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A STUDY ON THE ANTIMICROBIAL EFFECT OF *ACMELLA OLERACEAE* AGAINST DENTAL CARIES BACTERIA

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ABSTRACT

This study aimed to discover the antibacterial effect of *Acmella oleraceae* against dental caries bacteria and aware the populace about the importance of using phytomedicines. According to this study, the antibacterial effects of *Acmella oleraceae* was checked against the dental caries bacteria using well diffusion method. Phytochemical screening of *Acmella oleracea* checked with chloroform, ethanol, methanol and water and acetone extracts. Screening tests revealed that this plant is a big source of phytochemical compounds. The phytochemical analysis of *Acmella oleracea* is conducted using Thin Layer Chromatography (TLC).Acetone solvent system of *Acmella oleracea* showed two spots with highest Rf value 0.922. Three compounds were obtained in the chloroform solvent system with highest Rf value of 0.744.

INTRODUCTION: Dental caries is a multifactorial infection whose presence in a population requires extensive study. Recent studies have revealed that periodontal disease is also a statistically significant risk factor for cardiovascular disease. A link between the two diseases is the secretion and systemic appearance in periodontitis of pro-inflammatory cytokines capable of eliciting effects associated with atherosclerosis and coronary heart disease [1]. Caries are initiated by direct demineralization of the enamel of teeth due to lactic acid and other organic acids which accumulate in dental plaque.

Lactic acid bacteria in the plaque produce lactic acid from the fermentation of sugars and other carbohydrates in the diet of the host. *Streptococcus mutans* has most consistently been associated with the initiation of dental caries, but other lactic acid bacteria are probably involved as well. These organisms normally colonize the occlusal fissures and contact points between the teeth and this correlates with the incidence of decay on these surfaces.

Lactobacilli, *Actinomyces* and various proteolytic bacteria are commonly found in human carious dentin and cementum, that they are secondary invaders that contribute to the progression of the lesions [2]. Teeth get decayed due to a combination of causes that include bad oral hygiene, stagnation of food on or around the teeth, presence of plaque on the tooth structure and the presence of caries causing microorganisms [3].

Periodontal disease has long been recognized as a chronic disease, but literature describes it as a disease derived entirely from the effects of a microbial colonization of the gingival crevice. If this were so, it would mean that periodontal disease is unique among chronic diseases, all of which represent the long-term cumulative effects of interaction between a host biologic system and the surrounding environment [4]. The infection of bacteria can prevented using different antibiotics with reliable spectrum of action but some of them have the ability to withstand the effects of these antibiotics. It is a specific type of drug resistance.

Antibiotic resistance evolves naturally via natural selection through random mutation, but it could also be engineered by applying an evolutionary stress on a population. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange. The patterns of antibiotic usage greatly affect the number of resistant organisms which develop. Overuse of broad-spectrum antibiotics, such as second- and third-generation greatly hastens the development of resistance.

Other factors contributing towards resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients [5]. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals. Many of the herbs and spices used by humans to season food yield have useful medicinal compounds including those having antibacterial activity [6]. Plant derived drugs remain an important resource especially in developing countries to combat serious diseases.

MATERIALS AND METHODS:

Selection of Bacterial Strains: Bacterial strains of six different species (*Pseudomonas aeruginosa, Streptococcus salivarius, Streptococcus viridans, Streptococcus mutans, Bacillus megaterium* and *Neisseria catarrhalis*) with enhancing activity in caries formation were selected from Microbial Technology Laboratory, Malankara Catholic College, Mariagiri, Kaliakkivilai, Tamil Nadu.

Collection of Medicinal Plants: The medicinal plant sample was collected from the Maruthuvarmalai region of Western Ghats of Kanyakumari district. The different parts such as root, stem, leaves and inflorescence of *Acmella oleracea* were selected for testing its antibacterial studies and characterization of secondary metabolites of effective ones.

Preparation of Plant Extracts: Plant sample was shade dried and ground well. 10 gram of powdered sample was filled in screw cap bottles with 10 ml of different solvent systems (acetone, ethanol, chloroform, methanol and water). It was kept at 22^{o}C for fifteen days.

Antibacterial Effect Checking of Medicinal Plant Extracts: Antibacterial effect of medicinal plant extracts were checked by Well- diffusion method.

Well Diffusion Method: The bacterial isolates were effectively swabbed on the Mueller-Hinton agar plates. After allowing the inoculum to dry at room temperature, six mm diameter wells were bored on it. The extract was introduced (50 µl of a 100mg/ml concentration) into three duplicate wells. The plates were allowed to stand at room temperature for one hour for the extract to diffuse into the agar and then they were incubated at 37^{o}C for 18 hours. After incubation the plates were observed for the results.

Phytochemical Screening: A preliminary phyto-chemical analysis was conducted for the detection of steroids or terpenoids (Liebermann-Burchard Test), flavonoids (Shinoda's Test), Carbohydrates (Molisch's Test), saponins, tannins and phenolic compounds [7].

Phytochemical Analysis (TLC): Silica gel^{G} slurry 1: 2 (W/V) with thickness of 0.25 mm was prepared on a head glass plate. It was dried for 15 to 30 min followed by hot treatment in an oven at 100^{o}C for one to two hours. The samples were applied at one end (2.5 cm away from ends) of the gel plate with equal distance between them. The plates were dipped in solvent tanks to a depth of 1.5 cm from bottom and allowed to cover the solvent over the top. After that the plates were removed dried and processed for the identification of separated compounds (as colored spots) and the R_f *values* were calculated using the formula;

$$*R_{f=} \frac{\text{Distance (cm) moved by the solute (extract) from the origin}}{\text{Distance (cm) moved by the solvent from the origin}}$$

Where, [Rf= Retention Factor], * R_f - Retention Factor

RESULTS & DISCUSSION:

Antimicrobial effect of *Acmella oleracea* extracts: The antimicrobial activity of *Acmella oleracea* were checked with ethanol, acetone, chloroform, methanol and water extracts against bacterial isolates (*Pseudomonas aeruginosa, Streptococcus salivarius,*

Strptococcus viridans, Strptococcus mutants, Bacillus megaterium, Neisseria catarrhalis) using well diffusion method (**Table 1**). Acetone extract of *Acmella oleracea* showed high activity against *Neisseria catarrhalis* and *Streptococcus mutans (*27mm and 20mm respectively). Whereas its chloroform extract revealed high effect over *Streptococcus viridans* (17mm). Methanol extract showed high activity against *Bacillus megaterium* and *Pseudomonas aeruginosa*. But the water extract did not showed any inhibitory effect over the test organisms.

TABLE.1. DIAMETER OF ZONE OF INHIBITION OF DIFFERENT EXTRACTS OF *ACMELLA OLERACEA* AGAINST BACTERIA

Bacteria	Acetone (mm)	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
Neisseria catarrhalis	27	10	15	16	-
Streptococcus mutans	20	9	9	13	-
Streptococcus salivarius	9	7	9	7	-
Streptococcus viridans	19	17	15	9	-
Bacillus megaterium	14	12	13	15	-
Pseudomonas aeruginosa	13	12	12	18	-

Phytochemical Screening: In *Acmella oleracea,* the test for steroids and terpenoids give positive in chloroform, ethanol, methanol and water extracts and negative in acetone extracts (**Table 2**). The tests for flavonoids were positive in acetone, ethanol and methanol extracts. Test for carbohydrates, phenolic compounds and tannins showed negative response in all the extracts. Whereas, chloroform and water extracts comprised with saponins.

TABLE 2: RESULT OF PHYTOCHEMICAL SCREENING OF *ACMELLA OLERACEAE*

Experiment	Acetone Extract	Chloroform Extract	Ethanol Extract	Methanol Extract	Water Extract
Liebermann –Buchard test for steroids and terpenoids	Absent	Present	Present	Present	Present
Shinodas test for flavanoids	Present	Absent	Present	Present	Absent
Molisch's test for carbohydrates	Absent	Absent	Absent	Absent	Absent
Test for Phenolic compounds	Absent	Absent	Absent	Absent	Absent
Test for saponins	Absent	Present	Absent	Absent	Present
Test for tannins	Absent	Absent	Absent	Absent	Absent

Phytochemical Analysis by Thin Layer Chromatography (TLC): Acetone solvent system of *Acmella oleracea* showed two spots with highest Rf value 0.922. In water, ethanol and methanol solvent system of *Acmella oleracea* each produced two spots. Three compounds were obtained in the chloroform solvent system with highest Rf value of 0.744 (**Table 3**).

TABLE 3: RESULTS OF THIN LAYER CHROMATOGRAPHY OF *ACMELLA OLERACEAE*

Solvent system	No of spots obtained	R_f value
Acetone	Compound 1	0.390
	Compound 2	0.922
water	Compound 1	0.670
	Compound 2	0.790
Ethanol	Compound 1	0.470
	Compound 2	0.722
Chloroform	Compound 1	0.266
	Compound 2	0.670
	Compound 3	0.744
Methanol	Compound 1	0.711
	Compound 2	0.890

The zonation differences produced in each extracts were due to the polarity of solvents which determines the type of reaction and solubility of compounds. Most of the extracts have better extracting capacity which may be attributed to the ability to extract the natural antimicrobial compounds such as alkaloids, flavanoids, terpinoids and phenolic compounds from the plant. The spots on TLC gel plate revealed the presence of numerous secondary metabolites in *Acmella oleraceae*.

CONCLUSION: The results evidenced that *Acmella oleraceae* is capable and best remedy to inhibit the growth of dental caries bacteria with its copious source of secondary metabolites. This discovery leads to development of better treatment with herbal medicines to overcome the side effects caused by antibiotics.

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