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In-Vitro Evaluation of Antioxidant Activity and Total Phenolic Content of Methanolic Extract of *Convolvulus pluricaulis.*

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ABSTRACT

Cellular damage caused by reactive oxygen species (ROS) has been implicated in several diseases, and hence natural antioxidants have significant importance in human health. *Convulvulus pluricaulis* is an important traditional medicinal plant, the present study was carried out to determine the total phenolic content and antioxidant properties of methanolic extract of leaves of this plant. The total phenolic content for 50, 100 and 200 µg/ml extracts was 0.2092, 0.2380 and 0.3608 mg GAE/ gram. The radical scavenging activity of methanolic extract of *Convolvulus pluricaulis* and the standard was found to be highest at 100ug/ml which was 52.56% and 93.48% respectively. The concentration of *Convolvulus pluricaulis* needed for 50% inhibition (IC50) was found to be 90.56 µg/ml whereas 29.02µg/ml needed for BHT. Based on the results it can be concluded that methanol extract of *Convulvulus pluricaulis*, a natural herb may have potential antioxidant effects against several oxidants.

Keywords: Antioxidant activity, Gallic acid, BHT, Convolvulus pluricaulis, DPPH, Folin-Ciocalteu assay.

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INTRODUCTION

Convolvulus pluricaulis Choisy is a perennial wild herb commonly found on sandy and rocky areas under xerophytic condition. It is a reputed drug of ayurveda and reported to possess anti-oxidant, brain tonic, nerve tonic, and laxative and has been used in anxiety, neurosis, and epilepsy, insomnia, burning sensation, oedema and urinary disorders. The whole herb is used medicinally in the form of decoction with cumin and milk in fever, nervous debility and loss of memory [1]. It is very commonly found in north temperate regions such as India, Myanmar, Nepal, Sri Lanka and also in Malaysia. It is commonly known as Shankpushpi, Shankhini, Kambumalini and Sadaphuli [2]. It is known as Aloe weed or speed wheel in English. The herb has appearance like morning glory with blue flowers situated at alternate positions with flowers or branches. Herbalists believed that this herb calms the nerves by regulating the body's production of the stress hormones, adrenaline and cortisol.Even though Ayurvedic practitioners have used this herb for centuries, there is no hard scientific evidence as to the positive effects of this herb outside of few studies performed in 1970's and 1980's. In those studies people suffering from anxiety were given Shankpushpi for six weeks and claimed to have slept better, have more energy and better concentration. Others than that, it is one of the best herbs that are used for enhancing beauty and helps in nourishing all the layers of the skins. Furthermore, this herb has other therapeutic uses such as hypolipidemic, antiulcer, analgesic, antidiabetic, anticatatonic, antifungal, antibacterial and immunomodulatory action [3]. The present investigations is undertaken to estimate the total phenolic content and antioxidant potential of *Convolvulus pluricaulis* methanolic leaf extract though DPPH *in vitro* assay model.

MATERIALS AND METHODS

Plant materials and extraction

For the present study purpose, the shade dried leaf part of the study species was made into fine powder of 40 mesh size using the pulverizer. 100 g of the powder was filled in the filter paper and successively extracted using 500 mL methanol using the soxhlet extractor for 8 – 10 hours [4].Then the extract was filtered through Whatman No.1 filter paper to remove all undissolved matter, including cellular materials and other constituents that are insoluble in the extraction solvent.

Chemicals

All the chemicals used in the work were purchased from R&M Chemicals, Essex, UK. The chemicals used were of analytical grade.

Phytochemical Screening

Phytochemical components of the leaves of *P.betel* were screened by using the standard methods [6, 7]. The components analyzed were alkaloids, flavonoids, triterpenoids, antharacene glycosides, tannins, phenolics and saponins[5,6].

Total Phenolic Content

The determination of total phenolic content of methanolic extract of C.pluricaulis was done by Folin-Ciocalteau assay method [7,8]. For every 1ml of extract, about 1ml of Folin-Ciocalteau reagent and 2ml of 2.5% sodium carbonate were added. The mixture was mixed completely and allowed to stand for two hours. Then, the absorbance of the solution at 750nm was measured. The same procedure was repeated by replacing the extract with the gallic acid. Quantification of total Phenolic content was done using standard curve of gallic acid as a standard Phenolic compound (0.1-0.5 µg/ml), which was dissolved in methanol and expressed as mg gallic acid per gram of plant material. Sample measurement was done triplicates and the mean was calculated in each case.

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the fractions was measured in vitro by 1, 1- diphenyl- 2-picrylhydrazyl (DPPH) assay. About 0.3mM solution of DPPH in 100% methanol was prepared and 1ml of this

solution was added to 3ml of the extract dissolved in methanol at different concentrations. The mixture was shaken and allowed to was measured at 517nm using a shimadzu spectrometer. The percentage scavenging inhibition was determined and was compared with that of BHT, which was used as the standard [9, 10].

Statistical Analysis

Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD. Correlation analysis of free radical scavenging activity versus total phenolic content and reducing power was carried out using the correlation and regression program.

Table 1: Phytochemical screening studies of the methanolic extract of *C.pluricaulis*.

S.No.	Name of the compound	Result
1.	Alkaloids	+
2.	Glycosides	-
3.	Reducing sugar	+
4.	Flavonoids	-
5.	Tannins	+
6.	Phlobotannins	-
7.	Anthroquinones	+
8.	Saponins	-
9.	Terpenoids	+
10.	Steroids	-

+: Present - : Absent

Table: 2: Absorbance of Gallic Acid (Standard)

Concentration(µg/ml)	Absorbance	Mean absorbance
0.1	0.3262	0.3253±0.0002
	0.3256	
	0.3251	
0.2	0.3315	0.3314±0.0005
	0.3314	
	0.3314	
0.3	0.3779	0.3782±.0004
	0.3780	
	0.3788	
0.4	0.5326	0.5336±0.0005
	0.5336	
	0.5339	
0.5	0.5855	0.5858±0.0004
	0.5861	
	0.5864	

Absorbance of Different Concentrations of Methanolic Extract Of*convolvulus Pluricaulis* Leaves (Shankhpushpi)

Concentration(µg/ml)	Absorbance	Mean Absorbance
50	0.3310	0.3310±0.0003
	0.3315	
	0.3318	
100	0.3570	0.3570±0.0003
	0.3578	
	0.3577	
200	0.4679	0.4678±0.001

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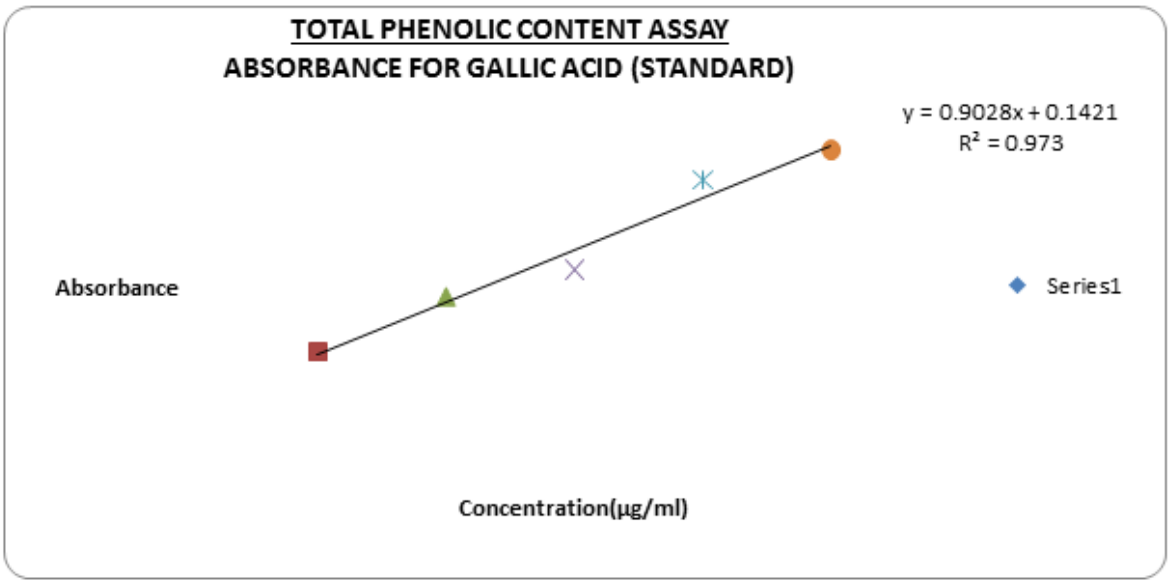
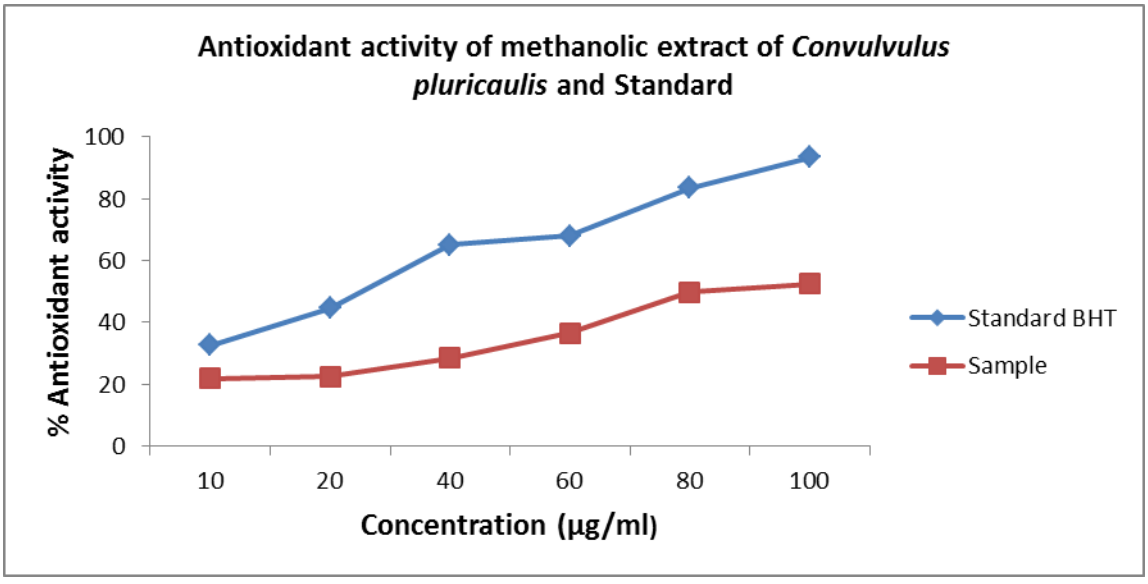


Figure 1: Standard graph of gallic acid (µg/ml)

Anti-Oxidant Activity Of Different Concentrations Of Methanolic Extract Of Convolvulus Pluricaulis (Shankhpushpi) Leaves And Standard

Concentration(µg/ml)	Standard	Sample
	BHT Anti-oxidant Activity (%)	*Convolvulus pluricaulis* Anti-oxidant Activity (%)
10	32.66	21.84
20	44.69	22.57
40	65.09	28.58
60	67.94	36.55
80	83.62	49.77
100	93.48	52.56



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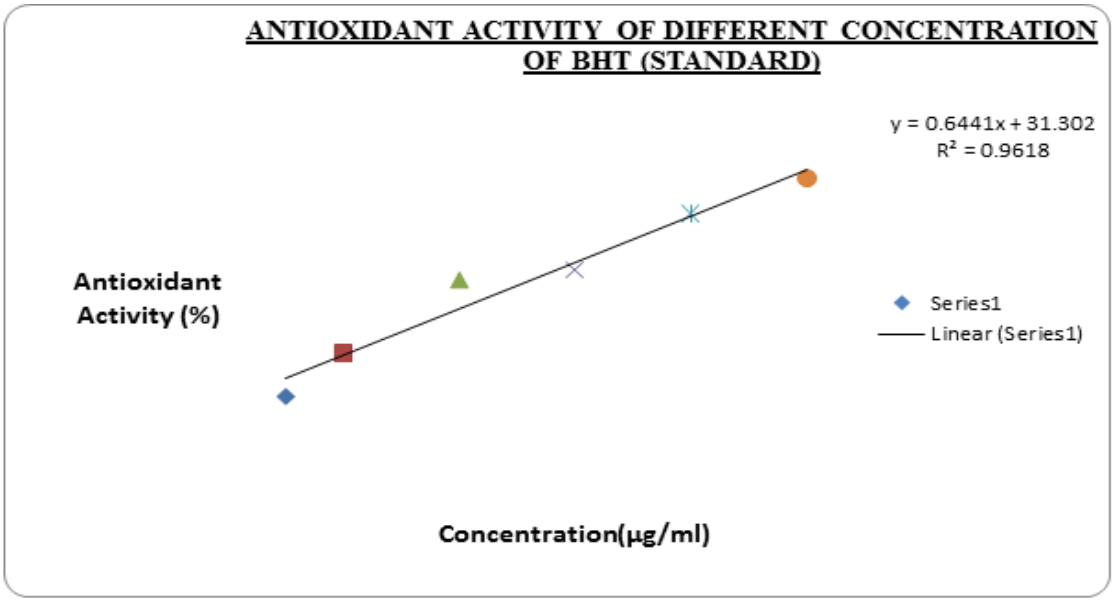

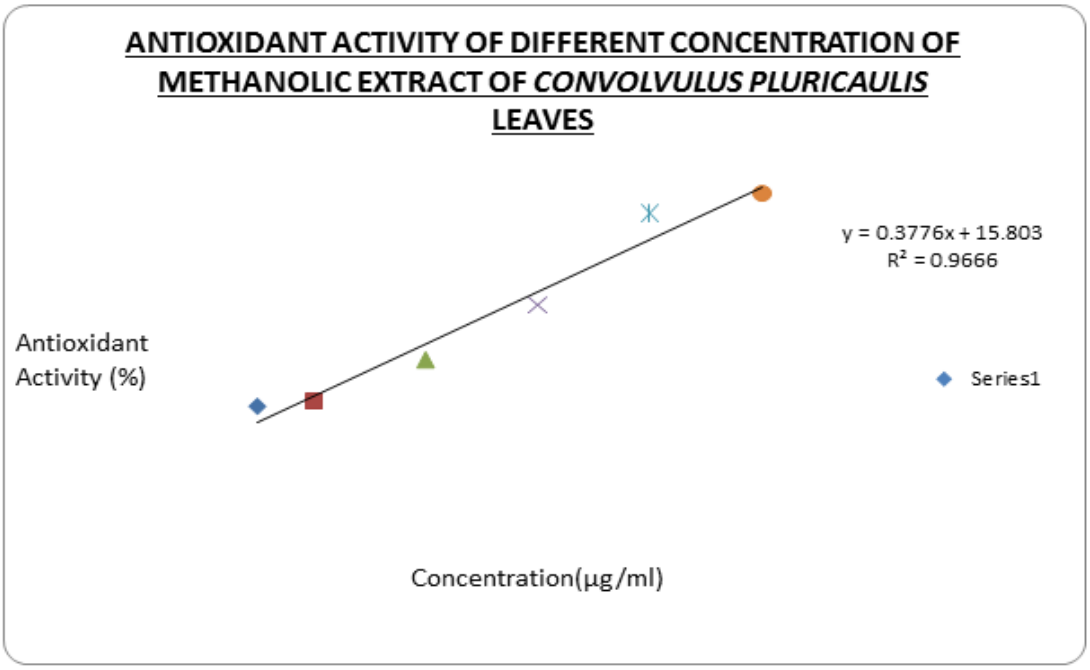

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the methanolic extract of leaves of *Convolvulus pluricaulis* was reported. (Table.1). The positive result for the presence of alkaloids, tannins, flavonoids, steroids and reducing sugars was observed. The total phenolic content of extract of *Convolvulus pluricaulis* was measured by Folin-Ciocalteu assay method. The absorbance of sample was measured at 750 nm using a UV-spectrophotometer Table 1. The amount of total phenolic compounds that are present in each of the sample was reported as mg GAE per gram sample. A standard curve was plotted to quantify phenolic compound in sample as mg GAE per gram sample that is methanol extract Fig.1. As the concentration of the extract increases, the mean absorbance value also increases. For the extract of Shankhpushpi, the concentration used was 50, 100 and 200µg/ml and the absorbance values were 0.3310, 0.3570 and 0.4678 correspondingly. The total phenolic content for 50, 100 and 200 µg/ml extracts was 0.2092, 0.2380 and 0.3608 mg GAE/ gram. Gallic acid was reported as a free radical scavenger and as an inducer of differentiation and apoptosis in leukemia, lung cancer, and colon adenocarcinoma cell lines, as well as in normal lymphocytes cells. DPPH is stable nitrogen centered free radical which can be effectively scavenged by anti-oxidants and shows strong absorbance at 518 nm. The change in absorbance of DPPH radicals caused by the extracts was due to the

reaction between anti-oxidant molecules and the extracts, which resulted in the scavenging of the radical by the hydrogen donation. The DPPH radical scavenging activity of methanolic extract of *Convolvulus pluricaulis* was found to be highest at 100ug/ml concentration which was 52.56%. Nevertheless, % DPPH scavenging activity of standard, BHT at same concentration was found to be 93.48%. The % DPPH scavenging activity increases with the increasing concentration. The concentration of Convolvulus pluricaulis needed for 50% inhibition (IC50) was found to be 90.56 µg/ml whereas 29.02µg/ml needed for BHT.

CONCLUSION

The total phenolic content of the methanolic extract *Convolvulus pluricaulis* of showed that it may account for its antioxidant properties. However, In DPPH assay, the methanolic extract showed antioxidant activity, however when compared to standard, it has shown moderate antioxidant activity. Further investigations are needed to establish the components of phenolics that have contributed to the antioxidant properties.

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