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Lack of Antibacterial Activity of *Capsicum annuum* and *Simarouba glauca* against *Streptococcus mutans* and *Streptococcus sobrinus*

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***Capsicum annuum* L. is an edible vegetable crop while *Simarouba glauca* L. is a medicinal plant. The study was taken up to evaluate the antibacterial activity of these extracted plant materials against *Streptococcus mutans* and *Streptococcus sobrinus*, the main dental caries agents. Three plant materials (fruit, seeds and leaves) of *Capsicum annuum* L. var. baydagi dabba and two plant materials (leaves and bark) of *Simarouba glauca* L. were collected in the month of November and February, respectively. The plant materials were cleaned, dried and prepared for extraction. The antibacterial susceptibility testing was performed using disc diffusion method. At maximum concentration of 100 mg/ml, it was observed that both the extracted plant materials did not possess antibacterial activity against *S. mutans* and *S. sobrinus*. The study revealed the lack of antibacterial activity of *C. annuum* and *S. glauca* against *S. mutans* and *S. sobrinus*.**

Keyword: Anticariogenic, Chili, Baydagi dabba, Medicinal plant, Plant extraction.

Dental caries is main oral infectious disease and yet no permanent cure or vaccine is being developed. *Streptococcus mutans* and *Streptococcus sobrinus* are closely related species of the group named mutans streptococci, primarily associated with dental caries in human beings[1]. However, among the therapeutic agents used, plants have become notable candidates against cariogenic bacteria[2-4]. Moreover, plants are the generous source of medicinal compounds[5].

Capsicum annuum **(**chili) belongs to the family Solanaceae. Chili is one of the spice crops that is considered to be the most important vegetable or spice plant in India and occupies a large area with an annual production of 8.5 lakh tonnes. Chili is generally planted for their organic products, which may be eaten fresh or cooked, utilized as a dried powder, or converted into oleoresins[6,7]. *C. annuum* var. baydagi dabba is one of the best varieties of chilli grown in India mainly

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in the state of Karnataka which is also known as Kashmiri chilli in other states and other parts of the world known as paprika. Chili has also been employed in folk therapy for dropsy, indigestion, diarrhea, asthma, arthritis, muscle cramps, and toothache. The active compounds in *C. annuum* are alkaloids (capsaicin), fatty acids, flavonoids, volatile oil, carotene pigments[8,9]. Studies reported the antibacterial activity of *C. annuum* against gram positive and gram negative bacteria. However, bioautographic test confirmed that the capsaicin was the main antibacterial component[10,11].

Simarouba glauca belongs to the family Simaroubaceae. *S. glauca* is an edible oil tree commonly known as a paradise tree or Laxmi Taru, originally from Florida, Brazil and Bahamas, the first plantation in India was in 1966[12]. *S. glauca* is one of the vital medicinal plants used against dysentery and therefore its bark is also known as dysentery bark. The leaves and bark of *S. glauca* are famous for its diverse pharmacological properties such as anticancer, antihelmenthic, anti-parasitic, anti-dysenteric, haemostatic and antipyretic[13].

The main active chemical group in Simaroubaceae family is called quassinoids. *S. glauca* possess antimicrobial, antifungal, anti-protozoa and insecticidal activity[14-16]. Among the different extracts tested, methanol extract of leaves showed significant antibacterial activities against gram positive and gram negative bacteria[17].

In this background, the aim of the study was to test the antibacterial activity of *C. annuum* (fruit, seeds and leaves) and *S. glauca* (leaves and bark) by using different extraction methods, against caries causative agents (*S. mutans* and *S. sobrinus*).

MATERIALS AND METHODS

Collection and processing of test plant materials

All the plant materials were collected from Bangalore and the botanical identity of the species were authenticated by Dr. Vasundhara M. Professor of the Horticulture Department, University of Agricultural Sciences, Bangalore, India. Three plant materials (fruit, seeds and leaves) of *Capsicum annuum* L. var. baydagi dabba were collected in the month of November, while, two plant materials (leaves and bark) of *Simarouba glauca* L. were collected in the month of February. Fig. 1 depicts the plant materials used. All the plant materials were rinsed with sterile distilled water and kept for oven to dry. The dried plant materials were ground to a fine powder and stored in airtight bottle until further uses.

Extraction of plant materials

Aqueous extraction

Two methods were employed for the aqueous extraction, heating and cooling method. The aqueous heating method was carried out according to the methodology described earlier[11]. Briefly, 100 ml of boiling distilled water was mixed with 10 g each of plant material powder and then boiled for 15 min. Whatman paper No. 2 was used to filter the mixture and then oven evaporated at 50 ºC. The dry extract was then suspended in sterile distilled water.

The aqueous cooling method was carried out following the methodology adopted by Diwan *et al*.[18] with a few modifications. Briefly, 10 g of each plant material powder was mixed with 10 ml of autoclaved distilled water to make a paste and stored at 4 ºC for 24 h. The paste was squeezed by sterile muslin cloth to obtain the extract and then filtered through Whatman paper No. 2. The final extract was evaporated at 50 ºC and dissolved in autoclaved distilled water.

Methanol extraction

Methanol extraction was prepared according to methodology explained by Koffi-Nevry *et al*.[11]] Briefly, 60 ml of methanol (HPLC grade) was added to 10 g each of plant material powder and shaken for 15 min. The supernatant was collected after filtering using Whatman paper No. 2, and mixed with 60 ml methanol and shaken again, the process was repeated thrice. The final extract was filtered through Whatman paper No. 2, and dried at 50 ºC. Sterile distilled water was added to the dried extract.

Extraction by Soxhlet apparatus

Forty gram of each plant material was loaded separately with 600 ml of HPLC grade acetonitrile for *C. annuum* (fruit, seeds and leaves) and HPLC grade methanol for *S. glauca* (leaves and bark). The temperature of the soxhlet apparatus was set at 25 ºC for 30 h. The extracts, then filtered using two sheets of Whatman paper. The rotary vacuum evaporator was used to concentrate the extract and then stored until further uses.

Inoculum preparation

Cultures of *S. mutans* MTCC 497 and *S. sobrinus* ATCC 33478 were used in this study. One colony was picked from 24 h cultured on brain heart infusion (BHI) agar and inoculated into BHI broth[19]. The test tubes were incubated at 37 °C for 24 h. The turbidity of the inoculum was adjusted to match 0.5 McFarland standards (HiMedia, India).

Antibacterial assay

The antibacterial susceptibility assay was determined by disc diffusion method[20]. Briefly, the inoculum was aseptically swabbed on BHI agar using a sterile cotton swab. The test was conducted using concentration up to 100 mg/ml of plant extracts dissolved in 5 % dimethyl sulfoxide and applied to the sterile disc papers (HiMedia, India). Negative control was prepared using relevant solvent. Ampicillin 10 µg/disc (HiMedia, India) was used as a positive control[21]. Impregnated discs of the plant extracts were aseptically placed on the swabbed agar medium and then incubated anaerobically for 24 h at 37 ºC. To confirm the reliability and reproducibility of the results, the test was repeated thrice.

RESULTS AND DISCUSSION

The extracts of all the plant materials of *C. annuum* (fruit, seeds and leaves) and *S. glauca* (Leaves and bark) did not show any antibacterial activity against *S. mutans* and *S. sobrinus* at maximum concentration of 100 mg/ml. This was in contrary to a report demonstrated that the ethyl acetate extract of *C. annuum* has antibacterial activity against *S. mutans* with minimum inhibitory concentration of 2.5 mg/ml[22]. It might be because of the different plant variety employed or the solvent used for the extraction. However, a study reported that methanol and aqueous extracts of *C. annuum* (bell pepper) possesses antibacterial activity against gram-positive and negative-bacteria[9]. Moreover, extract from *C. annuum* var. *annuum*

(a): *Capsicum annuum* Fruit (b):*Capsicum annuum* Leaves (c):*Capsicum annuum* Seeds

(d): *Simarouba glauca* Leaves (e): *Simarouba glauca* Bark

Fig. 1. Plant materials of *Capsicum annuum* and *Simarouba glauca*

fruit, have a potential antibacterial activity against gram-positive and gram-negative bacteria[23,24].

In the present study, acetonitrile solvent was used for the extraction of *C. annuum* (fruit, seeds and leaves) using soxhlet apparatus. That is due to a reported study demonstrated that extraction of *C. annuum* by acetonitrile provide a high quantity of capsaicinoid compound[25]. Furthermore, an earlier study reported that capsaicinoid in *C. annuum* inhibited the growth of *S. mutans*[22].

The methanolic extract of *S. glauca* (leaves and bark) did not exhibit antibacterial activity against the tested organisms. In contrary, the methanolic extract of *S. glauca* leaves showed significant antibacterial activities against gram-positive and gram-negative bacteria[17].

CONCLUSION

Further investigations are warranted to determine the antibacterial activity of *C. annuum* and *S. glauca* against cariogenic bacteria by using different extraction methods and solvents.

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