Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 1, 2020, Pages. 1212 -1223
Received 15 April 2020; Accepted 23 June 2020

Evaluation Of Bactericidal Efficacy Of Polyherbal Extract From Three Different Medicinal Plants

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ABSTRACT
Traditional healers right that their remedy is inexpensive andmore active than modern medicine. In developing countries low incomes people such as farmer's from segregated village people for the medicine of infectious diseases. In recent years the use of herbal medicines for the prevention and cure of diseases has tremendously increased in the recentyears. They are believed to be safe and free from serious adverse reactions. Polyherbal therapy is made used in Ayurvedic, Chinese and Unani medicines, yet scientific evidence of their therapeutic benefits are still lacking. Antimicrobial activity in combination gives a synergistic and boosted inhibition against pathogenic bacteria and fungi and thus leading towards developing more potent drugs.The existing study was assumed to evaluate the antimicrobial potential of polyherbal extract from medicinal plants such as *Areca catechu, Acalypha indica and Piper betel* against the microorganism.

Keywords: modern medicine, polyherbal extract, pathogens, bacteria and fungi

1. INTRODUCTION

Oral health has a great impact on the overall health and well being of an individual. Maintenance of oral health is particularly challenging in people with special needs.(Satcher 2000) Dental caries, also known as tooth cavities and tooth decay is the condition that describes holes or other structural damage in the tooth. Caries is a unique multifactorial infectious disease. A group of phenotypically similar bacteria known as mutants streptococci, has been implicated as the chief component responsible for the initiation and the development of dental caries (loesche, 1986).

Dental caries is a pathological process depending on source etiologic factors, which cause the destruction of the dental tissues and produces local and general complications.Dental caries is caused by action of acids on the enamel surface. The acid is produced by sugar in foods and drinks when it reacts with the bacteria present in the dental biofilm (plaque) on the tooth surface.

Demineralization occurs by the acid produced which removes calcium and phosphate from the enamel.

Role of Microorganisms and Dental plaque

Streptococcus mutans a gram-positive coccus (round bacterium) in the human oral cavity is a significant contributor to tooth decay (Loesche,1996). The acidic environment created by this process causes the highly mineralized tooth enamel to be vulnerable to decay. S. mutans is one of a few specialized organisms equipped with receptors that improve adhesion to the surface of teeth using sucrose as a substrate to form a sticky polysaccharide (Ryan, 2004).However, other sugar like glucose, fructose, lactose digested by
S. mutans, produce lactic acid which is the end product.

Lactobacillus spp. plays a vital role in the development of dental caries. Although effective methods are known for the prevention and management of dental caries, still it lacks its position.The dental caries is still increasing amongst school-age children. .Streptococcus mutans and Lactobacillus spp. are the most common pathogens isolated from human dental plaque are considered as the major etiologic agents of caries. Initiation of dental caries is by S. mutans and progression is by Lactobacillus spp. . In addition, risk factors such as host susceptibility, age, dietary habits socioeconomic and oral hygiene status have been associated with increased incidence of dental caries in human population.

Herbal extracts on oral health care

The Areca nut ,a seed of the Areca catechu grows in the tropical Pacific, Asia and parts of east Africa. Dried form of the Arecanut strengthens gums, sweetens the breath, eliminate bad taste and act as dentifrices. It has been reported that Arecanut exerts a direct antimicrobial effect againstoral bacteria including Streptococcus mutans,Streptococcus salivarius, Candida albicans and Fusiform nucleatum(Shwethaet al.,2017

An Anting-anting (*Acalypha indica*) plant is a species of plant having catkin type of inflorescence. This plant is traditionally used to treat dysentery, diarrhoea, malnutrition, and malarial (Arisandi et al, 2008). The ethyl acetate extract of Anting-anting was reported as an anti-bacterial against Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Streptococcus faecalis, and Pseudomonas aeruginosa(Govindarajan et al, 2008). Oral and dental disease which is one of the health problem is highly correlated to bacteria. On the tooth surface, bacteria makes solid biofilm called as plaque. Dental plaque adheres to the teeth and consists of many species microbe, salivary polymers and microbial extracellular products. The most common bacteria play a role in the formation of plaque is Streptococcus mutans (Tarigan,1990).

Piper betel belonging to the piperaceae family is one of the precious medicinal herbs.Betel leaves are very nutritive and contain substantial amount of vitamins and minerals (Pradhan et

al.,2013).Widespread use of drugs is leading to the development of resistance against them in the pathogen and also the side effects associated with them is urging people not to use them. Therefore there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious disease from medicinal plant.Here with we reveal, antibacterial screening of crude extracts and polyherbal extract of three medicinal plants, and their extract fractions were carried out for different assay and reported.

2. MATERIALS AND METHODS

Bacterial Culture

One ml of frozen LAB (*Lactobacillus* sp.) cultured in 20 ml MRS broth with pH 6.2, while *Streptococcus* sp. in M17 broth with pH 6.9, respectively, at 37 °C for 24 hours. 1 ml of LAB culture sub-cultured in MRS broth overnight and cells were harvested using a centrifuging at 14,000*g* for 5 min (Sorvall RC6 PLUS, Thermo-electron Corporation, Asheville, NC, USA). The cell pellet washed with saline solution (0.85% NaCl) was re-suspended using 0.85% NaCl to a final optical density (O.D.) of 0.4 at 600 nm measured with a spectrophotometer (Ultrospec 3100 Pro, Biochrom Ltd. England). The cell suspension was used as the inoculum for the growth curve experiments. (En Yang et al ,2012).

Antibacterial test

The antibacterial test against Streptococcus mutans and Lactobacillus was performed using disc-diffusion method. The disc diffusion method was adopted to assess the antibacterial activity of the prepared extract. A loop full of bacterial stock suspensions was thoroughly mixed with 100 ml of sterile nutrient agar and kept for overnight incubation. 0.1ml of overnight culture of Streptococcus mutans and Lactobacillus sp was spread on the surface of the disc and was placed in MHA medium and MRS medium respectively . The disc's were filled with extract of various concentrations of about 1000µl, 750µl, 500µl and a antibiotic chlorhexidine as a control. After 24 hours at 37 ºC , the agar plates were examined for the zone of inhibition and the zones were measured in millimeters. The zones were measured, averaged and the mean values were tabulated.(Dinesh et al, 2016).

Minimum Bactericidal Concentrations (MBC) on Streptococcus mutans and Lactobacillus

Dilutions and inoculations were prepared in the same manner as for the determination of MIC. The control tube without plant extract is immediately sub cultured (Before incubation) along with the tubes with plant extract at 37ºC overnight. The MIC of the control organism was to check the drug concentrations. The growth was compared with control which is the original inoculum. If similar number of colonies exists it indicates bacteriostatic only. Partial or slow bactericidal activity indicates a reduced number of colonies and if no growth then the whole inoculum is killed. The highest dilution showing at least 99% inhibition is taken as Minimum Bactericidal Concentrations (MBC). (Dinesh et al, 2016).

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3. RESULTS AND DISCUSSION

Invitro antibacterial activity of *Areca catechu, Acalypha indica, Piper betel* and combination (PAA) of all the three plant extracts assessed by determining the inhibition zone diameter is given in figure 1 consequently. The analysis of the all the plant extracts showed a positive inhibitory activity against the *Streptococcus mutans* and *Lactobacillus* .No strain in this study showed a resistance to these extracts .The inhibitory zone significantly increased in a dose dependent manner. The methanolic extract of PAA (Poly herbal compounds) turned out to be the most effective for its bactericidal activity against *Streptococcus mutans* and *Lactobacillus.* The widest zone of inhibition with a diameter of 12mm was observed in case of PAA (Poly herbal compounds)extract, followed by the extract of *Areca catechu* with the zone of inhibition extending upto 9mm. Among the *Streptococcus mutans* the PAA (Poly herbal compounds) extracts showed a higher activity (Fig 1,2,3).

Incase of Lactobacillus the extract of PAA (Poly herbal compounds) extract showed a higher activity. From this investigation it was observed that PAA (Poly herbal compounds) extracts at a concentration of 1000 µl/mL inhibited.It has been reported that the phenolic nature Areca catechu extract may be responsible for inhibiting bacterial growth (Graph 1,2,3,4).

In dilution method, the Minimum Inhibitiory Concentration for *Streptococcus mutans* by *Areca catechu, Acalypha indica, Piper betel* was 15.6 µg/mL (Fig 12,13,14) and for the polyherbal combination (PAA) was 7.2 µg/mL (Fig 15) respectively. (Table 13 & Graph 5).

The analysis extracts of PAA against *Lactobacillus* showed a minimum inhibitory concentration were at 7.2 µg/mL and that of *Areca catechu, Acalypha indica* and *Piper betel* were at 15.6 µg/mL respectively.The Minimum Bactericidal Concentration for *Streptococcus mutans* by *Areca catechu* was 62.5 µg/mL, *Acalypha indica was 15.6* µg/mL , *Piper betel* was 125 µg/mL and for the polyherbal combination (PAA) was 7.2 µg/mL respectively.

Oral health is essential for overall health. Several oral diseases and conditions count for most of the oral diseases burden that includes dental caries, periodontitis etc., might be due to the micro organisms like *Streptococcus* and *Lactobacillus.*To evidently prove the plant extracts biological activity against the oral pathogens, *Strepococcus mutans* and *Lactobacillus* antibacterial activity was carried out.The maximum zone of inhibition was found in the combination plant extracts of *Areca catechu, Acalypha indica* and *Piper betel*. Minimum Inhibition Concentration (MIC) was performed to arise the lowest concentration of an antimicrobial ingredient and to evaluate the antimicrobial efficacy of various compound by measuring the effect of decreasing concentration of plant extracts of *Areca catechu, Acalypha indica, Piper betel* and PAA which inhibits the microbial strain of *Streptococcus mutans* and *Lactobacillus* growth. The strain of *Streptococcus mutans* as an MIC of 15.6µg/mL for *Areca catechu* and an MIC of 15.6 µg/mL for *Acalypha*

indica and that of 15.6 µg/mL for *Piper betel* and that of 7.2 µg/mL for PAA.The strain of *Lactobacillus* has an MIC of 62.5µg/mL for *Areca catechu* and an MIC of 15.6 µg/mL for *Acalypha indica* and that 125 µg/mL for *Piper betel* and that of 7.2 µg/mL PAA.

As Minimum Bactericidal Concentration (MBC) of an antibacterial agent is necessary for screening a drug efficiency the methanolic exracts of *Areca catechu, Acalypha indica,Piper betel* and PAA were subjected to minimum bactericidal concentration as it a tool to simultaneously evaluate multiple antimicrobial agent for potency.The MBC of *Areca catechu,Acalypha indica,Piper betel* were 15.6 µg/mLand PAA were 7.2 µg/mL and various concentration such as ,62.5 µg/mL, 31.2 µg/mL, 15.6 µg/mL and 7.2 µg/mL respectively against *Streptococcus mutans* and *Lactobacillus* was 62.5 µg/mL,15.6 µg/mL,125 µg/mL and 7.2 µg/mL respectively . The *Streptococcus mutans* and Lactobacillus was found to be susceptible for PAA extract.

4. CONCLUSION

The plant extracts were considered as suitable candidates for antibacterial drug discovery. This combination in antimicrobial activity gives a synergistic and advanced inhibition against pathogens and thus foremost towards developing more potent drugs. Habitually used polyherbal creations offer new hope to cracking the microbial resistance issue.These poly-herbal mixtures for clinical and *in vivo* trials against respective diseases need attention. The ability of these therapies to inhibit the organisms is a sign that they are a potential broad-spectrum antimicrobial agent.

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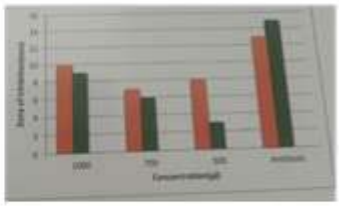
Figure 1: Antibacterial activity of *Areca catechu*



Zone of Inhibition of *Acalypha indica* Methanol extract		
Concentrations (µl)	*S. mutans*	*Lactobacillus sp.*
1000	10mm	9mm
750	7mm	6mm
500	8mm	3mm
Antibiotic	13mm	15mm

Figure 2: ANTIBACTERIAL ACTIVITY OF *Acalypha indica*

Zone of Inhibition of *Acalypha indica* Methanol extract		
Concentrations (µl)	*S. mutans*	*Lactobacillus sp.*
1000	10mm	9mm
750	7mm	6mm
500	8mm	3mm
Antibiotic	13mm	15mm

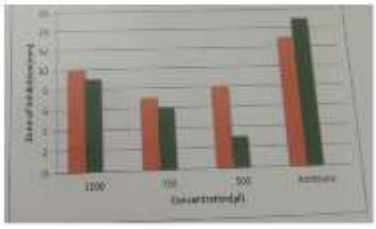


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Figure 3: Antibacterial activity of *piper betel*

Zone of Inhibition of Piper betel Methanol extract		
Concentrations (µl)	S. mutans	Lactobacillus sp.
1000	12mm	9mm
750	8mm	8mm
500	7mm	8mm
Antibiotic	17mm	15mm

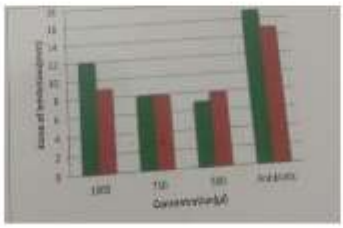

Figure 4: Antibacterial activity of PAA

Zone of Inhibition of PAA Methanol extract		
Concentrations (µl)	*S. mutans*	*Lactobacillus sp.*
1000	12mm	10mm
750	8mm	8mm
500	7mm	5mm
Antibiotic	12mm	13mm

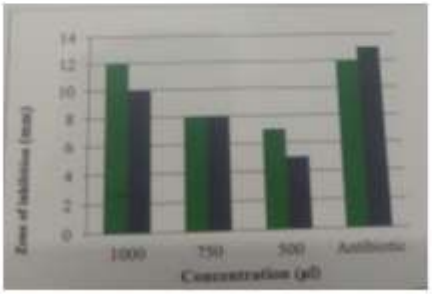

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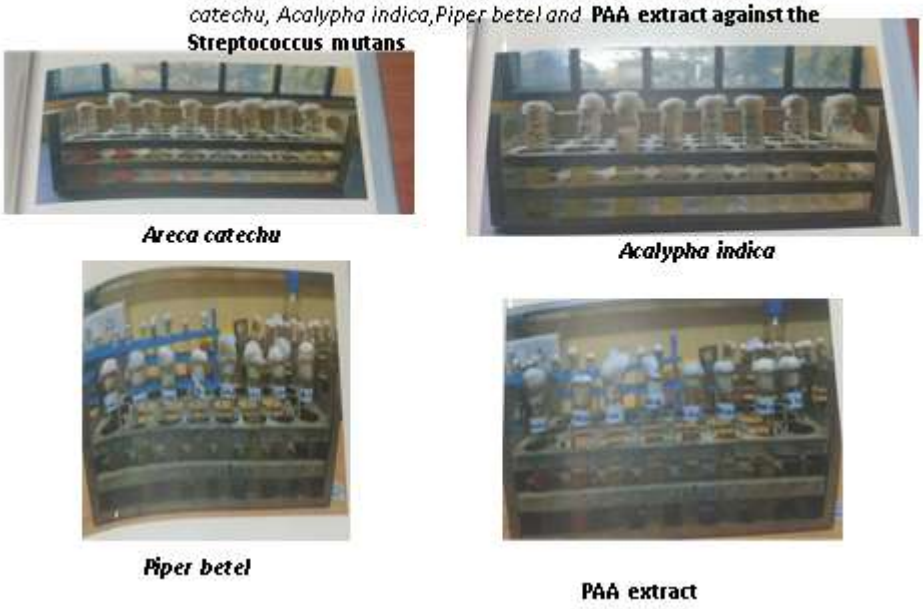
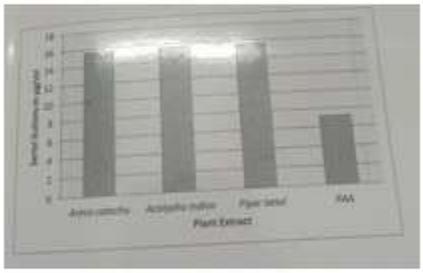
Figure 4 Minimum of Inhibitory Concentration (MIC) on *Areca catechu, Acalypha indica,Piper betel and* **PAA extract against the Streptococcus mutans**



Minimum of Inhibitory Concentration (MIC) on *Areca catechu, Acalypha indica,Piper betel and* **PAA extract against the Streptococcus mutans**



FUNDING
No funding sources

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The encouragement and support from Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India is gratefully acknowledged for providing the laboratory facilities to carry out the research work.

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