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Full Length Research Paper

Antimicrobial properties of leaves of *Calycopteris floribunda* Lam.

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The antimicrobial activities of leaf extracts of *Calycopteris floribunda* in three different solvents such as diethyl ether-methanol, aqueous 90% methanol extract and petroleum ether-butanol extract were tested against *Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus*. The diethyl ether-methanol extract of the leaves and its petroleum ether-butanol fraction showed significant antibacterial activity. The antioxidant property was maximum in petroleum ether-butanol extract and was minimum in diethyl ether-methanol extract.

Key words: Antibacterial activity, antioxidant, *Calycopteris floribunda*, plant extract.

INTRODUCTION

Calycopteris floribunda Lam. is an evergreen shrub, locally known as enjir. It is commonly referred as a life-saver by the forest dwellers who regularly depend on this vine during summer when streams dry up. Sections of the vine store water, which people often use to quench their thirst. The plant parts especially leaves being used medicinally for various complications such as intestinal worms, colic, leprosy, malarial fever, dysentery, ulcers and vomiting. The fruits are useful in jaundice, ulcers, pruritus and skin diseases. A number of phenolic and non-phenolic flavonoids including cytotoxic, anthelmintic and antiviral properties have been isolated from the plant, the tender copper coloured leaves ground into paste or dry powders administered for the expulsion of bacteria, free radicals and round worms (Nadkarni, 1927; Ratnagiriswaran et al., 1934). According to Nadkarni an extract of the leaves exhibits the colour reactions of santonin. Dey et al. (2005) conducted experiment on antibacterial activity and antioxidant property in *C. floribunda* using leaf extracts in different solvents. Sreekanth et al. (2007) in their studies on leaf extracts of *C. floribunda* revealed that the extracts were toxic to calf, rabbits and rat. The main objectives of the present research work was to study the effect of isolated plant extracts on the growth of bacteria *in vitro* for antibacterial and antioxidant activity.

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MATERIALS AND METHODS

Isolation of extracts from the leaves of *C. floribunda*

The leaves were collected from nearby forest, cleaned, air dried, finally dried in shade for a week and powdered. For sample preparation 100 g of leaf powder was extracted with diethyl ether – methanol (1:1). The extract was filtered, concentrated and evaporated to dryness. The residue was suspended in water and partitioned with diethyl ether yielding diethyl ether extract. The diethyl ether extract was again suspended in aqueous 90% methanol and partitioned with petroleum ether, yielding petroleum ether and aqueous 90% methanol extract. The aqueous part was also partitioned with 1-butanol to get 1-butanol soluble part. All the extracts were taken in separate test tubes and named accordingly. The bacterial cultures were collected from Microbiology Dept. of St. Aloysius College, Mangalore. The colonies of *Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus* were maintained on nutrient agar (Himedia, Mumbai) slants and sub-cultures were made every fifteen days, 24 h cultures were used for the present study.

Antibacterial activity

Antibacterial activity was studied by agar diffusion method. The nutrient agar plates were swabbed with each of the bacterial strains (*B. cereus, B. subtilis* and *S. aureus).* A sterile swab was dipped

Table 1. Antibacterial activity of the leaf extract of *C. floribunda.*

Name of bacteria	Name of extract	Zone of inhibition (mm)	
		Test sample	Reference drug
Bacillus subtilis	Diethyl ether-Methanol	18	
	Aqueous 90% Methanol extract	11	24
	Petroleum ether-Butanol extract	14	
	Diethyl ether-Methanol	19	
Bacillus cereus	Aqueous 90% Methanol extract	13	30
	Petroleum ether-Butanol extract	16	
	Diethylether-Methanol	21	
Staphylococcus aureus	Aqueous 90% methanol extract	09	10
	Petroleum ether-Butanol extract	16	

Activity key: 0 to 13 mm inactive, 13 to16 mm good activity, 16 to 24 mm significantly active.

into the broth and expressed any excess moisture by pressing the swab against the side of the tube. The filter paper disks were impregnated with the solvent extracts of the leaves of *C. floribunda.* Each free disc is then placed right onto the agar with the sterile forceps and incubated at 37°C for 3 to 4 days. The zone of inhibition was measured separately for each bacterial strain using a scale.

Antioxidant activity

The antioxidant studies were carried out by DPPH radical scavenging method (Sreejayan and Rao, 1997). Antioxidant activity of the diethyl ether-methanol extract, aqueous 90% methanol and petroleum ether- butanol soluble fractions were studied with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity. The radical scavenging activity was examined by the reduction of 1,1-diphenyl-2-picryl-hydrazil (DPPH) in methanol. To the methanol solution of DPPH (200 µl), 0.05 ml of test samples dissolved in methanol were added at different concentrations (10, 20 and 40 mg/ml). An equal amount of methanol was added to the control. After 20 min, the decrease in absorbance of test mixture due to quenching of DPPH radicals was read at 517 nm using a visible spectrophotometer.

RESULTS AND DISCUSSION

The antibacterial activity of leaf extracts of *C. floribunda* in three different solvents with three selected bacterial strains was showed in Table 1. The diethyl ether-methanol extract was possessed a significant antibacterial activity against *B. cereus, B. subtilis* and *S. aureus.* Whereas, the petroleum ether-butanol soluble fraction was found to be moderately active and the aqueous 90% methanol extract showed less antibacterial activity. Rajendran et al. (1998) tested extracts from *Acorus calamus, Zingiber officinalis, Cinnamomum zeylanicum, Moringa oleifera, Ocimum sanctum* and *Piper betel* against *S. aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella* spp. and concluded that *E. coli* exhibited higher resistance to antibiotics. They were also concluded that *Ocimum sanctum* extracts was the most effective against all the four microorganisms tested. Dey et al. (2005) used dichloromethane- methanol and 95% methanol as solvents for sample extraction from the leaves of *C. floribunda* and found that a significant antimicrobial activity observed against *B. subtilis, Streptococcus pyrogens, S. aureus* and *Salmonella typhi*. They were also reported the presence of antioxidant property. The copper-coloured tender leaves of *C. floribunda* are reputed to have antibacterial, antioxidant, anthelminthic and laxative properties (Nadkarni, 1927); ground into paste or dry powdered finely and administered for the expulsion of bacteria, free radicals and round worms. According to Nadkarni an extract of the leaves exhibits the colour reactions of santonin. There are other reports that supports the antimicrobial properties of extracts of different plants extracted with different solvents on bacterial strains (Raman et al*.,* 1998; Shang et al., 2001; Rambir et al., 2002; Farrukh and Iqbal, 2003; Sabitha et al., 2003; Adebolu et al., 2007; Lokhande et al., 2007; Thaakur and Rani, 2009: Mary et al., 2009; Abirami et al., 2009). The present work hence proves additional value to other antimicrobial activity of this plant compared to earlier work by Rao Bahadur and Koman (1919) who reported that the plant will prove to be a good antibacterial and anthelmintic and a very efficient substitute for santonin. The mode of extraction without organic solvent also proved antimocrobial in other studies. Sampurna and Nigam (1979) extracted five essential oils from the leaves of *Skimmia laureola*, *Cinnamomum zeylanicum, Cymbopogon flexuosus, Geranium* and *Eucalypta citridora* by steam and water distillation methods and were tested for their antimicrobial activity at different concentrations against *Vibrio cholerae, Salmonella paratyphi, Bacillus anthracis* and *Xanthomonas malvacearum* using paper disc agar diffusion technique and they obtained very encouraging

Table 2. Antioxidant activity of the leaf extracts of *C. floribunda*.

Plant part	Name of the extract	Scavenging activity (%)
Leaf	Diethyl ether-Methanol	56
	Aqueous 90% Methanol extract	79
	Petroleum ether-Butanol extract	88

DPPH free radical scavenging activity: Criteria – 0 to 30% inactive; 30 to 50%, little active; 50 to 70% moderate, above 70% highly active.

results. Abirami et al. (2009) worked on antibacterial activity of *Asteracantha longifolia* and *Adrographis paniculata* with 11 bacterial species mostly pathogenic and concluded that the presence of obscurinine (alkaloid) and octadecanoic acid are major responsible for its activity which showed in ethanolic extract.

The antioxidant properties of leaf extract was given in Table 2. The order of the antioxidant property was in the order of Diethyl ether-Methanol < Aqueous 90% Methanol extract < Petroleum ether-Butanol extract. The percent scavenging activity was maximum in the petroleum ether –butanol extract, which shows that it contains the major active principle of the extract. Dey et al. (2005) earlier reported the presence of antioxidant property in the dichloromethane-methanol and 90% methanol extracts of *C. floribunda* leaves. On the other hand, Murugani et al. (2009) showed the antioxidant active principle in the stem extracts of *Phyllanthus longiflorus* was due to steroids and flavanoids. These oxidative stress has been implycated in the pathology of many diseases and conditions including diabetes, cancer, inflammatory, cardiovascular diseases and ageing (Marx, 1987). Although Sreekanth et al. (2007) made an preliminary work on toxicity studies of *C. floribunda* on rat and calf and there is need of further *in vitro* and *in vivo* studies using active ingredients.

Mechanism of free radical scavenging activity

Rapid (DPPH) tests are often applied to classify the scavenging activity of the extracts (E) of the leaves of *C. floribunda.* Published analytical protocols differ in more than one experimental condition, and results for the relative order or magnitude of activity are often contradictory. In our work, parameters such as duration of test, [E]/[DPPH] molar ratio, and solvent effects were examined and discussed. The test duration and the value of the [E]/[DPPH] ratio did not influence the order of activity among tested antioxidants. Methanol, commonly used as solvent in our tests, was compared with aceto - nitrile and tertiary butyl alcohol. Solvent properties such as the ability to form hydrogen bonds with the [E] seem to influence the level of the relative activity (%RSA). Higher per cent RSA values were observed in ethanol. The activity of the most polar compounds was affected the most. Standardization of the analytical period should include a 20 min reaction period and a molar ratio that permits attainment of a 60 to 80% RSA value for the most potent antioxidant. Solvent choice is critical for classifying activity. Safe classification can be based only on results from kinetic studies. Antioxidants are micronutrients that have gained interest in recent years due to their ability to neutralize the actions of free radicals (Cadenas and Packer, 1996) Free radicals are potentially harmful products generated during a number of natural processes in the body and associated with ageing of cells and tissues. Failure to remove active oxygen compounds, over a long term, can lead to cardiovascular disease, cancer, diabetes, arthritis and various neurodegenerative disorders. Hence the recent research on development of healthy foods focuses on antioxidant properties. Consumers demand food products with fewer synthetic additives but expect increased safety, quality and shelf-life (Roller, 2003). This demand has led to renewed interest in the use of natural antimicrobials to preserve foods. Though there is wide range of potential antimicrobials available, only few are suitable for use.

The antioxidant can donate either an electron or hydrogen to cellular molecules oxidized by free radicals. They can thus prevent damage of cellular constituents, including DNA, proteins and lipids membranes from free radicals. Modulation of diseased states such as cardiovascular ailments, neurological disorders, cancer and diabetes using dietary components, including fruits and vegetables, natural products and medicinal plants as a possible therapeutic measure has become a subject of active scientific investigations (Hertog et al., 1997; Sur, 2002).

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