Original Article

"Total Polyphenolic Content and *In-vitro* Antioxidant Potential of Extracts of Creeping Herb *Ipomoea reniformis* (Roxb.) Choisy"

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ABSTRACT

Objective: The objective of this study was to screen the methanolic and aqueous solvent extract of creeping herb of *Ipomoea reniformis* to display the potent *in vitro* antioxidant activity, total phenolic contents (TPC) in order to find possible sources for future novel antioxidants.
Methods: A detailed study was performed on the antioxidant activity of the methanolic and aqueous extract of creeping herb of *Ipomoea reniformis* by in vitro antioxidant activity using DPPH and lipid peroxidation method. The total phenolic (TPC) were also determined in the methanolic and aqueous solvent extract.
Results: The Methanolic and Aqueous extract of *Ipomoea reniformis* show dose dependant antioxidant activity. The alcoholic extract show highest % inhibition of 81.40% at concentration of 20 µg/ml by DPPH assay and 82.32% at 1000 µg/ml by LPO. It is very clear that the total amount of phenol in methanolic extract was found to highest (61.11±0.1829) and in aqueous extract was found to be the least (19.15± 0.2406) respectively.
Conclusion: In the present study analysis of total phenolic content, free radical scavenging activity and lipid per oxidation show concentration dependant inhibition by methanolic and aqueous extract of *Ipomoea reniformis leaves.*

Keywords: TPC, LPO, DPPH, Antioxidant.

INTRODUCTION

In the last decade there has been a growing interest in understanding the role of free radicals in biomedicine. Free radicals are atoms with unpaired electrons such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). In popular scientific/biomedical literature the term 'free radical' is used in a broad sense and also

includes related reactive species such as 'excited states' that lead to free radical generation or those species that results from free radical reactions. In general, free radicals are very short lived, with half-lives in milli-, micro- or nanoseconds. Some of the biologically important reactive species. ROS are molecules that contain oxygen and have higher reactivity than ground state molecular oxygen[1]. The reactive oxygen species includes: the hydroxyl radical (•OH), the most damaging of this chemical species; which also include superoxide anions (O_2•-); singlet oxygen ($1O_2$); and hydrogen peroxide (H_2O_2). ROS can be formed under the aerobic condition not only during oxidative phosphorylation, through the action of mixed function oxidases, and as by-products of normal metabolism by enzymes such as superoxide dismutase (SOD), NADPH oxidase, and xanthine oxidase (XO) in neutrophils, but can also be generated from redox cycling of certain drugs and by radiation[2]. A well known example for RNS is nitric oxide (NO), a short-lived endogenous gas that acts as a signaling molecule in the body. NO was synthesized by nitric oxide synthase (NOS) and produced by almost all mammalian cells. Excessive or unregulated NO synthesis has been implicated as causal or contributor to some pathophysiological conditions including cancer. Expression of NOS has been distinguished in various cancers including cervical, breast, central nervous system, laryngeal, and head and neck cancers[3-7]. (See figure 1.)

Providentially, the mammalian cells have endowed by an antioxidant defense mechanism that allows equilibrium between the generation of oxidants and antioxidants. The interrupted condition in between these two factors develops an oxidative stress, and despite the antioxidant defense mechanism to counteract the reactive species-related deleterious effects, damage to macromolecules occurs as a result of these reactions. Oxidative damage accumulates during the life cycle and lead to different pathological progressions like myocardial infection, atherosclerosis, neurodegenerative disorders, rheumatoid arthritis, and cancer[8]. *Ipomoea reniformis* (convolvulaceae) is a creeping herb, rooting at nodes. The leaves are up to 1.9 cm long, kidney shaped or ovate-cordate, broader than long and toothed. The flowers are axillary and yellow with hairy sepals. The plant flowers in the rainy season and cold weather[9]. Phytochemical investigation of the plant showed the presence of resin, glycosides, reducing sugars, starch, caffeic, pcoumaric, ferulic and sinapic acid esters. In the Indigenous system of Medicine, *Ipomoea reniformis* has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of nose, fever due to enlargement of 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[10]. The aim of present study to explore the Polyphenol content and *In-vitro* antioxidant potential of extracts of plant *Ipomoea reniformis.*

MATERIAL AND METHOD

Collection and authentication of Plant material

Ipomoea reniformis (Roxb.) Choisy (Leaves) were collected from the tribal belt of Kunkuri Jashpur, Raigarh, Bilaspur and Amarkantak region of Chhattisgarh. The plants were identified and authenticated by Dr. HB Singh, Scientist, National Institute Scientific Communication and Research (NISCAIR), New Delhi (India). The plant material was dried, powdered and store in air tight containers for further studies.

Chemicals and reagents

All the chemicals including solvents were of analytical grade, obtained from Sigma-Aldrich, India.

Preparation of plant extract

The successive extraction procedure was adopted for the preparation of various extracts of plant material. The powdered material was subjected to successive extraction with solvents in their ascending order of polarity. In this process the substance, which is soluble in a solvent with particular polarity, is extracted in the solvent and remaining marc further extracted with next solvent of higher polarity index. The constituents, which are soluble in both polar and non-polar solvents, can be extracted separately by adopting this approach[1]. The powdered drug was taken and subjected for successive solvent extraction. The extraction was carried out for 16 hours with the following solvents in the increasing order of the polarity (i.e. Petroleum ether, methanol and chloroform water). A 1: 4 w/v ratio of drug and solvent was maintained. The extract obtained was concentrated by distilling the solvent and evaporating them to dryness at low temperature. Extract were then weighed and the percentage of different extractive values was calculated in term of air dried weight of the plant material. The color and consistency of the dried extracts were recorded.

Quantitative determination polyphenols

Total Phenolics Assay: Folin-Ciocalteu Method[11].

Reagent:

Folin-Ciocalteu: Folin-Ciocalteu reagent.

Na2CO3 solution: Na2CO3 solution (concentration 5 g/l).

Standard solution

Ascorbic acid was used as a standard for the calibration curve; the stock solution was prepared by dissolving 50 mg into 50 ml water (1000µg/m) then different dilutions were made as 5, 25, 50, 100, 150, 200, 250 µg/ml. 2 ml from these solution was taken and diluted with reagent to produce calibration curve 1 – 50 µg/ml.

Test solutions

test solutions of all the extracts, were prepared by dissolving 15 mg into 100 ml water (150µg/m) then 2 ml from these solution was taken and diluted with reagent to produce sample for the taking absorbance.

Procedure

The Folin-Ciocalteu method was used for the determination of the total phenolics. In brief, an aliquot (2 ml) of the appropriate diluted extracts was added to a 10 ml volumetric flask, containing 5 ml of distilled water. Then, 0.5 ml of Folin-Ciocalteu reagent was added and the contents mixed. After 3 min, 1.5 ml Na_2CO_3 solution (concentration 5 g/l) was added and made up to a total volume of 10 ml distilled water. After keeping the samples at 50°C (water bath) for 16 min in sealed flasks and subsequent cooling, their absorbance were read at 765 nm against distilled water as the blank. A calibration curve was constructed using gallic acid standard solutions (1–50µg/l). The concentration of total phenolics is expressed as the gallic acid equivalent (GAE). All samples were prepared in triplicate and result is tabulated in table.

In-vitro antioxidant activity

DPPH Method

This activity was measured using DPPH (1-1-diphenyl 2-picryl hydrazyl) the method[12] is based on the reduction of colored solution of DPPH in presence of test drug measured at 516 nm. The activity is expressed as $\log EC_{50}$, which is the concentration of the test solution required to give a 50% decrease

in absorbance compared to that of blank solution.

Reagents

DPPH Solution

A working solution of methanolic DPPH having an absorbance of 0.9 at 516 nm was used. This was prepared by taking 150 µl of stock solution (12.9 mg of DPPH in 10 ml of methanol) in 3 ml of methanol.

Standard solution

Ascorbic acid was used as a standard free radical scavenger. 50 mg of ascorbic acid was dissolved in 100 ml of methanol.

Test solutions

1 mg/ml test solutions of all the extracts, were prepared in methanol.

Procedure

To 150 µl of DPPH Solution in methanol, different concentrations of ascorbic acid were added and the volumes were made up to 3 ml with methanol. DPPH diluted to 3 ml was taken as blank. Decrease in absorbance in the presence of ascorbic acid was noted down at 516 nm after 15 min. linear graph of concentration Vs Absorbance was prepared and log EC_{50} values were calculated. The test solutions were treated in the similar manner and the log EC_{50} values were calculated.

Ferric chloride induced lipid per oxidation[13]

Male albino rat (200 to 250 g) was sacrificed by cervical dislocation. The skin over the abdomen was cut open and the liver was perfused with ice cold 0.15 M KCl via portal vein. After perfusion, the liver was isolated and 20 % (w/v) homogenate in 0.15 M KCl was prepared using tissue homogenizer under ice- cold (0º-4ºC) conditions. The homogenate was centrifuged at 1500 *g* for 5 min and clear supernatant was used for further study. Different concentrations of test extracts were taken in test tubes to which 1ml of 0.15 M KCl and 0.5 ml of cell free homogenate were added.

Peroxidation was initiated by adding 100 µl of 1mM ferric chloride. The mixture was incubated for 30 min at 37ºC. After incubation, the reaction was stopped by adding 2 ml of ice cold 0.25 N HCl containing 15 % trichloroacetic acid (TCA), 0.38% thiobarbituric acid (TBA), and 0.5% of 0.05% butylated hydroxyl toluene (BHT). The reaction mixture was heated for 60 min at 80ºC. The sample was cooled and centrifuged at 5000 *g* for 15 min and absorbance of supernatant was measured at 532 nm and result is tabulated in table.

An identical experiment (control induced) was performed in absence of test compounds to determine the amount of lipid peroxidation obtained in the presence of inducing agents without any test compounds. A blank was performed with all the reagents except ferric chloride and test extracts.

% Anti-lipid peroxidation effect (% ALP) was calculated by the following formula:

$$\% \text{ ALP} = 1 - \left\{ \frac{(\text{Sample absorbance} - \text{blank})}{(\text{Absorbance of control})} \right\} \text{X } 100$$

RESULT AND DISCUSSION

Total phenol content and absorbance at 760nm is shown in table 1 along with calibration curve in Fig.2.It is very clear that the total amount of phenol in methanolic extract was found to highest (61.11±0.1829) and in aqueous extract was found to be the least (19.15± 0.2406) by soxhlation extraction technique.

The DPPH anti-oxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of anti-oxidants. The Fig 3 showed % DPPH scavenging activity of Methanolic and Aqueous extract of leaf of *Ipomoea reniformis*. The Methanolic and Aqueous extract of *Ipomoea reniformis*

show dose dependant antioxidant activity. The alcoholic extract show highest % inhibition of 81.40% at concentration of 20 µg/ml by DPPH assay.

Table 3 and figure 4 showing ferric chloride induced lipid per oxidation activity of Methanolic and Aqueous extract of leaf of *Ipomoea reniformis*. The Methanolic and Aqueous extract of *Ipomoea reniformis* show dose dependant lipid per oxidation activity.

CONCLUSION

In the present study analysis of total phenolic content, free radical scavenging activity and lipid per oxidation show concentration dependant inhibition by methanolic and aqueous extract of *Ipomoea reniformis leaves*. The methanolic extract of *Ipomoea reniformis* having highest antioxidant potential and total phenolic content.

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Conflict of Interest

None.

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Table 1. Quantitative determination polyphenol at 760nm

Quantitative determination polyphenol				
S. No.	Sample	Absorbance	Gallic acid equivalent (µg/ml) (n=3)	% (n=3)
1	IPR.METH	0.332	18.33±0.0548	61.11±0.1829
2	IPR.AQS	0.108	5.75±0.0721	19.15± 0.2406

*Total phenolic content are expressed as gallic acid equivalent [mg/g] of the dried weight (mean values ± standard deviation).
IPR.METH: Methanolic extract of *Ipomea reniformis* Herb
IPR.AQS: Aqueous extract of *Ipomea reniformis* Herb

Table 2. IC_{50} value of DPPH scavenging activity of methanolic and aqueous extract of leaf of *Ipomoea reniformis*

Conc. (ug/ml)	Std. Ascorbic Acid	Meth. Ext. IPR	Aqu. Ext. IPR
0.25	12.4633±0.6212	7.10325±0.2367	2.9325±0.5068
0.5	19.1105±0.4701	15.7038±0.0627	5.1856±0.3504
1	39.0794±0.5784	33.3187±0.4886	10.0132±0.2634
2.5	55.4277±0.924	46.3465±0.78	13.7956±0.2946
5	67.8333±0.728	61.2976±0.2888	27.154±0.3899
10	80.6758±0.8773	73.2837±0.562	37.9538±0.4137
20	90.0845±1.1566	81.4022±0.273	40.6364±0.1024

Table 3. Lipid per oxidation data methanolic and aqueous extract of leaf of *Ipomoea reniformis*

Conc (ug/ml)	Standard	Meth. Ext. IPR	Aqu. Ext. IPR
3	13.1669±0.3573	10.2061±0.5746	2.529±0.7148
10	21.8369±0.4851	19.496±0.924	4.7133±0.5539
30	41.7025±0.3786	35.4973±0.9779	11.803±0.6413
100	62.2426±0.5707	55.2697±1.104	21.0435±0.3862
300	77.7096±0.4418	70.7259±1.0759	30.8939±0.0353
1000	91.6268±0.3604	82.3201±0.616	40.4897±0.4428

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Figure 1. Creeping herb *Ipomea reniformis*[14]

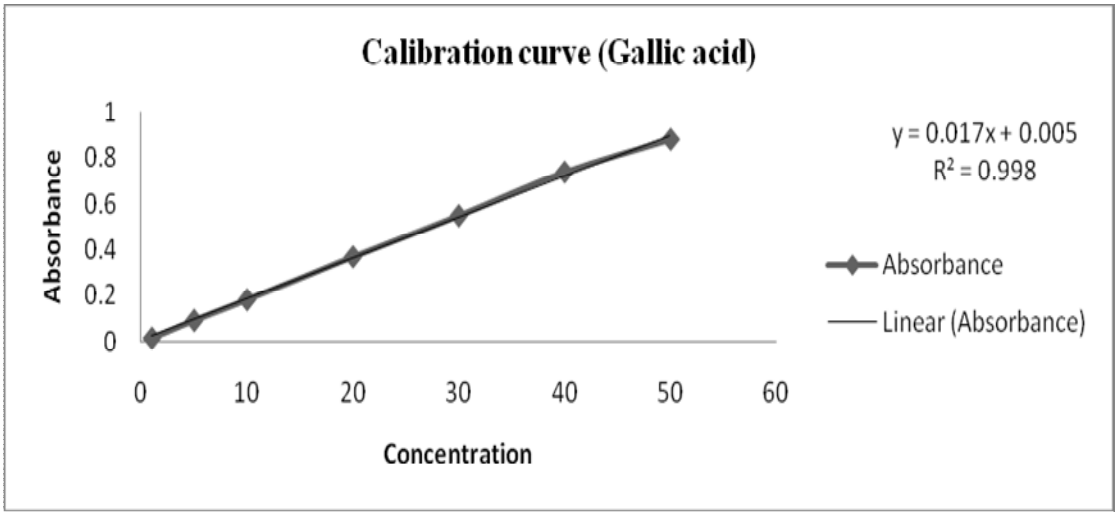

Figure 2. Calibration curve for standard (Gallic acid)

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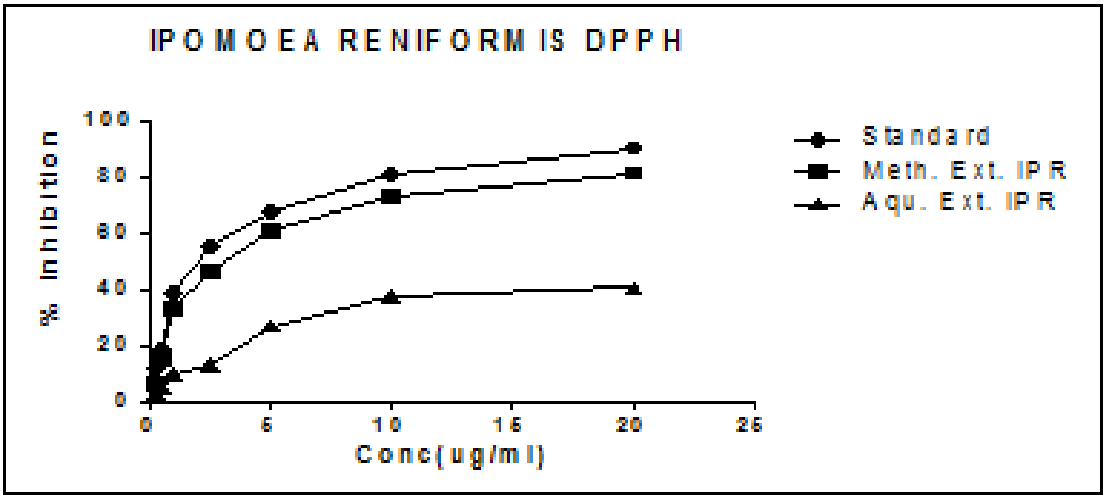

Figure 3. DPPH scavenging activity of methanolic and aqueous extract of leaf of *Ipomoea reniformis*

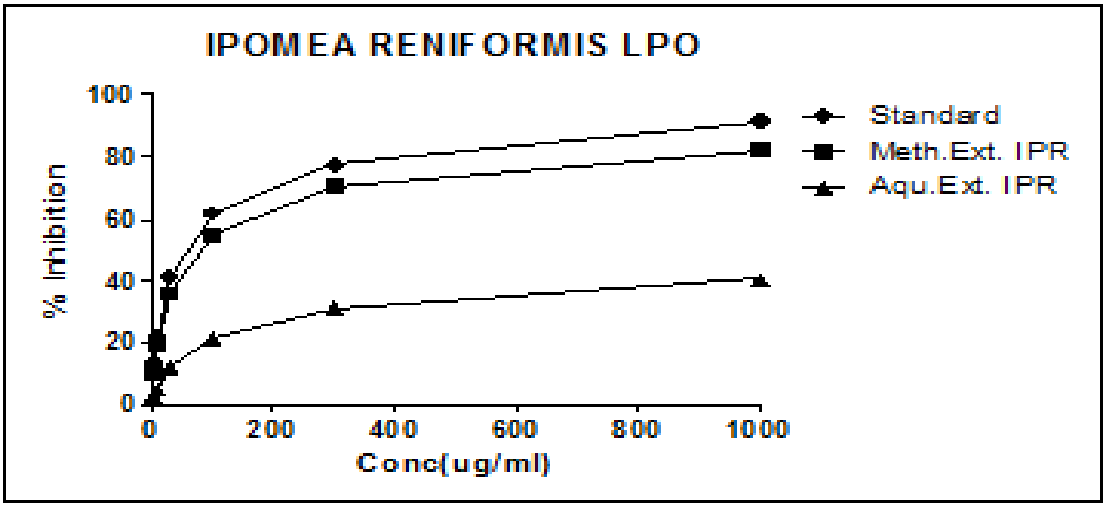

Figure 4. Lipid per oxidation activity of methanolic and aqueous extract of leaf of *Ipomoea reniformis*