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Evaluation of Antioxidant activity of *Ipomoea carnea* leaves

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ABSTRACT

Natural antioxidants have a wide range of biochemical activities including inhibition of ROS generation, direct or indirect scavenging of free radicals and alteration of intracellular redox potential. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health promoting effects in the prevention of degenerative diseases. In addition, it has been reported that there is an inverse relationship between dietary intake of antioxidant rich food and the incidence of human diseases. In searching for novel natural antioxidants, some plants have been extensively studied in the past few years for their antioxidant and radical scavenging components. Ipomoea species is one of them. Literature survey revealed its medicinal importance. Antioxidant activity of leaves and flowers extracts of I. carnea is performed due to phenolic and flavonoid contents, by employing radical scavenging assay ; 2,2 –diphenyl, 1-picryl hydrazyl (DPPH). Ascorbic acid is used as a standard. Quantitative determination of phenols and flavonoids are carried out using spectrophotometric method. Total flavonoid content is determined as quercetin equivalent according to the method described by Malik and Singh and total phenolic content is determined as pyrocatechol equivalent using Folin-Ciocalteu reagent. In the investigated range of concentrations (50 -500µg/ml), significant DPPH radical scavenging value was exhibited by the acetone extract (25-89%). The antioxidant activity of ethanol (10.55-88.11%) and ethyl acetate (5.5-79%) extracts were lower than that of the acetone extract. All the three extracts exhibited higher antioxidant activity than that of the fresh leaves itself (4.22-64%). This study was carried out for the first time from this plant source.

Keywords: Antioxidant, *Ipomoea carnea,* DPPH radical, Foline-Catechol and Quercetin.

INTRODUCTION

Antioxidants are a class of secondary metabolites of plant. The plant kingdom offers many polyphenolic compounds. Several isolated plant constituents as well as extracts have been recognized to possess antioxidant effects against free radicals in biological systems[1]. Natural antioxidants have a wide range of biochemical activities including inhibition of ROS generation, direct or indirect scavenging of free radicals and alteration of intracellular redox potential[2]. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health promoting effects in the prevention of degenerative diseases[3]. In addition, it has been reported that there is an inverse relationship between dietary intake of antioxidant rich food and the incidence of human diseases[4].

Plants (fruits, vegetables, medicinal herbs) contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites, that are rich in antioxidant activity[5-8].

Phenols are one of the main secondary metabolites present in the plant kingdom. They are commonly found in both edible and non-edible plants and have been reported to have multiple biological effects, including antioxidant activity[9].

Flavonoids, the most common group of polyphenolic compounds that are found ubiquitously in plants. These are widely distributed in plant fulfilling many functions. Flavonoids and other plant phenolics are especially common in leaves, flowering tissues and woody parts such as stems and bark[10]. They are important in plant for normal growth development and defense against infection and injury[11]. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and the concomitant lipid peroxidation, protein damage and DNA strand breakage[12].

Flavonoids are the most important secondary metabolites and pigments for flower coloration. They protect plants from attracts by microbes and insects. They exhibit anti-allergic, anti-inflamatory, anti-microbial and anti-cancer activity[13]. Ipomoea- a class of medicinally important plant species is reported in literature for their antimicrobial, anticancer, anti-inflammatory and for many other medicinal activities[14].

Ipomoea species such as *I. batata* and leaf extract of *I. aquatica* show antioxidant properties[15,16].

Preliminary qualitative phytochemical screening of *I. carnea* revealed the presence of phenolic compounds, terpenoids, flavonoids and steroids. Some of them have antioxidant and antimicrobial activities. This study presents the quantitative estimation of total flavonoid and total phenolic contents from the leaves of *I. carnea* by spectrophotometric methods. The data is compared with the contents of bark and flowers.

I. carnea which belongs to convolvulaceae family and fistulosa sub-family is reported for wound healing activity [17]**.** Antibacterial activities of *I. carnea* stem was reported in our earlier study [18]. However, there is no information available about the antioxidant activity including radical scavenging activity of *I. carnea* leaves extracts.

Quantitative determination of total phenols and flavonoids in dry leaves, stem and flowers of *Ipomoea carnea* using spectrophotometric methods was reported in another publication[19]. The aim of this study was to investigate the antioxidant properties of the different extracts of *I. carnea* leaves .1,1- diphenyl -2-picryl-hydrazyl (DPPH) radical scavenging activity of extracts of this plant part was investigated spectrophotometrically for the first time.

MATERIALS AND METHODS

The air shade dried and pulverized plant material was used for experiments. Folin-ciocalteau reagent and all chemicals used were of Merck. UV-Vis S1700 Pharmaspectrophotometer, Schimadzu was used for absorbance measurements. Accurately weighed powdered sample was ground with a pestle and mortar in the measured volume of solvents (80: 20 ethanol–water). The extracts were filtered through Whatman filter paper number 1. Fresh extracts were used for the analysis to prevent degradation.

Determination of total Phenolics

The total phenolic content of leaves and flowers extracts of *I. carnea* were determined according to the method described by Malik and Singh. Aliquots of the extract was taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added sequentially in each tube. A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in folin ciocalteau reagent in alkaline medium which resulted in a blue colored complex, molybdenum blue. The solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the blank reagent. A standard calibration plot was generated (**Figure 1**) at 650 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of samples.

Determination of total Flavonoids

The aluminum chloride method was used for the determination of the total flavonoid content of the sample extracts.[91] Aliquots of extract was diluted to 3ml with methanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water was added. The mixture was vigorously shaken. Absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated (**Figure 2**) at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test sample was calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

DPPH radical scavenging activity

Antioxidants react with DPPH radical and convert it to 1, 1- diphenyl -2-picryl hydrazine. The degree of change in colour from purple to yellow can be used as a measure of the scavenging potential of antioxidant extracts. Aliquots of extract solutions were taken and made up the volume to 3ml with methanol. 0.15ml of freshly prepared DPPH solution (98 µg/ml) was added, stirred and left to stand at room temperature for 30 minutes in dark. The control contains only DPPH solution in methanol instead of sample while methanol served as the blank (negative control). Absorbance was noted at 517 nm by using UV-Vis spectrophotometer. The capacity of scavenging free radicals was calculated as follows:

Scavenging activity (%) = {(Control abs. - sample abs.)/Control abs.} **X** 100.

The measurements were carried out in duplicates.

IC_{50} value was calculated from the plotted graph **(Figure 3)** of scavenging activity against the concentrations of the samples. IC_{50} is defined as the total antioxidant necessary to decrease the initial DPPH radical by 50%. Triplicate measurements were carried out and IC_{50} was calculated for all the extracts based on the percentage of DPPH radicals scavenged. Ascorbic acid was used as the reference compound (positive control) with concentrations 20 to 500 µg/ml.

RESULT AND DISCUSSION

Fig. 1 and 2 presents the calibration plot for the determination of phenols and flavonoids, respectively.

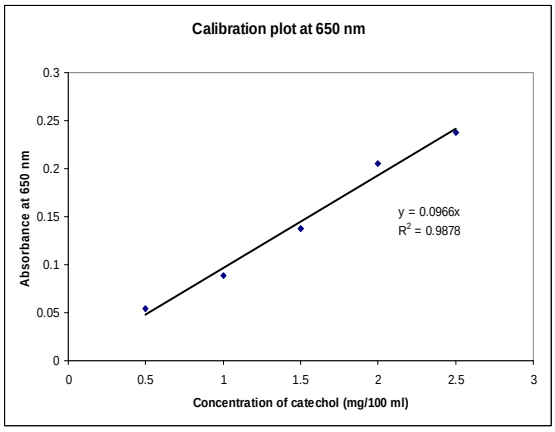

Fig. 1 Calibration plot for phenolic determination

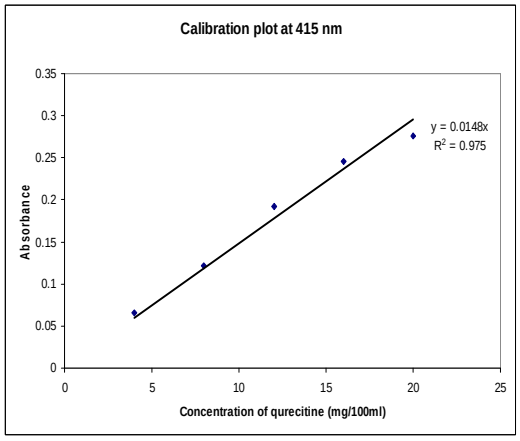

Fig.2 Calibration plot for flavonoid determination

Table 1 and **2** summarize the phenol and flavonoid content of leaves and flowers of *I.carnea*. The present study reveals the phenol contents of the leaves and flowers of *I. carnea* in terms of mg catechol equivalent/g of dry sample (standard plot: y =0.0966x, R^2=0.9878). The values are observed between 45 to 73 mg catechol equivalent/g. Phenolics present in the leaves have received considerable attention because of their potential biologigal activities.
Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural derivatives of phenols. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Using the standard plot of quercetin(y = 0.0148x, R2 =0.975), the flavonoid contents of *I. carnea* leaves and flowers were found mg quercetin equivalent/g of dry sample.

DPPH free radical scavenging activity of extracts of leaves of *Ipomoea carnea* is depicted (**Fig 3).** IC_{50} values for the extracts of the plants and ascorbic acid are recorded (**Table 3)**.

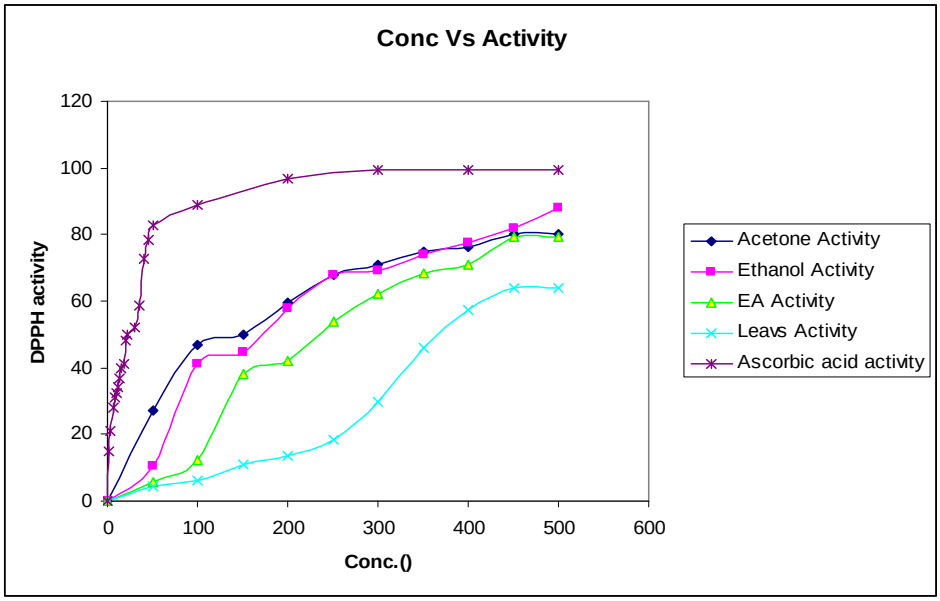

Fig 3

In the investigated range of concentrations (50 -500µg/ml), significant DPPH radical scavenging value was exhibited by the acetone extract (25-89%). The antioxidant activity of ethanol (10.55-88.11%) and ethyl acetate (5.5-79%) extracts were lower than that of the acetone extract. All the three extracts exhibited higher antioxidant activity than that of the fresh leaves itself (4.22-64%). The IC_{50} values for fresh leaves, ethyl acetate, acetone and ethanol extracts were found at 398 µg/ml, 270 µg/ml, 195 µg/ml and 215 µg/ml respectively. For ascorbic acid, the IC_{50} value was 25µg/ml.

Table 1 Phenol content

Plant part	Phenol content (mg catechol equivalent/g dry material)
Leaves	45
Flower	73

Table 2 Flavonoid content

Plant part	Flavonoid content (mg quercetin equivalent /g dry material)
Leaves	84
Flower	422

Table 3 DPPH radical scavenging activity

Extracts/Standard	IC_{50}(µg/ml)
Leaves	398
Ethyl acetate	270
Acetone	195
Ethanol	215
Ascorbic acid	25

CONCLUSION

The present investigation announces that the leaves and flowers of *I. carnea* contain significant amount of phenols and flavonoids. The objective of this study is to get information of the amount of phenolics and flavonoids in leaves and flowers of *I. carnea*. The flowers contain the maximum and the leaves contain the minimum amount of phenols. The flavonoid content of the flowers is quite high compared to that of the leaves. This is the first study determining the phenol and flavonoid contents of *Ipomoea carnea*. All the three extracts exhibited higher antioxidant activity than that of the fresh leaves itself which may act as a chemopreventative agent, providing antioxidant properties and offering effective protection from free radical. Further intention of this study is to correlate relationship of these secondary metabolites to possible biological activities and evaluate *I. carnea* as a potential source of natural bioactive chemicals. The work has been communicated for publication.

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