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ORIGINAL RESEARCH ARTICLE

Inhibitory Efficacy of *Croton gibsonianus* Nimm. Grah (Euphorbiaceae) Against Oral Isolates of *Streptococcus mutans*

Swathi D, Suchitha Y, Venugopal TM, Prashith Kekuda TR, Mallikarjun N*

Dept. of PG Studies and Research in Microbiology, Sahyadri Science College (Autonomous), Shivamogga-577203, Karnataka, India

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ABSTRACT

The present research was undertaken to investigate the anticariogenic activity of methanol extract of *Croton gibsonianus* Nimm. Grah (Euphorbiaceae) leaves. The shade dried leaves were powdered and extracted with methanol. The inhibitory efficacy of methanol extract was tested against 24 oral isolates of *Streptococcus mutans* by Agar well diffusion method. The extract caused a dose dependent inhibition of cariogenic isolates. Preliminary phytochemical analysis showed the presence of saponins, glycosides, tannins and terpenoids. The inhibitory efficacy of extract against cariogenic isolates could be due to the presence of these metabolites. In suitable form, the plant could be used to treat dental caries. Further study is needed to identify the bioactive components and their efficacy against cariogenic bacteria.

Key words: *Croton gibsonianus* Nimm. Grah, Anticariogenic activity, Viridans Streptococci, *Streptococcus mutans*, Agar well diffusion

INTRODUCTION

Croton gibsonianus Nimm. Grah is a shrub belonging to the family Euphorbiaceae that grows up to 1.5 to 2 meters in length. It is growing in under story of evergreen forests of Western Ghats. Branchlets are stellately pubescent; leaves opposite, elliptic oblong, glandular-serrate, apex shortly acuminate, base rounded with a pair of glands. Inflorescence is terminal racemes. Flowers are unisexual, male flowers are above and female at the base. Ovary is globose, densely hairy; fruit is tricarpellary and is berry. Flowering and fruiting occur in October to December [1]. In a previous study by Vinayaka *et al*. [2], the methanol extract of leaves was shown to exhibit antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*; antifungal activity against *Aspergillus niger*, *Trichophyton rubrum*, *Candida albicans* and *Chrysophyllum indicum*; and antioxidant activity in terms of scavenging activity against DPPH free radical with IC_{50} of 43.78µg/ml. An extensive literature review carried on the biological activities of *C. gibsonianus* revealed that no work is being carried out on anticariogenic activity of *C. gibsonianus*. Hence, in continuation of our previous work, the present study was undertaken to investigate anticariogenic activity of methanol extract of *C. gibsonianus* leaves against oral isolates of *Streptococcus mutans*.

MATERIALS AND METHODS

Collection and identification of plant material

The plant material was collected in Hulikal Ghat region (alt-2115, lat-13º 44' N to 75º 01' E), Karnataka (Western Ghats region of Southern India) during May 2009 and authenticated by Prof. KG Bhat, Udupi, Karnataka, India. Voucher specimen (KU/AB/KSV/2032) was deposited in the University herbaria at PG Department of Studies and Research in Botany, Shankaraghatta, Karnataka for future reference.

Extraction and phytochemical analysis

The leaves were washed thoroughly, shade dried, powdered and used for extraction. A known quantity of powdered leaf material (500gm) was subjected to soxhlation and exhaustively extracted with methanol (HiMedia, Mumbai) for about 48 hours. The extract was filtered and concentrated in vacuum under reduced pressure and dried in the desiccator [3]. Methanolic extract was subjected to

***Corresponding Author:** Dr. Mallikarjun N, **Email:**dr.mallikarjun2010@rediffmail.com,**Phone No:** +91-944992780

preliminary phytochemical screening to screen secondary metabolites namely alkaloids (Dragendorff's test and Mayer's test), saponins (frothing test and hemolysis test), flavonoids (Shinoda test), glycosides (Salkowski test and Keller-Kiliani test), tannins (ferric chloride test) and Terpenoids (Salkowski test) [4,5].

Anticariogenic activity of methanol extract

The anticariogenic efficacy of methanol extract was tested by Agar-well-diffusion method [6] against 24 oral isolates of *S. mutans* (S-1 to S-24) recovered from dental plaque and saliva samples of dental caries patients. The *S. mutans* isolates were maintained on sterile Brain heart infusion agar (HiMedia, Mumbai) slants. Briefly, 24 hours old Brain heart infusion broth (HiMedia, Mumbai) cultures of *S. mutans* isolates were swabbed uniformly on solidified sterile Brain heart infusion agar plates using sterile cotton swab. Then, wells of 6mm diameter were punched in the inoculated plates with the help of sterile gel puncher and the extract (10 and 20mg/ml of 10% DMSO), Standard (Chloramphenicol, 1mg/ml) and Control (10% DMSO) were added separately into respectively labeled wells. The inoculated plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition formed around the well was measured with a ruler. The experiment was carried in triplicates to get average reading.

RESULTS

The yield of extract was 4.5%. The phytoconstituents detected in the extract is shown in (**Table 1**).

Table 1: Phytoconstituents detected in methanol extract

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's test	-
	Mayer's test	-
Saponins	Frothing test	+
	Hemolysis tst	+
Flavonoids	Shinoda test	-
Glycosides	Salkowski test	+
	Keller-Kiliani test	+
Tannins	Ferric chloride test	+
Sterols	Burchard test	-
Terpenoids	Salkowski test	+

'+' Present; '-' Absent

The anticariogenic activity of methanol extract was investigated against 24 oral isolates of *S. mutans* recovered from dental plaque and saliva samples of dental caries patients. The result of inhibitory activity of extract is shown in (**Table 2**). Results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it as reported as positive and the absence of zone as negative. It was found that the extract caused inhibition of oral bacterial isolates in a dose dependent manner. The diameter of inhibition zone formed was in the range of 1.0 to 1.7cm and 0.8 to 1.4 cm at extract concentration of 20mg/ml and 10mg/ml respectively. Inhibition caused by standard antibiotic was higher than that of methanol extract. DMSO did not cause any inhibition of cariogenic isolates.

Table 2: Anticariogenic activity of methanol extract of *C. gibsonianus*

Bacterial isolates	Zone of inhibition in cm			
	Methanol extract		Standard	DMSO
	20mg/ml	10mg/ml	(1mg/ml)	(10%)
S-1	1.1	0.9	2.4	-
S-2	1.2	1.1	2.6	-
S-3	1.1	0.9	2.3	-
S-4	1.0	0.8	2.2	-
S-5	1.4	1.2	2.6	-
S-6	1.5	1.1	2.8	-
S-7	1.3	1.1	2.4	-
S-8	1.1	0.9	2.1	-
S-9	1.0	0.8	2.3	-
S-10	1.6	1.3	2.9	-
S-11	1.4	1.1	2.6	-
S-12	1.2	1.0	2.3	-
S-13	1.2	1.0	2.4	-
S-14	1.3	1.1	2.4	-
S-15	1.5	1.2	2.7	-
S-16	1.1	1.0	2.3	-
S-17	1.0	0.9	2.1	-
S-18	1.6	1.4	2.6	-
S-19	1.5	1.1	2.7	-
S-20	1.1	1.0	2.3	-
S-21	1.0	0.8	2.1	-
S-22	1.3	1.1	2.6	-
S-23	1.2	0.9	2.5	-
S-24	1.7	1.3	2.9	-

DISCUSSION

Dental caries is the most common infectious diseases in the oral cavity. Approximately 200 to 300 bacterial species colonize human dental plaques, but as noted above only a finite number have been associated with either dental caries or periodontal disease [7]. In the oral microbiota, *Streptococci* are the major group of microorganisms when compared to other genera. The study of the genus *Streptococci* is of clinical significance because of their pathogenic potential particularly in oral science as there is concern about the group called *viridans streptococci*. These streptococci form a significant part of the normal flora of the human oral cavity and are associated with several disease conditions including dental caries, infective endocarditis and septicaemia, as well as purulent infections of oral and other sites. The species most frequently isolated from the oral cavity are *Streptococcus salivarius*, *Streptococcus sanguis*, *Streptococus*

mitis, and *mutans Streptococci* [8,9]. Among the oral bacteria, mutans streptococci have been considered as the major cariogenic bacteria. Mutans streptococci are further divided into seven species namely *Streptococcus mutans*, *S. sobrinus*, *S. downei*, *S. rattus*, *S. cricetus*, *S. ferus*, and *S. macacae*. Among these, *S. mutans* is the major agent of this disease in man, followed by *S. sobrinus*, which has also been implicated in this process. Other species such as *S. cricetus* and *S. rattus* are less frequently isolated from humans as they are mainly related to dental caries in animals. *S. ferus* does not seem to be related to the etiology of dental caries [7, 10,11,12,13].

Several studies have been carried on the inhibitory role of natural compounds/extracts against mutans streptococci. Yanagida *et al.* [14] investigated the inhibitory effects of apple polyphenols on the synthesis of water-insoluble glucans by glucosyltransferases of streptococci of the mutans group and on the sucrose-dependent adherence of the bacterial cells. It was observed that the polyphenols markedly inhibited the activity of enzyme and the strongest inhibitors were condensed tannins. Lim *et al.* [15] reported that the leaf-extract from *Camellia sinensis* had an antimicrobial effect on mutans streptococci. Chung *et al.* [16] isolated macelignan from the methanol extract of *Myristica fragrans*. Macelignan showed potent anticariogenic activity against *S. mutans* and other oral pathogens. The minimum inhibitory concentration of macelignan against *S. mutans* was much lower than those of other natural anticariogenic agents such as sanguinarine, eucalyptol, menthol and others. The inhibitory activity of methanol extract of *Rheum undulatum* root against *S. mutans* and *S. sorbinus* was investigated by Song *et al.* [17]. The dichloromethane fraction showed the most active antibacterial activity. The fraction significantly inhibited the caries-inducing factors of these bacteria. A significant reduction of glycolytic acid production was also observed. The activity of the fraction was related to the presence of anthraquinones, cardiac glycosides, coumarines, sterols/terpenes, and phenolics. Esmaeelin *et al.* [18] investigated anticariogenic effect, in terms of inhibition of growth and acid production, of ethanol and chloroform extracts of *Alcea longipedicellata* against *S. mutans*, *S. salivarious*, *S. sorbinus* and *S. sanguis*. Both the extract were found to be bacteriostatic while malvidin-3,5-diglucoside, isolated from ethanol extract of flowers was found to be the principal constituent for antibacterial activity. In a study, Zheng *et al.* [19] observed inhibitory efficacy of methanol extract of *Aceriphyllum rossii* Engler root and its components aceriphyllic acid A and 3-oxoolean-12-en-27-oic acid against all cariogenic bacteria tested. Aceriphyllic acid A was found to possess faster bacteriostatic activity and the inhibitory action was shown to be membrane disruption leading to killing of bacteria. In our study, dose dependent inhibition of cariogenic isolates was observed. The leaf extract was found to contain secondary metabolites namely saponins, glycosides, tannins and terpenoids. Antibacterial activity of these secondary metabolites from plant extracts has been well documented and the inhibitory efficacy of extracts against cariogenic isolates was linked to the presence of these metabolites by several researchers. In this study also, the inhibitory activity of leaf extract of *C. gibsonianus* could be related to the phytoconstituents.

CONCLUSION

In this study, we report anticariogenic activity of *C. gibsonianus* leaf extract. The extract was shown to possess anticariogenic activity and the inhibitory efficacy could be related to the presence of secondary metabolites. In suitable form, the plant could be used to treat dental caries. Further study is needed to isolate and characterize the bioactive components present in the extract and their efficacy against cariogenic bacteria.

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