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ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF *CURCUMA LONGA* ON HUMAN PATHOGENS AND ANTIBIOTICS PATTERN

Microbiology

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ABSTRACT

Plant extracts and biological active compounds are receiving more attention due to antibiotic side effects and bacterial resistance build against antibiotics. Turmeric (*Curcuma longa*) belongs to the family Zingiberaceae, has been used for thousands of years as a flavouring agent, medicinal herb, antimicrobial, antioxidant and a dyeing agent. In India, it is commonly known as 'Haldi' and is broadly cultivated in all parts of India. Microorganisms such as *Staphylococcus aureus* and *Escherichia coli* are considered to be the most resistant species. Therefore; in the present study, twelve different concentrations of ethanolic extract of *Curcuma longa* rhizome were prepared and evaluated antibacterial activity by agar well diffusion method. Ethanolic extracts of *C. longa* rhizome showed good antibacterial activity. Most of bacteria found resistance to antibiotics but these pathogens were sensitive to *C. longa* rhizome. MIC of *E. coli* (78mg/ml), *Klebsiella pneumoniae* (87.77mg/ml), *Pseudomonas aeruginosa* (75.55mg/ml), *Staphylococcus aureus* (74.28mg/ml), *Streptococcus viridans* (80mg/ml) and *Enterococcus faecalis* (68.88mg/ml) were recorded. This study confirms the efficacy of *C. longa* extracts as natural antibacterial and suggests the possibility of employing them in drugs for treatment of infectious diseases caused by the test pathogens.

KEYWORDS

Curcuma longa, Ethanolic extracts, Agar well diffusion, Antibacterial activity, Antibiotics pattern.

INTRODUCTION

Plant extracts and biological active compounds are receiving more attention due to antibiotic side effects and bacterial resistance build against antibiotics. Antibiotic resistance is one of the biggest risks to global health, food safety and development today. The world needs to change the way of using antibiotics. Even if new medicines are developed, without behaviour change, antibiotic resistance and its side effects will remain a major threat (World health organization). Due to this problem now-a-days many researchers work on natural antimicrobial sources like plant extracts, phytochemical and secondary metabolites and try to make new drugs either synthetic or natural. Many plants in different country around the world have been extracted, semi-abstersion to explore individually their antimicrobial activity. However, very little information is available on such activity of medicinal plants and out of the four lacs plant species on earth, only a small amount has been systematically investigated for their antimicrobial activities (3,4). The use of plant compounds as natural sources for pharmaceutical purposes has constantly increased in Brazil. The ability of higher plants as sources for new drugs is still mostly unexposed (1).

Turmeric (*Curcuma longa*) belongs to the family Zingiberaceae, has been used for thousands of years as a flavouring agent, a medicinal herb, and a dyeing agent. It has antimicrobial, antioxidant, astringent, and other useful properties, it also useful in treatment of various infectious diseases. In India it is commonly known as 'Haldi' and is largely cultivated in all parts of India (2). Turmeric is an aromatic herb. Rhizomes are tuberous or horizontal, rarely aerial, short or elongated. Constituents of turmeric are curcuminoids, it includes mainly curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin compound is the responsible and important for the biological activities of turmeric. Curcumin is soluble in ethanol, methanol, ketone, acetone, acetic acid and chloroform and insoluble in water.

Currently, most bacteria isolated from various samples such as urine, pus, sputum, blood are either aerobic or anaerobic and gram negative or gram positive microorganisms. Microorganisms such as *Staphylococcus aureus* and *E. coli* are considered to be the most resistant species. Therefore, the motive of this study was to estimate the antibacterial activity of the ethanolic extracts of *Curcuma longa* rhizome against human pathogenic microorganisms.

METHODOLOGY

Material- Fresh rhizomes of *Curcuma longa*, ethanol, distilled water, whatman's filter paper number 1, air tight bottle etc (Micro bio lab, RNT medical College, Udaipur).

Sample collection- Six bacterial species were isolated from various samples such as urine, sputum, blood, pus arrived in Micro bio lab, RNT medical College, Udaipur.

Test Microorganisms- *E.coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus viridans* & *Enterococcus faecalis*.

Media used – MHA (Muller Hinton Agar).

Extract preparation – The rhizomes of *C.longa* were washed with distilled water and dried. They were cut into small pieces and dried in an oven at a temperature of 45±5 °C for a period of about 7-8 days till they were completely moisture-free. The small pieces were ground to make a powder form. For concentration of 200 mg/ml, 2 Gms powder of the Curcuma longa rhizomes was placed in a small flask and 10 ml ethanol was added in it. For evaporation flask was allowed to stand for 7 days at room temperature or used a water bath to evaporate it. Filtration was transferred in a beaker using a Whatman's filter paper number 1. Filtered was stored in a refrigerator at 4°C in a test tube or a beaker. 10 ml distilled water was added in it. It was stored in a refrigerator at 4 °C in a dark colored pre-sterilized airtight container until its further use. This solution was made again and diluted in different concentrations.

Preparation of microbial inoculum – 24 hours old culture was used for the preparation of bacterial inoculum. The turbidity of the culture was maintained according to the 0.5 MacFarland standards, so the number of microbes maintain within a given range.

Antibacterial susceptibility test – Agar well diffusion method was used to conduct the antibacterial susceptibility test. Twelve different concentrations 10mg/ml to 100mg/ml, 150m g/ml, and 200mg/ml of ethanolic extract of *C. longa* rhizome were prepared.

A sterile cotton swab was immersed into the bacterial inoculum. The MHA media plate was inoculated with the test microorganism by swabbing over the entire surface of the plate three times, rotating the plate approximately 60 degrees. Wells of 6 mm size were made with sterile borer or a tip of micropipette into agar plates containing the bacterial inoculums. 100µl volume of each concentration of the *Curcuma longa* extract was filled into the wells. Sterilized distilled water or ethanol used as a negative control which were introduced into the wells instead of extract. The plates were refrigerated or keep at room temperature for few minutes allowing the diffusion of the extract into the agar. After incubation for 24 hours at 37°C, plates were observed. If antibacterial activities were present, it was indicated by inhibition zones surrounding the agar well containing the extracts. Inhibition zones were measured by scale and expressed in millimetres. Antibacterial activity was recorded if the inhibition zones were more than 9mm. Less than 9mm diameter of zones were considered as

inactive, 9 to 12mm as partially active, while 13-18mm as active and more than 18mm as very active (1). The mean or average values of the diameter of inhibition zones were calculated.

Determination of MIC – Minimum inhibitory concentration (MIC) is the lowest concentration of a extract or a chemical or usually a drug, which prevents visible growth of bacteria and other microbes. It is depends on the microorganism which affected human being. Rhizome ethanolic extracts were prepared to the highest concentration of 200mg/ml (stock concentration) in ethanol and serially diluted by distilled water in others lower concentrations.

These different concentrations were used in agar well diffusion method to conduct the antibacterial susceptibility test. The least concentration where diameter of inhibition zone was near or equal to 9mm was the MIC value. For each bacterial species, MIC was determined by many times and then calculates the mean value of it.

RESULTS

Three major concentrations (200,150,100mg/ml) of ethanolic extracts of *Curcuma longa* rhizome showed good antibacterial activity against all tested microorganisms.

Concentrations between 50mg/ml to 90mg/ml showed less antibacterial activity and concentrations below 40mg/ml didn't show any antibacterial activity.

Mean value of diameter of inhibition zones of ethanolic extract of *Curcuma longa* rhizome at Concentration of 200mg/ml for *E. Coli* (18mm), *Klebsiella pneumoniae* (20mm), *Pseudomonas aeruginosa* (21mm), *Staphylococcus aureus* (20mm), *Streptococcus viridans* (23mm) and *Enterococcus faecalis* (22mm) were recorded (Table-1).

Maximum inhibition zones of ethanolic extract of *Curcuma longa* rhizome at concentration of 200mg/ml for *E.coli* (23mm) , *Klebsiella pneumoniae* (25mm), *Pseudomonas aeruginosa* (28mm), *Staphylococcus aureus* (25mm), *Streptococcus viridans* (29mm) and *Enterococcus faecalis* (30mm) were recorded (Table-2).

The negative controls, distilled water and ethanol did not show any inhibitory activity on the microorganisms.

MIC of *E.coli* (78mg/ml), *Klebsiella pneumoniae* (87.77mg/ml), *Pseudomonas aeruginosa* (75.55mg/ml), *Staphylococcus aureus* (74.28mg/ml), *Streptococcus viridans* (80mg/ml) and *Enterococcus faecalis* (68.88mg/ml) were recorded (figure-1).

Overall 70% of antibiotics used were resistant to tested pathogens and only 30% of antibiotics were sensitive. Ethanolic extracts of *Curcurma longa* rhizome showed 80% sensitivity and 20% resistant to tested pathogens (figure-3).

Most of bacteria found resistance to antibiotics but these pathogens were sensitive to ethanolic extract of *Curcuma longa*. Ethanolic extracts of *C.longa* rhizome showed more sensitivity than antibiotics used (Table-1&3 and figure-2&3).

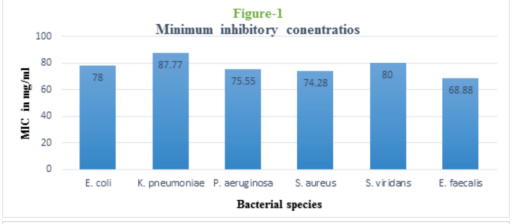
Table - 1		Mean values of diameters of inhibition zones (mm)					
		E. coli	***K. pneumoniae***	***P. aerugi nosa***	***S. aureus***	***S. viridans***	***E. faecalis***
Ethanolic extracts of *C. longa*	**100mg /ml**	12	13	15	14	18	14
	150mg /ml	15	17	17	18	21	18
	200mg /ml	18	20	21	20	23	22
Antibiotics	**Gen**	14	16	15	16	18	17
	Cef	16	-	17	-	-	14
	Amo	-	14	-	-	-	-
	Amp	-	-	16	-	15	14
	Amc	S	-	-	S	S	-
	Cip	19	18	16	-	15	16
	Dox	12	13	12	14	-	13
	Tet	14	15	15	17	-	15
Negative control (Distilled water)		-	-	-	-	-	-

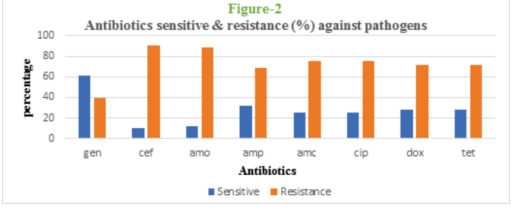
Gen- Gentamicin, Cef- Ceftazidime, Amo- Amoxicillin, Amp- Ampicillin, Amc- Amoxyclave, Cip- Ciprofloxacin, Dox- Doxycycline, Tet- Tetracycline

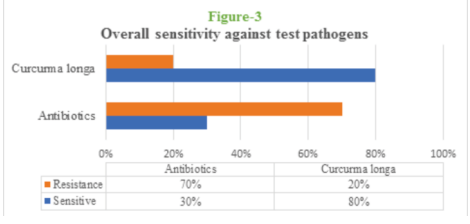
Table-2 **Microorganism**	**Maximum inhibition zones at different concentrations of ethanolic extracts of *C. longa* rhizome**		
	100mg/ml	**150mg/ml**	**200mg/ml**
E.coli	16	21	23
K. pneumoniae	17	21	25
P. aeruginosa	22	24	28
S. aureus	19	22	25
S. viridans	18	22	29
E. faecalis	24	28	30

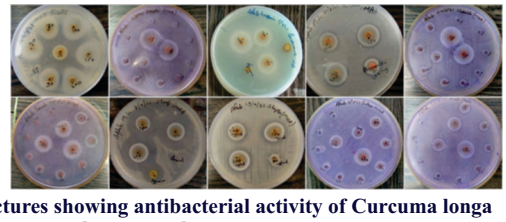
Table - 3	Antibiotics pattern (S- Sensitive and R- Resistance)															
Microorganisms	**Gen**		**Cef**		**Amo**		**Amp**		**Amc**		**Cip**		**Dox**		**Tet**	
	S%	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**
E.coli	40	60	10	90	0	100	0	100	30	70	10	90	30	70	20	80
K. pneumoniae	50	50	0	100	20	80	0	100	0	100	30	70	30	70	50	50
P. aeruginosa	80	20	30	70	0	100	40	60	0	100	30	70	30	70	10	90
S. aureus	70	30	0	100	0	100	0	100	20	80	0	100	30	70	50	50
S. viridans	100	0	0	100	50	50	100	0	100	0	50	50	0	100	0	100
E. faecalis	30	70	20	80	0	100	50	50	0	100	30	70	50	50	40	60

Gen - Gentamicin, Cef - Ceftazidime, Amo - Amoxicillin, Amp - Ampicillin, Amc - Amoxyclave, Cip - Ciprofloxacin, Dox - Doxycycline, Tet - Tetracycline









Pictures showing antibacterial activity of Curcuma longa rhizome on human pathogens

DISCUSSION

Due to antibiotic resistance and its side effects problems, we discussed on natural sources to estimate the antibacterial properties of plant extracts. We studied on some researches and only few studied were found systematically. Some research has shown very less antimicrobial activity and some have shown high. Various methodology were used which makes it complicated. Therefore, world needs more research on them and find more new ways which make it simple.

In the last few years, many studies have been conducted in different countries and areas to prove antimicrobial properties of plants (5,6,7,8,9,11,12,13). In 2012, a research (1) showed good antimicrobial activity but they showed different concentrations of aqueous and hydro alcoholic extract of *Curcuma longa* rhizome in percentage. They took only three readings of each Microorganism. So, percentage of concentration and three readings were insignificant. In the present study, we took many readings of each bacterial species and calculated the MIC in mg/ml. In 2013, a research article (10) showed very good inhibition zones of ethanolic extract of *C. longa* rhizome against *E.coli* (22.9mm), *Staphylococcus aureus* (27.8mm), *Pseudomonas aeruginosa* (23mm) and *Bacillus subtilis*(22.7mm). The present study also showed good antibacterial activity of ethanolic extracts of *C. longa* rhizome.

In our study, the antibacterial efficacy of the extracts of *Curcuma longa* rhizome was determined on the basis of inhibition zones, MIC and comparison with antibiotics pattern. Twelve different concentrations of ethanolic extract of *Curcuma longa* rhizome were prepared and evaluated antimicrobial activity by taking many readings of each bacterial species. MIC and antibiotics pattern also calculated.

In the present study, we found that ethanolic extracts of *Curcuma longa* rhizome revealed effective antibacterial properties. Data in the tables-1&3 and figures-2&3 reveals that ethanolic extract of *Curcuma longa* were more effective than antibiotics used.

This study showed very good inhibition zones of ethanolic extract of Curcuma longa rhizome against E.coli (18mm), *Klebsiella pneumoniae* (20mm), *Pseudomonas aeruginosa*(21mm), Staphylococcus aureus (20mm), *Streptococcus viridans* (22mm) and *Enterococcus faecalis* (23mm) (Table-1).

We found 70% of antibiotics were resistant to pathogens and only 30% of antibiotics were sensitive. Ethanolic extracts of Curcuma longa rhizome showed 80% sensitive and 20% resistance to tested pathogens. These results showed that antibiotics resistance bacteria were sensitive to ethanolic extract of Curcuma longa rhizome (Table-3 & figure-3).

MIC figure reveled that these minimum inhibition concentrations in mg/ml can be used for inhibits the tested bacterial species and treatment for many infectious diseases.

Limitation of our study was that it cannot described any method for isolation of antimicrobial components from these prepared extracts and formulation of drugs from extracts but this study and mechanism will help in future studies and may recommend for the isolation of bioactive constituents and biological methods for the standard drug preparations through natural sources.

CONCLUSION

Curcuma longa rhizome consists very good antimicrobial components and proved their effective antibacterial activity against all tested pathogens especially which causes skin diseases, pneumonia, urinary tract infections, diarrhoea, blood infection, dermatitis and bacteremia. Most of antibiotics resistance pathogens found sensitive to ethanolic extract of *Curcuma longa* rhizome. This study confirms the efficacy of *Curcuma longa* extracts as natural antibacterial and suggests the possibility of employing them in drugs for treatment of infectious diseases caused by the test pathogens. This study and mechanism will contribute greatly to the development new alternatives and further studies may recommend for the isolation of bioactive constituents and biological methods for the standard drug preparations from natural sources.

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REFERENCES

1. Hegde, M. N., Shetty, S., Yelapure, M., & Patil, A. (2012). Evaluation of antimicrobial activity of aqueous and hydro-alcoholic Curcuma longa extracts against endodontic pathogens. *IOSR Journal of Pharmacy*, *2*(2), 192-198.
2. Gupta, A., Mahajan, S., & Sharma, R. (2015). Evaluation of antimicrobial activity of Curcuma longa rhizome extract against Staphylococcus aureus. *Biotechnology reports*, *6*, 51-55.
3. Al-Manhel, A. J., & Niamah, A. K. (2015). Effect of aqueous and alcoholic plant extracts on inhibition of some types of microbes and causing spoilage of food. *Pakistan Journal of Food Sciences*, *25*(3), 104-109.
4. Varahalarao, V., & Chandrashekar, N. K. (2010). In vitro bioactivity of Indian medicinal plant Calotropis procera (Ait). *Journal of Global pharma technology*, *2*(2), 43-45.
5. Nascimento, G. G., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian journal of microbiology*, *31*, 247-256.
6. Anesini, C., & Perez, C. (1993). Screening of plants used in Argentine folk medicine for antimicrobial activity. *Journal of ethnopharmacology*, *39*(2), 119-128.
7. Almagboul, A. Z., Bashir, A. K., Farouk, A., Karim, A., & Salih, M. (1985). Antimicrobial activity of certain sudanese plants used in folkloric medicine. Screening for antibacterial activity. IV. *Fitoterapia*.
8. Ikram, M. (1984). Screening of medicinal plants for antimicrobial activities. III. *Fitoterapia*.
9. Izzo, A. A., Di Carlo, G., Biscardi, D., De Fusco, R., Mascolo, N., Borrelli, F., & Autore, G. (1995). Biological screening of Italian medicinal plants for antibacterial activity. *Phytotherapy research*, *9*(4), 281-286.
10. Yasodamma, N., Chaithra, D., & Alekhya, C. (2013). Antibacterial activity 0f Curcuma neilgherrensis WT. A Wild Curcuma Species. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(3), 571-576.
11. Araujo, C. A. C., & Leon, L. L. (2001). Biological activities of Curcuma longa L. *Memórias do Instituto Oswaldo Cruz*, *96*, 723-728.
12. Pundir, R. K., & Jain, P. (2010). Comparative studies on the antimicrobial activity of black pepper (Piper nigrum) and turmeric (Curcuma longa) extracts. *International Journal of Applied Biology and Pharmaceutical Technology*, *1*(2), 492-500.
13. Singh, N., Gupta, S., & Rathore, V. (2017). Comparative antimicrobial study of ethanolic extract of leaf and rhizome of Curcuma longa Linn. *Pharmacognosy Journal*, *9*(2).