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AN INVITRO COMPARATIVE EVALUATION OF ANTICANDIDAL HERBS (GINGER & TURMERIC) ON STREPTOCOCCUS MUTANS

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Abstract

Aim: To comparatively evaluate the antimicrobial effect of turmeric and ginger extracts on Streptococcus mutans in in-vitro conditions.

Material & Method: An in-vitro experimental study was conducted in a laboratory setting. Ethanolic extract of Ginger and Turmeric was prepared separately by cold masseration technique. The extract of each was then diluted with an inert solvent, Dimethyl Formamide, to obtain 5 different concentrations (2%, 4%, 6%, 8%, and 10%) of each. 0.2% chlorhexidine was used as a positive control and dimethyl formamide was used as negative control. The different extracts, along with controls, were then subjected to microbiological investigation to determine which gave a wider zone of inhibition against streptococcus mutans. The zone of inhibition was measured in millimeters.

Results: Turmeric extracts presented the largest zone of inhibition 33mm at the concentration of 8%, while Ginger extract showed a zone of inhibition of 34mm at the concentration of 10%.

Conclusion: Ethanolic extract of Turmeric demonstrated antimicrobial activity against Strptococcus Mutans at a lower concentration than that of Ginger.

Keywords: Ginger, Turmeric, Streptococcus Mutans.

Introduction:

The practitioners of traditional system of medicine treat about 80% of patients in India, 85% in Burma and 90% in Bangladesh [1].Medicinal plants rich in secondary metabolites (potential sources of drugs) and essential oils are of important advantage claimed for their therapeutic uses in various ailments besides being safe, economical, effective and easily available [1].

Moreover in today's world consumer and producer alike have become highly conscious about the health benefits of food leading to value added products in health sector and discovery of "Functional Food" that encompasses all edible items having a health-promoting and/or disease-preventing property beyond the primary function of providing nutrients. Some of these have already been used in successful management of both general and oral disease conditions like bronchitis, bronchial asthma, skin diseases, Oral thrush, Oral Cancer, Periodontal diseases, etc.

Several plants like Ginger, Tulsi, Garlic, and Turmeric have been used as Neutraceuticals in treatment of oral candidiasis by local and systemic routes[2-6]. When used locally or systemically they also affect the oral bacterial flora, some having proved their action against bacteria like Streptococcus Mutans, the main causative organism for dental caries.

Lack of comparative data on efficacy of Turmeric and ginger against Streptococcus Mutans need to be accounted owing to their extensive use in Oral cavity in oral thrush and other oral diseases. The aim of present study was to comparatively evaluate the antimicrobial effect of turmeric and ginger extracts on Streptococcus mutans in in-vitro conditions.

METHODS

Preparation of Ginger extract:

Fifty grams of sun dried finely powdered ginger rhizomes were macerated with 150 ml of 100% ethanol and then subjected to filtration with whatman filter paper to obtain a clear filtrate. The filtrate so obtained was reduced in a borosilicate glass beaker at a low temperature of less than 40 degree celcius with the help of a Soxhlet Extraction Unit (heating mantle) MSW-436 of MAC Macro Scientific Works Limited., to obtain semi solid residue of ginger extract. From 50 grams of powder dissolved in 150 ml of ethanol, 2 grams of residue extract was obtained, so the yield was 2 % w/w.

Preparation of Turmeric extracts:

Fifty grams of sun dried finely powdered ginger rhizomes were macerated with 150 ml of 100% ethanol and then subjected to filtration with whatman filter paper to obtain a clear filtrate. The filtrate so obtained was reduced in a borosilicate glass beaker at a low temperature of less than 40 degree celcius with the help of a Soxhlet Extraction Unit (heating mantle) MSW-436 of MAC Macro Scientific Works Limited., to obtain semi solid residue of turmeric extract. From 50 grams of powder dissolved in 150 ml of ethanol, 2 grams of residue extract was obtained, so the yield was 2 % w/w.

Preparation of different concentrations of Ginger and Turmeric extracts:

2 grams of extract was dissolved in 20 ml of Dimethylformamide to obtain 10 % concentration of extract, which was used as a stock solution. Subsequent serial dilution of the stock solution with Dimethylformamide was done to obtain 2 %, 4 %, 6 %, 8 % and 10% concentration of stock solution of Ginger and Turmeric extracts.

Furthermore 1% of extract of both the stock solutions was used as a starting point followed by other concentrations as mentioned above for Minimum inhibitory concentration (MIC) determination.

Controls:

Control of 0.2% Chlorhexidine was used as positive control, a gold standard for comparison and Dimethylformamide was used a negative

control to rule out its effect on Streptococcus mutans.

Collection of micro-organisms:

MTCC strain No 497 was obtained from Microbial Type Culture Collection (MTCC) and Gene Bank, Chandigarh. The strain belonged to genus Streptococcus while the species was mutans. i.e. streptococcus mutans., was used for the study purpose.

Preparation of Culture Media:

The Brain Heart Infusion agar powder (Special Infusion Agar) for invitro diagnostics, M211, was obtained from HiMedia Laboratories Limited, Mumbai. Fifty two grams of this powder was suspended in 1000ml of distilled water. It was then boiled to dissolve the medium completely and then sterilized by autoclaving at 15 lbs pressure and 121 degree Celsius for 15 minutes. The pH of the agar was maintained at 7.4 at 25 degree Celcius. The media was then mixed well and poured into petri-dishes. The process of making culture media was carried out as per the instructions provided by the manufacturer.

Streptococcus mutans MTCC was then added to nutrient broth which was incubated at 37 ^{0} C for 24 hours. It was sub-cultured onto nutrient agar plate and incubated at 37 ^{0} C for 24 hours. The inoculum for antimicrobial activity was prepared by adjusting the density of organism to approximately 10^8 colony forming units/ml with the help of 0.5 Mcfurland opacity standards. Then it was inoculated on agar plate by lawn culture method. The growth conditions were aerobic as specified by Gene bank, Chandigarh.

Determining Microbic sensitivity:

Determination of microbic sensitivity mainly can be done by two methods i.e. Dilution methods and Diffusion methods. Ditch plate diffusion method was used in the present study as it has been proven to be more suitable for research purpose[11]. In this method, ditches were made in Petri-dishes by using punch. These ditches were filled with the equal amount of prepared extract of Ginger & Turmeric extract. Six plates were used for six different concentrations of each. Chlorhexidine and Dimethylformamide were used as controls. Plates were then incubated at 37 ^{0} C for 48 hours, after which zone of inhibition was measured were measured.

MIC was determined by broth dilution method and values were determined by visual inspection of tubes. In each series of tubes, the last tube with clear supernatant was considered to be without any growth and taken as MIC value.

Antimicrobial susceptibility testing:

The ditch plate method was used to test the antimicrobial activity. Ditches were prepared on agar plates with the help of punch having 6mm diameter. On each petri dish 3 different ditches were labeled for one same concentration of ginger and turmeric extract. 50 microlitre of each of different concentrations were introduced in equal sized ditches made on petri dishes.

The plates were left for 1 hr at room temp & then incubated at 37 degree celcius for 48 hrs and later examined for zone of inhibition.

The zone of inhibition was measured with the help of Hi Antibiotic Zone scale from HiMedia Laboratories Limited, Mumbai which is certified to ISO and WHO GMP [12]. The scale used was of high quality, standardized, efficient and easy to use with high reproducibility of observations.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 15.0 windows evaluation trail version release 15.0.0, 6 September 2006, USA. Data was tested for normality using Kolmogrov Smirnov test following which unpaired student t test.was applied to compare the effect of turmeric, ginger and controls on S.mutans.

RESULTS

Table 1 shows zone of inhibiton of various concentrations of ehtanolic extracts of Ginger & Turmeric. **Table 2** shows zone of inhibition of the positive and negative controls. **Table 3** shows results for one sample Kolmogorov Smirnov test for normality. The results showed that data was

normally distributed in the both groups i.e. ginger and turmeric. **Table 4** shows results for intergroup comparison using unpaired t test. There was a non significant difference between the means of zones of inhibition of the Turmeric & Ginger, Turmeric & Chlorhexidine, Ginger & Chlorhexidine

Table 1: Zones of Inhibition of different Extracts

Sr. No.	Extract → Concentration ↓	Zones of Inhibition	
		Ginger	Turmeric
1	1%	R*	R*
2	2%	27mm	21mm
3	4%	24mm	25mm
4	6%	25mm	27mm
5	8%	25mm	***34mm***
6	10%	***33mm***	32mm

***R – Resistant**

Table 2: Zones of Inhibition of Controls

Sr. No.	Controls	Zones of Inhibition
1	0.2% Chlorhexidine	17 mm
2	Dimethyl Formamide	R*

***R – Resistant**

Table 3: One-Sample Kolmogorov-Smirnov Test for normality of data

Extract	chlorhexidine	ginger	turmeric
N	6	6	6
Mean	14.17	22.33	23.17
Std. Deviation	6.940	11.413	12.287
Kolmogorov-Smirnov Z	1.205	.959	.645
p	.110	.317	.800

Table 4: Inter group Comparison of effect of Ginger, Turmeric & Chlorhexidine on S. Mutans using Unpaired t-Test

xtract	N	Mean	Std. Deviation	T	p
Chlorhexidine Vs Ginger	6 6	17.0000 22.3333	.00000 11.41344	-1.145	.279
Chlorhexidine Vs Turmeric	6 6	17.0000 23.1667	.00000 12.28685	-1.229	.247
Turmeric Vs Ginger	6 6	23.1667 22.3333	11.41344 12.28685	-0.419	0.693

DISCUSSION

The continuous increase in dental caries signals a pending public health crisis[13]. Although there are differences of opinion regarding the cause of this global dental caries increase, the remedy could be shifting to functional food.

Functional foods have been introduced into the corporate mainstream owing to continuously increasing health care costs, rising consumer awareness about health aspects of foods and food regulations and above all an increased level of education and literacy[14]. Ginger (Zingiber officinale) and turmeric (Curcuma Longa) commonly used as food ingredients have been used for medicinal application also [2,3,6] .

Ginger a "maha aushadhi", meaning the great medicine [15] contains active compounds of volatile essential oils and fragrant or harsh phenol compounds[16] .The fresh ginger rhizome contain gingerols as major active components and the volatile oil consists of mainly mono and sesquiterpenes; camphene, beta-phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol, limonene, linalool, alpha-zingiberene (30-70%), beta-sesquiphellandrene (15-20%), beta-bisabolene (10- 15%) and alpha-farmesene[15]. These active components are reported to enhance GI tract functioning, inhibition of prostaglandin and leukotriene biosynthesis in inflammation process and have anti cancer effects [15, 17-25]. Gingerol and shagelol are reported to have strong antibacterial against Escherichia coli, Proteus sp, Staphylococci, Streptococci, Salmonella, A.niger, S.cerevisiae, Mycoderma SPP. and L. acidophilus [7, 9, 26] . The results seen in the present study can be attributed to the same.

The 10% ethanolic ginger extract showed higher antimicrobial activity against S.mutans with maximum zone of inhibition of 33 mm at 50µl which was almost twice as compared to Akihiro O Hara et al[9] and Anjan g et al[27] where zone of inhibition of 8.2 mm & 8mm was seen respectively with same concentration and same volume of extract. The difference observed could be attributed to variations in the quality of ginger used, differences in the microbiological techniques used, variation in temperature and solvent used to prepare ginger extract.

Curcuma longa L. commonly called as Turmeric or Indian Saffron is a shrub related to ginger. The rhizome (underground stem) of the turmeric plant contains up to 5% curcumin, in combination with essential oils and other compounds. Turmeric has been used for various medicinal uses in both Indian (Ayurvedic) and Chinese medicine systems for thousands of years [28]. It is only recently that modern medicine has begun to critically evaluate effects of turmeric and its extracts in various disease process such as inflammation, infection, cancer etc[29-34].

In the present study 8% ethanolic turmeric extract showed highest antimicrobial activity against S.mutans with maximum zone of inhibition of 34 mm at 50µl. This anti-bacterial activity of curcumin can be attributed to xanthorrhizol an active anti bacterial component of turmeric [35].

The negative control has shown no action on S. Mutans suggesting the bacterial resistance to its properies. Thus it can be stated that the action showed by ginger and turmeric were purely due to their own characteristics and not due to the vehicle used. The positive control did show the antimicrobial activity as expected. The zone of inhibition formed, though were statistically insignificantly different among the three it was higher clinically in turmeric and ginger extracts suggesting their superior effect against the S. Mutans as compared to Chlorhexidine.

It was concluded from results that with the known side effects of Chlorhexidine and no known side effects of turmeric and ginger they could be used as replacements for Chlorhexidine in various market products at the same time their regular consumption as food ingidients could also help in reducing the dental caries problem.

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