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INVITRO STUDIES ON THE ANTIOXIDANT ACTIVITIES OF EXTRACTS FROM THE FLOWERS OF *GOMPHRENA GLOBOSA*

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ABSTRACT

Oxidative stress leads to formation of free radicals, reactive oxygen species and nitrogen species, which leads to numerous pathophysiological disorders. The present study is aimed to evaluate the free radical scavenging potential of flowers of *Gomphrena globosa*. The extracts from the flowers of *Gomphrena globosa* were prepared in solvents *viz.* methanol, ethanol and butanol. Spectrophotmetric and HPLC analysis was performed to determine total phenols, flavonoids and antioxidants with gallic acid and quercetin served as standards, respectively. The antioxidant potential of flowers of *Gomphrena globosa* was investigated with free radical scavenging methods, including H_2O_2 and DPPH as free radicals. The present findings suggested that methanol extract was more potent and had a high content of phenols and flavonoids, emphasizing the predominance of flavonoids in the extracts from *Gomphrena globosa*. The scavenging inhibition of H_2O_2 and DPPH free radicals in methanol preparations from the flowers of *Gomphrena globosa* were 62.3% (IC_{50} =13.8µg/ml) and 72% (IC_{50} = 10µg/ml) at 30µg/ml of extract preparations. Furthermore, positive correlation was observed between polyphenols and free radical scavenging inhibition. These observations indicate that the flowers of *Gomphrena globosa* may possess antioxidant properties and further pharmacological investigations could provide an insight into the potential use of these flowers in the treatment of oxidative related disorders.

KEYWORDS: **Antioxidant, Flavonoids, *Gomphrena globosa*, Oxidative stress, Phenols**

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INTRODUCTION

Oxidative stress leads to the formation of the free radicals and reactive oxygen species, which may cause the pathophysiological disorders like insulin dependent Diabetes mellitus, cardiovascular diseases, neural disorders, Alzheimer's disease, Parkinson's disease, fatty liver associated with chronic alcoholism and aging [1-4]. For the past few decades, the herbal plants have been extensively explored for the natural source of antioxidants, which could combat the free radicals and are looked upon as persuasive therapy; with minimal side effect on the well being of the patient [5-10]. The natural product of plant origin phenolic compounds like flavonoids, phenols, flavonols and proanthocyanidins contribute towards antioxidant activity [5, 7, 10]. In China, flowers *viz. Osmanthus fragrans, Chrysanthemum morifolium, Gomphrena globosa, Chrysanthemum indicum,* etc are used as herb tea. Hung YC et al.[11] isolated five phenolic compounds from *O. fragrans* and observed that these compounds exhibit hydrogen peroxide and DPPH scavenging activity relative to green tea. Lin and Harnly [12] identified about ten phenolic compounds in the flowers of *C. morifolium*, which may contribute to its use as herbal medicine in several Asian countries for the treatment of headache, fever, hypertension, sore throat, inflamed eye, etc [13]. However, limited studies have focused on *Gomphrena globosa* (Family, Amaranthaceae), a medicinal herb with broad clinical significance in blood coagulation, jaundice, hypertension, diabetes, cancer, kidney and other urinary problems [14-19]. Himuduzzaman and Zafrul Azam[20] found that the extracts prepared from the whole plant of *G. globosa* possess antioxidant and cytotoxic potential. Sakia and Upadhayaya [14] suggested that the leaves of *G. globosa* have phenols and flavonoids and exhibits antioxidant activities, but it lacks any correlation between phenols and free radical scavenging activity. In view of these available reports, in the present study, the extracts of the flowers of *Gomphrena globosa* were prepared in different organic solvents, and were optimized for both total phenol and flavonoid content, and then *invitro* antioxidant activities were determined, in an attempt to investigate the potential antioxidant properties of flowers of *G. Globosa*.

MATERIALS AND METHODS

Chemicals

Quercetin and gallic acid were procured from CDH Ltd, India. 11, 1-diphenyl-2-picrul-hydrazil (DPPH), $NaNO_2$, $AlCl_3$ and H_2O_2 were purchased from Hi-Media Laboratories Pvt. Ltd., India. All the chemicals used were of analytical grade.

Plant Materials

Flowers of *Gomphrena globosa* were collected from the Botanical garden of Lovely Professional University, Phagwara (India). The dried powder of flowers of *G. globosa* was prepared in an oven at 25 °C and the powder was stored in a dry place.

Extraction

5 g of dried powder of *G. globosa* was extracted exhaustively in a soxhlet apparatus with methanol and ethanol, 200 ml each for 8 hrs at 45°C [21]. The concentrated ethanol extract was suspended in water and fractionated with butanol (10×50 ml) and filtered with Whatman no. 1 filter paper thrice. The solvent was completely removed by distillation process at 45°C and made volume of butanol extract to 10 ml with rotary evaporator (45-50°C).

Antioxidant Assays

Total Phenols

Total phenol content (TPC) of extracts of *G. globosa* was estimated by the Folin Denis method as described by Cetkovick et al.[22]. To 1 ml of the sample extracts suspended in solvent (i.e. methanol/ ethanol/ butanol), added 0.5ml of Folin-Denis reagent (2 N) and 1ml of sodium carbonate with final volume to 10ml with distilled water. After 30min, the absorbance was measured at 700 nm against the blank.

Absorbance was proportional to the content of tannins and was determined against standard gallic acid (5- 30 µg/ml).

Flavonoids

Flavonoid content of the extracts of the sample was estimated by aluminium chloride colorimetric assay as described by Chang et al.[23]. The extracts of *G. globosa* were taken in 10 ml of volumetric test tubes separately. To the above mixture 0.3ml of 5% $NaNO_2$ was added. After 5min, 0.3ml of 10% $AlCl_3$ was added, followed by 2 ml of 1M NaOH after 6min and final volume was made to 10ml with distilled water. Then solutions were mixed well and the absorbance was measured at 510 nm against the blank. Also run blank and standard (quercetin; 10- 100 µg/ml)) simultaneously.

Antioxidants

Reducing the antioxidant power of extracts from flowers of *G. globosa* was carried out following FRAP method as described by Lim and Murtijay [24]. The different alcoholic extracts of samples were taken in 10 ml test tubes separately. Each extract preparation was then mixed with 2.5 ml of potassium buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. After incubating at 50°C for 20min, the solution was mixed with 2.5 ml of 10% trichloroacetic acid to stop the reaction. To 2.5ml of above solution, 2.5ml of distilled water was added followed by 0.5ml of 0.1% of $FeCl_3$ and was allowed to stand for 30min before measuring the absorbance at 593 nm. Also run blank and standard (gallic acid; 10- 100 µg/ml) simultaneously.

High Performance Liquid Chromatography (HPLC)

HPLC was used to measure the phenols and flavonoids in methanol extracts of *G. globosa*[25].

Preparation of Standard

Gallic acid and quercetin were served as standards for antioxidants and flavonoids respectively. Standards were added at different concentrations in volumetric flasks. To each flask, added equal amount of methanol for their dilution. These solutions were used for the linearity study.

Preparation of Extract

10 ml extract of *G. globosa* was taken in volumetric flasks separately. To this, added methanol, sonicated for 5min; kept overnight for the extraction and were filtered through whatman filter paper no. 41.

Instrumentation and Method

The flavonoids and antioxidants were measured in methanol extract preparations of *G. globosa* by HPLC. 10 µl of the sample was injected through column (C18-UV detector). The mobile phase (i.e. 0.1% orthophosphoric acid and acetonitrile, 4:6) was diluted at the flow rate of 1.0 ml/min through the C18 column at the run time of 45' and the detection was performed at λ =270 nm.

Invitro Free Radical Scavenging Assays:

11, 1-diphenyl-2-picrul-hydrazil (DPPH) as a free radical

The total radical scavenging activity of the extracts and gallic acid as standard (10-100µg/ml) were determined spectro-photometrically following the protocol as described by Blois [26]. DPPH is a source of free radicals, absorbs at 517 nm; but upon reduction by antioxidant compounds its absorption is reduced. 1 ml solution of 1 mM DPPH was added to 15, 30 and 45µg/ml of extract preparations. Low absorbance of the reaction mixture implies higher free radical scavenging activity. The free radical scavenging activity of the extracts or standard was determined at 517nm by using formula:

$$\% \text{ DPPH radical scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The concentration leads to 50% inhibition (i.e. IC_{50}) was determined from the graph of inhibition percentage against sample concentration by linear regression analysis.

Hydrogen peroxide (H_2O_2) as a free radical

H_2O_2 scavenging activity was analyzed in samples following the method by Ruch et al. [27]. 100 mM H_2O_2 was prepared in phosphate buffer saline (pH 7.4) and the extracts of *G. globosa* and *C.morifolium* were added at different concentrations (15, 30 and 45µg/ml) to 2 ml of H_2O_2 solution and made equal volume with phosphate buffer saline. The absorbance was measured at 230 nm. The blank contained extract without H_2O_2. The percentage of H_2O_2 scavenging by extracts and gallic acid (standard; 10- 100 µg/ml) was calculated by using the formula:

$$H_2O_2 \text{ scavenging affect (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The concentration leads to 50% inhibition (i.e. IC_{50}) was determined from the graph of inhibition percentage against sample concentration by linear regression analysis.

Statistical analysis

Each test was expressed as a mean ± SD. At least triplicate experiments were conducted. Level of significance was performed by two sample t test with *Graphpad Software.*

RESULTS AND DISCUSSION

The oxidative stress leads to numerous pathophysiological conditions including cancer, cardiovascular diseases, neural disorders, Alzheimer's disease, Parkinson's disease, insulin dependent diabetes, fatty liver associated with chronic alcoholism and aging [1-4]. For the last few decades, an immense interest has been developed in plants with medicinal properties, with prime focus on therapeutic significance and research is carried out to identify and develop the plants with antioxidant properties to combat the oxidative stress. Reducing antioxidant properties of the flowers of *G. globosa* were estimated following the FRAP assay [24] in terms of gallic acid equivalent (standard curve equation: y = 0.0117x ± 0.1304, r^2= 0.9909) and data obtained is shown in Table I. Total antioxidants were 1.31± 0.001mg/g and 1.15± 0.007mg/g extract, respectively in methanol and ethanol preparations. The antioxidant content was found to be reduced markedly (97%) in the butanol extract (i.e. 0.03± 0.004 mg/g extract), suggesting the flowers exhibited the reducing abilities, although it varies markedly with the choice of solvent for extraction of polyphenols. The plant phenols and flavonoids are classified as the most abundant secondary metabolites, with high antioxidant properties, which could contribute to reduce oxidative stress [28-31]. Antioxidant properties of phenols attribute to their ability to stabilize and delocalize the unpaired electron (i.e. chain-breaking function); however flavonoids act by scavenging the free radicals or chelating the metal ions and/ or inhibiting the enzymes which leads to generation of free radical[29]. Table 1 presents data on total phenols that were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: y = 0.00068x ± 0.01, r^2= 0.9917). The total phenol content (TPC) of *G. globosa* was 1.11± 0.01mg/g extract in methanol, which was significantly higher ($p< 0.001$) compared to the TPC of ethanol and butanol preparations (i.e. 0.41± 0.01 and 0.27± 0.002mg/ g). The flavonoid content of the flower extracts from *G. globosa* in terms of quercetin equivalent (standard curve equation: y = 0.0009x + 0.0035, r^2 = 0.9918; Table 1) revealed that the concentration of flavonoids in the methanol extract of *G. globosa* was 13.32± 0.045mg/g extract ($p< 0.001$) as compared to ethanol and butanol preparations (5.03± 0.002mg/g and 3.88± 0.012mg/g extract respectively). The results clearly indicate that the nature of solvent influences the extractability of the polyphenols. Interestingly, the flavonoid

content was measured predominately in different solvent extract preparations from flowers of *G. globosa* (Table I). HPLC was also performed for quantitative analysis of methanol extracts prepared from *G. globosa* for phenols and flavonoids following the method described by Deshmukh and Prabhu[25]. Quantification was performed on the basis of calibration curve for standards of phenols and flavonoids (i.e. gallic acid and quercetin respectively) (Figure Ia). The percentage recovery of gallic acid was 0.093% (w/w) for *G.globosa*. However, for quercetin the percentage recovery is 0.363 % (w/w) for *G.globosa* (Figure Ib), corroborating the above observations that flavonoids were the predominant polyphenols in flower sample extracts, which are in agreement with the reports [32-35], suggesting the occurrence of flavonoids, isoflavonoids and flavones-C glycosides in the family Amaranthaceae.

Free radicals are released in biological systems including H_2O_2, a non radical oxidant, which is generally less-toxic; however it can lead to the formation of hydroxyl radical (highly reactive oxygen species) and may cause cell death[31]. The effective scavenging of H_2O_2 could protect cellular oxidative damage. Scavenging activity for H_2O_2 in methanol extracts of *G.globosa* in terms of gallic acid indicated that methanol extract had 62% inhibition at 30µg/ml with IC_{50} (13.8µg/ml) as illustrated in figure IIa. Similar results were observed with the methanol extracts from *G.globosa* (Figure IIb), exhibiting 72% scavenging inhibition at 30µg/ml (IC_{50} = 10µg/ml) for DPPH radical. H_2O_2 and DPPH scavenging inhibition activity of methanol extracts increased linearly with an increase in the concentration of extracts (i.e. 15 to 45µg/ml), which is consistent with earlier observations[14, 29], and thus signifies the free radical scavenging potential of flowers of *G.globosa*. Furthermore, the percentage scavenging inhibition of methanol sample extracts for free radicals (i.e. H_2O_2 and DPPH) are either same or markedly higher than the synthetic antioxidant, gallic acid (Table II), implying that flowers of G. globosa could be relatively good sources of antioxidants. Himuduzzaman and Zafrul Azam [20] found that extracts prepared from whole plant of *G. globosa* possess antioxidant and cytotoxic potential. Sakia and Upadhayaya [14] suggested that the leaves of *G. globosa* have phenols and flavonoids and exhibit antioxidant activities, but it lacks any correlation between polyphenols and antioxidant activities. In the present study, high correlations (i.e. r^2 = 0.99 and 0.98) were observed between phenols and IC_{50} for DPPH and H_2O_2, which corroborate the earlier investigations [27-31] that antioxidant properties of medicinal plants attribute to their phenolic compounds. Interestingly, there was a very high correlation (i.e. r^2 = 0.99) between phenolic content and flavonoid content, which imply that flavonoids may contribute profoundly toward antioxidant properties of *G. globosa*. We conclude from the present study that flowers of *G. globosa* may possess antioxidant properties. However, further isolation and identification of antioxidant component(s) in these flowers are necessary to understand whether the flowers of *G.globosa* and its flavonoids constituents have the potential as a source of antioxidant products, which could provide real and useful improvement in physiological antioxidant status.

Table I

Total phenols, flavonoids and antioxidants in the extracts from the flowers of G. globosa.

Extracts	Concentration (mg/g)		
	Phenols	Flavonoids	Antioxidants
Methanol	1.11± 0.01	13.32± 0.045	1.31± 0.001
Ethanol	0.41± 0.01*	5.03± 0.002**	1.15± 0.007*
Butanol	0.27± 0.002*	3.88± 0.012**	0.03± 0.004*

Mean± S.D (n=3); p*< 0.001 vs methanol extract

Table II

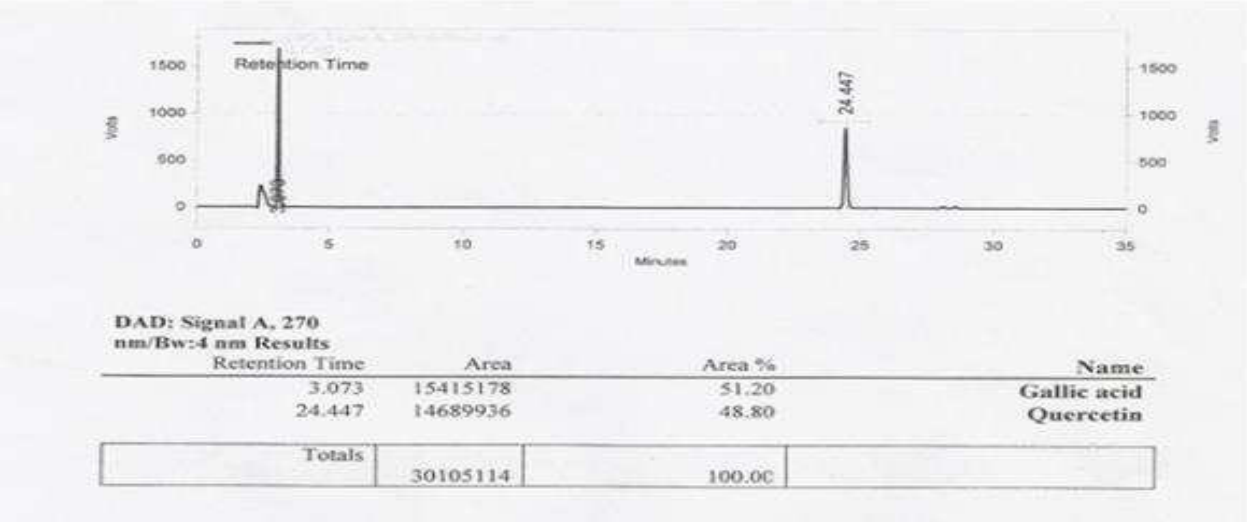
H_2O_2 and DPPH scavenging activities of methanol extract preparation from G. Globosa

Sample	H_2O_2 scavenging effect IC_{50} (µg/ml)	DPPH scavenging effect IC_{50} (µg/ml)
Gallic acid	13.2	10
Methanol extract of *G. globosa*	13.8	20

Figure I

HPLC profile of (a) gallic acid and quercetin (standards), (b) methanol extract from the flowers of G. globosa

(a)



(b)

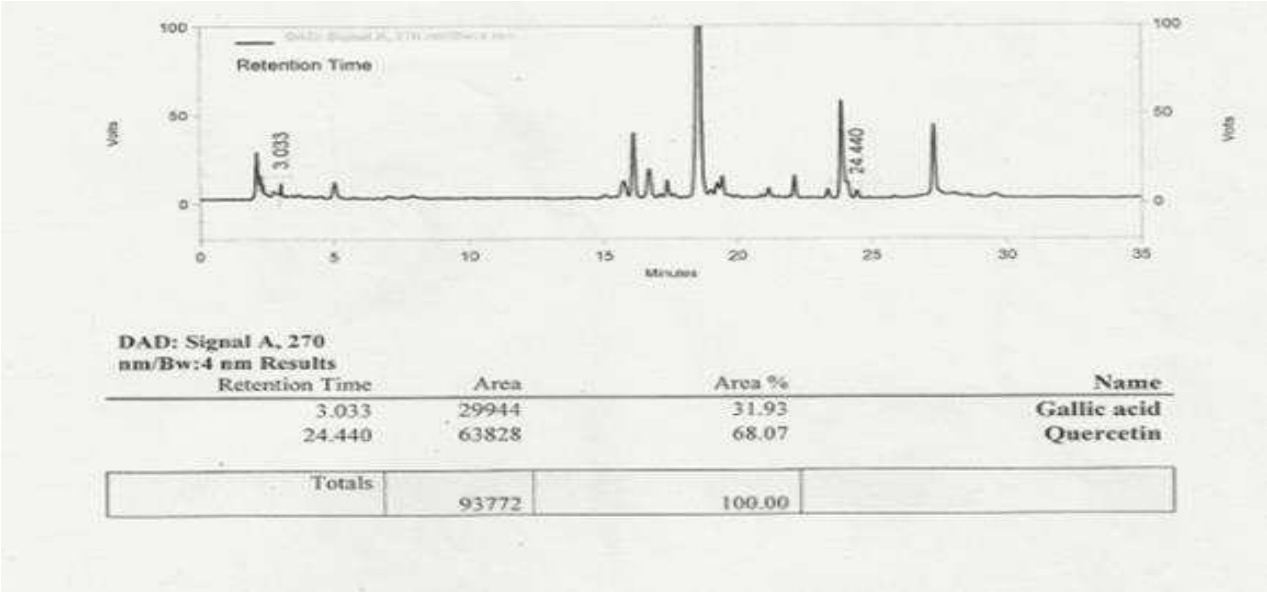
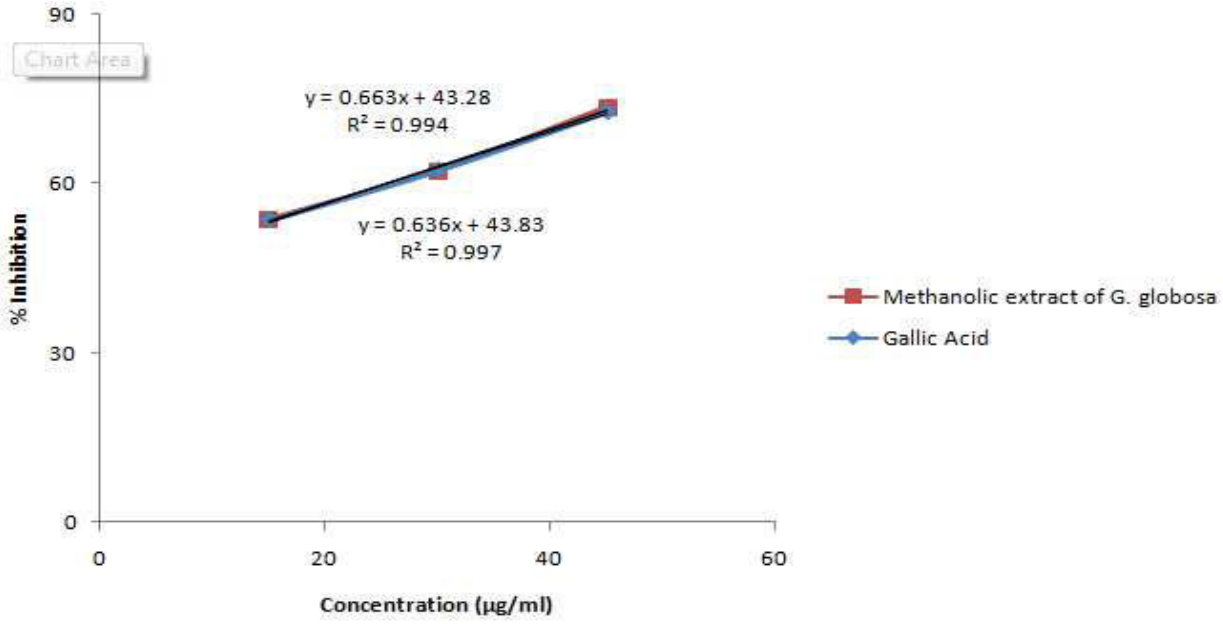


Figure II

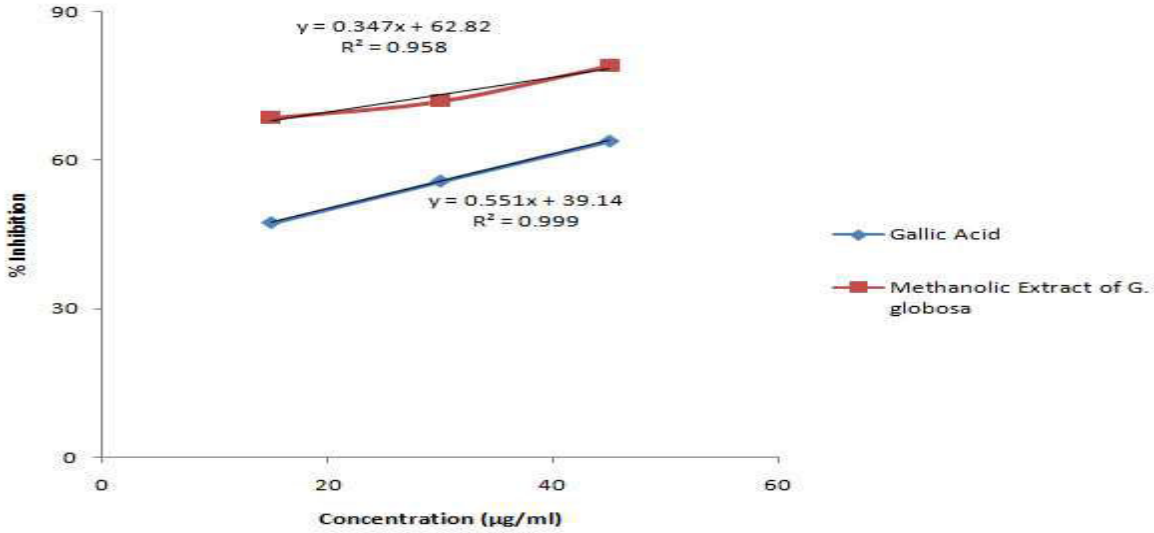
Scavenging effects of methanol extract of flowers of G. globosa on (a) H_2O_2, and (b) DPPH.

(a)



R^2 values represented mean data set of n=3

b)



R^2 values represented mean data set of n=3

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CONFLICT OF INTEREST

There is no conflict of interest among authors of this publication.

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