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Antibacterial Potential of Herbal Formulation

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Abstract: Natural drugs are boon to mankind. They have few side effects as compared to allopathic medicine. This invention relates to herbal composition, having potent anti-bacterial and wound healing property. The formulation prepared is a gel, which is used for effective treatment of wounds and exhibits broad spectrum antibacterial action. Crude extracts of *Punica granatum* pericarp and *Curcuma longa* showed antibacterial activity against different strains of gram positive such as *Staphylococcus aureus*, *Bacillus subtilis* and gram negative microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Enterobacter aerogenes*. The MIC is recorded as the lowest concentration of drug which showed clear fluid without turbidity. Minimum inhibitory concentration of *Punica granatum* peel ranged from 0.05 to 3.2 mg mL^{-1} and for *Curcuma longa* MIC ranged from 5 to 320 mcg mL^{-1}. Formulation containing these extracts, showed significant zone of inhibition for 0.5, 1, 2.5, 5% of which 5% showed maximum zone of inhibition (ranging from 20.2 to 26 mm) as compared to marketed preparation. The present investigation revealed that gel formulation has potential antibacterial activity.

Key words: Antibacterial activity, *Curcuma longa*, formulation, minimum inhibitory concentration, *Punica granatum* pericarp, zone of inhibition

INTRODUCTION

The various categories of advanced wound dressing products available today, gels are popular because they are effective, comfortable, easy to use and cost effective. Medicinal herbs have been used to heal wounds, burns, skin ulcers, pressure sores, bed sores for thousands years. Pomegranate fruit rind and turmeric rhizome were formulated in a combination and its activity was studied. *Curcuma longa* Linn family Zingiberaceae is native to Southern Asia and is cultivated through out India (Rajpal, 2006). It is also distributed in China, Thailand, Java and other tropical countries. In India, Maharashtra State (Sangli), Andhra Pradesh (Nizamabad), Tamil Nadu (Erode), Kerala (Cochin) are the prominent zones where good quality turmeric are grown for food and spice processing units and industrial sector. The key constituents in turmeric are curcumin, demethoxycurcumin, bisdemethoxycurcumin known for its well known anti-inflammatory, antioxidant, antibacterial and wound healing properties. *Punica granatum* belong to the family Punicaceae. A large deciduous shrub or a small tree, wild and cultivated almost throughout India up to an altitude of 2000 m in the hills. The rind consists of high content of phenolic compound such as punicaligin, ellagic acid, gallic acid etc. The extract of fruit rind show antibacterial, antihelmintic, antifungal and antiviral activity. Micro organisms have developed resistance to many antibiotics, hence there is a need to develop alternative antibiotic drugs from plants. The present study was carried out to investigate the antibacterial properties of two herbs. Zone of inhibition and MIC were carried out in this study.

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MATERIALS AND METHODS

The herbs used for study were obtained from Jadhavji Lallubhai and Co., 245, Kalbadevi road, Mumbai-2. The drugs were then sun dried and grinded into powder. The study was conducted at Bharati Vidhyapeeth's College of Pharmacy, Navi Mumbai.

Extraction

Materials were collected and washed with distilled water; it was then subjected for drying in the sun. The dried material was then grinded to powder. The individual drug weighing 15 g each of *Punica granatum* and *Curcuma longa* were subjected for methanolic extraction using a Soxhlet extractor. The methanolic extract was then concentrated and evaporated under water bath to get it in dry powder form. The dried powder form weighing 2 g of *Punica granatum* and resinous mass of 3.5 g in *Curcuma longa* were obtained and it was used further for the formulation of the gel.

Microorganism

The bacterial strains used in the study were both gram positive and gram negative bacteria such as *Escherichia coli* (Ananthnarayan, 2005)(Strain No. NCIM 2256 NCTC 9002), *Klebsiella pneumoniae* (Strain No. NCIM 2957 ATCC NO 10031), *Pseudomonas aeruginosa* (Strain No. NCIM 5031 ATCC NO 25619), *Proteus vulgaris* (Strain No. NCIM 2027 ATCC NO 13315), *Enterobacter aerogenes* (Strain No. NCIM 2694) and gram positive organism are *Staphylococcus aureus* (Strain No. NCIM 2079 ATCC NO 6538), *Bacillus subtilis* (Strain No. NCIM 2063 ATCC NO 6633). All the bacterial strains were grown and maintained on nutrient agar slants. The inoculum size of each test strain was 1×10^8 bacteria mL^{-1}. This was standardized by adjusting the optical density of the bacterial suspension to turbidity corresponding to spectrophotometric absorbance 0.6-0.8 at 540 nm. The individual drug extract was studied using Mueller Hinton Broth by Broth dilution (Sao, 2002) and the gel formulation using Mueller Hinton Agar by cup-plate method (with the help of borer a well is made in the agar plate i.e., cup) to determine zone of inhibition. The formulated gel is then compared with the marketed preparation for the activity.

RESULTS AND DISCUSSION

Extracts and formulations were tested for antibacterial activity against gram positive and gram negative bacteria. The extracts showed antibacterial activity against gram negative microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomon aeruginosa*, *Proteus vulgaris*, *Enterobacter aerogenes* and gram positive organism *Staphylococcus aureus*, *Bacillus subtilis*. MIC of *Punica granatum* peel ranges from 0.05 to 3.2 mg mL^{-1} and for *Curcuma longa* MIC ranges from 5 to 320 mcg mL^{-1}, which was compared against the marketed preparation. The formulation 5% showed maximum zone of inhibition ranging from 20.2 to 26 mm. The results of MIC for extracts are shown in Table 1 and 2. The results are the values in triplicates. The MIC of individual extracts was higher than marketed preparation. The results of zone of inhibition are shown in Table 2. Zone of inhibition of formulation was more than marketed preparation.

Minimum Inhibitory Concentration (MIC)

MIC of individual extracts of pomegranate rind and turmeric were determined by Mueller Hinton Broth using Broth dilution method (Suwipa *et al*., 2005). The bacterial suspension was used as a positive control and broth was used as negative control. The MIC is recorded as the lowest concentration of drug, which shows clear fluid without turbidity after 24 h of the incubation at 37°C (Collins, 2004). MIC of *Punica granatum* peel ranges from 0.05 to 3.2 mg mL^{-1} and for *Curcuma longa* (Sirirak *et al*., 2005) MIC ranges from 5 to 320 mcg mL^{-1}, which was compared against marketed preparation.

Table 1: MIC for *Pomegranate pericarp* and *Curcuma longa* extract

Drug concentration (mg mL^{-1})	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Proteus vulgaris*	*Enterobacter aerogenes*
Pomegranate pericarp							
0.05	+	+	+	_	+	+	_
0.1	_	_	_	_	+	_	_
0.2	_	_	_	_	_	_	_
0.4	_	_	_	_	_	_	_
0.8	_	_	_	_	_	_	_
1.6	_	_	_	_	_	_	_
3.2	_	_	_	_	_	_	_
Curcuma longa **(mcg mL^{-1})**							
5	+	+	_	_	+	_	+
10	_	+	_	_	+	_	_
20	_	_	_	_	_	_	_
40	_	_	_	_	_	_	_
80	_	_	_	_	_	_	_
160	_	_	_	_	_	_	_
320	_	_	_	_	_	_	_

+: Presence of growth; _: Absence of growth

Table 2: Zone of inhibition for formulation

		Formulation (%)			
Microorganism	Preparations	0.5	1	2.50	5
Staphylococcus aureus	Gel formulation	12 mm	14 mm	15.5 mm	21 mm
	Marketed silver sulfadiazine preparation	11.5 mm	12 mm	12.5 mm	12.5 mm
	Marketed turmeric preparation	12 mm	13 mm	12.5 mm	12.5 mm
Bacillus subtilis	Gel formulation	14 mm	15 mm	16 mm	25 mm
	Marketed silver sulfadiazine preparation	12 mm	11.5 mm	11mm	12 mm
	Marketed turmeric preparation	13 mm	12 mm	12 mm	12.5 mm
Escherichia coli	Gel formulation	12 mm	14 mm	15.5 mm	21 mm
	Marketed silver sulfadiazine preparation	11.5 mm	12 mm	12 mm	12 mm
	Marketed turmeric preparation	12 mm	13 mm	12.5 mm	12.5 mm
Klebsiella pneumoniae	Gel formulation	11.5 mm	12 mm	16 mm	24.4 mm
	Marketed silver sulfadiazine preparation	11 mm	13 mm	13 mm`	12.5 mm
	Marketed turmeric preparation	11 mm	12 mm	12mm	13 mm
Proteus vulgaris	Gel formulation	11 mm	15 mm	17 mm	20 mm
	Marketed silver sulfadiazine preparation	7 mm	12 mm	12.5 mm	13 mm
	Marketed turmeric preparation	10 mm	12.5 mm	12.5 mm	13 mm
Enterobacter aerogenes	Gel formulation	13 mm	15 mm	16 mm	20.2 mm
	Marketed silver sulfadiazine preparation	12 mm	11.5 mm	11 mm	12 mm
	Marketed turmeric preparation	11 mm	12 mm	11.5 mm	10.5mm
Pseudomonas aeruginosa	Gel formulation	16 mm	16.5 mm	18 mm	26 mm
	Marketed silver sulfadiazine preparation	12.5 mm	11 mm	12 mm	11.5 mm
	Marketed turmeric preparation	13 mm	12.5 mm	12 mm	12 mm

5% formulation show maximum zone of inhibition as compared to other formulations. After 5% there was no significant inhibition and therefore 5% is selected as the significant

Zone of Inhibition

Zone of inhibition was carried out for different formulations by cup-plate method to evaluate the antibacterial activity. This method was studied using Mueller Hinton Agar (Supayang *et al*., 2005) against gram negative microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Enterobacter aerogenes* and gram positive microorganism such as *Staphylococcus aureus*, *Bacilus subtilis*. The plates were incubated (Negi and Jayaprakasha, 2003) for 24 h at 37°C. The formulation containing these extracts showed significant zone of inhibition for 0.5, 1, 2.5, 5%, of which 5% showed maximum inhibition ranging from 20.2 to 26 mm as compared to marketed preparation.

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