IJPSR (2012), Vol. 3, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 August, 2012; received in revised form 07 November, 2012; accepted 24 November, 2012

PHYTOCHEMICAL STUDIES ON *CASUARINA EQUISETIFOLIA* AND INVESTIGATION OF ITS EFFECT AGAINST PATHOGENIC ORAL FLORA

Shafi Thompson T.*[1], Alen Paiva[1], Greeshma G. Mohan[1], Jincy Das [1], Arya Suresh [1], K.K. Dil Baseer Sabith [1], J. Densingh [2]

Department of Biotechnology, Science Campus [1], Nalanchira P.O, Thiruvanathapuram Dist-695 015, Kerala, India
Department of Microbial Technology, Malankara Catholic College [2], Kanyakumari Dist- 629 153, Tamil Nadu, India

Keywords:
Phytochemicals, *Casuarina*, Dental caries, Oral flora

Correspondence to Author:

Shafi Thompson T.

Chief Scientific Officer, Science Campus, Nalanchira, Thiruvananthapuram Dist-695 015, Kerala, India

E-mail: shafithompson@gmail.com

ABSTRACT

This research was targeted to reveal the phytochemical activity of *Casuarina equisetifolia* against susceptible pathogenic oral bacteria. Antibacterial effect of the plant was examined by well diffusion method. Different plant parts were screened for the phytochemicals using various polar solvents like ethanol, acetone, methanol, chloroform and water. Studies disclosed that *Casuarina equisetifolia* encompasses a wide range of phytochemicals with anticariogenic activity. During phytochemical analysis by thin layer chromatography four compounds were obtained in the acetone solvent system with highest R_f value of 0.911.

INTRODUCTION: Dental caries is an infection, predominantly bacterial in origin, which permanently affects the hard organic material of the tooth by acid hydrolysis and demineralization. Tooth ache associated with it is due to the exposure of the nerve of the tooth through the passages of the dentinal tubule. Dental caries can precede increased sensitivity (**Fig. 1**) of the tooth and even life- threatening complications like Ludwig's angina [1].

Worldwide, most children and an estimated ninety per cent of adults have experienced caries. In 1940's the prevalence of dental caries in India was 55.5%, while in 1960's it was reported to be 68%. Recent studies show an incidence ranging from 44-73%. It is the primary pathological cause of tooth loss in children. Conditions in the oral cavity may be indicative of systemic diseases such as osteoporosis, diabetes, or cancer [2]. Numerous studies have also revealed that gum disease is associated with an increased risk of diabetes, cardiac disorder, and preterm birth.

FIG. 1: PROGRESSION OF DENTAL CARIES

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Website:
www.ijpsr.com

Opportunistic oral flora like *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Streptococcus salivarius*, *Streptococcus viridians, Bacillus megaterium, Neisseria catarrhalis, Lactobacillus acidophilus, Actinomyces viscosus* and *Nocardia spp*.[3] are most susceptible for dental caries. Bacteria accumulate around the teeth and gums in an adhesive, creamy-coloured mass called plaque, which serves as a biofilm. Grooves on the occlusal surfaces of molar and premolar teeth provide microscopic retention sites for plaque bacteria. Fermentation of carbohydrates by these bacteria reduces the pH and the demineralisation of the tooth enamel instigate at pH 5.5 [4].

Lack of appropriate oral hygiene, presence of remnant fermentable carbohydrates, presence of pathogens, reduced production of saliva [5], certain medications and excessive use of tobacco [6] are some of the causes of dental caries.

Use of antibiotics is the most prevalent method of treatment of dental caries. Increased use of antibiotics can lead to the formation of the antibiotic resistant mutants. Phytomedicines can prove to be a reliable alterative in this context. Various plant species are ascertained to be effective phytomedicines against the dental caries causing pathogens. *Casuarina equisetifolia* is a dicot angiosperm with immense potential to combat dental caries [7].

Traditionally the decoction formulated from the tannin containing astringent bark of Casuarina used against tooth ache. Routine chewing of the root is also effective to maintain dental hygiene. Being a plant with anticariogenic properties, isolation of phytochemicals from *Casuarina equisetifolia* can prove to be an effective alternative for the side effects causing antibiotic drugs.

MATERIALS AND METHODS:

Selection of Bacterial Strains: Six different species (*Pseudomonas aeruginosa, Streptococcus salivarius, Streptococcus viridans, Streptococcus mutans, Bacillus megaterium, and Neisseria catarrhalis*) of bacterial strains which can enhance the formation of dental caries were collected from Microbial Technology Laboratory, Malankara Catholic College, Mariagiri, Kaliakkivilai, Tamil Nadu.

Collection of Medicinal Plants: The different plant parts such as root, stem, leaves and bark of *Casuarina equisetifolia* (**Fig. 2**) were collected from Maruthuvarmalai region of Western Ghats of Kanyakumari district to study its antibacterial efficiency against the selected strains of microbes and also to isolate secondary metabolites.

FIGURE 2: *CASUARINA EQUISETIFOLIA*

Preparation of Plant Extracts:

Powdered samples were prepared by shade drying and grinding the different plant parts. 10 grams of the samples were procured in screw cap bottles and mixed with solvent systems like acetone, ethanol, chloroform, methanol and water for the extraction of phytochemicals. These screw cap bottles were preserved at 20^{o}C for fifteen days.

Examination of Antibacterial Activity of Plant Extracts: Well diffusion method was employed for analyzing the antibacterial effect of extracts from the medicinal plant.

Well Diffusion Method: The bacterial isolates were effectively swabbed on the prepared Mueller-Hinton agar plates. After allowing the inoculum to dry at room temperature, wells of six mm diameter were bored on the medium. The extract was introduced (50 µl of a 100mg/ml concentration) into the three duplicate wells. The plates were allowed to stand at room temperature for one hour for the extract to diffuse into the agar and further incubated at 37^{o}C for 16-18 hours. After incubation the plates were observed for the results.

Phytochemical Screening: Screening for secondary metabolites like steroids or terpenoids (Liebermann-Burchard Test), flavonoids (Shinoda's Test), Carbohydrates (Molisch's Test), saponins, tannins and phenolic compounds [8] was conducted to reveal the phytochemical properties of the plant.

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 air 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=

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

* R_f - Retention Factor

RESULTS & DISCUSSION:

Antimicrobial effect of *Casuarina equisetifolia* extracts: Acetone extract of *Casuarina equisetifolia* showed inhibitory activity against *Streptococcus mutans* and *Pseudomonas aeruginosa* with 29mm and 26mm respectively. Ethanol extract showed activity against *Streptococcus viridians* (Fig:3) and *Neisseria catarrhalis* with a zone of inhibition of 16mm. Whereas the methanol extract showed activity against *Bacillus megaterium* with a zone of 16mm.Water extract gave activity only against *Streptococcus salivarius* with a zone of 9mm (**Table 1**).

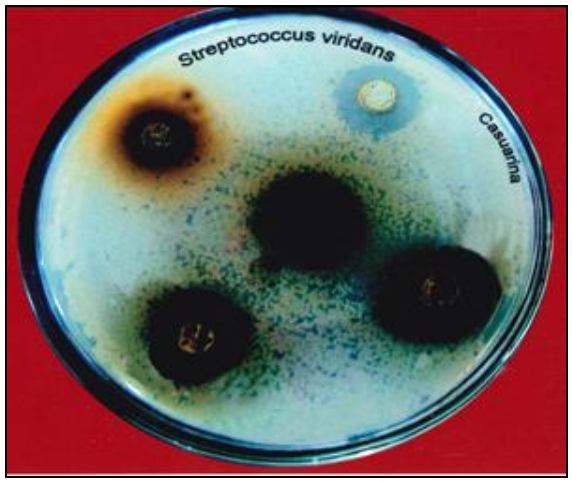

FIG. 3: ZONE OF INHIBITION (WELL DIFFUSION METHOD)

TABLE 1: DIAMETER OF ZONE OF INHIBITION OF DIFFERENT EXTRACTS OF *CASUARINA EQUISETIFOLIA AGAINST* BACTERIA

Bacteria	Acetone (mm)	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
Neisseria catarrhalis	12	9	16	10	-
Streptococcus mutans	29	15	14	11	-
Streptococcus salivarius	10	11	10	9	9
Streptococcus viridans	17	10	16	12	-
Bacillus megaterium	15	9	12	16	-
Pseudomonas aeruginosa	26	12	21	12	-

Phytochemical Screening: The chloroform, methanol and water extract of *Casuarina equiestifolia* revealed the presence of saponins. The test for steroids and terpenoids were positive in acetone, ethanol and methanol extracts. The test for flavonoids gave positive result only in ethanol extract. The test for phenolic compounds was positive in acetone and ethanol extracts. Whereas the test for tannins were positive in acetone and methanol extracts (**Table 2**).

TABLE 2: RESULT OF PHYTOCHEMICAL SCREENING OF *CASUARINA EQUISETIFOLIA*

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

Phytochemical Analysis by Thin Layer Chromatography (TLC): *Casuarina equisetifolia* produced four compounds in acetone solvent system. Compound 4 showed highest R_f value of 0.911 and the compound 1 showed the least R_f value of 0.333. In water solvent system three compounds were obtained with highest R_f value of 0.900. Ethanol solvent system showed two spots with highest R_f value of 0.612. Chloroform solvent system produced two spots with highest R_f value of 0.691. Methanol solvent system gave two compounds with highest R_f value 0.780 (Table 3).

The ill effect of antibiotics includes the formation of multiple drug resistant mutants, emphasizing the necessity of alternative therapeutics. The use of plant compounds to treat infections is an age-old practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics.

TABLE 3: RESULTS OF TLC DONE ON *CASUARINA EQUISETIFOLIA*

Solvent system	No. of spots obtained	R_f value
Acetone	Compound 1	0.333
	Compound 2	0.380
	Compound 3	0.622
	Compound 4	0.911
Water	Compound 1	0.533
	Compound 2	0.800
	Compound 3	0.900
Ethanol	Compound 1	0.333
	Compound 2	0.612
Chloroform	Compound 1	0.366
	Compound 2	0.691
Methanol	Compound 1	0.470
	Compound 2	0.780

CONCLUSION: The research accomplishes that the plant extract from *Casuarina equisetifolia* has antibacterial action against microbes accountable for the tooth caries formation and thus helps avert the caries formation. The treatment involving the phytomedicines obtained from this plant can be employed as an alternative for the antibiotics that generates various side effects.

ACKNOWLEDGEMENTS: The authors are thankful to the authorities of Malankara Catholic College, Mariagiri, Tamilnadu and Science Campus, Nalanchira, Trivandrum for providing facilities and support.

REFERENCES:

1. Hartmann R.W: Ludwig's Angina in Children. American Family Physician. 1999; 60(1), 109-112.
2. Norman O. Harris: Historical Perspectives of Oral Biology: A Series. Critical Reviews in Oral Biology and Medicine. 1990; 1(2), 135-151
3. Hardie J.M: The microbiology of dental caries. Dent Update. 1982; 9(4), 199-208.
4. Dawes C: What is the critical pH and why does a tooth dissolve in acid. Journal of Canadian Dental Association. 2003; 69 (11), 722–724.
5. Allen C., Bouquot J., Damm D., Neville B.W: Oral & Maxillofacial Pathology 2nd edition. 2002; 347,398.
6. DM Winn: Tobacco use and oral disease. Journal of Dental Education. 2001**;** 65(4), 306-312
7. Vaughn H.S., Robinson P.G: The oral health-related experiences, attitudes and behaviours of the carers of Aboriginal children of Groote Eylandt. International Dental Journal. 2003; 53(3), 132-140
8. Fransworth N.R: Biological and Phytochemical screening of plants. J. Pharm. Sci. 1966: 35; 225-276.

How to cite this article:
Thompson ST, Paiva A, Mohan GG, Das J, Suresh A, Sabith KKDB, Densingh J: Phytochemical studies on *Casuarina equisetifolia* and investigation of its effect against pathogenic oral flora. *Int J Pharm Sci Res*. 3(10); 4807-4810.