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Potencial Anticariogenic Activity of Tabernamontana Divaricata

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Abstract :
Background:*Herbal medicine is always appreciated for its unadulterated and pristine nature. However Allopathy is considered as a modality to fix the ailment that too in a very short span of time and hence its very popular.Nevertheless plant based medicines has its own value which cannot be countered by any other.Tribals have an elaborate knowledge regarding this.Adivasis in Wayanad still uses medicinal products from plant for different ailments including oral diseases.In the present study anticariogenic potential of Tabernamontana divaricata(nandyar vattom) is evaluated .Various parts of this plant is used amongst tribals for treating dental diseases*
Materials and Methods: *Agar well diffusion method is used to find the anticariogenic potential of ethyl alchaholic extract of Tabernamontana divaricata leaf against Streptococus mutans and Lactobacillus acidophilus*
Results: *Study showed that Tabernamontana divaricata has significant(p<.001) anticariogenic potential against Streptococus mutans and Lactobacillus acidophilus.*
Conclusion: *Since that Tabernamontana divaricata is found to have substancial anticariogenic nature ,it can be used in products pertaining oral care.Further research is needed with respect to this matter.*
Keywords: *Tabernamontana divaricata,Zone of Inhibition,Anticariogenic potencial.*

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I. Introduction

Many a times we intentionally ignore the use of herbal medicines assuming that allopathy is panacea or sure cure for any ailment.Many plants around us have medicinal properties however we are totally unaware of this fact and are least bothered to know about it.In age old medicinal practise native plant and plant products were extensively used.The details regarding medicinal plants are familiar amongst tribal population of the area

In traditional medicines[1], Chinese [2700 BC]; advocated hygiene, dietetics, hydrotherapy and massage along with medicine to cure ailments.However Egyptians believed that disease is caused by absorption of harmful elements from intestine. Mesopotamian'sused magic and necromancy to cure diseases [2100 BC].Greeks rejected supernatural theory and believed that ailment occurs when equilibrium among humors [earth, air, fire and water] in the body is violated. Indiansgained their medicinal knowledge from Harappan civilization. In Rig-Veda , Indian medicine is elaborately scripted. In the course of time Ayurvedic medicines evolved and sages propagated it. Since that it has high moral grounds it gained rapid and wider acceptance. In Kerala Ayurveda is very popular in main stream community .Nevertheless many Adivasis still rely on tribal medicines in Kerala.

With the acceptance of Allopathic medicine as a miracle cure for ailments ,ethnomedicinal practices are relatively side lined or marginalized[2,3,]. Many a times we fail to acknowledge its authenticity and potential as an unadultered medical practise that is relatively free of side effects.In Waynad a major population in the tribal belt completely rely on their age old wisdom for health and treatment for diseases.[4]. To clean teeth they use small twigs obtained from medicinal plants, shrubs or trees . Simlarily many plant parts are used for oral health care extending from cleaning the tooth to treatment of mucosal lesions,gingivitis and periodontitis[5].Dr Deepa etal[6]noted the usage of Tabernamontana divaricatalatex (nandyar vattom) for dental caries. Though Nandyar vattom is extensively grown as a beautiful garden plant ,not much of research is done to tap its medicinal properties.In this study its anticriogenic potential against common caries causing bacterias like Steprococus .mutans and Lactobacillus.acidophilus is evaluated.

II. Materials and Methods

Institutional ethical Committee no:(IEC/M/15/2018/DCK)

Preparation of leaf extract**:** Maceration of Tabernamontana leaves was done to produce the ethylalchaholic extract.Leaves were shade dried until they are completely dry[7,]these leaves were then ground

to fine powder (100g) and soaked in 100% ethyl alcohol (250ml) for seven days in a sealed conical flask and shook occasionally. The solution was filtered using Whatman's filter paper no1 and the solvent allowed to evaporate completely to obtain the viscous extract. The extract was stored in sterile glass vials at 4 ^{0}C until use.

Antimicrobial activity-(agar- well diffusion method)[8]:Petriplates containing 20ml Muller Hinton Agar Medium are seeded with bacterial culture of Lactobacillus acidophilus (L.acidophilus) and Streptococus mutans(S.mutans)(growth of culture adjusted according to McFarlands Standard0.5%). Wells of approximately 10mm was bored using a well cutter and different concentrations of sample such as 250µg/ml, 500µg/ml, 1000µg/ml were added. The plates are incubated at 37°C for 24 hours. The antibacterial activity was assessed according to the diameter of the inhibition zone (ZOI)formed around the well (NCCLS, 1993). Positive control used in the test is streptomycin.

Statistical Analysis

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released in 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses.

Descriptive Statistics:

Descriptive analysis expression of different study parameters in terms of Mean & SD.

Inferential Statistics:

Independent Student Ttest was used to compare the mean ZOI (in mm) between Extracts and Positive Control for Lacto Bacillus and S. Mutans.

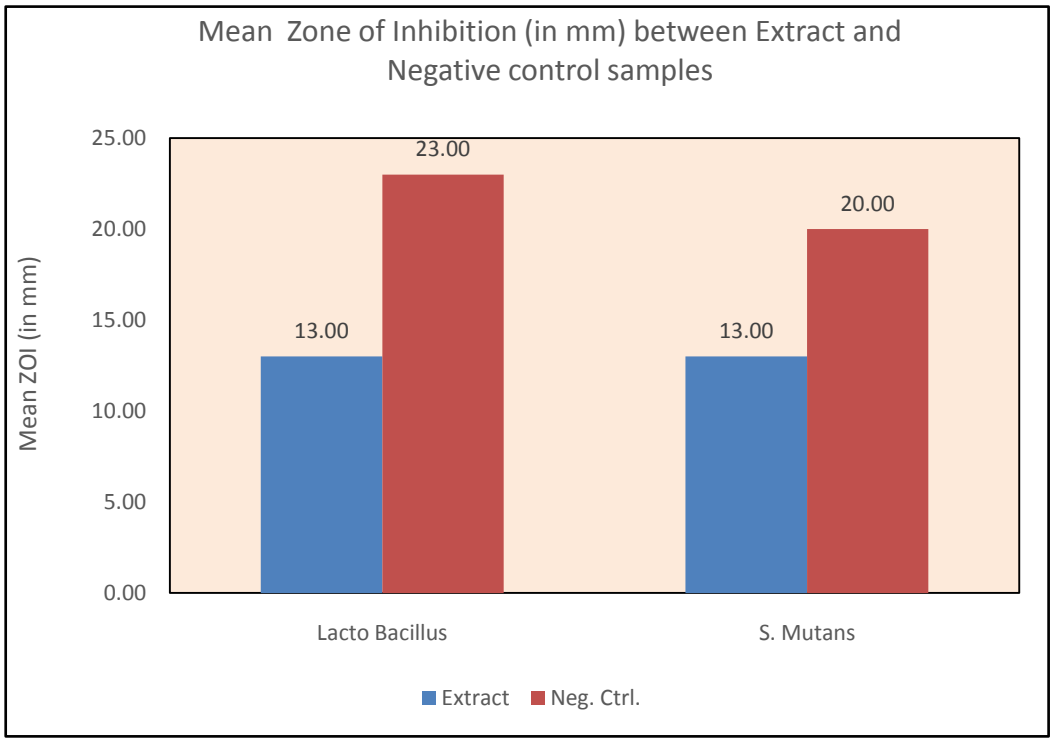
111. Results

Figures and Tables

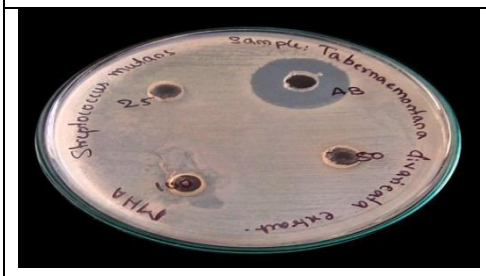
Table 1-Comparison of mean Zone of Inhibition (in mm) between Extract and Positive control samples using Independent Student t Test

Orgs.	Groups	N	Mean	SD	Mean Diff	P-Value
Lacto Bacillus	Extract	5	13.00	0.35	-10.00	<0.001*
	Posi. Ctrl.	5	23.00	0.35		
S. Mutans	Extract	5	13.00	0.71	-7.00	<0.001*
	Posi. Ctrl.	5	20.00	0.71		

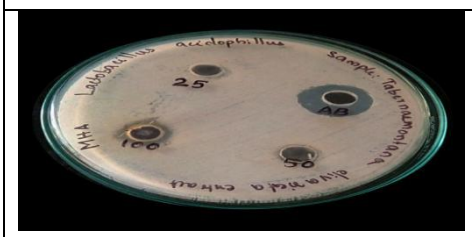
* - Statistically Significant



Streptococus Mutans (Zone of Inhibition)

Sample	Concentration µgm/ml	Zoneof inhibition(mm)
	Streptomycin (100µg)	23
	Extract	
	250	Nil
	500	Nil
	1000	13

Lactobacilus Acidophilus(Zone of Inhibition)

Sample	Concentration µgm/ml	Zone of inhibition(mm)
	Streptomycin (100µg)	20
	Extract	
	250	Nil
	500	10
	1000	13

Note: Concentration of Stock 10mg/mlDMSO -taken as micro liter from 10mg/ml of the stock

Tabernamontana sap and macerated leaves are commonly used by tribal population as a cure for tooth ache. Based on the documentation regarding this aspect a research was done to evaluate the action of macerated leaf on S.mutans and Lactobacillus acidopilus(common caries causing bacteria's).

As a basic level test to ascertain the anticariogenic nature, agar well diffusion method is used with leaf extract in three concentrations(250µg/ml, 500µg/ml, 1000µg/ml) against Streptococos mutans and Lactobacillus acidoplilus . With S.mutans the zone of inhibition is less for lesser concentration but at higher concentration(1000 µg/ml) ZOI is 13mm. For L. acidophilus (1000 µg/ml) zone of inhibition is 13mm ,meanwhile for positive control it is 23mm.With positive control the mean difference for L.acidophilus is -10.00 and p value <.001*,for S.mutans the mean difference with positive control is -7 and hence p<.001*.In this initial phase evaluation, it is evident that T.divericata has an antibacterial activity against common caries causing bacteria's(Table 1).

III. Discussion

In the present study initial level tests were done to evaluate the antimicrobial potency of the leaf extract on S.mutans and L.acidophilus using Agar well diffusion method.Various concentration of extract were taken and incubated for 24hrs,concentration of 1000µg/ml of both extracts showed a zone of inhibition of 13mm.This reading is in agreement with Rahamath Unissas study[9].

Studies have proved that many parts of T. divaricata has medicinal values.Root extract shows inhibitory action on oxidative stress [10].The leaves show an anti-inflammary potential [,11,12] Scientists have isolated a compound conolidine from the bark of the plant that has analgesic property similar to that of opioid[13.] .Its stem shows antimicrobial effect on many of the tested organisms with maximum antibacterial activity against Staphylococcus aureus MTCC 96 (zone of inhibition at 800µg/ml is 12.17±0.124 mm)[14]. About 66 alkaloids is isolated and identified from T. divaricata. Non-alkaloids including the enzymes, pyrolytic oil,

hydrocarbons, terpenoid and phenolic acids are also isolated from plant parts [15]. T. divaricata has antioxidant, anti-infection, anti-tumour action, analgesic potential and it enhances cholinergic activity in both peripheral and central nervous system[16]

Many parts of T.divericatais used for oral care ,stem to clean the teeth , the latex is directly applied on dental caries to relieve pain.The root is a local anodyne and chewed for the relief of toothache[17] . Stalin etal [18] studied the ethanolic extract of leaves and flowers of T. divaricata and found that it has significant antioxidant potential.

IV. Conclusion

The present study shows that leaf extract is bactericidal at higher and bacterio-static at lower concentrations . Further research is needed to isolate the active ingredientof plant extract that has anticariogenic potential . This study may lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial compound of natural origin in the treatment of dental diseases.

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