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Antioxidant and Antimicrobial Properties of *Galega purpurea* Root

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Abstract: This study was designed to determine the antioxidant and antimicrobial properties of methanol extract of *Galega purpurea* (MEGP) (Papilionaceae) root in various systems. The antioxidant property was evaluated by two methods, namely the DPPH (1,1-diphenyl-2-picryl hydrazyl) test and the lipid peroxidation assay by using rat liver homogenate. In lipid peroxidation assay MEGP showed significant activity in all the tested concentrations and the results were dose dependent and comparable to that of the standard drug α-tocopherol. Also the extract inhibited DPPH radical at all concentration in a dose-dependent manner with a calculated IC_{50} = 51.5±0.02 μg mL^{-1}. Further the extract was evaluated for its antimicrobial activity using disc diffusion method, with three gram positive, three gram negative and two fungal species. MEGP showed broad-spectrum antimicrobial activity against all the tested microorganisms. The results suggest that the methanol extract of *G. purpurea* root possesses significant antioxidant and antimicrobial properties.

Key words: Lipid peroxidation assay, antimicrobial activity, DPPH, *Galega purpurea*

INTRODUCTION

Free radical reactions have been implicated in the etiology of several human diseases including atherosclerosis, ischemic heart disease, the ageing process, inflammatory lesions, diabetes mellitus, different immunosuppressive disorders, metabolic disorders, neuromuscular degenerative conditions etc (Berlett and Stadtman, 1997). Antioxidants may act by decreasing oxygen concentration, intercepting singlet oxygen, preventing first chain initiation by scavenging initial radicals, binding metal ion catalysts, decomposing primary products to non-radical compounds and chain-breaking to prevent continued hydrogen abstraction from substrates. There are two basic categories of antioxidant namely synthetic and natural ones. Restriction on the use of synthetic antioxidants is being imposed because of their carcinogenicity (Bronen, 1975; Ito *et al*., 1983). Thus the interest in natural antioxidants has been increased considerably. As resources of natural antioxidants much attention has been paid to plants (Couladis *et al*., 2003; Linn *et al*., 2003).

Galega purpurea (Papilionaceae) a plant popularly known as kolinji in Tamil and vempali in Telugu, which thrives in Southern parts of India. It grows on hard stony ground too difficult to be rooted. The various parts of the plant are widely used in the folk medicine for the treatment of cough, asthma, bilious febrile attacks, arthritis and rheumatism. Decoction of the root useful in the management of enlargement and obstruction of the liver, spleen and kidney. Also the root is useful in the treatment of dyspepsia, chronic diarrhoea and ulcers (Nadkarni, 1976). The objective of the present investigation is to evaluate the antioxidant and antimicrobial properties of methanol extract of *Galega purpurea* root.

MATERIALS AND METHODS

Plant material: The roots of the plant *Galega purpurea* (Family: Papilionaceae) were collected from Erode district of Tamilnadu, India. The plant material was taxonomically identified by Botanical Survey of India, Kolkata. A voucher specimen (No. GMG 03/05) has been preserved in the laboratory for future reference. The collected plant material was dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the leaves was defatted with petroleum ether and the marc thus obtained was then

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extracted with methanol in a soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained. The dried MEGP was dissolved in water and used for the present study. All the studies were conducted in Division of Pharmaceutical Chemistry and Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India in the month of December 2006.

Chemicals: 1,1-diphenyl-2-picryl-hydrazyl (DPPH), α-tocopherol, ascorbic acid, thiobarbituric acid, dodecyl sulphate, ferrous ammonium sulphate were purchased from Sigma Chemical Co. Ltd., USA. All other unlabeled chemicals and reagents were analytical grade.

Phytochemical screening: The extract was screened for the presence of various constituents employing standard screening test (Trease and Evans, 1983). Conventional protocol for detecting the presence of steroids, alkaloids, tannins, flavonoids, glycosides, etc., was used.

Lipid peroxidation assay: The peroxide formation was measured by the method of Ohkawa *et al.* (1979) by measuring the color of thiobarbituric acid reactive substances (TBARS) formed at the end of the reaction. Malondialdehyde (MDA), which is formed as the end product in lipid peroxidation will react with thiobarbituric acid (TBA) to give TBARS, which is pink in color measured at 530 nm. The reaction mixture contained rat liver homogenate (0.1 mL, 25% w/v) in Tris-HCl buffer (20 mM, pH 7.0), KCl (150 mM), ferrous ammonium sulphate (0.8 mM), ascorbic acid (0.3 mM) and various concentrations of MEGP (10-100 μg mL^{-1}) in a final volume of 0.5 mL was incubated for 1 h at 37°C (Bishayees and Balasubramanian, 1971).

The incubated reaction mixture (0.4 mL) was treated with 0.2 mL of 8% sodium dodecyl sulphate (SDS), thiobarbituric acid (1.5 mL, 8%) and acetic acid (1.5 mL, 20%, pH 3.5). the total volume was then made up to 4 mL by adding distilled water and kept in a water bath at 100°C for 1 h. After cooling, 1 mL of distilled water and 5 mL of a mixture of n-butanol-pyridine (15:1, v/v) were added and shaken vigorously. The absorbance of the organic layer was measured at 560 nm after centrifugation. The percentage inhibition of lipid peroxide formation was determined by comparing the results of the extract-treated and untreated samples. Alpha tocopherol was used as a reference compound.

DPPH scavenging assay: The free radical scavenging activity of MEGP was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Blois (1958). Used as a reagent, DPPH evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants (Cao *et al.*, 1997). 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of MEGP dissolved in water at different concentrations (10, 25, 50, 75 and 100 μg). After 30 min, absorbance was measured at 517 nm. Alpha tocopherol was used as a reference material. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the absorbance values of control and samples.

Antimicrobial activity

Microorganisms and media: *Bacillus subtilis* (ATCC 6633 Gram positive), *Staphylococcus aureus* (ATCC 6538 Gram positive), *Micrococcus luteus* (ATCC 10240 Gram positive), *Escherichia coli* (ATCC 9837 Gram negative), *Pseudomonas aureginosa* (ATCC 9027 Gram negative), *Salmonella typhimurium* (ATCC 43579 Gram negative) were used to determine antibacterial activity. Fungal organisms such as *Aspergillus niger* (ATCC 16404), *Candida albicans* (ATCC 10231) strains were employed for the determination of antifungal activity.

Bacteria and fungi were obtained from the stock cultures of the Central Drugs Laboratory (CDL), Kolkata and Indian Institute of Chemical Biology (IICB), Kolkata. The bacterial and fungal stock cultures were maintained on Muller Hinton agar and Sabouraud-dextrose agar slants respectively, which were stored at 4°C. Eight microorganisms maintained on nutrient agar base were used to assess the antimicrobial activity of the plant extract. The fungi were maintained on Sabouraud-dextrose agar, which is often used with antibiotics for the isolation of pathogenic fungi.

Antimicrobial screening: Agar cultures of the test microorganisms were prepared as described by Mackeen *et al.* (1997). Three to five similar colonies were selected and transferred to 5 mL broth with a loop and the broth cultures were incubated for 24 h at 37°C. The MEGP was dissolved in dimethyl sulfoxide with a magnetic stirrer. For screening, sterile 6 mm diameter filter paper discs were impregnated with 100-1000 μg of the MEGP and placed in Muller Hinton agar medium. The inoculums for each organism were prepared from broth cultures. The concentration of cultures was to 1×10^5 colony forming units mL^{-1}. The results were recorder by measuring the

zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicate the presence of antimicrobial activity. All data regarding antimicrobial activity are the average of triplicate analyses. The antibacterial amikacin (10 μg mL^{-1}) and antifungal griseofulvin (20 μg mL^{-1}) were used as reference standards as recommended by the National Committee for clinical laboratory standards.

Statistical analysis: The experiments were done in triplicate. The results are given as the mean±SD. Student t-test was used for comparison between two means.

RESULTS

Phytochemical screening: The extraction of *Galega purpurea* root with petroleum ether ad methanol gave yields of 3 and 7.3%, respectively. The results of preliminary phytochemical screening of the methanol extract revealed the presence of steroids, flavonoids, tannins, alkaloids and glycosides (Table 1). Further separation of the specific phytochemical is in progress.

Antioxidant activity: Two *in vitro* tests, the DPPH test for direct free radical scavenging activity and the lipid peroxidation assay for antioxidant activity were used to assess the antioxidant properties of methanol extract of *Galega purpurea*.

In lipid peroxidation assay α-tocopherol was used as a standard drug. MEGP at all the tested concentrations inhibited the lipid peroxides formed from rat liver homogenate and the results were comparable to that of the standard drug (Table 2). In the DPPH test the ability of a compound to act as donor for hydrogen atom or electrons was measured spectrophotometrically. MEGP was able to reduce the stable radical DPPH to the yellow colored diphenyl hydrazine and the activity increased dose-dependently at the tested concentrations ranging from 10-100 μg mL^{-1}. The strongest effect was measured for MEGP 100 μg mL^{-1} with an IC_{50} of 51.50±0.02 μg mL^{-1} (Table 3).

Antimicrobial activity: The initial screening of antibacterial activities of methanol extract was assessed by the agar disc diffusion method (Table 4). The extract inhibited the growth of Gram-positive bacteria at a concentration of 100 μg mL^{-1} which was the lowest concentration used in this study. The diameters inhibitory zones were in the range of 7.46 to 16.88 mm. However, 100 μg mL^{-1} concentration of the extract did not inhibit the growth of *Escherichia coli* and *Pseudomonas aureginosa*. It was also observed that the methanol extract exhibited antifungal activity against *Aspergillus niger* and *Candida albicans* in a dose dependent manner

Table 1: Preliminary phytochemical screening of extracts of *Galega purpurea*

Test	Petroleum ether extract	Methanol extract
Alkaloids	-	++
Glycosides	-	++
Tannins	-	++
Flavonoids	-	++
Steroids	++	+
Saponins	-	+
Carbohydrates	-	++
Proteins	-	+
Pentoses	-	+

(-): Absent; (+): Present; (++): Abundant

Table 2: Effect of methanol extract of *Galega purpurea* on lipid peroxidation assay

Concentration (μg mL^{-1})	Percentage inhibition	
	α-tocopherol	MEGP
10	4.83±0.02	3.25±0.10
25	11.93±0.03	8.37±0.08
50	25.83±0.01	19.33±0.10
75	32.50±0.01	28.45±0.20
100	40.53±0.05	37.61±0.06

Table 3: Effect of methanol extract of *Galega purpurea* on DPPH radical scavenging activity

Concentration (μg mL^{-1})	Percentage inhibition	
	α-tocopherol	MEGP
10	41.03±0.1	29.55±0.10
25	47.73±0.1	40.32±0.08
50	59.21±0.4	48.54±0.09
75	73.42±0.2	57.16±0.30
100	86.35±0.1	63.81±0.10

Table 4: Antibacterial activity of methanol extract of *Galega purpurea* and standard antibiotics

Samples	Concentration (μg mL^{-1})	Diameter of zone of inhibition (mm)					
		BS	SA	ML	EC	PA	ST
MEGP	100	9.31±1.2	7.52±1.4	8.47±0.7	-	-	8.13±0.8
	250	11.15±0.3	9.34±0.5*	10.31±0.3	8.08±1.1	7.46±1.2	9.87±0.4
	500	13.49±1.0	12.77±1.0	13.02±1.5	11.71±1.4	9.25±1.1	11.40±1.0
	1000	16.88±1.4	15.23±1.2	15.73±0.6	13.36±0.4	10.01±1.1	13.36±1.4
Amikacin	10	24.24±0.6	22.81±1.0	17.45±1.0	19.31±0.6	23.42±1.4	22.32±0.6

- No Inhibition zone, BS-*Bacillus subtilis*, SA-*Staphylococcus aureus*, ML-*Micrococcus luteus*, EC-*Escherichia coli*, PA-*Pseudomonas aureginosa*, ST-*Salmonella typhimurium*; Values are mean±SD (mm) of five separate experiments; Statistical value *$p<0.05$ when compared to standard

Table 5: Antifungal activity of methanol extract of *Galega purpurea* and standard antibiotics

Samples	Concentration (μg mL^{-1})	Diameter of zone of inhibition (mm) AN	CA
MEGP	100	8.22±0.6	12.74±1.3
	250	11.36±0.9	15.47±1.5
	500	13.61±0.7	18.09±0.5
	1000	17.28±1.2	21.35±1.1
Griseofulvin	20	21.43±0.8	22.81±1.1

AN-*Aspergillus niger*, CA-*Candida albicans*. Values are mean±SD (mm) of five separate experiments

(Table 5). When compared to standard commercial antifungal drug the methanol extract with concentration 1000 μg mL^{-1} exhibit the zone of inhibition similar to that of griseofulvin against *Candida albicans* (21.35±1.1 mm) at the dose of 1000 μg mL^{-1}.

DISCUSSION

About 5% or more of the inhaled oxygen (O_2) is converted to Reactive Oxygen Species (ROS) such as O_2^-, H_2O_2 and OH by univalent reduction of O_2 (Maxwell, 1995). Antioxidant can act by scavenging reactive oxygen species by inhibiting their formation (e.g., by blocking activation of phagocytes), by binding transition metal ions and preventing formation of OH and/or decomposition of lipid hydroperoxides by repairing damage (e.g., α-tocopherol repairing peroxyl radicals and so terminating the chain reaction of lipid peroxidation) or by any combination of the above (Niwa *et al*., 1994).

In this study the antioxidative activity of the root extract of *Galega purpurea* was measured using lipid peroxidation assay and DPPH radical scavenging assay method. This good antioxidant activity of MEGP might be attributed to the presence of phytochemicals, such as flavonoids and biflavones (Wang and Wixon, 1995). The DPPH radical is considered to be a model for a lipophilic radical. A chain reaction in lipophilic radicals was initiated by the lipid autoxidation. The radical scavenging activity of the crude plant extract was determined from the reduction in the optical absorbance at 517 nm due to scavenging of stable DPPH free radicals. Positive DPPH test suggests that the samples are free radicals scavengers.

The result obtained from antimicrobial study demonstrated that methanol extract of root of *Galega purpurea* produced *in vitro* antibacterial and antifungal activity against some medically important microorganisms. The bacteria inhibited in this study, have been associated with infections which the local tribes treat with *Galega purpurea* extract. Also the fact that the extract inhibited *Staphylococcus aureus* which is associated with respiratory tract infections and chest complaints, provides some scientific rationale for the use of the extracts for bronchitis, chronic asthma etc. The Gram-positive bacteria appeared to be more susceptible than the Gram-negative bacteria (Khunt *et al*., 1994). The weak activity shown by the methanol extract against the Gram-negative bacteria could be due to the presence of compounds in the extract possessing lipophilic characteristics (Werner *et al*., 1979).

Thus, the lipid peroxidation assay, DPPH radical scavenging activity and antimicrobial activity strongly suggest that MEGP has antioxidant and antimicrobial properties. Further studies are in progress in our laboratory to evaluate the *in vivo* antioxidant potential of this extract in various animal models and phytochemical studies are required to establish the types of compounds responsible for the bioactivity of this medicinal plant and to determine the value of the ethnobotanical approach for the screening of plants as potential source of bioactive substances.

REFERENCES

Berlett, B.S. and F.R. Stadtman, 1997. Protein oxidation in aging, disease and oxidative stress. J. Biol. Chem., 272: 20313-20316.

Bishayees, S. and A.S. Balasubramanian, 1971. Assay of lipid peroxide formation. J. Neurochem., 18: 909-920.

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature, 29: 1199-1200.

Bronen, A.L., 1975. Toxicology and biochemistry of butylated hydroxyl anisole and butylated hydroxyl toluene. J. Am. Oil Chem. Soc., 52: 59-63.

Cao, G., E. Sofic and R.L. Prior, 1997. Antioxidant and pro-oxidant behavior of flavonoids; structure-activity relationships. Free Rad. Biol. Med., 22: 749-760.

Couladis, M., O. Tzakou and E. Verykokidou, 2003. Screening of some Greek aromatic plants for antioxidant activity. Phytother. Res., 17: 194-196.

Ito, N., S. Fukushima, A. Hasegawa, M. Shibata and T. Ogiso, 1983. Carcinogenecity of butylated hydroxyl anisole in F344 rats. J. Natl. Cancer Inst., 70: 343-344.

Khunt, M., A. Probstle, H. Rimpler, R. Baner and M. Heinrich, 1994. Biological and Pharmacological activities and further constituents of *Hyptis verticillata*. Planta Med., 61: 227-232.

Linn, C.C., S.J. Wu and C.H. Chang, 2003. Antioxidant activity of *Cinammomum cassia*. Phytother. Res., 17: 726-730.

Mackeen, M.M., A.M. Ali, S.H. El-Sharkawy, M.Y. Salleh, N.H. Lajis and K. Kawazu, 1997. Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables. Int. J. Pharmacol., 35: 237-243.

Maxwell, S.R.J., 1995. Prospects for the use of antioxidant therapies. Drugs, 49: 345-361.

Nadkarni, K.M., 1976. Indian Materia Medica. Vol. I, Popular Prakashan, Bombay, pp: 561-563.

Niwa, T., U. Doi, Y. Kato and T. Osawa, 2001. Antioxidant properties of phenolic antioxidants isolated from corn steep liquor. J. Agric. Food Chem., 49: 177-182.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Trease, G.E. and M.C. Evans, 1983. Text Book of Pharmacognosy. 12th Edn., Oxford University Press, Oxford, pp: 343-383.

Wang, C. and R. Wixon, 1999. Phytochemicals in soybeans: Their potential health benefits. Inform., 10: 315-321.

Werner, R.G., K.R. Appel and W.M.A. Merk, 1979. Gunacin, a new quinine antibiotic from *Ustilaga* sp. J. Antibiotic., 32: 1104-1111.