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A COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF DIFFERENT MEDICINAL PLANT AN IN VITRO STUDY

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Abstract: Many trees and plants shows medicinal effects. It has many chemical constituents for the biological function which prevent against microorganism, fungi and herbivorous mammals. Many different compound can be isolated from plants and trees. The study was carried out in the Mehsana district. In current studies, we can concentrate in studying various alternative ways to treat mouth infection caused by resistant microorganism. Biofilm form by bacteria creating many infection. They attach to surface of tooth and not easily breakdown, especially those species that form the deep layer of thick coating biofilm. This is in-vitro antimicrobial studies shows non-toxic and eco-friendly products, there are different uses of plants, trees and spices as its pharmacological effects. Finally we can see the phytochemical screening of extracts so that the bioactive compound which are responsible for antimicrobial activity. It also have secondary metabolites such as alkaloids, phenolic compounds, which shows antimicrobial activity. Different parts of plants and trees like leaves, bark, stem, root, flower and seeds used in medicinal therapy.

Keywords: Antimicrobial activity, medicinal plant, streptococcus mutans, phenolic compounds, medical therapy

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INTRODUCTION

Mouth infection recognized as vast public health problem. Tooth problem is common disease that affects poorly. Clove has been used for many years as preservation of food and for many medicinal purposes **(de Souza CR, et al 2014).** Which can be available easily and used by people always. There are lots many studies have been managed to explore medicinal purpose of the clove, pertaining to streptococcus mutans species. Oral biofilms are compositionally not so simple bacterial groups. Dental caries is an infectious disease that results in dissolution and destroy the calcified tissues of teeth **(Gamboa F, et al.2004).**

Streptococcus mutans are main agent of dental caries in humans. It develops various mechanisms in tooth surface to colonize and that's why become an important organism in cariogenic biofilm formation.The acid released by organisms causes the demineralization of tooth. Specially Streptococcus species, it colonize the surface of tooth and startsthe formation of plaque by producing extracellular polysaccharides from sucrose. Due to the formation of Biofilm there is much higher chances of infection occur.

Guava leaves shows important antibacterial activity against Staphylococcus,Streptococcus mutans, Bacillus, E.coli, Protius vulgaris and Pseudomonas. It has also potency of antifungal, antiyeast (Candida), antibacterial, anti-amoebic and antimalarial effects.In research studies there are total nine components found in ginger which binds to human serotonin receptor which proves its essential effects on gastro-intestinal tract as well as anxiety **(Nievergelt et al., 2010).**Herbs were the basis for nearly all medicinal therapy. Now-a-days, we are using herbal medicines instead of other chemical so that gives lesser side effects. Many bacteria are resistant to synthetic antibacterial agents,There is search on the pharmacological action of different parts of this plants helps in anti-tumor, antipyretic, anti-inflammatory, antiulcer, antispasmodic, antihypertensive, lower down the cholesterol, antioxidant, anti-diabetic properties **(InNikkon et al.,2003).**

Materials and Methods

Plant material Collection:

The plant material i.e. **guava leaves** (Psidiumguajava) were collected from the nursery in the Ganpat University campus.

Preparation of Stock solution:

Dried plant leaves extract were stored at refrigerator and Prepare the 10µg/100ml stock solution of streptomycin. Then prepare working solution for experimental purpose.

Cell counting determination:

First of all, drop 0.1ml of bacterial culture and make suspension from broth culture then put on neubaer chamber to make smear and dry this smear in air. After that stain it with dyeaccording to organism and place covering glass on smear carefully so that it can not form any bubble in chamber. Observe it under the compound microscope at 40X power. Count the bacteria in five square of the middle square **(K.R Aneja et al. 2003).**

Extraction process

Methanolic extract: The leaves of Psidiumguajava, camellia sinensis, zingiberofficinale, syzygiumaromaticum, are dried-up accurately in natural light, then grinded to fine powder & filter the powder through a mesh to get equal particle size. The powder (10gm) of the leaves is drenched in 100 ml of methanol and kept in rotatory shaker overnight at 60^{o}C. then the filtrate is collected by filtering through Whatman filter paper & evaporated till dehumidification comes in an evaporating dish. Then added 10ml DMSO in evaporated plant extract. And the left powder is again sundried and weighted and drenched in 100ml of methanol and do the same process two time **(Abhishek Sharma et al. 2014).**

Ethanolic extract: The leaves of **Moringaoleifera(50gm)** are dried-up accurately in natural light, then grinded to fine powder & filter the powder through a mesh to get equal particle size. The powder is drenched in 200 ml of ethanol 70% and kept in flask stoppered with rubber cork and left for 7-8 days with occasional shaking. Then the filtrate is collected by filtering through Whatman filter paper & evaporated till dehumidification comes in an evaporating dish. Then added 10ml DMSO in evaporated plant extract.**(Akueshi, et al. 2002).**

The leaves of **Chicko**(Manilkarazapota) are dried-up accurately in natural light, then grinded to fine powder & filter the powder through a mesh to get equal particle size. The powder (25gm) is drenched in 250 ml ofPetrolium ether < Toluene < Ethyl acetate < acetone < methanol < Water successively and kept in rotatory shaker overnight at appropriate temperature. Then the filtrate is collected by filtering through Whatman filter paper & evaporated till dehumidification comes in an evaporating dish. Then added 10ml DMSO in evaporated plant extract. And the left powder is again sundried and weighted and drenched in 250ml of methanol and do the same process two time **(MitalKaneria and Sumitrachanda et al. 2012)**

Determination of MIC (Minimum Inhibitory Concentration)

For determination of minimum concentration of our extract which shows inhibitory effects on the bacteria, we are used to make ten tubes of N-broth and one tube which is blank. These ten tubes are inoculated with same bacteria which is most ensitive to our extract as determinedby the zone of inhibition, the inoculation of Streptococcus mutans in ten tubes. After that

inoculate the other organism in different amount of our extract which is showing highest antimicrobial activity here It usedMethanolic extract of psidiumguajava and Moringaoleifera leaves extract. For example 5,10,15,20,25,30,35,40,45,50µl of eachextract in each inoculated n-broth test tube and added 0.1ml of each organism in each sample **(K.R Aneja et al. 2003).**

Filling the plant extract into the well

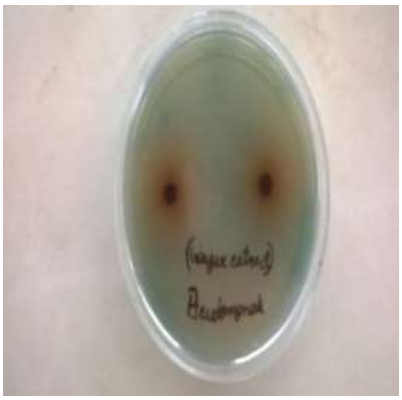
Make two wells with the help ofcork borer size of 0.6mm. Fill the 60-70µl of standard drug and test solution into the separate well. Then the standard drug is allowed to dispel for 20 minutes. Incubate all the petri dish into the incubator at 37°C temperature for 18-24hrs. After 24-28hrs of incubation, each and every plate is examined. If the plate was streaked carefully and the inoculum was also correct, then given zone of inhibition will be uniformly circular and there will be a concurrent lawn of growth.

Examination of plates and results

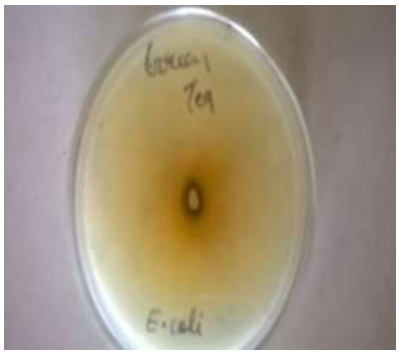
Camellia sinensis and Psidium guajava leaves extract on S.mutans in BHI medium.

camellia sinensis and Psidium aromaticum extract on B.subtilis in N-agar.

Moringaoleifera extract on S.mutans Manilkarazapota extract In M-H agar.



Zingiber officinale extract onPsidium guajava on *P.aeruginosa* in agar.



Camellia sinensis extract on *E.coli.*

Syzygium aromaticum extract on *S.aureus* in N-agar.

Moringa oleifera extract on *S.mutans* in M-H agar.

Psidium guajava extract on *P.vulgaris* in N-agar plate.

Diameter of zone of inhibition

Bacteria	Petrolium ether	Toluene	Acetone	Ethyl acetate	Methanol
E. coli	-	12±0.4	14±0.5	20±0.4	25±0.6
Pseudomonas aeruginosa	-	12±0.5	12±0.6	21±0.7	22±0.8

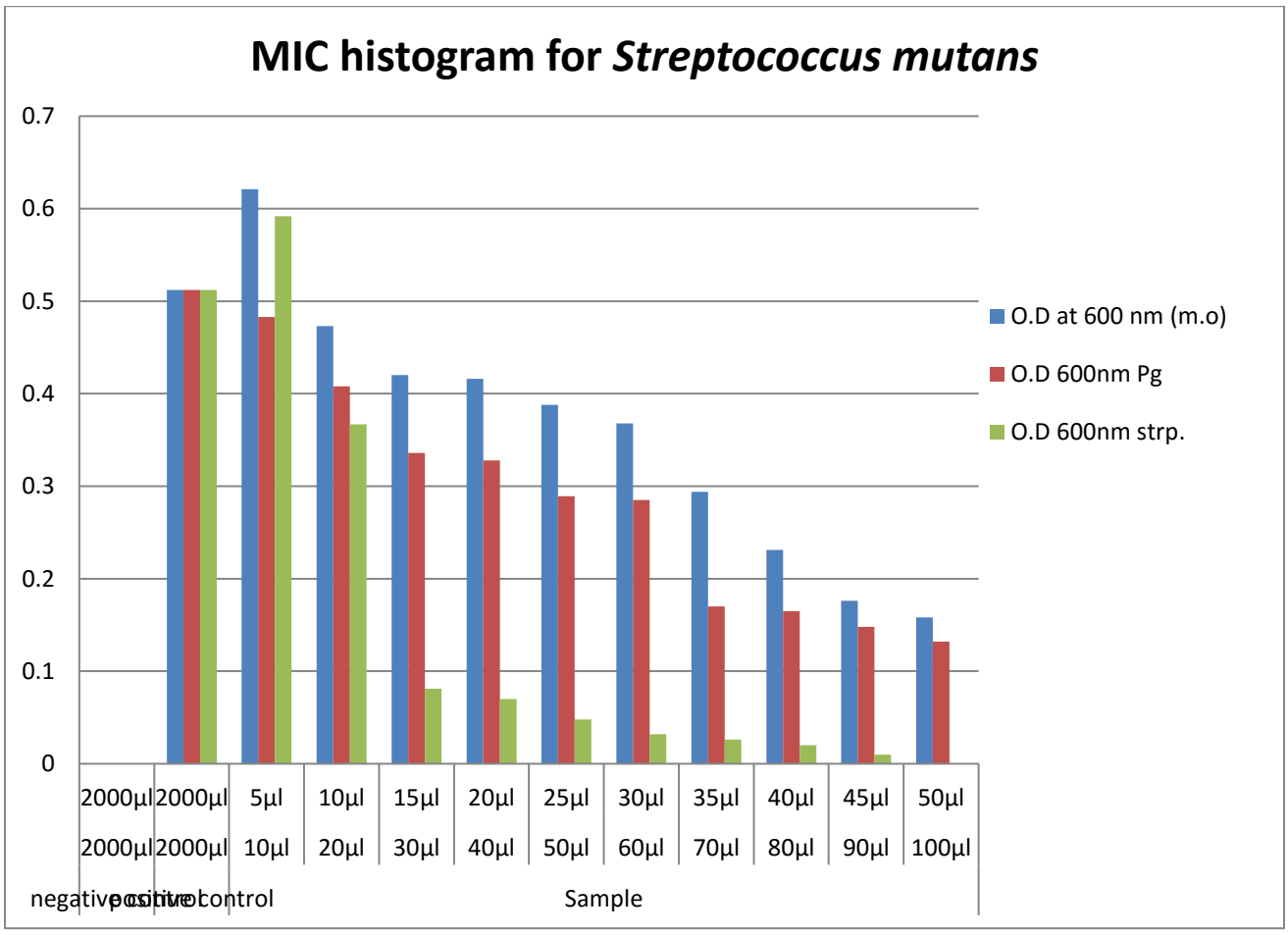
B. subtitis	-	13±0.3	13±0.4	21±0.3	22±0.8
S.mutans	-	12±0.2	15±0.5	22±0.5	23±0.6
P.vulgaris	-	14±0.5	12±0.8	21±0.6	23±0.4
S. aureus	-	11±0.4	14±0.3	18±0.3	21±0.5

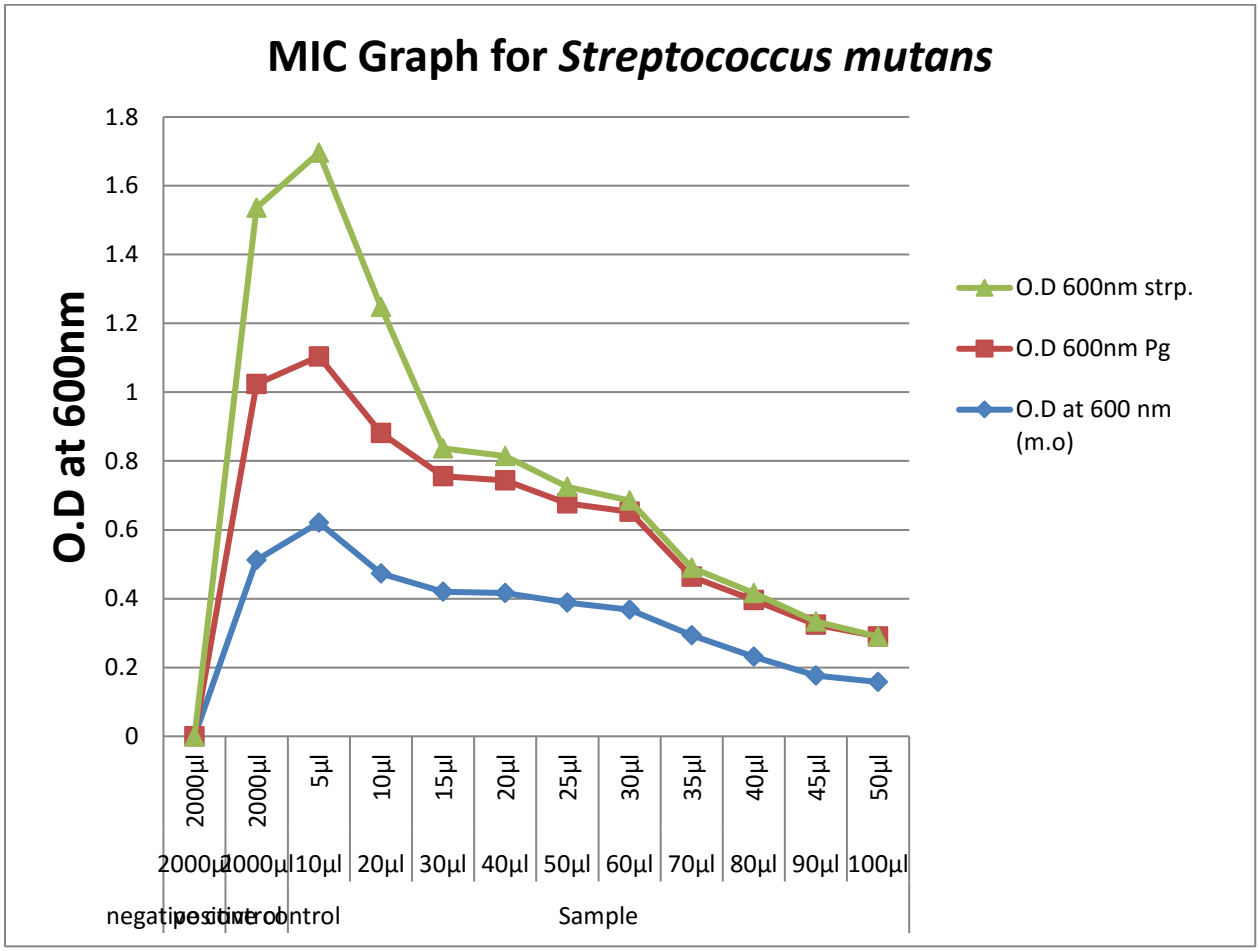
Bacteria	Aquous ginger	Methanol ginger	Methanol Drumstick leaves	Methanol Green tea	Methanol clove	Methanol Guava leaves
E. coli	24±0.7	27±0.2	26±0.6	25±0.4	26±0.6	25±0.4
Pseudomonas aeruginosa	21±0.4	24±0.4	25±0.3	19±0.6	24±0.4	24±0.6
B. subtitis	22±0.8	28±0.6	28±0.5	16±0.7	25±0.4	27±0.2
S.mutans	23±0.7	27±0.5	27±0.6	26±0.5	24±0.4	27±0.7
P.vulgaris	22±0.5	25±0.7	25±0.6	25±0.4	25±0.7	25±0.4
S. aureus	24±0.5	25±0.4	26±0.2	24±0.6	24±0.5	24±0.3

O.D of streptomycin and concentration of psidiumguajava extract

group	conc.	conc.	M.orifera leaf	Guava leaves	Streptomycin
negative control	2000µl	2000µl	0	0	0
positive control	2000µl	2000µl	0.512	0.512	0.512
Sample	10µl	5µl	0.621	0.483	0.592
	20µl	10µl	0.473	0.408	0.367
	30µl	15µl	0.42	0.336	0.081
	40µl	20µl	0.416	0.328	0.07

	50µl	25µl	0.388	0.289	0.048
	60µl	30µl	0.368	0.285	0.032
	70µl	35µl	0.294	0.17	0.026
	80µl	40µl	0.231	0.165	0.02
	90µl	45µl	0.176	0.148	0.01
	100µl	50µl	0.158	0.132	0





Discussion:

From the above table, we can observe that there is maximum zone of inhibition in guava leaves extract in Methonol solvent on the six different organisms, S.mutans. Protius vulgaris, Bacillus subtilis, E.coli, Pseudomonas aerugenosa, S.aureusalso organisms in which Psidiumguajava shows its antimicrobial property.

According to observation the Minimum Inhibitory Concentration of psidiumguajava leaves extract on highly pathogenic S.mutans organism, by comparing it with the standard Streptomycin antibiotic and observed the highest zone of inhibition shown in methanol solvent. The working concentration of Psidiumguajava is approximately 123µg/µl and Minimum Inhibitory Concentration for Psidiumguajava is 6150µg/µl on Streptococcus mutans. The working concentration of Moringaoliefera is 33µg/µl. and Minimum Inhibitory Concentration of Moringaoleiferais approximately 1650µg/µl.

CONCLUSION:

From the above research work, the antimicrobial activity of five different extracts, in which Psidiumguajava and Moringaoleifera extract showed most effective result on Streptococcus mutans and Bacillus subtilis. This may be due to presence of polyphenols and flavonoids which are biologically active compounds.

(Hsin-chun, Ming-jensheuet al.2006).

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