

Comparative evaluation of antimicrobial efficacy of cow urine, extracts of garlic, curcumin and virgin coconut oil against oral pathogens - An in vitro study.¹Dr. Sathya R, AECS Maaruti College of Dental Sciences, Bangalore²Dr. Sapna Konde, AECS Maaruti College of Dental Sciences, Bangalore³Dr. Manisha Agarwal, AECS Maaruti College of Dental Sciences, Bangalore⁴Dr. Preetha Peethambar, AECS Maaruti college of Dental Sciences, Bangalore**Corresponding Author:** Dr. Sathya R, AECS Maaruti College of Dental Sciences, Bangalore**Citation of this Article:** Dr. Sathya R, Dr. Sapna Konde, Dr. Manisha Agarwal, Dr. Preetha Peethambar, “Comparative evaluation of antimicrobial efficacy of cow urine, extracts of garlic, curcumin and virgin coconut oil against oral pathogens - An in vitro study”, IJDSIR- April - 2023, Volume – 6, Issue - 2, P. No. 517 – 523.**Copyright:** © 2023, Dr. Sathya R, et al. This is an open access journal and article distributed under the terms of the creative commons’ attribution non-commercial License. Which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.**Type of Publication:** Original Research Article**Conflicts of Interest:** Nil**Abstract**

Background: Medicinal plants have been used traditionally to cure numerous diseases. Recently, there has been a sudden increase in the use of herbal extracts as an alternative approach to modern day medicines due to the advantages of being safe besides being affordable and ease of procurement. Garlic, Curcumin and Coconut oil possess antimicrobial properties against oral pathogens.

AIM: To evaluate the antimicrobial efficacy of:

Garlic, Curcumin, Virgin Coconut Oil (VCO) and Cow urine against oral pathogens.

To compare the antimicrobial efficacy of these extracts and Cow Urine with the standard antimicrobials Amoxycillin and Nystatin

Methodology: Cow urine samples from normal cow were collected using sterile container. The fresh forms of Curcumin and garlic were powdered, and extracts were obtained by Soxhlet’s extraction process. VCO was

produced from fresh endosperm of coconut by wet extraction process. The antibacterial efficacy was assessed by broth dilution and disc diffusion assay.

Results: Garlic and Cow urine exhibited the maximum antibacterial efficacy with the highest zone of inhibition against oral pathogens followed by curcumin and the difference between these were statistically significant.

Keywords: VCO, Amoxycillin, Nystatin

Introduction

Oral health is an inseparable part of general health and relates to the quality of life. Health promotion measures are deemed incomplete without promotion of oral health and prevention of oral diseases ¹.

The global need for prevention and alternative treatment of oral diseases that are safe, effective and economical comes from the rise in disease incidence (particularly in developing countries), increased resistance by pathogenic bacteria to currently used antibiotics and Che

motherapeutics, opportunistic infections in immunocompromised individuals and economic considerations in developing countries. Traditional medicine is known to be a fertile ground for the source of modern medicines (Corson, Crews, 2007) and have been used to treat many infectious diseases since ages 6. Recently there is more inclination towards traditional or non-antibiotic approaches due to the upsurge in antibiotic resistance. Researchers are exploring several herbal products for their antimicrobial efficacy.

In ancient system of medicine, urine of cow was used as an antibiotic in the treatment of various ailments. CU possess antimicrobial activity due to the presence of certain volatile and non-volatile components. It contains urea, creatinine, carbolic acid, phenols, calcium, and manganese which are responsible for the antimicrobial and germicidal properties⁷.

Garlic botanically known as *Allium sativum* is very effective against antibiotic resistant organisms. Allicin being the main active component of garlic destroys the cell wall and cell membrane of bacteria⁸.

Turmeric which is mainly composed of curcuminoids has a wide range of beneficial properties, including anti-inflammatory, antioxidant and chemotherapeutic activity. These curcuminoids are polyphenols with a strong antioxidant function^[9-11].

Virgin coconut oil is extracted directly from coconut milk which predominantly comprise of lauric acid that exerts antibacterial efficacy against Gram-positive bacteria and lipid-coated viruses and inhibition of microbial signal transduction and transcription^[12-13].

The aim of the present study was to evaluate and compare the anti-microbial efficacy of cow urine, garlic, curcumin and coconut oil against oral pathogens.

Materials and methods

Preparation of extracts: The fresh forms of garlic cloves and Curcumin were collected, made into pieces, dried at room temperature, finely powdered and subjected to Soxhlet's extraction process. The dried extracts thus obtained were collected and stored in sterile containers. Virgin coconut oil was produced by wet extraction process of the fresh endosperm of the coconut. The flesh from coconut was extracted, grated and pressed using a sterilized sieve to produce coconut milk which was allowed to ferment for 48 hours. After fermentation, 3 layers were formed from which the protein coat and water layer were separated from the oil. The oil was passed through 1.5 mm pore size filter, collected in a sterile vial and stored at 40 Celsius. Fresh Cow Urine was collected from a healthy cow.

The standard antimicrobial discs, Amoxycillin and Nystatin was procured and used for analysis.

Microbiological analysis

The bactericidal activity was determined by agar well diffusion method. Nutrient agar and Nutrient broth were used for the four bacterial cultures whereas the Potato Dextrose agar and broth was be used for *Candida albicans* (fungi). Standard fungal and microbial cultures were sourced from Microbial Test Collection Centre, Chandigarh. The organisms under the study were *Streptococcus mutans* (MTCC890), *Enterococcus faecalis* (MTCC 439), *Lactobacillus acidophilus* (MTCC10307), *Escherichia coli* (MTCC44), *Candida albicans* (MTC C227)

Determination of MIC

Bacterial strains were grown overnight on MHA (Mueller-Hinton Agar) plates at 37°C before being used. The anti-microbial activity of extract was examined using the standard broth dilution method. The MIC was determined in MHA broth using serial two-fold dilutions of

extract in concentrations ranging from 200 to 12.5% (V/V) initial bacterial inoculums of 10⁶ CFU/ml and the measurements were obtained in triplicates to confirm the value of MIC for each tested bacterium.

Disc diffusion assay

3 Agar plates were inoculated with a standardized inoculum of the test microorganism and filter paper discs (about 6 mm in diameter), containing the test compound at the desired concentration, were placed on the agar surface using sterile forceps. The antimicrobial agent diffused into the agar and inhibited germination and growth of the test microorganism and the diameters of inhibition growth zones were measured using a millimetre scale. The antibiotic disc (Amoxycillin) was kept as control against the bacteria and Nystatin against *Candida* and incubated. Reading was taken at 600 nm using ELISA plate reader (TECAN -MODEL)

Statistical analysis

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. Zone of Inhibition was expressed in terms of Mean & SD for each study group. Kruskal Wallis Test followed by Dunn's Post hoc test was used to compare the mean ZOI between different groups against study organisms. The level of significance was set at $P < 0.05$.

Results

Table no 1 shows that Garlic was effective against all the tested organisms. The Zone of inhibition was significantly higher than turmeric and the standard drug used (Amoxycillin and Nystatin). Cow Urine was also effective against all the organisms except *L. acidophilus*. No statistically significant difference was seen between Cow Urine and Garlic. Coconut oil did not show any anti-microbial properties.

Discussion

Infectious diseases caused by bacteria, fungi, viruses and parasites are a major threat to the health of human beings. Due to the upsurge in the incidence of antibiotic misuse and overuse, researchers are focusing towards naturally available products to combat infections in human beings. Since ancient times, the use of natural products has been in practice 14. In Ayurveda, various herbs and medicinal plants are used as an adjunct for oral health care which has been proved to be safe and effective. Due to the beneficial effects of herbal products as they possess antibacterial, anti-inflammatory and anti-cariogenic effects, dentistry has witnessed the footprints of these products in the form of dentrifice, mouth rinses, root canal irrigants, storage media for avulsed tooth etc. Cow urine, in ancient system of medicine was used as a broad-spectrum antibiotic. The presence of amino acids and urinary peptides in cow urine enhance the bactericidal effect by increasing the bacterial cell surface hydrophobicity. The higher amounts of phenols in fresh CU make it 4 more effective against microbes. Studies have also shown that pregnant cow urine is more efficient due to the presence of endogenous peptides⁷. In the present study, Cow urine exhibited excellent antifungal as well as anti-bacterial properties. It was effective against all the tested microorganisms except *Lactobacillus acidophilus*. The Zone of inhibition was much higher than both Amoxycillin and Nystatin which was statistically significant. No statistically significant difference was seen between Garlic and Cow urine. This was in accordance to the study done by Sanyogita et al. They had evaluated the anti-fungal activities of cow urine of both the indoor feeding cow and an outdoor grazing cow. The results showed that the urine of the outdoor grazing cow that consumes variety of green grass including medicinal herbs showed better anti-

fungal properties 15. The cow selected for our study was also an outdoor feeding cow and were fed on medicinal herbs. The antibacterial activity of cow urine is attributed to the presence of different components like urea, uric acid, nitrogen, sulfur, copper, iron, sodium, other salts, carbolic acid, ammonia, sugar lactose, Vitamin-A, B, C, D, E, gonadotropin⁷.

The study showed that Garlic has excellent antibacterial efficacy against all the tested organisms. Allicin and other components such as thiosulfinates present in the garlic are considered to be responsible for the therapeutic benefits reported 17. The component of Allicin is produced from the enzymatic activity of Alliinase. It destroys the cell wall and cell membrane of bacteria. Allicin has ability to prevent the germination of spores 18. The results of this study demonstrated the anti-microbial efficacy of garlic against all the oral pathogens which was in accordance with the studies conducted by Devraj et al, SG et al., K shirsagar MM et al., Fatemeh AM et al., Mansour A et al. and Groppo FC et al.

Curcumin (diferuloylmethane) is the main yellow bio active component of turmeric. It possesses antibacterial property against a number of Gram positive and Gram-negative bacteria. It has been suggested that curcumin inhibits bacterial cell division, by perturbing the cytokinetic Z-ring through a direct interaction with FtsZ 21. In a study conducted by Praveenkumat et al, it was found that Curcumin exhibited antibacterial activity against all pathogens namely S mutans, Lactobacilli, Actinomyces, P gingival is but not against E faecalis. In our study Curcumin was effective against all pathogens including E faecalis at concentration of 250 mg/ml and more.

Coconut oil is a rich source of beneficial medium chain fatty acids (MCFAs), particularly, lauric acid, capric acid, caprylic acid, and caproic acid. The antimicrobial

effect of coconut oil was first reported by Hierholzer and Kabara. Recent studies have shown that coconut oil has antimicrobial activity against various gram positive and gram-negative organisms such as Escherichia vulneris, 5 Enterococcer spp, Helicobacter pylori, Staphylococcus aureus, Streptococcus mutans and Candida albicans. However in the present study Coconut oil did not show antimicrobial activity against any of the oral pathogens. Coconuts from different sources and varying degrees of ripeness might show varying antibacterial effects²².

The results of the present study state that extracts of garlic, curcumin and cow urine were effective in decreasing the viability of common oral pathogens and more effective than standard antibiotic Amoxycillin and anti-fungal Nystatin.

Conclusion

The present study compared the antimicrobial effect of Cow Urine and various herbal extracts with the standard Amoxycillin and Nystatin. The antimicrobial efficacy of Cow urine and garlic was significantly higher than the other extracts including the standard antimicrobials Amoxycillin and Nystatin. Amoxycillin was effective only against E coli. Virgin coconut oil did not show any antimicrobial efficacy against any of the tested microorganisms.

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Legend Tables and Graph

Table 1: Comparison of mean ZOI (in mm) for various organism’s b/w different study groups using Kruskal Wallis Test

Bacteria	Turmeric		Garlic		Cow's Urine		Coconut Oil		Std. Drug		p-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
E Coli	2.33	4.04	20.67	1.53	19.33	1.53	0.00	0.00	4.00	6.93	<0.001*
E Faecalis	7.33	0.58	19.67	1.53	17.00	1.00	0.00	0.00	0.00	0.00	<0.001*
S Mutans	7.33	0.58	15.00	1.00	20.33	0.58	0.00	0.00	0.00	0.00	<0.001*
L Acidophilus	9.00	1.00	24.33	1.53	0.00	0.00	0.00	0.00	0.00	0.00	<0.001*
C Albicans	5.00	4.36	13.33	0.58	26.67	1.16	0.00	0.00	16.67	5.86	<0.001*

* - Statistically Significant

Note: a. Kruskal Wallis Test & b. Mann Whitney Test

Standard Drug implies Amox for all organisms except for C. Albicans, whereas Nystatin was used for the C. Albicans

Table 2: Multiple comparison of mean diff. in ZOI for various organism’s b/w groups using Dunn's Post hoc Test

Groups	T vs G	T vs CU	T vs CO	T vs SD	G vs CU	G vs CO	G vs SD	CU vs CO	CU vs SD	CO vs SD
E Coli	0.001*	0.002*	0.93	0.98	0.99	<0.001*	0.002*	0.001*	0.004*	0.69
E Faecalis	<0.001*	<0.001*	<0.001*	<0.001*	0.02*	<0.001*	<0.001*	<0.001*	<0.001*	1.00
S Mutans	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	1.00
L Acidophilus	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	1.00	1.00	1.00
C Albicans	0.04*	<0.001*	0.40	0.01*	0.004*	0.004*	0.74	<0.001*	0.03*	0.001*

* - Statistically Significant

T – Turmeric Group; G – Garlic Group; CU – Cow’s Urine Group & CO – Coconut Oil Group; SD – Standard

