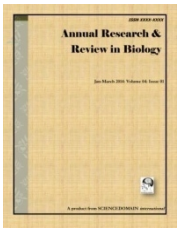

Annual Research & Review in Biology
4(6): 840-855, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of *in Vitro* Antibacterial Activity of *Caralluma umbellata Haw* Used in Traditional Medicine by Indian Tribes

K. Suresh Babu[1*], Sireesha Malladi[2], R. Venkata Nadh[3] and S. Siva Rambabu[4]

[1]Department of Chemistry, Mallareddy Engineering College, Hyderabad, India.
[2]Department of Science and Humanities, Vignan University, Vadlamudi-522213, and Department of Chemistry, JNTU-Anantapur, India.
[3]School of Biotechnology, Vignan University, Vadlamudi-522213, India.
[4]Department of Chemistry, Acharya Nagarjuna University, Guntur-522006, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author SSR prepared the protocol and supervised the work in all its aspects. Authors SM and KSB collected the plant sample and worked in the practical part. Author RVN analyzed the results and written the draft. All authors read and approved the final manuscript.

Original Research Article

Received 14th August 2013
Accepted 17th October 2013
Published 3rd December 2013

ABSTRACT

Aims: To find out a scientific validation for the traditional knowledge of tribals of Chittoor District, India for their usage of *Caralluma umbellata Haw* to cure stomach disorder and pain.
Methodology: Antibacterial activity of *Caralluma umbellata Haw* was studied on a few Gram positive and Gram negative bacteria. The dry roots and stems were extracted using hexane, benzene, diethyl ether, chloroform, acetone and methanol and were tested for their antibacterial activity.
Results: The root extracts were found to be effective against most of the organisms than the stem extracts. The extracts were highly effective against *Bacillus subtillis, Bacillus cereus, Escherichia coli* and *Staphylococcus aureus*. Chloroform extracts of both roots and stems exhibited good antibacterial activity against Gram positive and Gram negative bacteria except *Pseudomonas aeruginosa*.

**Corresponding author: Email: sivarambabu_anu@yahoo.co.in;*

Conclusion: The demonstration of antibacterial activity of *C. umbellata* against Gram positive (*B.subtilis* and *B.cereus*) and Gram negative bacteria (*E.coli*) provides the scientific basis for its use in the traditional treatment of stomach disorder.

Keywords: Asclepiadaceae; Caralluma umbellate; antibacterial activity; stomach disorder; traditional medicine; Indian tribals.

1. INTRODUCTION

1.1 Plants as Sources of Medicine

Plants have been known for their healing potential from primordial times. Throughout the globe, the medicinal usage of plant products is passed from generation to generation and vivid folk systems of medicine have been developed. Microorganisms have developed resistance to many antibiotics as a result of indiscriminate use of antimicrobial drugs in the treatment of infectious diseases. Hence, in the present trend, awareness on traditional medicine is enhanced and natural bioactive molecules have been drawing much attention in order to develop alternate antibiotics from various natural resources including plants. The pharmacological activity of plants can be attributed to secondary metabolites produced by them [1]. According to World Health Organisation [2], a medicinal plant is defined as any plant in which, one or more of its organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Since ancient times, herbal drugs have been used as medicines for the treatment of a range of diseases and many new drugs are provided by plants [3]. Different chemical compounds of plant origin demonstrate antibacterial activity, as they are capable of damage the *bacterial* cytoplasmic membrane [4]. As plants are rich sources of anti microbial agents, screening of local medicinal plants is required [5].

1.2 Medicinal Usage of *Asclepiadaceae* Family

Several plants belonging to *Asclepiadaceae* family are claimed to be useful in specific disease conditions in folkloric medicine as well as in ancient system of medicine like Ayurveda and Unani, which can be attributed to the presence of various compounds like alkaloids, steroids and their glycosides, cardiac glycosides, flavonoids and their glycosides [6]. For centuries, in semi-arid areas of Pakistan and India, *Caralluma* species have been used as emergency foods [7]. The genus *Caralluma* belongs to the family Asclepiadaceae, which comprises 200 genera and 2500 species [8,9] and is found in dry regions of the world [10]. The species of *Caralluma* found in India are edible and form a part of traditional medical system of country [11]. *Caralluma* is also regarded as the synonym of Boucerosia but it differs from Boucerosia by its habit of inflorescence primarily [12]. *Caralluma* has significant anti inflammatory and antitumor activity [10], anticancer, cytoprotective and antiulcer activity [13], antinociceptive [14], antioxidant, hypolipidemic [15], antihyperglycemic [16], treating paralysis and joint pains and antipyretic [17] properties. The presence of pregnane glycosides [14], stogmasterol and other further constituents [18] in *caralluma* species explains a range of biological activities including antimicrobial.

1.3 Sources and Uses of *Caralluma umbellata Haw*

Caralluma umbellata Haw grows wild in dry and arid regions of Chittoor District and several Districts of Andhra Pradesh, in India. It is a thick, erect, leafless, branching, and succulent a

perennial herb [19]. It is medicinally important and rich in pregnane glycosides, which may possess different biological activities [20] including anti-inflammatory activity [19,21]. A significant analgesic was exhibited by Carumbelloside-I, isolated from *C. umbellata* [22]. Previously, the tribal people of Chittoor District, Andhra Pradesh, India used *Caralluma umbellata Haw* stem juice warmed and mixed with turmeric powder for alleviation of stomach disorder and abdominal pains [23-25].

However to the knowledge of authors a little is known about antibacterial activity of *Caralluma umbellata* extracts. Hence, in the present investigation, an effort is made to identify the antibacterial activities of extracts using spectrum of solvents with varying polarities (chloroform, benzene, acetone, methanol, hexane, diethyl ether) against three each Gram positive and Gram negative bacteria. The results are reported in this paper along with assigning the reasons there off.

2. MATERIALS AND METHODS

All chemicals used in this study are Analytical Reagent grade of Merck India Co. Ltd., and purified according to the standard procedures [26]. Bacteria of the present study were provided by Institute of Microbial Technology (IMTECH), Chandigarh.

2.1 Preparation of Extract

Fresh whole plants of *Caralluma umbellata* (Asclepiadaceae) were collected from Tirumala Hills, Tirupati, Chittoor District, Andhra Pradesh, India in December 2010. Dr K. Madhava Chetty (Department of Botany, Sri Venkateswara University, Tirupati, India) identified and voucher specimen of the plant was deposited in Herbarium, Department of Botany, Sri Venkateswara University. Separately, the roots and stems were dried under shade, powdered and sieved through sieve No.14 and stored in air tight containers. The weighed quantity (200g) of dried powdered was subjected to successive solvent extraction method by using Hexane, Diethyl ether, Benzene, Chloroform, Acetone, Methanol in soxhlet extractor. All the extracts were concentrated and last trace of solvent was removed by applying vacuum [27,28]

2.2 Screening of Antibacterial Activity

The anti bacterial screening was evaluated for the root and stem extracts of the *Caralluma umbellata* by agar disc diffusion method [29]. All the extracts at the concentration of 750 µg/ml and 1,000 µg/ml were tested against Gram (+) bacteria such as *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96) and *Bacillus cereus* (MTCC 430) and Gram (–) bacteria such as *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424) and *Proteus vulgaris* (M1CC 142). 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 37ºC and microbial growth was determined by measuring the diameter of zone of inhibition after 24 h pure solvents were used as control and inhibitory zones were almost negligible compared to the inhibition zones of the samples. Chloramphenicol was used as standard drug for the purpose of comparison of antibacterial activities of *C. umbellata* extracts. The antibacterial activities were carried out in triplicate and average values were compiled in Tables 1 and 2 and shown in Figs. 1 to 4.

Table 1. Antibacterial activity of crude extracts of root of *Caralluma umbellata*

Solvent extract	Concentration (μg/ml)	Zone of inhibition (mm)*					
		Gram positive organisms			Gram negative organisms		
		B. subtilis **(MTCC 441)**	***S. aureus*** **(MTCC 96)**	***B. cereus*** **(MTCC 430)**	***E. coli*** **(MTCC 40)**	***P. aeruginosa*** **(MTCC 424)**	***P. vulgaris*** **(M1CC 142)**
Hexane	750	00.0	00.0	00.0	00.0	00.0	00.0
	1000	00.0	00.0	00.0	00.0	00.0	00.0
Diethyl	750	21.2	21.2	00.0	00.0	00.0	00.0
Ether	1000	31.4	48.6	00.0	00.0	00.0	00.0
Benzene	750	21.4	31.4	00.0	00.0	00.0	00.0
	1000	31.6	48.6	12.2	21.2	00.0	00.0
Chloroform	750	56.6	21.2	43.2	48.6	00.0	31.4
	1000	128.7	71.5	97.5	112.2	00.0	56.6
Acetone	750	21.3	31.4	00.0	00.0	00.0	00.0
	1000	42.6	56.5	14.2	00.0	00.0	00.0
Methanol	750	00.0	00.0	00.0	00.0	00.0	00.0
	1000	12.2	00.0	00.0	21.2	00.0	00.0
Chloram-	750	6	11	11	12	4	-
phenicol	1000	11	18	19	20	10	-

** indicates average of triplicate*

Table 2. Antibacterial activity of stem extract of *Caralluma umbellate*

Solvent extract	Concentration (μg/ml)	Zone of inhibition (mm)*					
		Gram positive organisms			Gram negative organisms		
		B. subtilis **(MTCC 441*)***	***S. aureus*** **(MTCC 96)**	***B. cereus*** **(MTCC 430)**	***E. coli*** **(MTCC 40*)***	***P. aeruginosa*** **(MTCC 424*)***	***P. vulgaris*** **(M1CC 142).**
Hexane	750	00.0	00.0	00.0	00.0	00.0	00.0
	1000	00.0	00.0	00.0	00.0	00.0	00.0
Diethyl	750	12.7	12.4	21.2	00.0	00.0	00.0
Ether	1000	22.4	14.6	46.4	00.0	00.0	00.0
Benzene	750	20.2	20.6	00.0	00.0	00.0	00.0
	1000	70.4	41.2	12.0	14.2	00.0	00.0
Chloroform	750	52.2	20.2	00.0	42.4	00.0	12.4
	1000	112.2	64.2	21.2	110.6	00.0	52.6
Acetone	750	25.6	20.9	00.0	00.0	00.0	00.0
	1000	30.2	42.6	14.4	00.0	00.0	00.0
Methanol	750	00.0	00.0	00.0	00.0	00.0	00.0
	1000	00.0	00.0	00.0	00.0	00.0	00.0
Chloram-	750	6	11	11	12	4	-
phenicol	1000	11	18	19	20	10	-

** indicates average of triplicate*

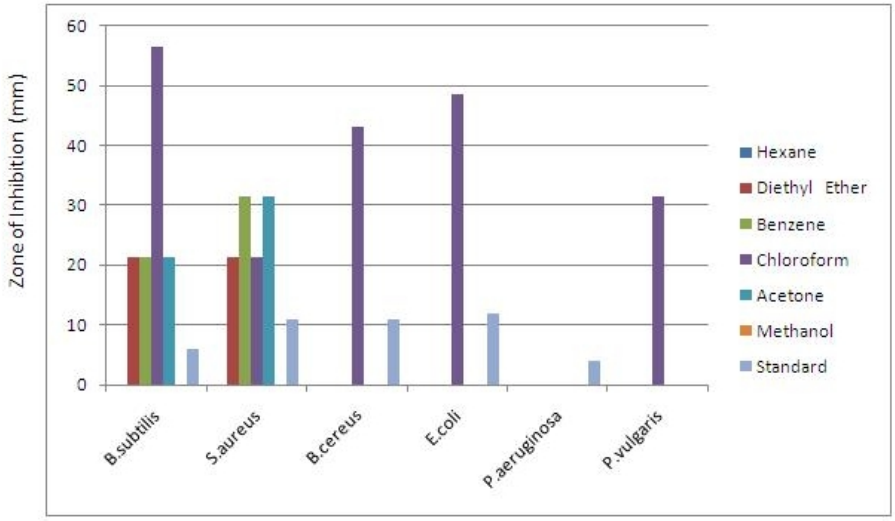

Fig. 1: Inhibition zones of *C. umbellata* root extracts having concentration of 750 µg/ml

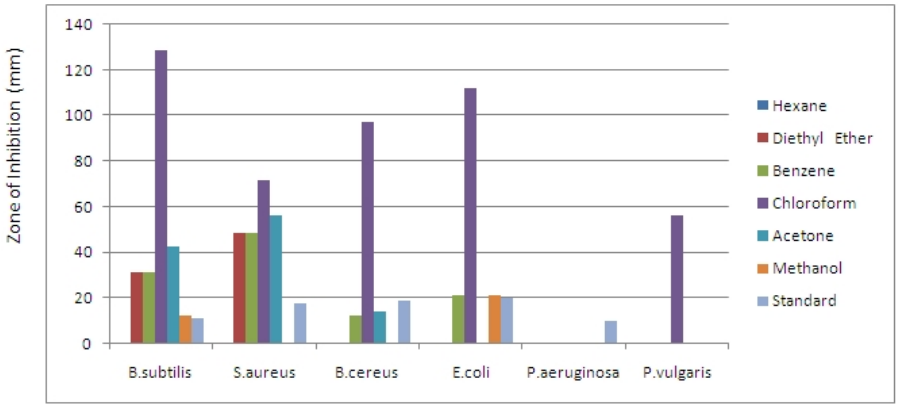

Fig. 2: Inhibition zones of *C. umbellata* root extracts having concentration of 1000 µg/ml

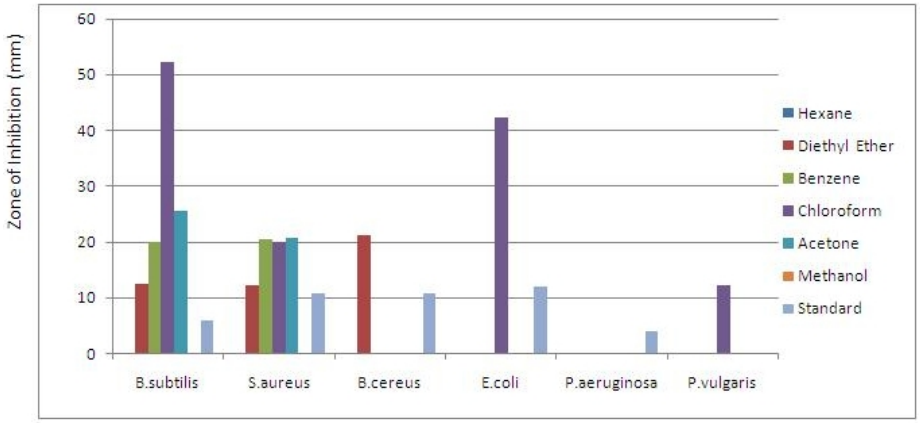

Fig. 3: Inhibition zones of *C. umbellata* stem extracts having concentration of 750 µg/ml

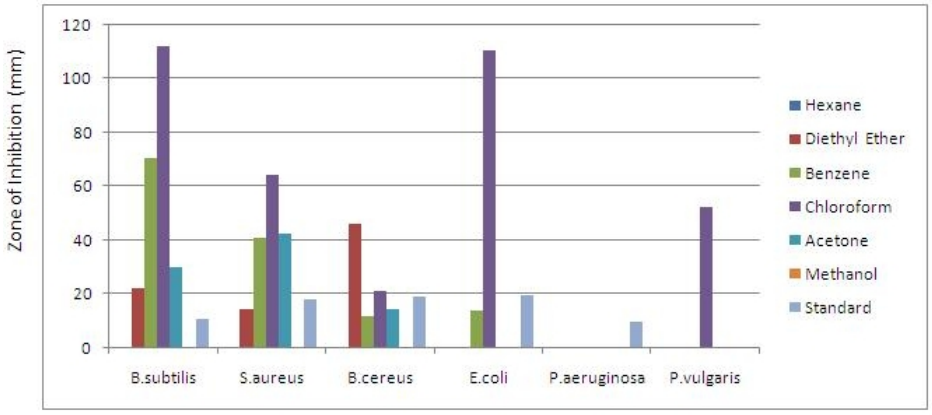

Fig. 4: Inhibition zones of *C. umbellata* stem extracts having concentration of 1000 μg/ml

3. RESULTS

All the extracts obtained from both root and stem by successive solvent extraction method were studied for their antimicrobial activity against Gram positive (*B. subtilis, S. aureus, B. cereus*) and Gram negative (*E. coli, P. aeruginosa, P. vulgaris*) bacteria in two different concentrations (750 and 1000 μg/ml) (Tables 1 and 2). Chloroform extracts of both root and stem were ineffective against *P. aeruginosa*, whereas, against other tested bacteria, root extracts made in chloroform were found to be effective compared to those of stem extracts. Extracts of diethyl ether, benzene, chloroform and acetone from both root and stem were found to be effective against the tested three Gram positive bacteria (*B. subtilis, S. aureus, B. cereus*) and out of the six solvents used for extraction, Chloroform extract showed the highest activity. The order of extract activity for the solvents was Chloroform > Benzene > Acetone > Diethyl Ether. In the case of Gram negative bacteria, only chloroform extracts of root and stem were active against *E. coli* and *P. vulgaris,* and inactive against *P. aeruginosa.* Hexane and methanol extracts were inactive against the tested both Gram positive and negative bacteria. The orders of sensitivity of the tested bacteria against *C. umbellata* extracts obtained from root and stem using different solvents were given in Table-3.

The plant extracts gave red color in Libermann-Burchard test [30] and gave violet color in Molisch test but no response to Shinoda test [31], indicating the presence of steroidal glycosides.

Table 3. Orders of bacteria sensitivity against *C. umbellata* extracts

Solvent used for extraction	Root/ stem	Order of bacteria sensitivity
Chloroform	Root	*B. subtilis* > *E. coli* > *B. cereus* > *S. aureus* > *P. vulgaris*
	Stem	*B. subtilis* > *E. coli* > *S. aureus* > *B. cereus* > *P. vulgaris*
Benzene/Diethyl ether/Acetone	Root	*S. aureus* > *B. subtilis* > *E. coli* > *B. cereus*
	Stem	*B. subtilis* > *S. aureus* > *B. cereus*> *E. coli*

4. DISCUSSION

In addition to undesirable side effects of some of antibiotics [32], there has been increasing incidence of multiple resistances to synthetic antibiotics in human pathogenic microorganisms [33] and hence, researchers are concentrating on the use of plant based drugs in management and treatment of microbial diseases [34]. As folk medicinal usage of *Caralluma umbellata Haw* extract is an indicative of the exhibition antibiotic nature of extracts, it was expected that *C. umbellata* would show activity against pathogenic bacteria. In the present investigation, the crude extracts of roots and stem of *C. umbellata* were screened for better management of microbial infections and multiple resistances in bacteria.

4.1 Differential Antibacterial Activity of Extracts

Chloroform extracts of both root and stem were found to be active against all the bacteria tested except *P. aeruginosa*. The biological activity of extracts depends on chemical composition or active constituents of the plants which in turn depends on season [35], geographical location [36] and time of collection of plant sample [37]. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent [38] which explains the variation in the antimicrobial activities of extracts using different solvents [39-41]. Compounds like C21 steroidal glycosides were reported with polar solvents like ethylacetate, methanol, ethanol and water [6,9,42-47]. Though, Madhuri Vajha et al. [48] reported anti *B.s ubtilis* activities for methanolic extracts of four different species of *Caralluma,* in the present work, the absence of antibacterial activity for methanol extracts indicate that the active ingredient(s) of *C. umbellata* might be extracted in to the earlier used polar solvents like chloroform and acetone.

In the present study, substantial antibacterial activity of chloroform and acetone extracts was observed against *B. subtilis, S. aureus, B. cereus and E. coli*. Similarly, extracts of Diethyl Ether and Benzene inhibited the growth of *B. subtilis* and *S. aureus. B. subtilis* is a food borne pathogen, capable of forming an emetic toxin and cause diarrhoea, abdominal pain, cramps [49]. *B. cereus* is involved in food borne poisoning and cause diarrhoea through production of enterotoxins in the intestine by ingested bacterial cells [50]. *E. coli* causes diarrhoea, traveller's tummy (which include abdominal pain and diarrhoea) and inflammation of the urinary bladder [51]. The current results disclose the usage of *C. umbellata* juice by tribal's for mitigation of stomach disorder and abdominal pains. This conclusion can be further supported by the reports of Kulkarni Aditi et al. [52], who proved that inhibitions shown by n-butanol extract of *Caralluma adscendends* against *E. coli* and *S. aureus* is the basis of its antidiarrhoeal potentiality.

4.2 Phytochemicals of *Caralluma Umbellata* and their Pharmacological Activities

The observed medicinal properties of *Caralluma umbellata* can be attributed to glycosides contained therein. The glycosides contained in *Caralluma* belong to pregnane group of glycosides. The key phytochemical ingredients of *Caralluma umbellata* are steroidal glycosides viz, Carumbelloside I and II [42], Carumbelloside III-V [9] (Figs. 5 and 6). In addition, recently, Suresh Babu et al. [47] reported a pregnane steroid with formyl group (Fig.7.) from the stems of *Caralluma umbellata.* Researchers show a great interest in *Caralluma* as it exhibits an array of immunostimulating activities due to predominant

presence of saponins and flavonoids [53]. Plant metabolites like steroids, steroidal alkaloids and triterpenoids glycosylated with one or more sugar chains come under the category of saponins [54]. Literature survey shows that in Ayurvedic medicine the major bioactive compounds are saponins, flavonoids and polyphenols [55]. Saponins have hemolytic activities. Previous studies on important medical plants reveal that saponins have potential anti-inflammatory [56] and antimicrobial activity [57], in particularly antibacterial activity [58,59]. The defense mechanism of plants can be explained on the basis of release of strong antibiotic compounds by the hydrolysis of saponins, when pathogens attack a plant [60,61]. The anti-microbial activities of even other species of *Caralluma* were attributed to the presence of tannins, flavonoids and sterols [62] as the aqueous extracts of *Caralluma adscendens* were effective against *S. typhi, E. coli* and *Pseudomonas aeruginosa* [63], whereas, petroleum ether extract was effective against *S. aureus and E. coli* [52]. In the current study, the presence of saponins in *C. umbellata* explains the antimicrobial activity in terms of specific interaction of saponins with the cell membrane leading to changes in cell permeability [64]. This is further supported by the fact that saponins are resposible for antimicrobial nature of extracts of different medicinally important plants [57]. Moreover, a stronger hemolytic property can be expected due to the presence of sugar in saponin molecule [65].

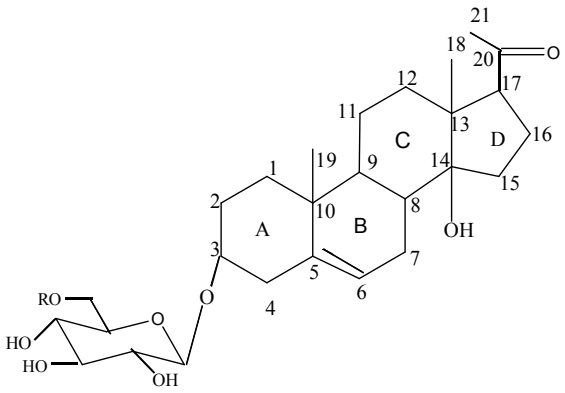

Fig. 5. Carumbelloside I and II
Carumbelloside I R = glu
Carumbelloside II R = H

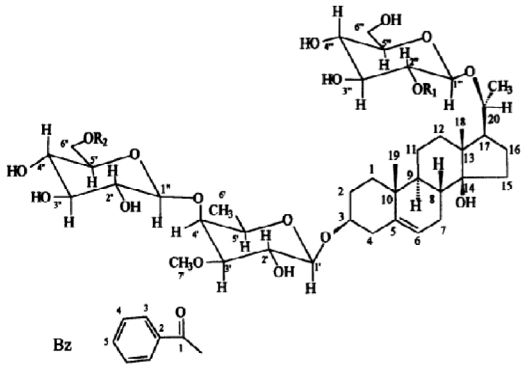

Fig. 6. Carumbelloside III, IV and V
Carumbelloside III $R_1 = R_2 = H$
Carumbelloside IV $R_1 = Bz; R_2 = H$
Carumbelloside V $R_1 = R_2 = Bz$

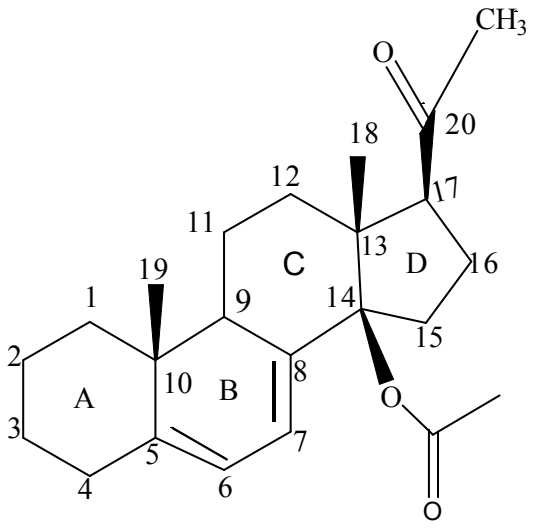

Fig. 7. Pregnane steroid with formyl group

4.3 Antibacterial Nature of Steroidal Glycosides

Sterols are a subgroup of the steroids and also known as steroid alcohols. Sterols form complexes with primarily phospholipids of the membrane and hence sterols are incorporated into biological membrane. Variation of epimers reactivities of sterols can be explained based on the different conformations of the 3-hydroxyl group. Due to low shielding of equatorial 3-hydroxyl groups compared to axial groups, esters of equatorial hydroxyl groups are readily hydrolysed and hence high reactivity in certain cases is observed. Stable complexes are formed by epimers having equatorial hydroxyl groups as they are readily sorbed on many carriers [66,67] due to hydrogen bonding [68].

4.4 Antibacterial Nature of Flavonoids and Flavone Glycosides

Phenols and their derivatives are some of the well-known aromatic compounds that are synthesized by most of the traditional medicinal plants [69]. In the present study, the antibacterial activity can be explained in terms of presence of active components in *C. umbellata* extracts. Literature survey shows that the main constituents of medicinal plants, such as saponins, flavonoids, and polyphenols are known to be major bioactive compounds in Ayurvedic medicine [55]. Flavonoids, flavones and flavonols are known to be synthesized by plants in response to microbial infection [70] and hence, it is expected that the produced components exhibit antimicrobial activity against a spectrum of microorganisms [70]. Flavonoids are phenolic compounds consisting one carbonyl group. Irreversible complexes are formed between phenolic compounds and extracellular/soluble proteins [71]. Such complexation inhibits the protein synthesis in bacteria cells [72] and hence exhibit antibacterial activity [3,73]. Moreover, microbial membranes may be disrupted by lipophilic flavonoids [74]. In addition, a flavonoid without hydroxyl group (on b-ring) targets the membrane having hydroxyl groups [75]. The relative toxicity of flavonoids towards microorganisms depends on the site(s) as well as number of hydroxyl groups on the molecule. The increased hydroxylation results in increased toxicity [69]. At physiological pH, in aqueous solutions certain flavonoids produce hydrogen peroxide via a superoxide intermediate [76,77].

The presence of a known flavone glycoside (Luteoline-4-O-neohesperiodoside) in *C. umbellata* (Fig. 8) explains [43] the both results of present study (1) effective antibacterial activity against diarrhoea causing bacteria (viz., *S. aureus*, *B. cereus* and *E. coli*) and (2) good and poor antibacterial activities against Gram positive and Gram negative bacteria respectively. Flavonoids presence imparts antibacterial activity to the plant extracts [3,73] . The exhibition of antibacterial activity by flavonoids can be explained on the basis of complex formation between the carbonyl group of flavonoid and extracellular and soluble protein [71]. In particular, (+)-catechin, the monomeric flavan sub-unit links with the lipopolysaccharide existing on the bacterial cell surface [78] and such polyphenols of plant origin exhibit vivid biological activities including anti bacterial and anti inflammatory [79].

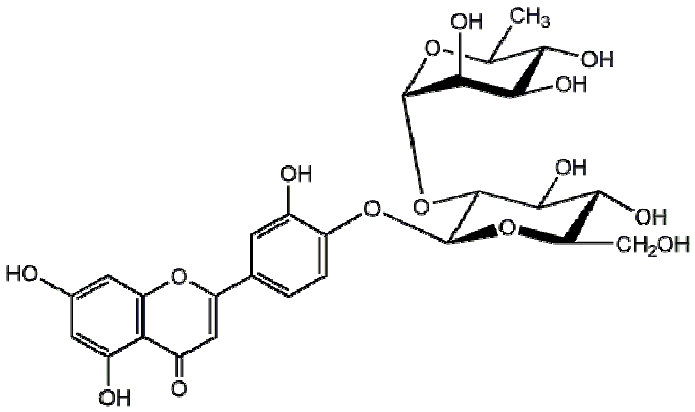

Fig. 8. Flavone glycoside (Luteoline-4-O-neohesperiodoside)

Flavone glycoside contribution to antibacterial activity in the present study is akin to the earlier reports suggesting that tea flavonoids exhibit remarkable activity against food-borne pathogenic Gram positive bacteria in comparison with Gram-negative bacteria [80,81]. Glycosylated flavonoids interfere with the polyphenols, thus playing a vital role in enzyme inhibition and protein precipitation of microorganisms [82,83]. A majority of phosphor kinases are inhibited by flavonoids and interaction with ATP binding site explains the inhibition of ATP [84]. The preferable activity shown against Gram positive bacteria by *C. umbellata* extracts might be due to selective inhibition of topoisomerases by glycosylated flavonoids which results in hampering of replication and transcription mechanics [85].

E.coli causes inflammation of the urinary bladder [51]. Excellent antimicrobial activity against *E.coli* by the chloroform extracts of *C. umbellata* as noticed in the present investigation helps to envisage its potent nature to treat the inflammation of the urinary bladder due to the presence of flavone glycoside in *C. umbellata* [43] , as potent anti-inflammatory activity by flavones glycoside was reported by Ramesh et al. [6].

4.5 Differential Antimicrobial Activity against Gram (+) and Gram (–) Bateria

One more observation in the present study is the exhibition of good activity against all the studied Gram positive bacteria and very poor activity against Gram negative bacteria (except *E. coli*) by the extracts of *C. umbellata,* which can be explained in terms of specificity of saponins towards Gram positive bacteria. Tava and Avato [65] also reported that saponins of *Medicago* species displayed good efficacy against Gram positive bacteria (viz., *S. aureus*,

B. subtilis and *B. cereus*) and ineffective against Gram negative bacteria. The good and poor antibacterial activities of plant extracts against Gram positive and Gram negative bacteria can be explained based on their cell outer layers. Gram positive bacteria have an ineffective and permeable outer barrier made of peptidoglycan layer, which is responsible for permeability of drug constituents. However, Gram negative bacteria have an impermeable outer membrane to drug constituents, as cell wall contains multilayered peptidoglycan and phospholipidic [86].

5. CONCLUSION

The demonstration of antibacterial activity of *C. umbellata* against Gram positive (*B. subtilis,* and *B. cereus*) and Gram negative bacteria (*E. coli*) provides the scientific basis for its use in the traditional treatment of stomach disorder. Further studies are required to establish the exact mechanism of antibacterial activity of phytochemicals extracted from *C. umbellata* so that better and safer chemotherapeutic agents can be developed from this plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hartmann T. The lost origin of chemical ecology in the late 19th century. Proc Natl Acad Sci. USA. 2008;105(12):4541-6.
2. World Health Organization. Guidelines for the assessment of herbal medicines. Programme on traditional medicines. WHO. Geneva; 1991.
3. Bruneton Jean. Pharmacognosy, Phytochemistry, Medicinal plants. 2nd edition. France Lavoisier publishing; 1999.
4. Deininger R. Neves aus der Terpenf or schung. Excerpta phytotherapeutika. Lectures of the Medical Congress. Berlin: Firma Klosterfrau, Koln, 1984.
5. Patel NB, Patel KC. Molecular Docking Study On Anisomeles Indica Linn. Wild Ethnomedicinal Plant for Therapeutic Purpose. Life Sci Leaflets. 2012;11:33-9.
6. Ramesh MY, Appa Rao AVN, Prabhakar MC, Murlidhar N, Seshagiri Rao C, Madhava Reddy B. Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuata*. J Ethnopharmcol. 1998;62:63-6.
7. Atal CK, Sharma BM, Bhatia AK. Search of emergency foods through wild flora of Jamu and Kashmir state. Sunderbani area– 1. The Indian Forester. 1980;106:211–19.
8. Rajendra Ramaswamy, Kamala, 2004; USP filed – 4: 6376657.
9. Qiu SY, Lin LZ, Cordell GA, Ramesh M, Ravi Kumar B, Radhakrishna M, et al. Acylated C-21 steroidal Bisdesmosidic glycosides from *Caralluma umbellata*. Phytochem. 1997;46:333-40.
10. Zakaria MNM, Islam MW, Radhakrishnan R, et al. Antinociceptive and anti-inflammatory properties of *Caralluma arabica.* J Ethnopharmacol. 2001;76:155–8.
11. AL-Yaha MA, Abdel-Sattar E. Pregnane Glycoside from *Caralluma russeliana*. J Nat Pro Chemical constitue. 2000;63(45):1451-53.
12. Stewart RR, Eds Nasir E, Ali S. Annolated Catalogue of Vascular Plants of West Pakistan and Kashmir; Fakhri Print Press, Karachi; 1972.
13. Zakaria MNM, Islam MW, Radhakrishnan, et al. Antigastric ulcer and cytoprotective properties of *Caralluma arabica*. Pharm Biol. 2002;40:225–30.

14. Abdel-Sattar E, Ahmed AA, Hegazy MEF, Farag MA, Al-Yahya MA. Acylated pregnane glycosides from *Caralluma russeliana*. Phytochem. 2007;68:1459–63.
15. Tatiya AU, Kulkarni AS, Surana SJ, Bari ND. Anti oxidant and hypoglycemic effect of *Caralluma adscendens Roxb*. in Alloxanized diabetic rats. Int J Pharmacol. 2010;6(4):362-68.
16. Venktesh S, Reddy GD, Reddy BM, Ramesh M. Appa Rao AVN. Anti hyper glycemic activity of *Caralluma attenuate*. Fitotherapia. 2003;74:274-79.
17. Khan SW, Khatoon S. Ethnobotanical Studies on some Useful Herbs of Haramosh and Bugrote Valleys in Gilgit. Northern Areas of Pakistan. Pak J Bot. 2008;40:43-58.
18. Bader A, Braca A, De Tommasi N, Morelli I. Further constituents from *Caralluma negevensis*. Phytochem. 2003;62:1277–81.
19. Qiu SY, Cordell GA, Ravi Kumar B, Nageswara Rao Y, Ramesh M, Kokate C, et al. Bisdesmosidic Pregnane glycosides from *Caralluma Lasiantha*. Phytochem. 1999;50:485-91.
20. Anitha K, Jayalakshmi G, Siva Rambabu S, Kiranmayee P. Antibacterial effect of *Caralluma attenuata Wt*. on Gram positive and Gram negative bacteria. Int Cong Chem Environ Sci. 2005;546-547.
21. Ray S, Nagaiah K, Khan NF. Anti-inflammatory activity of Carumbelloside-III, isolated from *Caralluma umbellata*. NSHM J Pham Health Mgt. 2011;2:83-8.
22. Sawant BM, Sayad TD. n-Pentatriacontane from *Caralluma fimbriata*. J Shivaji Univ Sci. 1978;18:87-91.
23. Vedavathy S, Mridula V, Sudhakar A. Tribal Medicine of Chittoor District of Andhra Pradesh, India, I edition, Herbal Folklore Research Centre; Tirupati; 1997.
24. Pullaiah T. Encyclopedia of world medicinal plants. Regency Publications New Delhi India. 2006;2:437-9.
25. Basavaraju R, Vennel Raj J, Bhiravamurthy PV, Medicinal Plant Resources of Puttaparthi Mandal, Taxonomic Overview and Need for Conservation. Ethnobotanical Leaflets. 2009;13:1382-1400.
26. Vogel AI, A Text Book of Practical Organic Chemistry. 3rd edition. London ELBS. 1971;46.
27. Trease and Evans Pharmacognosy. 15th edition. U.K. W.B. Company. 2002;135.
28. Gupta AK. Introduction to Pharmaceutics-1. New Delhi. CBS publication. 2004;145.
29. Benson HJ. Microbiological applications. 5th ed. USA. WMC Brown Publications. 1990.
30. Jefrey B. Harborne. Phytochemical Methods - A Guide to Modern Techniques of Plant Analysis. 3rd edition. London. Chapman & Hall. 1998;129.
31. Krishnaswamy NR. Chemistry of Natural Products: A Laboratory Handbook. 1st Edition. Hyderabad. Universities Press,India, Private Limited. 2003;48.
32. Marchese A, Shito GC. Resistance pattern of lower respiratory tract pathogens of Europe. Int J Antimicrobl Agent. 2001;16:25-29.
33. Aliero AA, Afolayan AJ. Antimicrobial activity of *Solanum tomentosum*. African J Biotech. 2006;5(4):369-2.
34. John D. One hundred useful raw drugs of the Kani tribes of Trivandrum forest division, Kerala. Int J Crude Drug Res. 1984;22:17–39.
35. World Health Organization. Traditional medicine. Fact sheet Number 134, Revised May, 2003.
36. Adoum OA, Akinniyi JA, Omar T. The effect of geographical location on the antimicrobial activities and trace element concentrations in the root of *Calotropis procera (Ait.) R. Br*, Annals of Borno. 1997;13(14):199-207.
37. Odugbemi T. A textbook of medicinal plants from Nigeria. University of Lagos Press. Nigeria. 2008;20-323.

38. Marjorie MC. Plants products as antimicrobial agents. Clin Microbial Rev. 1999;12:564-82.
39. Kawo AH, Water purification potentials and in-vivo toxicity evaluation of the aqueous and petroleum-ether extracts of *Calotropis procera (Ait.F.) Ait.F.* latex and *Moringa oleifera LAM* seed powder. PhD thesis. Microbiology Unit, Department of Biological Sciences, Bayero University, Kano, Nigeria. 2007;184.
40. Kawo AH, Mustapha A, Abdullahi BA, Rogo LD, Gaiya ZA, Kumurya AS. Phytochemical properties and antibacterial activities of the leaf and latex extracts of *Calotropis procera (Ait.F.). Bayero.* J Pure Appl Sci. 2009;2(1):34-40.
41. Yusha'u M, Bukar A, Balarabe AI. Prevalence and sensitivity of enterobacterial isolates from patients with urinary tracts infections to *Acalypha wilkisenia* extracts. Biol Environ Sci J Trop. 2008;5(3):72-76.
42. Lin LJ, Lin LZ, Gil RR, Cordell GA, Ramesh MB, Appa Rao AVN. Pregnane glycoside from *C. umbellate* Phytochem. 1994;35(6):1549-53.
43. Ramesh M, Nageswara Rao Y, Rama Kumar M, Krishna Mohan G, Ravi Kumar B, Appa Rao AVN, et al. Flavone glycosides from three *Caralluma* species. Bioche Syst Eco. 1999;27:85-86.
44. Ramesh M, Nageshwar Rao Y, Kumar MR, Venkata Narasimha Appa Rao A, Prabhakar MC, Reddy BM. Antinociceptive and anti-inflammatory activity of carumbelloside-I isolated from *Caralluma umbellata*. J Ethnopharmacol. 1999b;68:349–52.
45. Ramesh M, Ravi Kumar B, Chandra K, Venkatesdh S, Appa Rao AVN. C21 Pregnane Steroid from *Caralluma umbellata*. Nat Prod Sci. 2005;11(2):115-17.
46. Krishna Mohan G, Reddy BM, Nageswara Rao Y, Srinivas B, Sunil Thomas N, Appa Rao AVN. Acylated C-21 steroidal bisdesmosidic glycosides from *Caralluma umbellata*. Phytochem. 1997;46(2):333-340.
47. Suresh Babu K, Subba Rao R, Radhakrishnan Sriveda VV, Madhusudana Rao J, Siva Rambabu S, A new pregnane steroid from the stems of *Caralluma umbellata'*, J Asian Nat Prod Res. 2008;10(11):1013 -16.
48. Madhuri V, Amrutha VA, Murthy KSR. Evaluation of Immuno stimulating Activities of *Caralluma spp*. Int J Pharmacognosy and Phytochem Res. 2010;2(4):1-4.
49. Bennett R. The 'Direct Costs' of Livestock Disease: The Development of a System of Models for the Analysis of 30 Endemic Livestock Diseases in Great Britain. J Agri Eco. 2003;54(1):55-71.
50. Granum PE. Bacillus cereus in Doyle, M. P. e. a. (Ed), Food Microbiology; 2001.
51. Peter Josling. Stabilised Allicin, Power, Performance, Proof. 1st edition. HRC Publications, 2010.
52. Kulkarni A, Mute Vaishali SM, Dhamane Suchita P, Gadekar AS. Evaluation of Antibacterial Activity of *Caralluma adscendens Roxb*Stem. Int Res J Pharm. 2012;3(9):269-70.
53. Kamil MA, Fjayaraj F, Ahmad C, Gunasekhar S. Samuel Chan K, et al. Identification and quantification of flavonoids from *Caralluma arabica* and its quality control studies. J Pharm Pharmacol. 1999;51:225-9.
54. Dewick PM. Medicinal Natural Products – A Biosynthetic approach. Wiley, New-York, 2001.
55. Andrzej LD, Wianowska D, Barbara. The antioxidant properties of alcoholic extracts from *Sambucus nigra* L. LWT- Food Sci Technol. 2006;39(3):308-15
56. Tanaka O, Tamura Y, Masuda H, Mizutani K. Application of saponins in food and cosmetics. Saponins of Mohave yucca and *Sapindus mukurossi* In Waller, GR, Yamasaki, K eds., Saponins used in food and Agricultural, Plenum Press, New York. 1996;1-11.

57. Ali Y, Ahmet M, Ayse AK, Antioxidant and antimicrobial activities of *Polygonum cognatum Meissn* extracts. J Sci Food Agric. 2000;83:64−69.
58. Gronhaug TE, Glaeserud S, Skogsrud M, Ballo N, Bah S, Diallo D, Paulsen BS. Ethnopharmacological survey of six medicinal plants from Mali, West Afr J Ethnobiol Ethnomed. 2008;4(26):1186-1746.
59. Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhan P, Padmanabhan N, Krishman MRV. Phytochemicals investigation on a tropical plant, Syzgium cumini from Kattuppalayam, Erode District. Tamil Nadu, South India. Pak J Nutr. 2009;8(1):83-85.
60. Osbourn A. Saponins and plant defense - a soap story. Trends in Plant Sci. 1996;1:4-9.
61. Osbourn A, Saponins in cereals. Phytochem. 2003;62:1-4.
62. Harish H, Ravikumar A, Ramkrishana R. Phytochemical and antibacterial properties of *Calotropis igantea*, Int J Pharmacol Bio Sci. 2007;1(1):75-7
63. Naik JB, Jadge DR. Anti-bacterial and anti-fungal activity of *Actiniopteris radiata* and *Caralluma adscendens*. Int J PharmTech Res. 2010;2(3):1751-53.
64. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol. 1966;45(4): 493-6
65. Tava A, Avato P. Chemistry and biological activity of triterpene saponins from *Medicago* species. Nat Prod Commun. 2006;1:1159-80.
66. Fieser LF, Fieser M. "Reagents for Organic Synthesis" (Translated into Russian), Izd.Mir, Moscow. 1971;5:107.
67. Gibbons GF, Mitropoulos KA, Myant NB. Biochemistry of Cholesterol. Elsevier, New York,1982;63.
68. McHais I, Macek K. "Papirova Chromatografie" (Translated into Russian), Izd.Inostr.Lit. Moscow. 1962;336.
69. Geissman TA. Flavonoid compounds, tannins, lignins and related compounds. In: Florkin M, Stotz EH (eds). Pyrrole Pigments, Isoprenoid Compounds and Phenolic Plant Constituents, New York: Elsevier. 1963;9:2653.
70. Dixon RA, Dey PM. Lamb CJ. Phytoalexins: enzymology and molecular biology. Adv Enzymol. 1983;55:1–69.
71. Cowan MM, Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12:564-582.
72. Scalbert A. Antimicrobial properties of tannins. Phytochem. 1991;30:3875-83.
73. Tharan NT, Vaidvu R, Palanisamy M, Justin V. Antibacterial activity of Evolvulus alsinoides, Indian Drugs. 2003;40(10):585-86.
74. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, et al. Comparative study on the antibacterial activity of phytochemical flavonones against methicillin-resistant Staphylococcus aureus. J Ethnopharmacol. 1996;50:27–34.
75. Chaurasia SC, Vyas KK. In vitro effect of some volatile oil against Phytophtora parasitica var. piperina. J Res Indian Med Yoga Homeopath. 1997;1:24–6.
76. Miura YH, Tomita I, Watanabe T, Hirayama T, Fukui S .Active oxygen generation by flavonoids. Biol Pharm Bull. 1998;21:93–96.
77. Long LH, Clement MV, Halliwell B .Artifacts in cell culture: Rapid generation of hydrogen peroxide on addition of (−)-epigallocatechin, (−)-epigallocatechin gallate, (+)-catechin, and quercetin to commonly used cell culture media. Biochem Biophys Res Commun. 2000;273:50–53.
78. Delehanty JB, Johnson BJ, Hickey TE, Pons T, Ligler FS. Plant Proanthocyanidins Bind to and Neutralize Bacterial Lipopolysaccharides. Nrl Review. 2008;101-07.

79. Matsui T, Tanaka T, Amura S, Toshima A, Miyata Y, Tanaka K, et al. Alpha-glucosidase inhibitory profile of catechins and the aflavins. J Agri Food Chem. 2007;55:99-105.
80. Ikigai HT, Nakae Hara Y, Shimamura T. Bactericidal catechins damage the lipid bilayer. Biochim Biophys Acta. 1993;1147:132–36.
81. Toda MS, Okubo Hiyoshi R, Shimamura T. The bactericidal activity of tea and coffee. Lett Appl Microbiol. 1989;8:123–5.
82. Naz S, Siddiqi R, Ahmad S. Rasool S, Sayeed S. Antibacterial activity directed isolation of compounds from *Punica granatum*. J Food Sci. 2007;72:341–45.
83. Fan W, Chi Y, Zhang S. The use of a tea poly phenol dips to extend the shelf life of silver carp during storage of ice. Food Chem. 2008;108:148–53.
84. David Newman J, Natural Products as Leads to Potential Drugs: An Old Process or the New Hope for Drug Discovery. J Med Chem. 2008;51:2589–99.
85. Winter J, Lillian H, Moore VR, Dowell JR, Bokkenheuser VD. C-ring cleavage of flavonoids by human intestinal bacteria. Appl Environ Microbiol. 1989;55:1203–8.
86. Ravikumar S, Syed Ali M, Ramu A, Ferosekhan M. Antibacterial Activity of Chosen Mangrove Plants Against Bacterial Specified Pathogens, World Appl Sci J. 2011;14(8):1198-1202.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=365&id=32&aid=2630