



ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACT OF *CURCUMA LONGA* IN THE MANAGEMENT OF FISTULA IN ANO

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ABSTRACT

Ayurvedic parasurgical treatment procedure *Ksharasutra* was one of the effective treatment for management of the fistula in ano. *Curcuma longa* was the one of the main ingredient in a thread. In the present work an attempt has been made to analyze the antibacterial and potential of methanol, ethanol and aqueous extracts of *Curcuma longa* against *E.coli*, *S. aureus*, *S.agalactiae* and *P.aeruginosa* among bacterial strains by disc diffusion method and the antibacterial were determined by measuring the diameter of zone of inhibition. The study reports on extraction method using soxhlet extractor The methanol extracts of *Curcuma longa* possessed the highest antibacterial with a zone of inhibition of 34 mm for, *S. aureus* and lowest 13mm for *P.aeruginosa*, ethanol extract has range of zone of inhibition 31mm-10mm while lowest antibacterial activities was reported aqueous extract range 28mm-11mm zone of inhibition.

KEYWORDS: *Curcuma longa*, Agar well diffusion method Antibacterial activity.

INTRODUCTION

According to World Health Organization medicinal plants serve as the best source for a variety of drugs. Therefore, such plants are being investigated for better understanding of their medicinal properties (Alo, et al., 2012). Plants have been used since ancient times to treat common infectious diseases whether locally within the dermis or a blood infection (Ozcan et al., 2009). Resistance towards drugs developed by pathogenic microorganisms due to their indiscriminate use and side effects due to synthetic drugs, has recently drawn much attention towards plant extracts and biologically active compounds isolated from plant species used in herbal medicine. Medicinal plants represent an alternate to synthetic drugs for the treatment of several non-severe infectious diseases and can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. (Mathur et al., 2011).

Anal fistula a condition is chronically inflamed, abnormal tunnel between the anal canal and the outer skin of the anus. It often drains watery pus, which can irritate the outer tissues and cause itching, pain and discomfort. Fistula in ano is one of the commonest ailments pertaining to the ano-rectal area and is very

difficult to cure by surgical intervention where side effects like incontinence and high chance of recurrence are commonly experienced.

The *Ksharasutra* refers to a medicated thread described in the Ayurvedic text 3000 years back. This thread is prepared by repeatedly smearing special medicines. The technique of treatment involves ligating the entire fistulous tract with a caustic ligature without performing excisional therapy.

During the *Ksharasutra* treatment no antibiotic or antimicrobial drug is administered to the patients. But the chronic granulating tract which usually occurred in ano rectal region frequently gets contaminated with faecal matter and harbor the microorganism. *Curcuma longa* is one of main ingredient in *Ksharasutra* is responsible for the antimicrobial activity.

Curcuma longa has hundreds of molecular constituents, each with a variety of biological activities. For instance, there are at least 20 molecules that are antibiotic, 14 are known cancer preventives, 12 that are anti-tumor, 12 are anti-inflammatory and there are at least 10 different anti-oxidants. Infect, 326 biological activities of curcuma

longa are known. This also testimony to the use of whole herbs and not just isolated molecules. The important alkaloids in *Curcuma longa* are curcuminoids, cucumin, demethoxycurcumin and bisdemethoxycurcumin

METHODOLOGY

Antibacterial activity of *Curcuma longa*

Preparation of the plant sample: These rhizomes were identified by Department of *Dravyaguna*, Gampaha Wickramarachchi Ayurveda Institute. Selected rhizomes were washed thoroughly, kept for sometimes for drying and sliced the rhizome kept in oven for 72 hours at 50°C temperature for drying. The dried rhizome was crushed using crusher to make a coarse powder was then stored in air tight container and kept in cool, dark and dry place for the investigation.

Preparation of Aqueous extraction of *Curcuma longa*

rhizome: Finely powdered of 10g sample of *Curcuma longa* was taken in to thimble and placed in a Soxhlet apparatus. Then 250ml of distill water, was added and extracted at the (boiling point-70°C to 90°C). After completion of the extraction the dark brown extract was stored in refrigerated condition until use. The dried residue was dried over anhydrous sodium sulphate in a desiccator to remove moisture. Finally the extract was stored in a brown glass bottle and kept in refrigerator at 4°C for anti-bacterial analysis.

Preparation of Methanol extraction of *Curcuma longa*

finely powdered 10g of *Curcuma longas* sample was placed in a Soxhlet apparatus. Then 250ml of Methanol was added and extracted .Soxhlet apparatus was run for 03 hrs and 07 cycles at a temperature not exceeding the boiling point(65°C) of the solvent. Color of extraction was found to be dark grey. Then the supernatant was evaporated to dry at 70°C in laboratory water bath for 12 hours. The residue was dried over anhydrous sodium sulphate in desiccator to remove trace of methanol. Finally the extract was stored in a brown

glass bottle and kept in refrigerator at 4°C for anti-bacterial analysis.

Preparation of Ethanol extraction: weigh 10g finely powdered *Curcuma longa* sample in to thimble and placed in a Soxhlet apparatus.250ml of ethanol was added and extracted Soxhlet apparatus was run for 03 hrs and 07 cycles at a temperature not exceeding the boiling point (55°C) of solvent. Color of extraction was found in dark grey. Then the supernatant was evaporated to dry at 60°C in laboratory water bath for 12 hours. The extract in water bath was at the syrup consistency. The residue was dried over anhydrous sodium sulphate in desiccator to remove trace of ethanol. Finally extract was stored in a brown glass bottle and kept in refrigerator at 4°C for anti-bacterial analysis.

Antimicrobial Assay: Bacterial broth culture was prepared to a density of 10^8 cells ml^{-1} according to 0.5 McFarland standards. The aliquot was spread evenly onto Muller Hinton agar by sterile cotton swab. Then, the plated medium was allowed to dry at room temperature for 30 minutes. On each plate, equidistant wells were made with a 6 mm diameter sterilized, cork borer, 2 mm from the edge of the plate. Fifty micro liter of each methanol, ethanol and aqueous extract (100 mg/mL) was aseptically introduced into a respective agar well. Amoxicillin (100 mg/mL) were used as positive controls and the distilled water was included as negative controls. This was followed by allowing the agar plate on the bench for 40 minutes pre-diffusion followed by incubation at 37 °C for 24-48 h. The formation of clear inhibition zone of ≥ 7 mm diameters around the wells were regarded as significant susceptibility of the organisms to the extract. The experiment was performed in duplicate. Experiments that gave contradicting results were done for the third time for an easy decision (Shanmugam *et al.*, 2014) the antibacterial activity was evaluated by agar well diffusion method.

OBSERVATION AND RESULTS

Table 1: Antibacterial activity of *Curcuma longa* Ethanol, methanol and Aqueous Extracts against Gram positive and Gram negative bacteria strains.

Name of the bacteria tested	ZOI (mm)			
	Standard	Ethanol Extract	Methanol extract	Aqueous extract
<i>S. aureus</i> (ATCC 25923)	30.0±2.61	31.5±1.05	34.5±0.54	28.8±1.7
<i>E. coli</i> (ATCC 25922)	20.8±2.48	22.5±0.55	23.66±0.5	15.6±1.03
<i>S. agalactiae</i> ATCC (19615)	22±0.63	22.5±0.55	24.5±1.38	20.5±1.05
<i>P. aeruginosa</i> ATCC (27853)	6±0	10.83±0.98	13.6±0.5	11.3±1.03

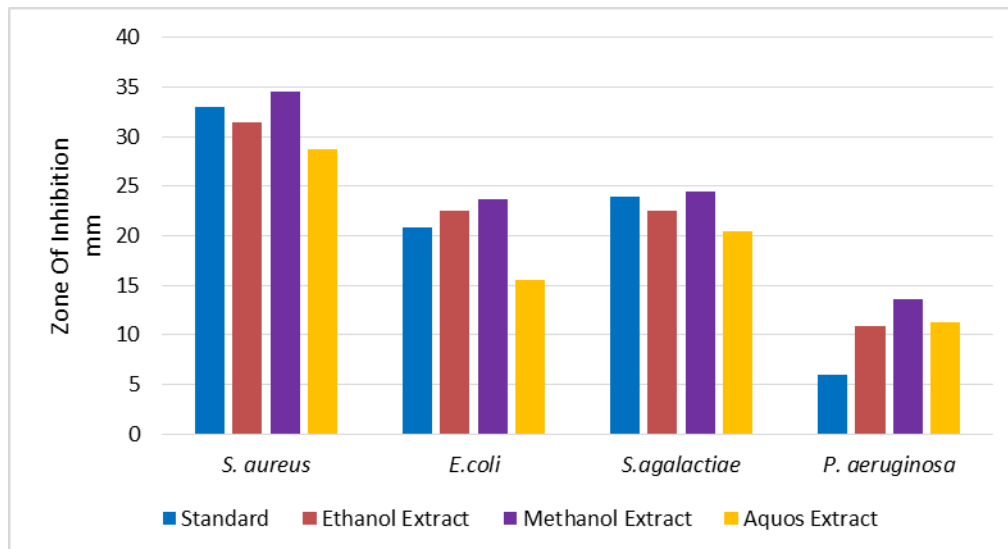


Fig 1: Antibacterial activity of *Curcuma longa* Ethanol, methanol and Aqueous

Extracts against Gram positive and Gram negative bacteria strains.

Antibacterial activity of *Curcuma longa* Ethanol, Methanol and Aqueous extract were evaluated using amoxicillin as the standard antibiotic and ATCC type cultures of the Gram positive and Gram negative bacteria were tested for the antibacterial sensitivity. It was observed that all three extract had antibacterial activity for the four organisms tested, but standard antibiotic did not show antibacterial activity against *P. aeruginosa*.

Among the three extracts highest ZOI was observed for the Methanol extract of *S. aureus* 34.5 ± 0.54 , *E. coli*, 23.66 ± 0.5 , *S. agalactiae*, 24.5 ± 1.38 and *P. aeruginosa*, 13.6 ± 0.5 . Aqueous extract reported relatively low ZOI for *S. aureus* 28.8 ± 1.7 , *E. coli*, 15.6 ± 1.03 , *S. agalactiae*, 20.5 ± 1.05 and *P. aeruginosa*, 11.3 ± 1.03 . Ethanol extract showed much better ZOI than the Aqueous extract; *S. aureus* 31.5 ± 1.05 , *E. coli*, 22.5 ± 0.55 , *S. agalactiae*, 22.5 ± 0.55 and *P. aeruginosa*, 10.83 ± 0.98 . Standard antibiotic possess relatively low ZOI compared to the methanol extract for *S. aureus* 30.0 ± 2.61 , *E. coli* ZOI inhibition was 20.8 ± 2.48 and for *S. agalactiae* 22 ± 0.63 . Furthermore slightly higher ZOI were observed in antibiotic against aqueous extract and Ethanol extract for; *S. aureus* but for *E. coli* only in Aqueous extract. For *S. agalactiae* higher inhibition was observed in both aqueous and ethanol extracts. In this study by using the mean separation method of least significant differences (LSD), methanol extract was observed significant difference from the other extract as well as for the standard antibiotic. This difference was statistically highly significant

DISCUSSION

Curcuma longa aqueous, methanolic and ethanolic extract were tested for their antibacterial activity all these extract possess significant zone inhibition against *S. aureus* type *E. coli* *S. agalactiae* and *P. aeruginosa*.

Moreover methanolic extract observed highest ZOI; *S. aureus* 34.5 ± 0.54 *E. coli* 23.66 ± 0.5 *S. agalactiae* 24.5 ± 1.38 and *P. aeruginosa* 13.6 ± 0.5 followed by the Ethanol ZOI; *S. aureus* 31.5 ± 1.05 *E. coli* 22.5 ± 0.55 *S. agalactiae* 22.5 ± 0.55 and *P. aeruginosa* 10.83 ± 0.98 and aqueous extract Ethanol ZOI; *S. aureus* 28.8 ± 1.7 *E. coli* 15.6 ± 1.03 *S. agalactiae* 20.5 ± 1.05 and *P. aeruginosa* 11.3 ± 1.03 . There was statistically significant $P < 0.001$ ZOI observed for methanolic extract in all the four bacteria species. There were significant difference was observed in comparison of mean ZOI for between three *C. longa* extracts (Table 1).

Curcumin, an important constituent of *Curcuma Longa*, is known for various biological activities including antibacterial and anti-oxidant etc. The present study focused on the antibacterial activity of curcumin as a significant component of *Curcuma Longa*, against four genera of bacteria, including those that are Gram-positive (*S. aureus* and *S. agalactea* and Gram-negative *E. coli* and *P. aeruginosa*). This study shows the strong antibacterial potential of *Curcuma Longa* against all the tested bacteria of Gram-positive as well as Gram-negative groups. The fine powder of *C. longa* has been used for the *ksharasutra* preparation.

It has been observed that methanol extract of *C. longa* samples showing much better antibacterial activities in contrast to aqueous extract, which may be because of organic nature of methanol for the reason of its high capacity to dissolve more organic and active antimicrobial compounds (Cowan, *et al.*, 1999). The antimicrobial action of the aqueous extracts could be ascribed to the anionic components such as thiocyanate, nitrate, chlorides and sulfates besides other water soluble components which are naturally occurring in the plant material (Darout *et al.*, 2000). These results confirmed that the substantiation of previous studies which have reported that methanol is a better solvent for more consistent extraction of antimicrobial substances from

medical plants compared to other solvents, such as water (Ahmad *et al.*, 1998, Eloff, 1998, Lin *et al.*, 1999).

Curcuma longa extract contained alkaloids, tannin, flavonoid, glycoside and carbohydrate. There are reports showing that alkaloids and flavonoids are the responsible compounds for the antibacterial activities in higher plants (Cordell *et al.*, 2001). In this study alkaloids and flavonoids are detected in *Ksharasutra* extract.

CONCLUSIONS

It can be concluded that methanolic plant extracts give the maximum inhibition against several pathogenic bacterial species, the ethonolic extracts were also reported to be having moderate antibacterial properties but aqueous extract, had lesser extent as compared to those of the methanolic and ethonolic extracts. Thus, it can be said that *curcuma longa* have great potential of antimicrobial activity hence, it contribute to the success of *Ksharasutra* treatment in the management of fistula in ano.

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