDTA), Nitro blue tetrazolium (NBT), and All other chemicals and reagents used were of analytical grade.

Preliminary phytochemical screening

The methanolic extract was taken for various qualitative chemical tests to determine the presence of various phyto constituents like alkaloids[8], glycosides[9], carbohydrates[10], phenolics and tannins [11], phytosterols, fixed oils [12], protein and amino acids[13], flavanoids[13], saponins gums and mucilage [13] using reported method.

Total Phenolic content

Total Phenolic content was determined using Folin-Ciocalteau method[14-15]. The powdered extract of MEIP was dissolved in methanol to obtain a concentration of 1 mg/ml. The 100µl of this solution was taken in to 25ml volumetric flask, to which 10ml of water and 1.5ml of Folin Ciocalteau reagent were added. The mixture was then kept for 5 min. and to it 4 ml of 20% w/v sodium carbonate solution was added the volume was made up to 25ml with double distilled water. The mixture was kept for 30 minute until blue color develops. The samples were then observed at 765 nm in UV- visible spectrometer Shimadzu, UV-1601, Japan. The % of total phenolic was calculated from calibration curve of Gallic acid plotted by using similar procedure. [16- 19]

* The powdered extract of MEIP was dissolved in methanol to obtain a concentration of 1 mg/ml and taken as a test sample to study various *In-vitro* activities i.e. the DPPH free radical scavenging activity, Super oxide free radical scavenging activity and Nitric oxide scavenging activity and were calculated using the using following formula:

$$\% \text{ Reduction} = \frac{\text{Control}_{\text{absorbance}} - \text{Test}_{\text{absorbance}} \text{ X } 100}{\text{Control}_{\text{absorbance}}}$$

The result of Total phenolic content, DPPH free radical scavenging activity, Super oxide free radical scavenging activity, Nitric oxide scavenging activity were compared with standard by using paired t-test.

Determination of DPPH Radical Scavenging Activity

4.3mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) was dissolved in 3.3 ml methanol; it was protected from light by covering the test tubes with aluminum foil. 150µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 516nm for control reading. Different volumes of MEIP measuring 30µl, 60µl, 90µl, 120 µl, 150 µl were taken and the volume was made uniformly to 150µl using methanol. Each of the samples was then further diluted with methanol up to 3ml and to each 150µl DPPH was added. Absorbance was taken after 15 min. at 516nm using methanol as blank on UV-visible spectrometer Shimadzu, UV-1601, Japan. IC_{50} values for MEIP were then calculated and compared with value of Ascorbic acid taking it as a positive control.[20, 21]

Determination of Nitric Oxide Radical Scavenging Activity

50µl, 60µl, 70µl, 90 µl of the samples of MEIP were taken in separate tubes and the volume was uniformly made up to 150µl with methanol to each tube 2.0 ml, of sodium nitroprusside (10 mM) in phosphate buffer saline was added. The solutions were incubated at room temperature for 150 minutes. The similar procedure was repeated with methanol as blank which served as control. After the incubation, 5 ml of Griess reagent was added to each tube including control. The absorbance of chromophore formed was measured at 546 nm on UV-visible spectrometer Shimadzu, UV-1601, Japan. Curcumin was used as positive control IC_{50} values were compared by paired t-test.[22, 23]

Super oxide free radical scavenging activity

100µl Riboflavin solution [20 µg], 200µl EDTA solution [12mM], 200µl methanol and 100µl NBT (Nitro-blue tetrazolium) solution [0.1mg] were mixed in test tube and reaction mixture was diluted up to 3ml with phosphate buffer [50mM] The absorbance of solution was measured at 590nm using phosphate buffer as blank after illumination for 5min.This is taken as control. Different volumes of 50µl, 70µl, 100µl, 125 µl, 150 µl of samples of MEIP were taken and volume was made up to 150µl with methanol, to each of this, 100µl Riboflavin, 200µl EDTA, 200µl methanol and 100µl NBT was mixed in test tubes and further diluted up to 3ml with phosphate buffer. Absorbance was measured after illumination for 5min. at 590nm on UV visible spectrometer Shimadzu, UV-1601, Japan. IC_{50} value for MEIP were calculated and compared with ascorbic acid which was used as positive control in this assay. [24, 25]

Result and Discussion

Preliminary phytochemical screening:

It was found that methanolic extract of Ipomoea palmate extract contained alkaloid, carbohydrate, proteins, amino acids, phenolic compounds and flavanoid.

Table 1. Preliminary phytochemical screening

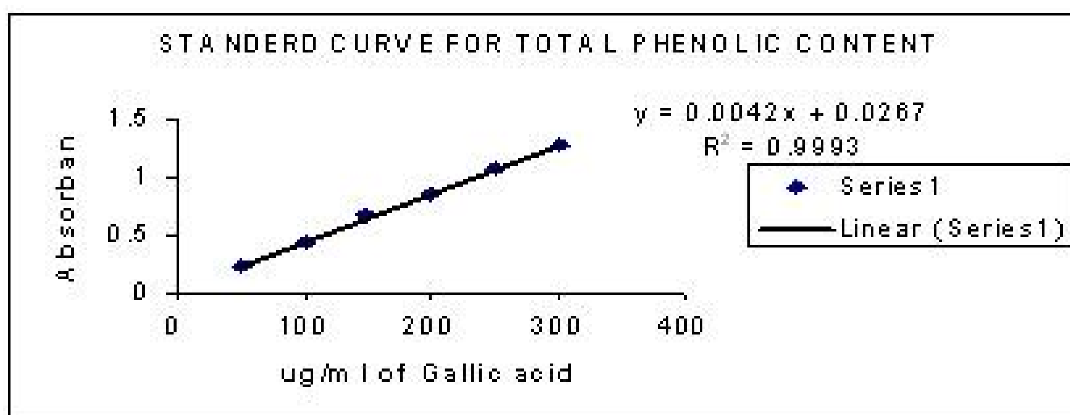
Class	Methanolic extract of *Ipomoea palmata*
Alkaloid	+
Glycosides	-
Carbohydrates	+
Phytosterols	-
Protein and Amino Acid	+
Flavanoids	+
Saponins	-
Phenolic compound	+

(+) Indicate Present and (-) Indicate Absent

Table 1. Preliminary phytochemical screening

The total phenolic content:

Figure 1. The total phenolic content



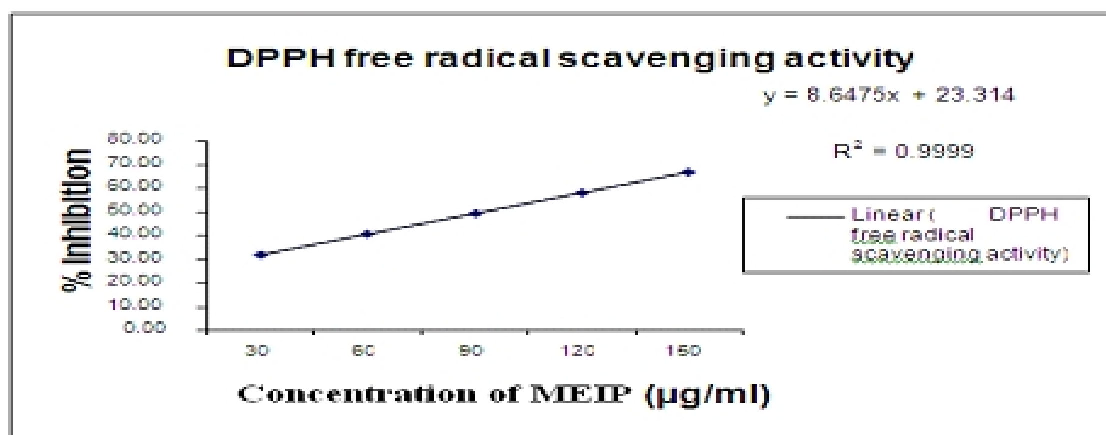
*The total phenolic content of methanolic extract flowering tops of Ipomoea palmata was 114.65 µg/mg calculated as Gallic acid equivalent of phenols was detected.

Free radicals are produced under certain environmental conditions and 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 create free radical, in turn, looks out for another molecules and withdraws or donates an electron, setting off a chain reaction that can damage hundred of molecules. Antioxidants halt this chain reaction. Some antioxidants are themselves free radical, donating electrons to stabilize and neutralize the dangerous free radicals. Other antioxidants work against the molecules that form free radicals destroying them before they can begin the domino effect that leads to oxidative damage.

DPPH free radical scavenging activity:

Figure 2. DPPH free radical scavenging activity

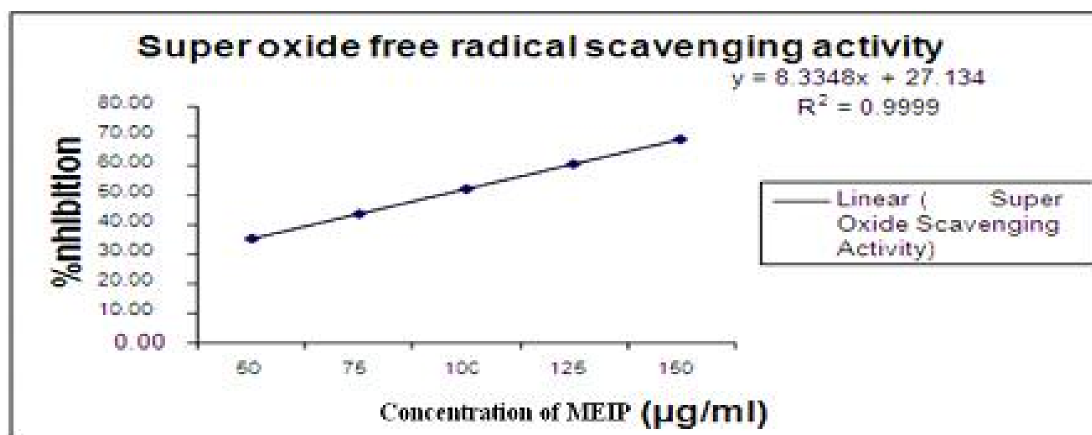


*The activity of methanolic extract flowering tops of Ipomoea palmata was compared with ascorbic acid as standard 18.53μg/ml (y = 1.786x – 0.009,0.9996) and result is 92.6μg/ml. Data were compared by student t – test and t- value found to be 30.239 and $p < 0.0001$ which is considered extremely significant.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH either transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character or convert it to 1 – 1 diphenyl – 2 – picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug. The reduction capacity of DPPH radical is determine by the decrease in its absorbance at 516nm induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. Hence DPPH is usually used as a substance to evaluate the antioxidant activity.

Super oxide free radical scavenging activity:

Figure 3. Super oxide free radical scavenging activity

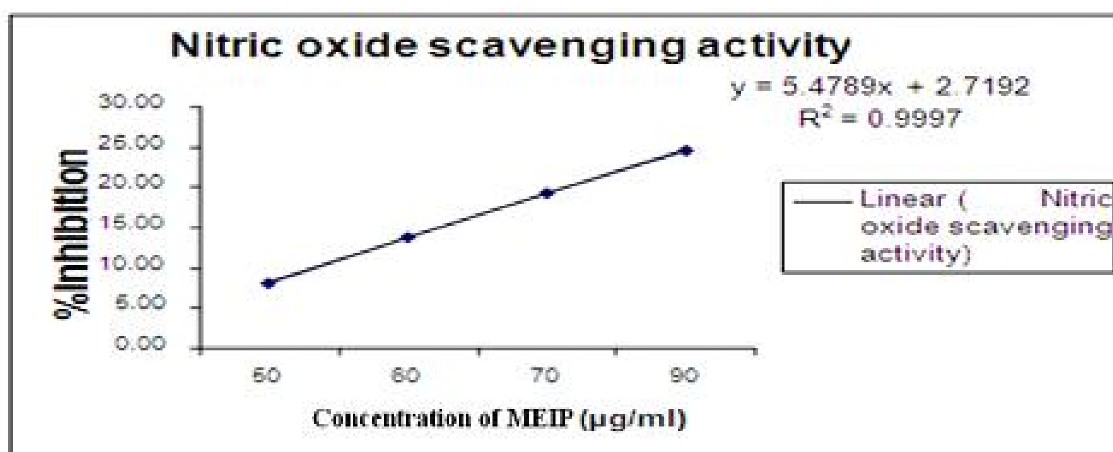


*The activity of methanolic extract flowering tops of Ipomoea palmata was compared with ascorbic acid as standard 18.16µg/ml (y = 1.345x + 1.23,0.9998) and result is 93.58µg/ml. Data were compared by student t – test and t- value found to be 46.185 and $p < 0.0001$ which is considered extremely significant.

Super oxide radical known to be very harmful to the cellular component. Superoxde free radical was formed by alkaline DMSO which react with Nitro blue tetrazolium (NBT) to produced coloured diformazan. The MEIP scavange superoxide radical and thus inhibit foamazan formation.

Nitric oxide radical scavenging activity:

Figure 4. Nitric oxide radical scavenging activity



*The activity of methanolic extract flowering tops of *Ipomoea palmata* was compared with Curcumin as standard 10.52µg/ml (y = 1.234x – 8.112, 0.9996) and result is 193.54µg/ml.

Data were compared by student t – test and t- value found to be 112.08 and $p < 0.0001$ which is considered extremely significant.

Nitric oxide was generated from sodium nitroprusside and measured by the greiss reagent reduction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrate ions that can be estimated by use of greiss reagent. Scavangers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by MEIP.

Conclusion

The methanol extract of (MEIP) flowering tops showed antioxidant activity by inhibiting DPPH and hydroxyl radical, nitric oxide and super oxide anion scavenging, hydrogen peroxide scavenging, and reducing power activities. In addition, the MEIP found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. The results of this study show that the MEMP can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. However, the components responsible for the antioxidant activity of MEIP are currently unclear. Therefore, further works should be performed on the isolation and identification of the antioxidant components in MEIP.

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