

EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF STEM AND LEAF EXTRACTS OF *COSCINIUM FENESTRATUM*

SANTHOSH W.GOVEAS* AND ASHA ABRAHAM

Department of Biotechnology, St.Aloysius College (Autonomous), Mangalore-575 003,Karnataka, India . Email: swgoveas@gmail.com

Received: 7 May 2013, Revised and Accepted: 24 May 2013

ABSTRACT

In the present study, antioxidant activity and antibacterial activity leaf and stem extracts of *Coscinium fenestratum* was investigated. To assess the antioxidant activity, methanolic leaf and stem extracts were used. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS. For antimicrobial activity, aqueous, acetone, ethanol and methanolic extracts of stem and leaf extracts were tested for its potent antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Total content of phenol and flavonoid was quantitatively estimated in leaf and stem extracts of *Coscinium fenestratum*. Total phenolic content in the stem and leaf was found to be 8.35 ± 0.56 and 3.35 ± 0.67 mg GAE/g extract. While the total flavonoid content in the stem and leaf were found to be 12.8 ± 0.88 and 3.2 ± 0.78 mg QE/g extract respectively. Among the different extracts, methanolic stem extract showed moderate activity against *E.Coli* (17 ± 0.33), *P.aeruginosa* (12 ± 0.20), *B.subtilis* (13 ± 0.45). Methanol leaf extract had maximum activity against *S.aureus* (6.4 ± 0.67) and lowest against *B.subtilis* (3.9 ± 0.58) respectively.

Keywords: *Coscinium fenestratum*, Antioxidant, Antimicrobial, DPPH, Medicinal plant, Phenol, Free radical.

INTRODUCTION

Reactive oxygen species (ROS) are class of highly reactive molecules derived from the metabolism of oxygen. Rapid production of free radicals may lead to oxidative damage to biomolecules and results in disorders such as degenerative disorders, cancer, diabetes, neural disorders and ageing [7, 21]. These free radicals occur in the body during an imbalance between ROS and antioxidants. Many medicinal plants have large amount of antioxidants such as Vitamin C, Vitamin E, polyphenols etc. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases like heart, cancer [16]. There are many synthetic antioxidants but they have side effects [8], hence there is a need for more potent, less toxic antioxidants. It has been found that plants having polyphenolic compounds such as flavonoids possess antioxidant activity [6]. Polyphenols have antioxidative properties which is due to their high reactivity as hydrogen donor or electron donor which stabilize and delocalize the unpaired electron [20].

Medicinal plants are rich sources of antimicrobial agents. Many infectious diseases have been known to be treated with herbal extracts. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. Although many plant species have been tested for antimicrobial properties, the majority of them have not been sufficiently evaluated [1]. As an alternate source to the existing antibiotics, there is an urgent need to discover new antimicrobial compounds from various medicinal plants which can be used to treat many infectious diseases.

Coscinium fenestratum (Gaertn.) Colebr commonly known as tree turmeric [24] is a dioecious woody liana belonging to the family Menispermaceae [19]. The active chemical ingredient of this plant is reported to be berberine. It is a natural isoquinoline alkaloid having wide variety of pharmacological activities [15]. Berberine is present in both vegetative and reproductive parts, indicating the synthesis of berberine in all parts of the plant. The plant extracts have been shown to have antimicrobial effect [11] and have been used in the ayurveda and siddha medicine [17]. The *in vitro* antioxidant activity of methanolic stem extract was studied by [22]. Although earlier reports shows the antimicrobial and antioxidant potential only the stem extract of *C.fenestratum*, there has been no report regarding the leaf extract. Hence in the present study we have studied the

antimicrobial and antioxidant activity of leaf and stem extracts of *Coscinium fenestratum*.

MATERIALS AND METHODS**Chemicals**

1,1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid were purchased from Sigma chemicals Co.(St.Louis,USA). Gallic acid, Folin Ciocalteu reagent, Quercetin were purchased from Merck Co(Germany). All other chemicals and reagents used in the study were of analytical grade.

Collection of plant material

The stem and the leaves of *C. fenestratum* obtained from the foot hills of Western Ghats of Karnataka were used for the investigation. The plant was authenticated by Dr.G.K Bhat, a well known taxonomist from Udupi, Karnataka, India. The material was brought to the laboratory and air dried at room temperature ($25 \pm 2^\circ\text{C}$). The dried samples were powdered using grinder mill and stored in desiccators. Herbarium samples have been deposited in the dept. of Botany, St. Aloysius College, Mangalore, Karnataka.

Preparation of Plant extracts

About 50grams of stem and leaf powder of *Coscinium fenestratum* was placed in soxhlet extractor separately. It was extracted with methanol for approximately 72 hours. After this extraction period the solvent collected from the extractor was evaporated using flash evaporator. The concentrated residue obtained which contains the plant extract was further concentrated by placing it in an incubator at 37°C for 24hours and then stored in desiccator for subsequent experiments.

Antioxidant activity**Preparation of *Coscinium fenestratum* leaf and stem stock solution**

Coscinium fenestratum leaf and stem stock solutions were prepared in methanol at a concentration of $1000 \mu\text{g/ml}$ (1mg/ml). From the stock solution various concentrations viz 2, 4, 8, 16, 32, 64, 128, 256, 512 and $1000 \mu\text{g/ml}$ were prepared in methanol and used for antioxidant studies.

DPPH Assay

The free radical scavenging activity of the stem extract was measured using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as described by ⁴. Briefly, to 1ml of different concentrations of methanolic stem and leaf extract, 1ml of DPPH 0.1mM was added. The mixture was mixed and left to stand for 30min in the dark and the absorbance was recorded at 517nm. An equal amount of DPPH and Methanol served as control. The experiment was done in triplicate. Ascorbic acid was used as standard control. The percentage scavenging was calculated using the following formula,

$$\text{DPPH Scavenging effect (\%)} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \right]$$

ABTS radical scavenging

The ABTS radical scavenging assay of stem extract was carried out using the standard protocol described by ¹⁸ with slight modifications. Briefly, stock solutions of ABTS (7mM) and potassium persulfate (140mM) in water were prepared, and mixed together to a final concentration of 2.45mM potassium persulfate. This mixture was left to react overnight (12-14hrs) in the dark, at room temperature. The solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734nm. To 20 μ l of the various concentrations (2-1000 μ g/ml) of test compound, 2.0ml of diluted ABTS solution added and the absorbance was taken after 6min. For the control, methanol was used instead of the test compound. Ascorbic acid was used as standard.

Total Phenolic Content (TPC)

The total phenolic compounds in the stem extract and leaf extracts were determined by Folin-Ciocalteu's method described by ¹². Briefly, 0.1ml of methanolic extracts, 2ml of 2% (w/v) sodium carbonate solution was added and vortexed vigorously. After 5min, 0.1ml of 50% Folin-Ciocalteu's reagent was added and incubated for 30mins; absorbance was measured at 750nm against blank. The same procedure was followed for the standard solution of gallic acid. The total phenolic content in the extract were expressed as mg of gallic acid equivalent (GAEs) per g of extract (GA mg/g).

Total Flavonoid (TF)

Total flavonoid content was measured by Aluminium chloride colorimetric assay as described by [5]. The extract (0.5ml of 1:10g/ml) in methanol was mixed with 1.5ml of methanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. After 30min of incubation, the absorbance was measured at 415nm with UV/Visible spectrophotometer. Quercetin was used as standard and the flavonoid content is expressed in terms of mg of quercetin equivalents (QE) per g of extract.

Antibacterial Assay

Extraction of plant material

Aqueous extract of stem and leaf was prepared by adding 15g in 100ml distilled water separately, boiled for 1hr on low heat and filtered through muslin cloth and evaporated to dryness using rotary flash evaporator. The methanolic extracts were separately prepared by adding 15g of stem and leaf powder to 100ml methanol in screw capped bottles, shaken at 200-300rpm on a horizontal shaker. After 48hrs, it was filtered, evaporated to dryness in rotary evaporator [10]. Dried extracts were stored in labeled sterile screw capped bottles at 4°C and later used *in vitro* study.

Bacterial strains

Standard bacterial cultures like *Staphylococcus aureus* (MTCC 96), *Escherichia.Coli* (MTCC 739), *Pseudomonas aeruginosa* (MTCC 424), *Bacillus subtilis* (MTCC 441) were procured from IMTECH, Chandigarh, India and used in the present study. The bacteria rejuvenated in Nutrient broth (Hi-media) at 37°C for 18 h and then stocked at 4°C in Nutrient Agar. Subcultures were prepared from the stock for bioassay. The bacterial culture was inoculated into sterile nutrient broth and incubated at 37°C for 2h until the culture attained a turbidity of 0.5 McFarland units [13].

Antibacterial assay

The nutrient agar plates were prepared and were inoculated with 100 μ l of the inoculums (1×10^8 CFU/ml). The aqueous and methanol extracts were prepared in their respective solvents and the sterile blotting paper disc (0.7 mm Hi-Media) was saturated with 50 μ l of the test compound. The prepared disc was dried and was introduced on the upper layer of the seeded agar plate. Streptomycin 10 mcg/disc (Hi-Media) was used as positive control while disc soaked in sterile distilled water and methanol solvent were used as negative control. The plates were incubated for 24 h at 37°C. The antibacterial activity was evaluated for 5 mg/disc and diameter of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded.

Statistical analysis

antioxidant activity, total phenolic content, and flavonoid content are expressed as the mean \pm Standard Error of Mean (S.E.M). Statistical analysis was carried out with GraphPad InStat version 3.0 using ANOVA ($p < 0.05$ is considered significant).

Results and Discussion

The free radical scavenging activity of *Coscinium fenestratum* was studied by its ability to reduce the DPPH, a stable free radical. The DPPH inhibition of leaf and stem extracts are shown in Fig.1. In DPPH assay, the methanolic stem extract showed more antioxidant activity when compared to methnolic leaf extract. DPPH scavenging activity was ranging from $4.7 \pm 0.67\%$ to $71.3 \pm 0.36\%$ in the case of methanolic stem extract, whereas in the case of leaf it was $4.6 \pm 1.2\%$ to $49 \pm 0.88\%$. In case of methanolic stem extract, the highest scavenging activity was found at a concentration 256 μ g/ml and the lowest was found at a concentration of 2 μ g/ml. The leaf extract showed maximum scavenging activity at a concentration of 512 μ g/ml and minimum was at 2 μ g/ml. The reduction in the number of DPPH molecule can be correlated with the available number of hydroxyl groups. Hence the significant scavenging activity may be due to the presence of hydroxyl groups present in the extracts.

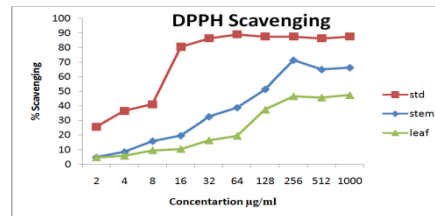


Figure 1: DPPH radical scavenging activity of different concentrations of *C.fenestratum* leaf, stem extracts and ascorbic acid.

In the ABTS assay, the maximum scavenging activity was seen at a concentration of 1000 μ g/ml and lowest was at 2 μ g/ml. the scavenging activity was ranging from $9.3 \pm 0.56\%$ to $69.3 \pm 1.76\%$ in case of stem extract and the leaf extract it was ranging from $3.6 \pm 0.27\%$ to $46.3 \pm 0.88\%$ (Fig.2.). This wide range of antioxidant activity may be attributed to the wide variety of bioactive compounds like phenolics, flavonoids, tannins etc. present in the plant. In both the antioxidant assays the extract showed efficient activity as compared to standard ascorbic acid.

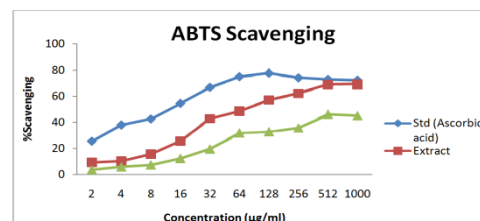


Figure 2: Scavenging activity of different concentrations of *C.fenestratum* stem, leaf extracts and Ascorbic acid in ABTS radical scavenging.

The antioxidant activity of the extracts might be due to the presence of phenolic compounds [23]. In this study, the total phenolic content (TPC) was determined using the Folin-Ciocalteu method, using gallic acid as standard. The stem methanolic extract of *C.fenestratum* contained large amount of phenolics 18.35 ± 0.56 mg GAE/g extract when compared to leaf 9.35 ± 0.67 mg GAE/g extract (Fig.3).

Flavonoids are responsible for the antioxidant properties of the medicinal plants. The antioxidative property of flavonoids may be due to different mechanism like scavenging of free radicals, chelation of metal ions [3]. The flavonoids also contain broad spectrum of biological activity [14]. The highest amount of flavonoid was found in the stem 12.8 ± 0.88 mg QE/g extract and in the leaf extract of *C.fenestratum* it was found to be 3.2 ± 0.78 mg QE/g extract (Fig.4). These results indicate that the flavonoid and phenolics are responsible for the free radical scavenging activity of *C.fenestratum*.

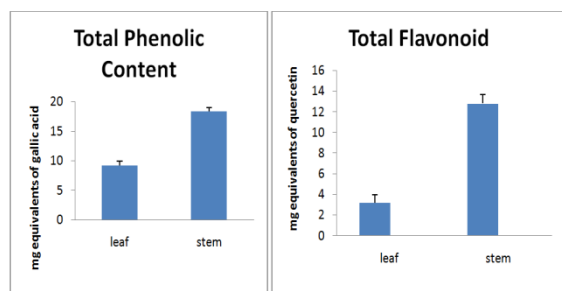


Figure 3 & 4: Total Phenolic content and Total Flavonoid content

Antibacterial activity

The antibacterial activity of aqueous and methanolic extracts of leaf and stem of *C.fenestratum* was assayed *in vitro* by agar disc diffusion method against four different bacterial strains. The methanolic extract had greater antibiotic activity when compared to aqueous extract. The methanol extract of stem showed maximum antibacterial activity against *E.coli* (17 ± 0.33 mm) whereas the methanolic leaf extract had maximum zone of inhibition for *S.aureus* (6.4 ± 0.67 mm) (Fig.5). In the aqueous extracts of leaf and stem of *C.fenestratum*, both extracts exhibited greater antibiotic activity (11 ± 0.3 mm and 6.4 ± 0.45 mm) against *B.subtilis*. The significant antibacterial activity of the plant extracts was compared with the standard antibiotic, streptomycin (10mcg/disc). These results indicate that the *C.fenestratum* plant can be used for developing antimicrobials which can act against various bacterial strains.

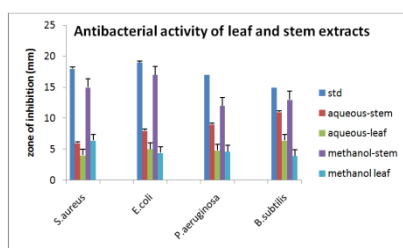


Figure 5: Antibacterial activity of stem extract of *C.fenestratum* against different bacterial strains. Values are mean inhibition zone (mm) \pm S.D. of three replicates

The herbal based phytomedicine have large therapeutic applications since they can have less side effects when compared with synthetic antimicrobials. Although earlier reports show the *in vitro* free radical scavenging activity and antibacterial activity of methanolic stem extract there has been no report on the leaf extract of *C.fenestratum*. In our studies we have found that both leaf and stem has antioxidant and antibacterial activity which can be comparable.

Acknowledgement

The authors thank the Principal and Management of St. Aloysius College (Autonomous), Mangalore for the facilities for carrying out the work.

REFERENCES

- Balandrin MF, Kjocke AJ, Wurtele E. Natural plant chemicals: sources of industrial and mechanical materials. *Science* 1985; 228:1154-1160.
- Bandow JE, Brotz H, Leichert LIO. Proteomic approach to understanding antibiotic action. *Antimicrob Agents Chemother.* 2003; 47: 948-955.
- Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del Rio JA. Uses and properties of Citrus flavonoids. *J Agric Food Chem* 1997;45: 4505-4515.
- Blois MS. Antioxidant determinations by the use of stable free radicals. *Nature* 1958; 26:1199-1200
- Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. *J.Food Drug Anal* 2002; 10: 178-182.
- Cook NC, Samman, S. Flavonoids- chemistry, metabolism, cardioprotective effects, and dietary sources. *Nutritional Biochemistry* 1996; 7: 66- 76.
- Hyun DH, Hernandez JO, Mattson MP, de Cabo R. The plasma membrane redox system in aging. *Aging Res. Rev.* 2006; 5: 209-220.
- Ito N, Fukushima S, Hagiwara A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyanisole in F344 rats. *J. Natl.Cancer Inst.* 1983;70: 343-347.
- Iwu MM, Duncan AR, Okunji CO. New Antimicrobials of plant origin. In Janick, J. (ed) Perspectives in New crops and New uses. ASHS Press, Alexandria, V.A. 1999; p 457-462.
- Jigna Parekh and Sumitra Chanda. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research* 2007; Vol. 10: 175 - 181
- Kumar D and Hyde KD. Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii*. *Fungal Diversity* 2004; 17, 69-90
- McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chem.* 2001; 73:73-84.
- McFarland, J. Standardization of bacterial culture for the disc diffusion assay. *Journal of American Medical Association* 1987;49, 1176-1178.
- Miliauskas G, Venskutonis PR and van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.* 2004; 85: 231-237
- Piyanuch R, Sukhthankar M, and Baek SJ. Berberine, a natural isoquinoline alkaloid, induces NAG-1 and ATF3 expression in human colorectal cancer cells. *National Institute of Health* 2007; 258(2), 230-240.
- Prior RL, Cao G. Antioxidant phytochemicals in fruits and vegetables. Diet and health implications. *Hortic. Sci.* 2000; 35: 588-592.
- Punitha ISR, Rajendran K, Shirwaikar A and Shirwaikar A. Alcoholic stem extract of *Coscinium fenestratum* regulates carbohydrate metabolism and improves antioxidant status in streptozotocin-nicotinamide induced diabetic rats. *Oxford Journal* 2005; 2(3), 375-381.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad. Biol. Med.* 1999; 26: 1231-1237.
- Remashree AB, Jayanthi A, Thushar KV, Ravindran PN and Indira Balachandran. Anatomy of *Coscinium fenestratum* (Gaertn.) Colebr. A critically endangered medicinal plant from Western Ghats. *Phytomorphology* 2005; 55, 249-258.
- Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* 1997; 2: 152-159.
- Sas K, Robotka H, Toldi J, Vecsei L. Mitochondrial, metabolic disturbances, oxidative stress and kynurenine system, with focus on neurodegenerative disorders. *J. Neurol. Sci.* 2007;257: 221-239
- Shirwaikar A, Shirwaikar A and Punitha ISR. Antioxidant studies on the methanol stem extract of *Coscinium fenestratum*. *Natural Product Sciences* 2007;13(1):40-45.

23. Tepe B, Sokmen M, Akpulat HA, Sokmen A. Screening of the antioxidant potentials of six *Salvia* species from Turkey. *Food Chem.*2006; 95:200-204
24. Tushar KV, George S, Remashree AB and Balachandran I. *Coscinium fenestratum* (Gaertn.) Colebr. A review on this rare, critically endangered and highly-traded medicinal species. *Journal of Plant Sciences* 2008;3(2), 33-145