ORIGINAL ARTICLE

Phytochemical and antioxidant evaluation of *Ipomoea reniformis*

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Abstract

Objective: *Ipomoea reniformis* was studied for its traditionally claimed activity, and still, it is unexplored. The plant can be further explored for the study of traditionally claimed unexplored activities, as well as isolation and identification of active constituents may lead to new findings. The objective of our study is to investigate antioxidant activity of the extracts of *I. reniformis* using *in vitro* models. The measured quantity of extract of *I. reniformis* was evaluated for its antioxidant activity as compared with standard by different models. The present investigation may be concluded that the plant *I. reniformis* is endowed with significantly antioxidant activity due to the presence of phenolics and flavonoids, thereby justifying its use in the indigenous system of medicine. **Materials and Methods:** *I. reniformis* was collected, dried, and extracted with different solvents, and finally, five extracts were studied for their total phenolics and flavonoids content followed by determination of antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl, superoxide radical scavenging, and α-amylase inhibitory activity. **Results:** The obtained results showed that ethanolic extract contains a higher concentration of phenolics and flavonoids and showed highest antioxidant activity among all the samples. **Conclusion:** The plant showed the antioxidant activity, and we believe that antioxidant activity of *I. reniformis* was due to synergistic effect of phytochemicals present in it. Further evidencing the need to conduct studies that can identify the active components responsible for the activity.

Key words: Antioxidant activity, *Ipomoea reniformis*, phenolics and flavonoids

INTRODUCTION

Many plants, particularly medicinal plants, have been extensively studied for their antioxidant activity in the recent years. It is believed that an increased intake of food rich in natural antioxidants is associated with lower risks of degenerative diseases, particularly cardiovascular diseases and cancer.[1] Many diseases in a human organism such as Alzheimer disease, arthritis, and cancer may be the due to increased levels of free radicals. Secondary metabolites from plants have important biological and pharmacological activities, such as antioxidative, anti-allergic, antibiotic, hypoglycemic, and anticarcinogenic.[2-4] The herbals are a good source for production of wide range of natural antioxidants. However, still, there is not enough knowledge and data about the practical usefulness of most of them. There are three major classes of plant chemicals: Terpenoids, phenolic metabolites, and alkaloids.[5] Among these three groups, phenolic compounds are the most important for dietary applications and the most extensively researched.[6] Phenolic compounds consist of acids such as hydroxybenzoic and hydroxycinnamic acid, hydrolyzable polyphenols, condensed tannins, and flavonoids. Plant species are generally protected by these compounds from oxidative damage and have been used as antioxidants by humans. Novel antioxidants from natural sources are of great importance for applications, functional foods, and nutraceuticals. Phytochemical screening is one of the methods that have been used to explore antioxidant compounds in

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plants. *Ipomoea reniformis* (Convolvulaceae) is a perennial, much branched herb (creeper). It is found widely distributed all over the India, especially in damp places in upper Gangetic plain, Gujarat, Bihar, West Bengal, Western Ghats, ascending up to 900 m in the hills, Goa, Karnataka in India, Ceylon, and Tropical Africa. It is reported to have many important medicinal properties. In the indigenous system of Medicine, *I. reniformis* has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of the nose, fever due to enlargement of the liver, and also in kidney diseases. Powder of leaves is used as a snuff during epileptic seizures, juice acts as purgative, and the root is having diuretic, laxative, and applied in the disease of the eyes and gums.[7] The plant is reported to contain resin, glycosides, reducing sugars and starch. Petroleum ether extract was reported to contain fats and fixed oil while aqueous extract was reported to contain amino acids and starch.[8] Chemical investigation of *I. reniformis* shows the presence of caffeic, p-coumaric, ferulic, and sinapic acid esters identified in seeds.[9]

The main aim of the investigation was determination the total phenolic content and concentrations of flavonoids in various extracts of the species *I. reniformis* using spectrophotometric methods, as well as to examine antioxidant activity of plant extracts using *in vitro* model system.

MATERIALS AND METHODS

Plant Material

The plant material used in this study was a whole plant of *I. reniformis*, collected from Narmada valley, Maheshwar, Madhya Pradesh, India, during August 2012 and was authenticated by the Taxonomist Dr. S. K Mahajan, Botany Department, Government P. G. College, Khargone, Madhya Pradesh.

Extraction

The plant materials were initially rinsed with distilled water and dried on paper towel in the laboratory at 37 ± 1°C for 24 h and milled into coarse powder by a mechanical grinder. The coarse powder was extracted with n-hexane, aqueous, benzene, ethyl acetate, chloroform, and ethanol in a Soxhlet extractor. The solvent was completely removed by distillation and dried in a vacuum desiccator. The standard extracts obtained were then stored in a refrigerator at 4°C for further use.[10]

Determination of Total Phenols Content

The total phenolic level in the crude extracts was determined using Folin-Ciocalteu reagent and external calibration with gallic acid. Exactly 0.2 mL of extract solution and 0.2 mL of Folin-Ciocalteu reagent were mixed thoroughly.[11] After an interval of 4 min, 1 mL of 15% Na_2CO_3 was added, and mixture was allowed to stand for a time period of 2 h at room temperature. The measurement of absorbance was taken at 760 nm using a Spectro (Thermo Fisher Scientific) spectrophotometer. Total phenolics concentration was calculated as mg of gallic acid equivalent using an equation obtained from gallic acid calibration curve. The determination of total phenolic compounds was carried out in triplicate, and the results were averaged [Table 1].

Determination of Total Flavonoids

The flavonoids content of *I. reniformis* extracts was determined using spectrophotometric method.[12] The sample contained 1 ml of ethanolic solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λ_{max} = 415 nm. The samples were prepared in triplicate for each analysis, and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin, and the calibration line was constructed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU/g of extract) [Table 2].

In vitro Antioxidant Activity

Antioxidant activity should not be reported based on a solo antioxidant test model. Many *in vitro* test procedures

Table 1: Phenols content (as gallic acid equivalent) extracts of *I. reniformis*

Extracts	mg of GA/g of extract
Benzene	6.21±0.13
Chloroform	21.40±0.32
Ethyl Acetate	12.58±0.26
Ethanolic	28.12±1.08
Aqueous	16.84±0.19

The values are mean±SD of three replicates, *I. reniformis: Ipomoea reniformis*, SD: Standard deviation

Table 2: Total flavonoid content extracts of *I. reniformis*

Extracts	mg of RU/g of extract
Benzene	6.64±0.12
Chloroform	52.32±0.69
Ethyl acetate	29.15±0.43
Ethanolic	59.29±1.87
Aqueous	24.54±1.87

The values are mean±SD of three replicates, *I. reniformis: Ipomoea reniformis*, SD: Standard deviation

are carried out to estimate antioxidant activity.[13] Another characteristic is that antioxidant test models vary in different respects. Therefore, it is not practical to compare one method to another one. In general, *in vitro* antioxidant tests using free radical traps are relatively straight forward to perform. Among free radical scavenging methods, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method is quick, simple, and cheap in comparison to superoxide radical scavenging activity and α-Amylase inhibitory activity.

DPPH Scavenging Activity

The molecule 1, 1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH) is characterized by the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize. The delocalization of electron results in deep violet color, characterized by an absorption band in ethanol solution at about 517 nm.[14] When a solution of DPPH is mixed with that of a substrate that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.

To assess the antioxidant potential by free radical scavenging of the test samples, the change in optical density of DPPH radicals is observed. The plant extracts and fractions in different concentration (0.2 ml) are diluted with methanol, and 2 ml of DPPH solution (0.5 mM) is added. After 30 min, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using the equation as given below:

$$\text{DPPH radical scavenging activity (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} .100$$

Superoxide Radical Scavenging Activity Method

Superoxide radicals were generated *in vitro* by non-enzymatic system and determined spectrophotometrically (560 nm) by nitro blue tetrazolium (NBT) photo reduction method.[15] The assay mixture consists of 6.6 mM EDTA containing 3 μg of NaCN, 2 μM of riboflavin, 50 μM of NBT, crude extract, and 67 mM of phosphate buffer (pH 7.8) in a final volume of 3 mL. The optical density at 560 nm was measured before and after 15 min illumination. The superoxide radical scavenging activity of the crude extracts was expressed in inhibitory concentration 50% (IC_{50}) values.

α-amylase Inhibitory Activity

The α-amylase activity was measured using the dinitrosalicylic acid (DNS) method[16,17] developed by Bernfeld (1955), improved by Jamieson *et al.* (1969), and adopted for testing α-amylase inhibitory potential[18] using 1% soluble starch as substrate. The test substance was pre-incubated with amylase (100 μL) at room temperature for 20 min before the addition of 100 μL of the substrate solution followed by incubation at 37°C for 10 min. The reactions were stopped by the addition of 200 μL of DNS reagent followed by color development by placing the tubes in boiling water for 5 min and then added 3.6 mL of distilled water. Acarbose was used as positive control. The absorbance was read at 470 nm, and experiments were carried out in duplicates [Table 3].

The IC_{50} values were determined 3 times, ±standard error of mean

RESULTS AND DISCUSSION

The *in vitro* antioxidant activity by different models for the extracts of *I. reniformis* exhibited significant activity with different methods. The results of the above studies showed significant antioxidant activity for ethanolic extract of *I. reniformis*. From Figure 1 it was clear that in the DPPH method, the ethanolic extract of *I. reniformis* showed potent antioxidant activity, with IC_{50} values 9.87 μg/mL^{-1} followed by ethyl acetate extract 23.65 μg/mL^{-1}. However, the standard

Table 3: Effect of various extracts of *I. reniformis* on IC_{50} values by DPPH, superoxide radical scavenging, and α-amylase inhibitory activity

Extracts/standard	IC_{50}±SEM* (μg/mL^{-1})		
	DPPH	Superoxide radical scavenging	α-amylase inhibitory activity
Benzene	58.26±0.67*	88.01±0.59	135.72±1.05
Chloroform	42.21±1.31	47.12±1.23*	192.25±1.54*
Ethyl acetate	23.65±1.98*	52.11±1.28	352.12±0.65
Ethanolic	9.87±0.54	96.66±1.41	102.32±1.78*
Aqueous	36.41±1.54	87.42±0.89**	258.21±1.52
Vitamin C	4.12±0.21	165±1.02	-
Acarbose	-	-	10.4±1.25

*Average of three independent determinations, three replicates, values are mean±SEM. *Indicates a significant difference at *P*<0.005. **In the same column with different superscripts differ significantly at *P*<0.005, IC_{50}: Inhibitory concentration 50%, SEM: Standard error of mean, DPPH: 1, 1-diphenyl-2-picrylhydrazyl, *I. reniformis: Ipomoea reniformis*

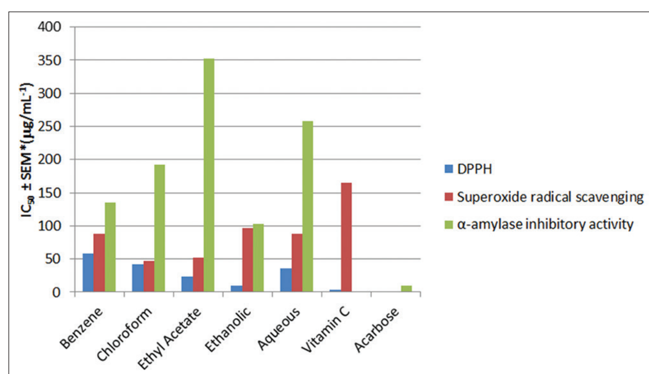

Figure 1: Graphical representation of all extracts on inhibitory concentration 50% (IC_{50}) value by different models.

Vitamin C exhibited better results with lower IC_{50} values. In the superoxide radical scavenging method, the ethanolic extract again showed highest activity 96.66 µg/mL^{-1} among all the samples used followed by benzene extract with a value of 88.01 µg/mL^{-1}. In the α-amylase inhibitory activity method, all the extracts exhibited potent antioxidant activity, but ethanolic extract again was most potent among all 102.32 µg/mL^{-1}, followed by benzene extract with 135.72 µg/mL^{-1}. The phenolic and flavonoids content of ethanolic extract was much higher than other extracts used for the study, and this might be the main reason behind its antioxidant activity.

CONCLUSION

The present study indicates that the *I. reniformis* possesses antioxidant activity. Based on the result, we can correlate that phenolic and flavonoid content may affect antioxidant activity of any extract. Further studies might be carried out to explore the lead molecule responsible for aforesaid activity from this plant.

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