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Research Article

Evaluation of Immunostimulating Activities of Caralluma spp.

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ABSTRACT
Anti oxidant, anti inflammatory, anti obesity and anti microbial activities in four different species of Caralluma, include *Boucerosea lasiantha* (BL), *Caralluma adscendens var. attenuata* (CAA), *C.stalagmifera* (CSF) and *C.longipetala* (CL) were investigated. Whole plant is used for the extraction of compound by using methanol. These selected species of Caralluma are used for the study of saponins and flavanoids, their bioactive nature. Variations in the bioactivity of cactus species are compared as a preliminary survey for future pharmacological drug development. Methanolic extracts of CAA exhibited highest free radical scavenger activity (FRSA) followed by BL, CSF, CL respectively with IC_{50} values 50,37,32, 27 µg/ml. CAA also exhibited highest anti inflammatory activity (5-Lox assay) followed by BL, CL, CSF with IC_{50} values 27 ,17, 12.8, 11.8 µg/ml. Methanolic extracts of four species also show anti diabetic activities (α- amylase property assay). Extracts of four species show anti microbial activity, however CSF and CL show highest activity than CAA and BL. These results suggest that the methanol extract of Caralluma species show significant anti oxidant, anti inflammatory, anti diabetic, anti microbial activities, which could be used as a potential source of pharmaceutical materials.

Keywords: *Caralluma spp*, anti-oxidant, anti-inflammatory, anti-obesity, anti-microbial.

INTRODUCTION

Medicinal plants are good sources of antioxidants, and used as adjunctive therapy in the treatment of diabetes, diarrohoea, hyperlipidemia[1]. The main constituents of medicinal plants, such as saponins, flavanoids, and polyphenols are known to be major bioactive compounds in Ayurvedic medicine[2]. Inflammation is typically a protective mechanism that is triggered in response to noxious stimuli, trauma or infection to guard the body and to hasten-up the recovery process. However, inflammation that is unchecked leads to chronic inflammatory disorders. Arachidonic Acid (AA) metabolism plays a crucial role in inflammatory process and associated diseases. Some of the anti-inflammatory drugs inhibit the lipoxygenase pathway and some inhibit cyclooxygenase pathway, can be used for potential interventions against inflammation. Unfortunately most of the anti-inflammatory drugs, particularly steroids and cycloxygenase inhibitors are often associated with adverse side effects including, gastro intestinal irritation, ulcers, hypertension and cardiac abnormalities[3,4]. Most antioxidants isolated from medicinal plants are polyphenols, which show biological activities include anti bacterial, anti inflammatory, anti obesity, antiviral, anti carcinogenic and immunostimulating effect. The use of herbal remedies for arthritis treatment has been gaining momentum in recent years[5].

Caralluma, a cactus plant belongs to family Asclepiadaceae is a succulent, perennial herb, grow to a height of 1 to 10 ft and grow in different regions of India. The members of genus Caralluma are erect and fleshy. They have quadrangular stem, devoid of leaves and small flowers in several varieties of dark colour. The species of Caralluma found in India are edible and form a part of traditional medical system of country[6]. The present study aims to evaluate bioactivities of four species of Caralluma, *Boucerosea lasiantha*(BL), *Caralluma adscendens var.annuata* (CAA), *Caralluma stalagmifera* (CS), *Caralluma longipetale* (CL), distributed in peninsular India. This succulent Cactus

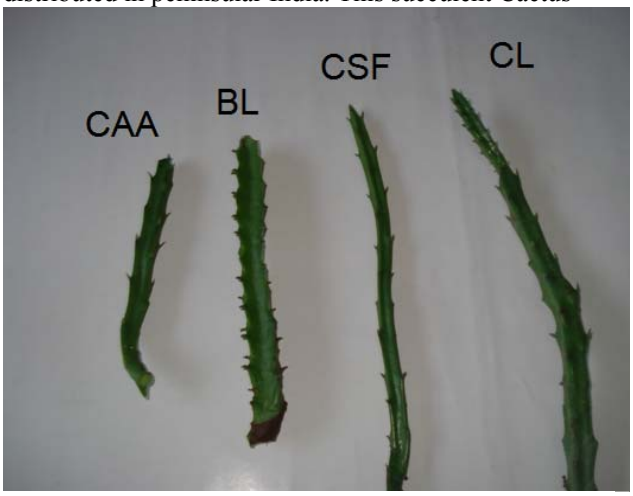

Plate 1: Different species of Caralluma (BL:*Bocserosea lasiantha*, CAA: *Caralluma adscendens var. attenuata* , CSF: *C.stalagmifera* and CL: *C.longipetala*)

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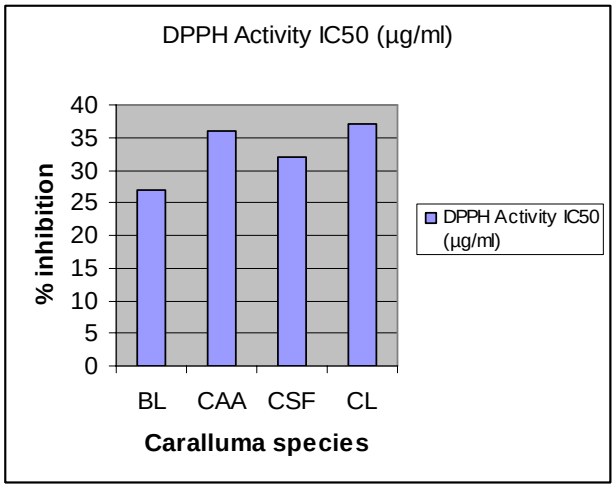

Fig1: FRSA of Caralluma species.
* Values are mean of triplicate.

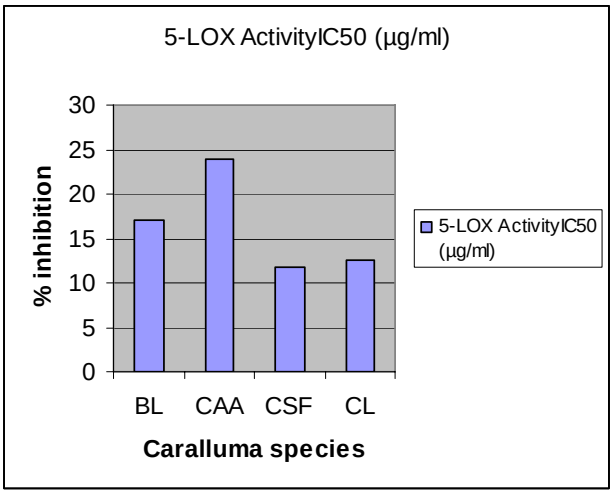

Fig 2: 5- Lox activity of Caralluma species
* Values are mean of triplicate.

contain glycosides, hydrocarbons, saponins as major phytoconstituents and reported for various biological activities such as rheumatism, diabetes, leprosy, antipyretic, anti-helminthetic activities[7,8]. Pregnane glycosides, saponins and flavanoids are the main constituents of Caralluma species, in which saponins are mainly ascribed to their specific interaction in their cell membrane causing changes in cell permeability[9]. Studies also revealed on the antimicrobial potential of saponins from various other medicinally important plants[10]. Presence of sugar in saponin molecule has a stronger hemolytic property[11].

Saponins are typical secondary metabolites widely present in the plant kingdom which include steroidal and triterpenoid glycosides classified according to the nature of their aglycones. Most saponins are hemolytic and display a range of various biological and pharmacological properties, such as molluscicidal, anti-inflammatory, anti-microbial and cytotoxic activities[12]. Commercial products containing plant saponins are available and used in the pharmaceutical, cosmetic and food industries[13]. For example triterpene saponins from *Quillaja saponaria* have immunostimulatory properties and are included as adjuvants in vaccine formulations[14]. However very few investigations are carried out on methanolic extracts of Caralluma species for their biological activities such as anti-oxidant, anti-obesity, anti-inflammatory and anti-microbial. In order to search for possible new compounds from methanolic extract, the present investigation is taken off and results are reported in this paper.

In the present investigation, *Boucerosea lasiantha* (BL), *Caralluma adscendens var* attenuata (CAA), *Caralluma stalagmifera* (CSF), *Caralluma longipetala*(CL) of Caralluma genera are focused for their immunostimulating effects such as anti oxidant anti inflammatory anti obesity and anti microbial activities .Whole plant is used for extraction of compound by using methanol . These selected species of Caralluma are used for the study of saponins and flavanoids, their bioactive nature. Variations in their bioactivity are compared as a preliminary survey for future pharmacological drug development from this cactus.

MATERIALS AND METHODS

Plant material: The plant material Caralluma species were collected from Gooty, Tadiparthy, Penukonda areas of Ananthapur district of Andhra Pradesh, in April, 2010. They were identified by Prof. Pulliah. A voucher specimen was deposited in the depository of the Taxonomy Division at SK University.

Free radical scavenging activity (FRSA):DPPH scavenging radical activity was determined on the basis of reduction of coloured methanolic solution of DPPH[15,16]. FRSA of the test substances added to the methanolic solutions of DPPH is inversely proportional to the differences in initial and final absorption of DPPH solution at 570 nm. Drug activity was expressed as that 50% inhibitory concentration (IC_{50}). The reaction mixture contained 1X 10^{-4} M methanolic solution of DPPH and various concentrations of the test substances. Percentage inhibition was determined by comparing the absorbance values of test and control tubes. IC_{50} values were obtained from the best fit line drawn concentration (µg) vs. percentage inhibition.

α -Amylase activity:Twenty µl of α-amylase (0.05 U/ µl was pre mixed with 20 µl of sample and 250 µl of 2% starch solution in 0.1M sodium phosphate buffer (pH 6.9) was added as a substrate to start the reaction. The reaction was carried at 37^0 C for 10 min. and terminated by the addition of 200 µl of DNS reagent (1% 3,5 Dinitro salicylic acid sand 12% sodium potassium tartarate in 0.4M NaOH). α-amylase activity was determined by measuring absorbance at 540 nm. IR=$\{ 1- (A_i - A_{ib})/A_o - A_{oB})\}$ X 100%; A_i is the A_{540} of the sample reactive solution, A_o is control the A_{540} of control reactive solution, A_{iB} is the blank of sample and A_{oB} is the blank of control[17].

In vitro 5-Lipoxygenase inhibition: 5-LOX enzyme inhibitory activity of Caralluma was measured using the method[15,16]. The assay mixture contains 80 mM linoleic acid and 10 µl potato 5-LOX in 50 mM phosphate buffer

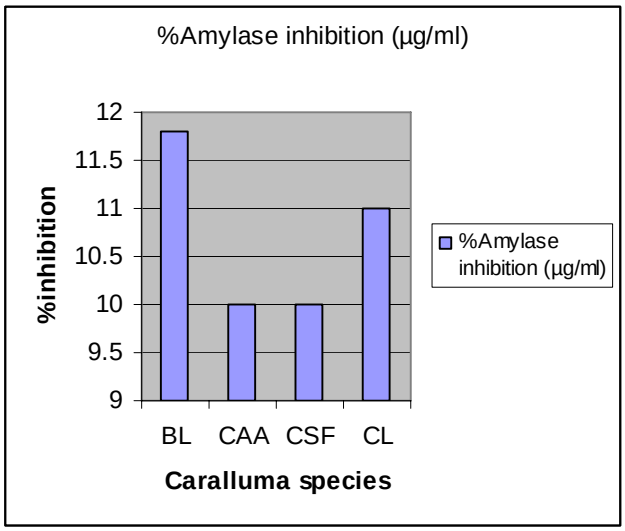

Fig 3: Anti obesity activity of Caralluma species
* Values are mean of triplicate.

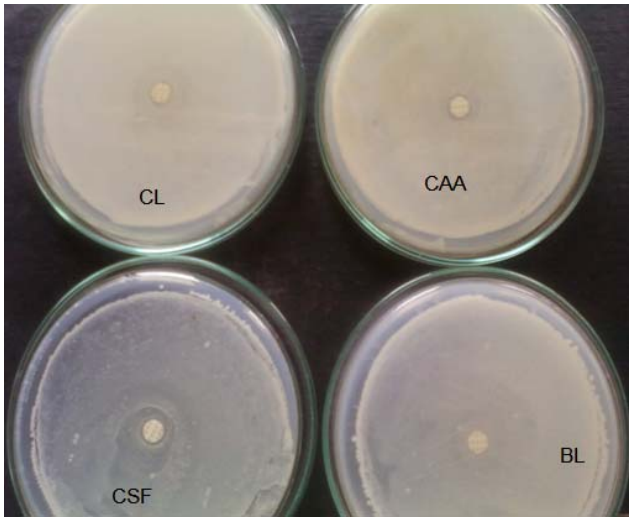

Plate 2: Anti microbial activity of methanolic extracts of Caralluma species
against *Bacillus subtilis*

(pH 6.3). The reaction was initiated by the addition of enzyme buffer mix to linoleic acid and the enzyme activity was monitored as increase in absorbance at 234 nm. The reaction was monitered for 120 sec and the inhibitory potential of test substances was measured by incubating various concentrations of test substances for two minutes before addition of linoleic acid. All assays were performed in triplicate. Percentage inhibition was calculated by comparing slope of test substances with that of enzyme activity.

Screening of Antimicrobial activity: The antimicrobial screening was evaluated against *Bacillus subtilus*. The antimicrobial assay was performed by agar disc diffusion method[18]. The molten nutrient agar was inoculated with 100 µl of the inoculum (1 x 10^8 cfu/ml) and poured into the Petri plate, the disc (0.7cm) (Hi-Media), was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated over 37^0 C and microbial growth was determined by measuring the diameter of zone of inhibition. Pure solvents were used as control.

RESULTS AND DISCUSSIONS

BL, CAA, CS and CL, widely available species of Caralluma were showing variations in their morphological characters (Plate 1). Antioxidant refers to any substance that hinders the reaction of a substance with dioxygen or any substance that inhibits free radical reaction. Nowadays Antioxidants have gained more importance on account of their positive effects, as health promoters in the treatment of cardiovascular problems, atherosclerosis, many forms of cancer, the ageing process, etc. many antioxidant compounds which are naturally occurring in plant sources have been identified as free radical scavengers[19]. In present study, invitro antioxidant activity (FRSA) of methanolic extracts of four different species of Caralluma show potential free radical scavenging activities expressed in IC_{50} (µg/ml) 27,36,32,37 respectively. Results revealed that CL has greater antioxidant property when compared to BL, CAA, and CS (Fig.1). These results suggesting that the antioxidant capacity of methanolic extracts of four species of Caralluma is due to the presence of phenolic compounds to a great extent and indicates that phenolic compounds, likely to contribute to the free radical scavenging activity[19].

Phenolic compounds are also known to inhibit plant lipoxygenase in the process of inflammation[20]. Phenolic compounds may block the cascade process of Arachidonic acid metabolism by inhibiting lipoxygenase activity and may serve as scavenger of reactive free radical which is produced during Arachidonic acid metabolism[21,22]. Anti-inflammatory activities of the methanolic extracts of these four species were studied by 5-Lox activity. BL, CAA, CS and CL are showing IC_{50} values 17, 24,11.8,12.5 µg/ml respectively. (Fig. 2)

Many natural resources have been investigated with respect to suppression of glucose production from carbohydrates. α- amylase activity inhibition is considered to be effective to control diabetes and obesity. Therefore effective and nontoxic inhibitors of alpha amylase have been sought. In this study anti-obesity potential of Caralluma species has also been investigated. The results clearly established that α-amylase activity of CS is greater than the remaining three species. However it is clearly shown that the methanolic extracts of BL, CAA, CS, CL, have antiobesity potential (Fig. 3)

Chemical compounds produced biosynthetically that could destroy or usefully suppress the metabolism of pathogenic microbes are referred as antibiotics which are extensively studied in various higher plants in recent times. However to our knowledge a little is known about antimicrobial activity of methanolic extracts of Caralluma species. In present study antimicrobial assays were also investigated (Plate 2; Table 1) against *Bacillus*

subtilus. The results emphasized that CS and CL showed greater antimicrobial potential than CAA, BL.

Therefore the methanolic extracts of Caralluma species show a significant, FRSA, inhibition of 5- Lox, α- amylase and also antimicrobial activities and suggesting that the plant contain potential bioactive compounds. Further bioactive studies and the identification of these compounds in Caralluma species are more useful in studies of immunostimulating effects.

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