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In vitro antioxidant activity of *Bougainvillea glabra and Mucuna pruriens*

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ABSTRACT

The purpose of the contemporary *in vitro* work implemented was to examine the antioxidant effect of *Bougainvillea glabra* and *Mucuna pruriens* methanol extracts. Methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* were screened individually for different *in vitro* antioxidant activity. Commercial antioxidants such as vitamin E, Ascorbic acid were used as reference standard. For $ABTS^+$ assay IC_{50} value of *Bougainvillea glabra & Mucuna pruriens* methanol extract were found to be 17.1μg/ml and 23.2μg/ml correspondingly. Likewise, the IC_{50} values for xanthine oxidase inhibitory activity for *Bougainvillea glabra* and *Mucuna pruriens* was found to be 46.7μg/ml and 95.1μg/ml, respectively. For scavenging of various assay models like nitric oxide, hydroxyl and superoxide anion scavenging activity, the IC_{50} values for *Bougainvillea glabra* were found to be 45.3μg/ml, 70.1μg/ml and 38.1μg/ml respectively. The IC_{50} values for *Mucuna pruriens* for nitric oxide, hydroxyl and superoxide anion scavenging activity, were found to be 92.1μg/ml, 43.4μg/ml and 50.3μg/ml respectively. *Bougainvillea glabra* and *Mucuna pruriens* methanol extracts showed 50 % inhibition effect at concentrations 118 μg/ml and 121 μg/ml respectively for lipid peroxidation assay. The IC_{50} values for DPPH scavenging assay for *Bougainvillea glabra* and *Mucuna pruriens* was found to be 13.24μg/ml and 14.4μg/ml correspondingly. For DMPD assay *Bougainvillea glabra* and *Mucuna pruriens* possess the IC_{50} value 16.34 μg/ml and 17.4 μg/ml correspondingly. Methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* exhibited potent *in vitro* antioxidant activity.

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INTRODUCTION

At molecular and cell level, antioxidants have a tendency to deactivate injurious free radicals. There is widespread proof to comprise reactive oxygen species in expansion of progressive diseases. Free radicals are produced due to oxidation reactions which initiate chain reactions that lead to destruction of cells and innumerable degenerative diseases like cancer (Aruoma, J.O, 1999; Reaven and Witztum, 1996)Antioxidants prevent these sequence responses by eradicating free radicals and act like oxygen scavengers (Shahidi F, Wanasundara P.K, 1992) Antioxidant is a molecule which possesses the ability of reducing or impeding the oxidation

of molecules. Natural antioxidants, predominantly in vegetables and fruits extended growing attention among consumers. Since, epidemiological studies has specified that frequent intake of antioxidants from natural sources is related to a reduced risk of diseases (Temple, 2000; Renaud *et al.*, 1998). The aim of the contemporary study was to assess the *in vitro* antioxidant activity of *Bougainvillea glabra* and *Mucuna pruriens* by evaluating various parameters.

MATERIALS AND METHODS

Collection & authentication of Plants

The whole plant of *Bougainvillea glabra* & *Mucuna pruriens* were obtained individually from the botanical garden, Hanamkonda, Warangal district, Telangana, India in the month of July. The plant materials were taxonomically recognized by Dr. V S Raju, Professor, Botany Department, Plant Systematics Lab, Kakatiya University, Warangal district, Telangana, India. A voucher specimen of *Bougainvillea glabra* (4610) and *Mucuna pruriens* (4612) were deposited in the herbarium.

Extraction

Powder of *Bougainvillea glabra* and *Mucuna pruriens* were extracted individually with methanol by soxhlet method of extraction continuously. By rotary vacuum evaporator the solvent was separated, the remaining mass of extract was concentrated and dried. The extracts were stored in desiccator for further studies.

DPPH radical scavenging assay

DPPH assay of methanol extracts of plants, *Bougainvillea glabra* and *Mucuna pruriens* was evaluated (Tailor C.S and Goyal A, 2014) model. By adding methanol extract of both the plants in distilled water a stock solution was made. Working solutions of dissimilar concentrations (5, 10, 20, 40, 80 μg/ml) prepared individually from above. Solution was made by dissolving 0.1 milli molar DPPH in ethanol. To 3 ml of methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* add 1ml of working solution was added at dissimilar concentrations. The mixture was shaken forcefully and put a side for twenty mins. At 518 nm absorbance was recorded by means of spectrophotometer. Standard compound quercetin was taken.

Nitric oxide radical inhibition (NO) assay

Different concentrations (5, to 160μg/ml) of *Bougainvillea glabra* and *Mucuna pruriens* methanol extract were prepared individually. Saline phosphate buffer with sodium nitroprusside (10 mM) in dissimilar compositions of *Bougainvillea glabra* & *Mucuna pruriens* methanol extracts were placed at 30°C for two hrs. Later, 1 milli liter of Griess reagent, ethylenediamine dihydrochloride, H_3PO_4 2% and phosphate buffer were poured into it. For further thirty minutes the mixture was incubated at room temperature and at 550nm its absorbance was recorded. Rutin served as standard (Parul *et al.*, 2013).

Hydroxyl radical scavenging activity

In distilled water, stock solutions of $FeCl_3$ (10mM), EDTA (1mM), H_2O_2 (10mM), deoxyribose (10 mM), and ascorbic Acid (1mM), were arranged. Reaction was executed by the addition of 0.1ml EDTA, 0.01ml of $FeCl_3$, 0.1ml Hydrogen peroxide, deoxyribose 0.36mL, 1mL of the plants extract of diverse (10 to 160 μg/ml) concentrations were prepared separately and liquefied in distilled water, phosphate buffer of 0.33mL (50 mM, pH 7.9), ascorbic acid 0.1mL in order. Fusion mix heated to 37^{o}C for 1 hour. Standard is ascorbic acid, (Halliwell *et al.*, 1972).

Superoxide anion radical scavenging assay

Determination of SOD assay of *Bougainvillea glabra* and *Mucuna pruriens* methanol extract was performed by (Nishikimi *et al.*, 1972). Serial dilutions of 5 to 160 μg/ mL were prepared separately from methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens*. Each concentration was added by nitroblue tetrazolium 1mL and 1 milli litre of (NADH) nicotinamide adenine dinucleotide. The reaction was initiated by pouring 100μl of phenazine methosulphate reagent. At 25°C for five minutes the reaction mixture was incubated and compared to a blank sample at 560 nm absorbances was evaluated. As, reference compound curcumin was taken.

Cupric ions reducing assay

Working solution concentrations of dissimilar range from 100 to 500 μg/mL were made separately from the stock solution of methanol extracts of both plants. One millilitre of $Cucl_2$, ethyl alcohol 7.5 mM, neocuproine reagent & 1M ammonium acetate buffer solution. Lastly, entire mixture was adjusted to two milli litre with H_2O and heated for thirty minutes at normal room temperature. Absorbance was recorded against a blank at 450 nm. For reference gallic acid was taken (Apak *et al.*, 2004).

N, N-dimethyl-p-phenylene diamine dihydrochloride (DMPD) assay

This study executed as per manner of (Fogliano *et al.*, 1999). Dissimilar concentrations from 200 to 1000 μg/mL were made individually from *Bougainvillea glabra* and *Mucuna pruriens* methanol extracts. 1

mL of H_2o and one mL DMPD were added to every dilution extract of methanol. The samples were agitated and incubated in dark at room temperature for 15 min. At 505 nm absorbance was recorded. As reference gallic acid was taken.

ABTS$^+$ Assay

In the same quantity ABTS (7 mmol/L) aqueous solution along with $K_2S_2O_8$ (2.4 mmol/L) were used for study. At room temperature the mixture was reacted for 12 h in the dim light. 2.5 mL of the prepared solution was added to 1 mL of *Bougainvillea glabra* & *Mucuna pruriens* methanol extracts (5-160 μg/mL) individually and using spectrophotometer, at 734 nm absorbance was recorded. For reference butylated hydroxyl toluene was taken.

Xanthine oxidase inhibitory activity

Assay was executed in accordance with (Chiang *et al.*, 1994) method. Reaction mixture was made by adding 1 mL of *Bougainvillea glabra* and *Mucuna pruriens* methanol extract serial dilutions (10-160μg/mL) separately, along with 12% v/v DMSO and 2 mL buffer of phosphate. Then, xanthine oxidase enzyme solution 0.1 mL & xanthine was poured to assay mixture. Later it was incubated. By mixing 1M HCl (1ml) to it, the reaction was terminated and against blank solution, at 295 nm the absorbance was measured. Allopurinol standard was selected.

Lipid peroxidation assay

TBARS was evaluated by this assay. By following the procedure of (Bouchet *et al.*, 1998) rat liver microsomal fraction was made. Serial dilutions (10-160 μg/ml) of methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* were prepared separately. Five hundred micro litre of liver microsomal fraction, three hundred micro litre of working solution of methanol extract of *Bougainvillea glabra, Mucuna pruriens* and 100 μl of $FeCl_3$ (1mM) were added. Using the reaction with TBARS lipid peroxidation was evaluated. In triplicate the reactions were completed. For standard Vitamin E was selected.

RESULTS AND DISCUSSION

DPPH assay

The above reaction was experimentally detected by a small variation of colour to yellow from purple. The reduction in absorbance of solution was associated to yellowing. DPPH activity of radical by *Bougainvillea glabra* and *Mucuna pruriens* methanol extracts were monitoredFigure 1. The IC_{50} value (13.2 μg/mL) of *Bougainvillea glabra* and *Mucuna pruriens* (14.4 μg/ml) were found to be more than quercetin (10 μg/mL) standard.

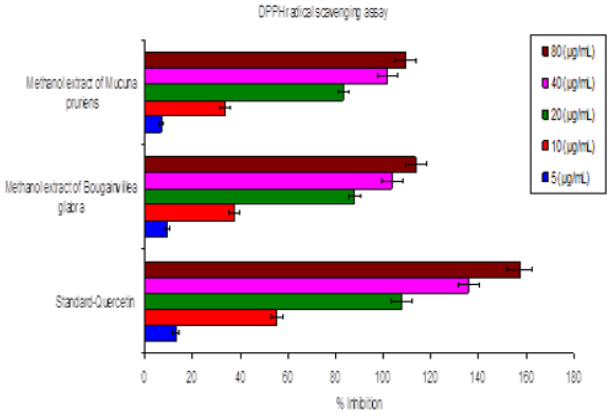

Figure 1: Scavenging outcome of *Bougainvillea glabra & Mucuna pruriens* methanol extracts and Standard quercetin

Nitric oxide radical inhibition assay

Methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* by antioxidant ability reduced the concentration of nitric oxideFigure 2. The IC_{50} values were calculated by plotting the obtained values. The scavenging activity with the IC_{50} values were 45.3μg/mL, were 92.1 and 17.2 for extracts of *Bougainvillea glabra, Mucuna pruriens* and rutin respectively.

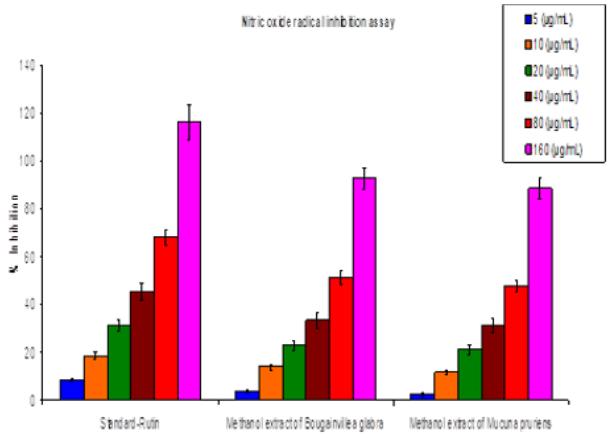

Figure 2: Scavenging result of *Bougainvillea glabra & Mucuna pruriens* methanol extracts and Standard rutin

Hydroxyl radical scavenging assay

Consequence of methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* increases with cumulative concentration of extract. The IC_{50} value (70.1 μg/mL) of *Bougainvillea glabra* was higher than the standard. Similarly, IC_{50} value (43.4μg/mL) of *Mucuna pruriens* extract was also higher than ascorbic acid standard (IC_{50}value 25 μg/mL) Figure 3.

Superoxide radical scavenging activity (SOD)

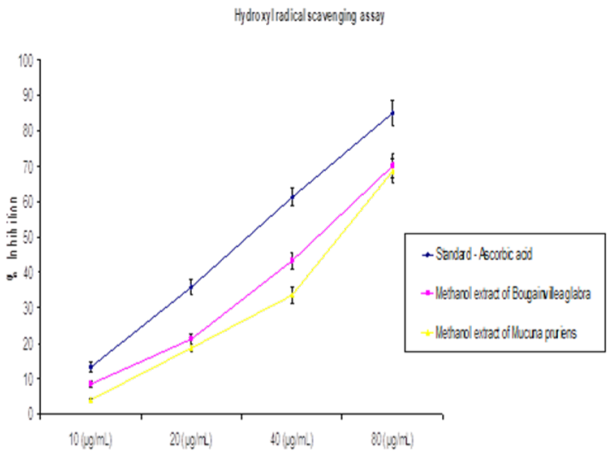

Figure 3: Scavenging outcome of methanol extracts of *Bougainvillea glabra & Mucuna pruriens* and Standard ascorbic acid

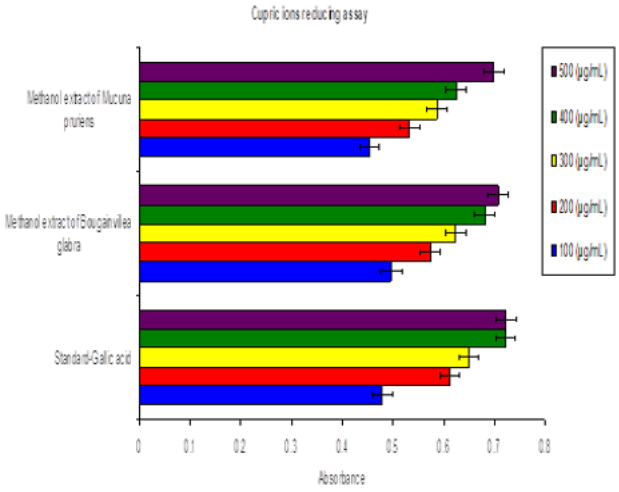

Figure 5: Effect of methanol extracts of *Bougainvillea glabra & Mucuna pruriens* and Standard gallic acid

The depletion of superoxide anion in the reaction composition showed decline in absorbance, by methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens*. Methanol extracts of *Bougainvillea glabra, Mucuna pruriens* and also curcumin exhibited good activity with IC_{50} values, 38.1, 50.3 and 15.7μg/mL, respectively Figure 4.

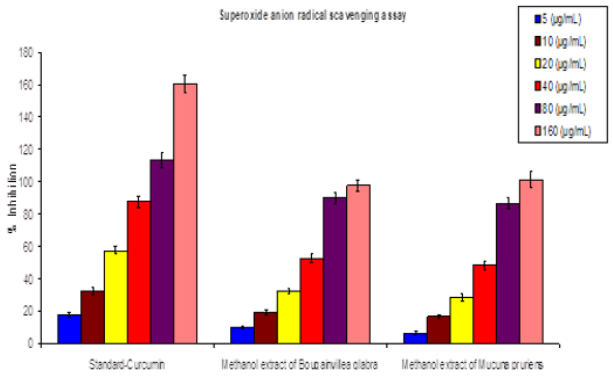

Figure 4: Scavenging influence of *Bougainvillea glabra & Mucuna pruriens* methanol extracts and Standard curcumin

Cupric ions assay

Methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens,* antioxidant activity was determined by cupric ions (Cu^{2+}) reducing assay. Methanol extracts of *Bougainvillea glabra, Mucuna pruriens* and also the scavenging activity of gallic acid revealed absorbance of 0.467, 0.353 and 0.616 correspondingly Figure 5. Nevertheless, the activity of plant extracts was less than that of the standard Gallic acid.

DMPD assay

Bougainvillea glabra and *Mucuna pruriens* diminished the DMPD radical concentration. The hydrogen donating ability of *Bougainvillea glabra* and *Mucuna pruriens* methanol extracts are good. *Bougainvillea glabra* and *Mucuna pruriens* possess the IC_{50} value 16.34 μg/mL and 17.4 μg/mL correspondingly. The IC_{50} value of *Bougainvillea glabra* is more than standard, gallic acid (IC_{50} value 8.5 μg/mL) and less than *Mucuna pruriens Figure 6.*

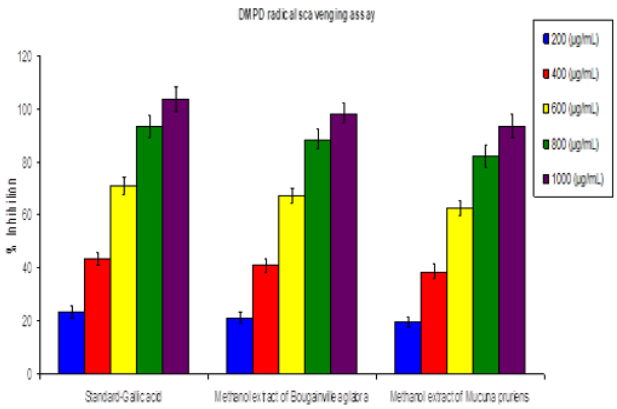

Figure 6: Influence of extracts of methanol of *Bougainvillea glabra & Mucuna pruriens* and Standard gallic acid

$ABTS^{+}$ radical scavenging assay

As mentioned in Figure 7 the decline was concentration dependent. Methanol extract of *Bougainvillea glabra* and *Mucuna pruriens* scavenged $ABTS^{+}$ radical potently. IC_{50} value of *Bougainvillea glabra & Mucuna pruriens* methanol extract was found to be 17.1μg/ml and 23.2μg/mL correspondingly. Under similar experimental conditions the standard butylated hydroxyl toluene displayed IC_{50} value of 12.8 μg/mL

Xanthine oxidase (XO) inhibitory activity

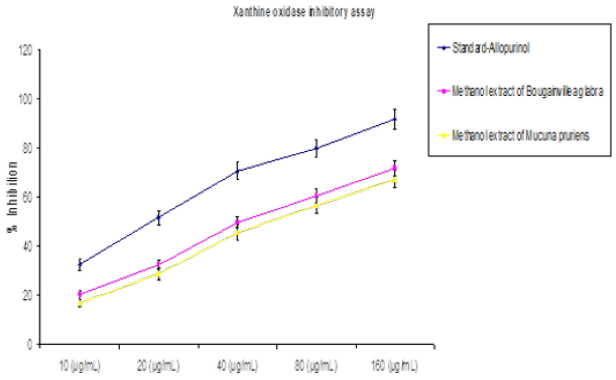

Figure 7: Consequence of methanol extracts of *Bougainvillea glabra & Mucuna pruriens* and Standard butylated hydroxy toluene

XO assay of *Bougainvillea glabra* and *Mucuna pruriens* methanol extracts were performed. Dissimilar concentrations of extracts of methanol & standard are verified and graph was obtained. The IC_{50} value of *Bougainvillea glabra* and standard was 46.7μg/mL and 19.4 μg/mL individually Figure 8. *Mucuna pruriens* IC_{50} value was 95.1μg/mL.

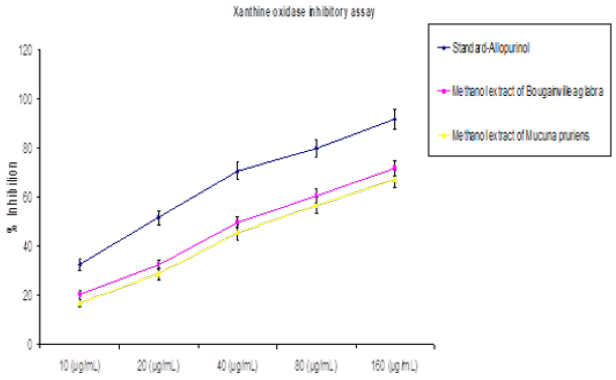

Figure 8: *Influence of Bougainvillea glabra & Mucuna pruriens* methanol extracts and Standard allopurinol

Lipid peroxidation

The influence of *Bougainvillea glabra* methanol extracts and vit E on lipid peroxidation was highly effective. *Bougainvillea glabra and Mucuna pruriens* methanol extracts showed 50 % inhibition effect at concentrations 118 μg/mL and 121 μg/mL respectively Figure 9 the outcome of standard vitamin E was 113.4 μg/mL.

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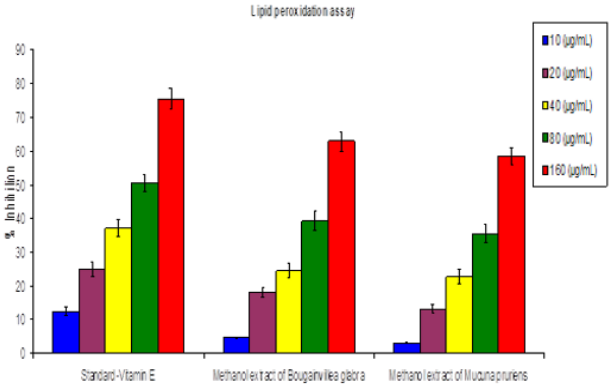

Figure 9: Outcome of methanol extracts of *Bougainvillea glabra & Mucuna pruriens* and Standard Vitamin E

Oxidative stress has been involved in the pathology of numerous diseases and disorders (Velioglu *et al.*, 1998) The lessening ability of radicals of DPPH was evidenced by the decline in its absorbance (Ganapaty S, Chandrashekhar V.M, Chitme H.R, Lakshmi N.M, 2007). Methanol extracts of both the plants revealed potent radical scavenging activity against DPPH assay in concentration reliant way. The IC_{50} value of the extract was comparable to that of the standards used (Thambiraj *et al.*, 2012). Methanol extract of *Bougainvillea glabra* and *Mucuna pruriens* inhibit nitric oxide in a dose dependent mode when associated to standard rutin. The effective reactive oxygen species in the living system was hydroxyl radical (Hallwell *et al.*, 1987). In this assay the inhibitory concentration was compared to that of standard rutin.

Even though superoxide anion causes the creation of hazardous hydroxyl radicals and also singlet oxygen, which leads to oxidative stress (Meyer and Isaksen, 1995). The current study demonstrated that methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* possess efficient superoxide scavenging activity. CUPRAC assay was grounded on decline of Cu (II)-neocuproine to extremely colored Cu (I)-neocuproine, it is measured at 450 nm absorbance. The development of cuprous ions (Cu^{1+}) from cupric ions (Cu^{2+}) may be due to reduction of cupric chloride from cuprous chloride in the chromogenic redox reagent bis (neocuproine) copper(II) chloride by antioxidants compounds. This copper (I) is extremely selective towards neocuproine, the resultant Cu(I)-neocuproine complex contains 2 moles of neocuproine (Apak *et al.*, 2008). It can be concluded that the methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* possess worthy reducing power when matched to that of the standard.

DMPD is appropriate for fast and sensitive evaluation of antioxidant activity of hydrophilic combinations. DMPD assay is correspondingly applied to both lipophilic and hydrophilic antioxidants. This method is rapid and inexpensive and reproducible, hence used in screening a large number of fruit samples (Nathan and Hibbs, 1991). This study evaluates the effectiveness of methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens*. The assay of $ABTS^{+}$ depends on the decline of the absorbance of radical cation by antioxidants. The methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* was effective scavenger of ABTS radical ions. The present examination shown that methanol extract of *Bougainvillea glabra* and *Mucuna pruriens* is an effective inhibitor of xanthine oxidase (XO). XO enzyme catalyzes the reaction of hypoxanthine to xanthine as well as xanthine to uric acid (Valko *et al.*, 2004). Xanthine oxidase inhibition of the methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* may be ascribed to their dissimilar compositions. The methanol extracts of *Bougainvillea glabra*

Mucuna pruriens and Vitamin E revealed a constant influence at various concentrations. The methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* showed noticeable decline of lipid peroxidation and it is because of existence of various phytoconstituents.

CONCLUSION

In the current study, a number of *in vitro* studies were performed to evaluate the antioxidant activity of *Bougainvillea glabra* and *Mucuna pruriens.* The good antioxidant potency will be credited to the existence of phytoconstituents like glycosides, flavonoids and steroids etc in methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens*. Thus, *Bougainvillea glabra* and *Mucuna pruriens* extracts, as auspicious natural sources of antioxidants and can be used in nutritional or pharmaceutical fields for the elimination of diseases mediated by free radicals. However, additional investigations and the different antioxidant studies are in advancement.

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