

## EVALUATION OF ANTIBACTERIAL AND ANTIHELMINTHIC ACTIVITY OF POLYHERBAL GEL

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### ABSTRACT

One half of deaths worldwide is due to infectious diseases and is leading to mortality and morbidity mainly in immune-compromised patients. The main existing problems are multi drug resistance and scarcity of novel antibiotics. This adds urgency for the development of novel, effective and affordable medicines that act against pathogens without causing any adverse effects to the host. Present investigation is carried out on following herbal extracts- *Azadirachta indica* (Leaves), *Cinnamomum zeylanicum* (bark) and *menthe longifolia* (Leaves). The aim of the present study is to formulate and evaluate antibacterial and antihelmintic activity of polyherbal topical gel formulation. The prepared polyherbal gels was subjected for antibacterial assay using both gram positive and gram negative bacterial strains through disc diffusion method; antihelmintic activity with the determination of

paralysis and death time using earthworms. The zone of inhibition produced by azithromycin was between 19 to 25 mm and by the poly herbal formulations PF1 and PF2 is between 8 to 18 mm and 14 to 29mm respectively. PF2 gel caused paralysis at  $6.16 \pm 1.94$  min and death at  $37 \pm 3.68$  min, while albendazole caused paralysis and death at  $11.5 \pm 1.64$  min and  $44 \pm 3.52$  min respectively. The above results confirmed antibacterial and antihelmintic activities of polyherbal gel therefore it may be processed for further research to find out the underlying mechanism involved.

**KEYWORDS:** Multi- drug resistance, Immune- compromised, Antibacterial activity, Antihelminthic activity, Disc diffusion method.

## INTRODUCTION

Multi-drug resistance and novel antibiotics scarcity has been acknowledged long back.<sup>[1, 2]</sup> One half of deaths worldwide is due to infectious diseases<sup>[3, 4]</sup> and is leading to mortality and morbidity mainly in immune-compromised patients.<sup>[5]</sup> This adds urgency for the development of novel, effective and affordable medicines that act against pathogens without causing any adverse effects to the host. Streptococcus, staphylococcus, pseudomonas species are involved in pathogenesis of respiratory, dermal, gastrointestinal tract and urogenital tract infections, contaminates the wounds caused by burns, causes noscomial infections and are resistant to all the existing antibiotics.<sup>[6]</sup> Multi-drug resistance is due to indiscriminate prescription of antibiotics and addition of antimicrobial agents as growth enhancers in poultry and live stock production.<sup>[7]</sup> Secondary metabolites obtained from plants are highly effective in treatment of infectious diseases.<sup>[8]</sup> Therefore, the phytomedicine may be a potent source of new antibacterial agents. Helminthiasis is a disease caused by round worms, tape worms and flukes.<sup>[9]</sup> These worms reside in the gastrointestinal tract but the larvae migrates the liver and other organs.<sup>[10]</sup> These helminthes developed resistance to existing antihelminthic drugs. Hence, the present study targeted to screen antibacterial and antihelminthic activity of plants.

Present investigation is carried out on following herbal extracts- *Azadirachta indica* (Leaves), *Cinnamomum zeylanicum* (bark) and *menthe longifolia* (Leaves). The aim of the present study is to formulate and evaluate antibacterial and antihelminthic activity of polyherbal topical gel formulation.

## MATERIALS AND METHODS

### Collection, identification and authentication of plants

Plant material of *Azadirachta indica* (Leaves) and *Mentha longifolia* (Leaves) were collected in the month of January- February from medicinal garden of Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda. *Cinnamomum zeylanicum* (bark) were procured from local market, Nalgonda. All the plant materials were identified and authenticated by pharmacognosist- Karnati. Sushma, Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda and a voucher specimen (No:SRTIPS/COG/2350) was deposited in department of Pharmacognosy. All the collected plant material was dried thereafter reduced to powder form.

### Preparation of Herbal extracts

Herbal extracts were prepared by maceration process. In this method, the dried leaves of *Azadirachta indica*, *Mentha longifolia*, bark of *Cinnamomum* & ethanol was taken in 1:3 ratios for each plant. They are macerated for about 1 week. After that the ethanolic extracts were collected.

### Formulation of Gel

Gel was prepared by using 1% and 2% concentration of the extracts. In a separate beaker, Carbopol 934 was dispersed uniformly in distilled water with continuous stirring and it was soaked for 24 hours. In another beaker, propyl paraben and methyl paraben was dissolved in distilled water. To this solution the extracts were added and triturated well. The above mixture was then added to the carbopol mixture which was soaked for 24 hours and stirred well. Finally propylene glycol and triethanolamine was added and the pH was adjusted to 6.8-7. Compositions of gel formulations are depicted in table 1.

### Evaluation of Gel

#### Physical evaluation

Physical parameters such as colour, appearance and consistency and feel were tested. The colour of the formulations was tested against white background. The consistency and greasiness was checked by applying on skin.

#### Spreadability

Spreadability is expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel, placed in between the slides under the direction of certain load. It is calculated by using the formula.

$$S = M \cdot L / T$$

Where M = weight tied to upper slide, L = length of glass slides, T = time taken to separate the slides.

#### Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

### Viscosity study

The measurement of viscosity of the prepared gel was done using Brookfield digital viscometer. The viscosity was measured using spindle no. 64 at 10 rpm and 25°C. Before measurement deaeration of gel was done and the gel was filled in appropriate wide mouth container. Samples of the gels were allowed to settle over 30 min at the assay temperature (25 ± 10°C) before the measurements.

### Determination of pH

The pH of formulation was determined using digital pH meter. One gram of gel was dissolved in 100 ml of demineralised water and stored for two hours. The measurement of pH of formulation was done in triplicate. Instrument was calibrated before use with standard buffer solutions at pH 4, 7, and 9.

### Evaluation of the antibacterial activity

#### Collection of microorganisms

Five pathogenic bacterial strains were used as the test organisms for antibacterial screening of the polyherbal formulation. Among them *Staphylococcus aureus* were gram positive and *Escherichia coli*, *Salmonella typhi* & *Shigella dysenteriae* were gram negative. All the stock cultures were collected from Department of Botany, Osmania University, Hyderabad.

#### Preparation of Nutrient Agar

The weighed amount of NaCl, peptone, Beef extract are dissolved in 1000 ml of the water, then agar is added slowly on heating with continuous stirring until agar is completely dissolved and pH is adjusted to 7.2 to 7.4. This nutrient agar medium is then sterilized by moist heat sterilization method using autoclave at temperature of 120°C at 15 lb pressure maintained for 15 minutes.

#### Evaluation of antibacterial activity

The antibacterial activity was determined by modified agar well diffusion method. In this method, nutrient agar plates were seeded with 0.2 ml of 24 h broth cultures of bacterial strains. The plates were allowed to dry for 1 h. A sterile 8 mm borer was used to cut four wells of equidistance in each of plates; 0.5 ml of solution of poly herbal formulations (PF1, PF2) and standard drug azithromycin was introduced in to the wells randomly. The plates were incubated at 37°C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of zones of inhibition in mm (Lovian 1980).

### **Evaluation of antihelmintic activity**

The antihelmintic evaluation of plant extracts was done by using earth worms. Fifteen earthworms of 5 to 8cms in length and 0.2 to 0.3cms in width were used. Animals were divided into 3 groups containing 5 earthworms in each group. Poly herbal formulations (PF1, PF2) and standard drug albendazole was applied to the three petri dishes containing earth worms. All petridishes were maintained at room temperature. The time of paralysis was noted when no moment of any sort could be observed except when the worms were shaken vigorously and the time for death recorded after ascertaining that worms neither moved when shaken vigorously or when they dipped in warm water (50°C) followed by fading away of their body colours (Adate et al., 2012).

## **RESULTS**

### **Evaluation of gel**

The pH was found to be  $6.7 \pm 0.4$  and  $6.9 \pm 0.5$  for PF1 and PF2 gel which was near to the neutral pH, thus the formulations can be used without the risk of skin irritancy. By this we can infer that the selected ingredients for gel formulation did not alter the pH of the formulation. The spreadability of gel formulations was found to decrease with increasing the concentration of gelling agent. The values of spreadability for PF1 and PF2 gel was found out to be 8.9 and 8.6cm indicating that the gel is easily spreadable by small amount of shear. The results concluded that the formulation can be applied easily without being runoff. This assures that the formulation maintains a good wet contact time when applied to the targeted site. The two prepared gel formulations were good in appearance and homogeneity.

### **Antibacterial activity of Polyherbal formulation**

The results of antibacterial activity of polyherbal formulations were depicted in Table 2. The zone of inhibition produced by azithromycin was between 19 to 25 mm and was larger than that produced by the poly herbal formulations PF1 and PF2 is between 8 to 18 mm and 14 to 29mm respectively. In the present the prepared poly herbal gels showed better antibacterial effect against gram negative strains than Gram positive organisms.

### **Antihelmintic activity of Polyherbal formulation**

The prepared polyherbal gels were compared with standard drug albendazole to assess the antihelmintic activity. The results were depicted in Table 3. Poly herbal gel formulation PF2 demonstrated shortest time of paralysis and death. PF2 gel caused paralysis at  $6.16 \pm 1.94$  min and death at  $37 \pm 3.68$  min, while albendazole caused paralysis and death at  $11.5 \pm 1.64$  min and

44±3.52 min, respectively. From the study, it revealed that the time for paralysis and death decreases as the concentrations of the plant extracts increases. Therefore, these results demonstrate that polyherbal gel PF2 possess antihelmintic effects.

**Table 1: Compositions of Polyherbal gels.**

S.No	Ingredients	Quantity for PF1	Quantity for PF2
1.	Ethanollic extract of neem	1%	2%
2.	Ethanollic extract of cinnamon	1%	2%
3.	Ethanollic extract of mint	1%	2%
4.	Carbopol	2%	2%
5.	Triethanolamine	2%	2%
6.	Propylene glycol	2%	2%
7.	Methyl paraben	0.1%	0.1%
8.	Propyl Paraben	0.1%	0.1%
9.	Distilled Water	Q.S	Q.S

**Table 2: In-vitro antibacterial activity of Polyherbal gels.**

Bacterial Strain	Test organism	Zone of Inhibition (mm)		
		PF1	PF2	Azithromycin
<b>Gram positive</b>	<i>Staphylococcus aureus</i>	18±3.03 <sup>a</sup>	29.17±3.97 <sup>d, f</sup>	23±2.85
<b>Gram negative</b>	<i>Escherichia coli</i>	14.83±3.71	20±3.28 <sup>d, g</sup>	25.5±3.32
	<i>Salmonella typhi</i>	8.0±2.75 <sup>b</sup>	14.5±3.88 <sup>d, g</sup>	19.5±2.66
	<i>Shigella dysenteriae</i>	10.6±2.50 <sup>c</sup>	15.5±4.08 <sup>e, g</sup>	23.1±2.31

Values are expressed as mean ± SD of 6 animals. Superscript letters represents the statistical significance done by ANOVA followed by Tukey multiple comparisons test.

<sup>a</sup>  $p < 0.0001$ ; <sup>b</sup>  $p < 0.01$ ; <sup>c</sup>  $p < 0.05$  indicates the significance on comparison of PF1 with Azithromycin

<sup>d</sup>  $p < 0.05$ ; <sup>e</sup>  $p < 0.01$  indicates the significance on comparison of PF2 with Azithromycin

<sup>f</sup>  $p < 0.05$ ; <sup>g</sup>  $p < 0.001$  indicates the dose dependent significance on comparison of PF1 with PF2.

**Table 3: In-vitro Antihelmintic activity of poly herbal gel.**

Groups	Time taken for paralysis (min)	Time taken for death (min)
Control (Distilled Water)	-	-
PF1	16.3±4.08 <sup>a</sup>	53±3.77 <sup>a</sup>
PF2	6.16±1.94 <sup>b, d</sup>	37±3.68 <sup>c, e</sup>
Albendazole	11.5±1.64	44±3.52

Values are expressed as mean ± SD of 6 animals. Superscript letters represents the statistical significance done by ANOVA followed by Tukey multiple comparisons test.

<sup>a</sup>  $p < 0.05$  indicates the significance on comparison of PF1 with Albendazole

<sup>b</sup> $p < 0.05$ ; <sup>c</sup> $p < 0.001$  indicates the significance on comparison of PF2 with Albendazole  
<sup>d</sup> $p < 0.001$ ; <sup>e</sup> $p < 0.01$  indicates the dose dependent significance on comparison of PF1 with PF2.

## DISCUSSION

Plants consist of abundant drugs and many plants have been screened for compounds with therapeutic activity (Rosy *et al.*, 2010). Therefore, it is very essential to evaluate the antibacterial and antihelminthic activity of polyherbal gel. Extensive and frequent use of antibiotics resulted in developing resistance by the bacterial strains. In the present investigation resistant bacterial species were selected and it was found that the mean zone of inhibition produced by the commercial antibiotic azithromycin was larger than that produced by polyherbal gels. This is due to the fact that crude extract obtained from plants contain a lower concentration of bioactive compounds (Baravalia *et al.*, 2009). However, in the present study it was found that both poly herbal gel extracts was effective against both gram positive and gram negative bacteria which suggest that the plant extract may possess broad spectrum of antibiotic compounds (Mohammed *et al.*, 2010). Previous studies reported that presence of several phytochemicals like terpenoids, flavonoids, tannins, alkaloids, steroids and phenolic compounds are responsible for the antibacterial activity of the plant extracts and the same mechanism might have involved in antibacterial activity of poly herbal gel (Ramzi *et al.*, 2008; Sule *et al.*, 2011). Parasitic helminthes affect human beings intestine causing chronic and devastating diseases which ultimately lead to death due to septicaemia. In vitro and in vivo studies reported that several plants possess antihelminthic activity (Kumar *et al.*, 2010). In the current investigation observations were made for the time taken to paralysis and death of worms against the polyherbal gel formulations PF1, PF2 and albendazole. The present study also revealed that polyherbal gel formulation PF2 showed potent antihelminthic activity. A significant dose dependent antihelminthic activity ( $p < 0.001$ ;  $p < 0.01$ ) was found on administration of polyherbal gel PF2. This may be described by the fact that several compounds like alkaloid, polyphenol, flavonoid and terpenes etc. may be responsible for the antihelminthic activity of the polyherbal gel. These compounds interferes with the energy generation in the helminthes by uncoupling the oxidative phosphorylation or they bind to free proteins in the gastrointestinal tract of the host animal or to glycoprotein on the cuticle of the parasite and causes death or it may act on the CNS of the parasites causing paralysis and death of worms (Salhan *et al.*, 2010).

## CONCLUSION

In conclusion from the recorded data, it is demonstrated that the polyherbal gel PF2 has promising antibacterial and antihelmintic effects. The polyherbal gel PF2 may be further explored for its phytochemical profile to recognize the active constituents accountable for its versatile activities and to find out the underlying mechanism involved.

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## CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

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