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Research Article

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### Formulation and Evaluation of Poly Herbal Antiseptic Powder for its Antimicrobial Activity

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#### ABSTRACT

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life. Natural products have gained popularity as traditional medicine because of its pharmacological activity and low toxicity. The present study was to investigate the poly herbal powder formulation (SRJM-1) for its antimicrobial activity. The peels of fruits of orange, pomegranate, lemon and rhizome of ginger were selected and grinded into coarse powder. The extraction was then carried out using soxhlet apparatus with ethanol as extraction solvent. The extract was concentrated using rotavapour. The poly herbal extract was then formulated into a powder using suitable preservative. The poly herbal formulation (SRJM-1) was screened qualitatively and showed the presence of phenolics, glycosides, alkaloids and flavonoids. The poly herbal formulation (SRJM-1) was further evaluated for its antimicrobial activity against gram negative *Escherichia coli* and fungal strain *Aspergillus niger* and the respective zone of inhibition was calculated. The poly herbal powder formulation (SRJM-1) was found to have antimicrobial activity.

**Keywords:** Antimicrobial activity, *Aspergillus niger*, *Escherichia coli*, poly herbal formulation, zone of inhibition.

#### ARTICLE INFO

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## 1. Introduction

Antibiotics provide the basis for the therapy of microbial (bacterial and fungal) infections. The discovery of antibiotics and their use as chemotherapeutic agents eventually were indicated for infectious diseases [1]. Natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs owing to their pharmacological activity [2]. The popular use of antibiotics as therapeutic agents to cure many infectious diseases forwarded the search for plants containing antimicrobial substances<sup>3</sup>. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties [4]. The antimicrobial constituents are present in all parts of the plant viz. bark, stalks, leaves, fruits, roots, flowers, pods, seeds, stems, latex, hull and fruit rind [5].

The scientific investigations have revealed the consecutive rich sources of antimicrobics, especially among fruits and vegetables, but only few of them involve waste parts of fruits, i.e. seeds and peels. The fruits and vegetables skins are thrown in the garbage or fed to livestock. Fruits and vegetables wastes and by-products are very rich in bioactive components have a beneficial effect on health. Since last decade, efforts are being made to improve the methods and ways of reusing fruits and vegetables wastes. The important purpose of using this agro waste therapeutically is a new approach which is slowly gaining popularity. These high value products when recovered will be economically attractive. These may act as novel, natural; eco friendly and economic sources of antimicrobials for prevention of diseases caused by pathogenic microbes and also reduce pollution [6]. Recent research has revealed that fruit peels and seeds, such as grape seeds and peels [7], pomegranate peel<sup>8</sup> and wampee peel may potentially possess antimicrobial property [9].

The Citrus sinensis orange peel extract was evaluated for Larvicidal, pupicidal, repellent and adulticidal activity was against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus [10]. This study on pomegranate peel extract (rind), seed extract, juice and whole fruit on the selected bacteria and fungi described the antibacterial and antifungal activities [11]. The Lemon was reported to have anticancer activities and the antibacterial potential in the crude extracts of different parts [12].

Researches carried out on ginger established that it is concentrated with active substances that have anti-bacterial, anti-flatulent, antimicrobial, anti-inflammatory, antiseptic, anti-spasm, anti-viral and antioxidative properties [13]. The present literature survey paved a way to identify antimicrobials from the plants especially for the agro wastes such as peels. The idea of formulating a powder composed of poly herbal peels is a new approach. The present study is to evaluate the antimicrobial activity of a poly herbal dusting powder formulation composed of peels of orange, pomegranate and lemon.

## 2. Materials and Methods

**Plant material:** Poly herbal formulation consists of 4 ingredients, viz., orange fruits, pomegranate fruits; lemon fruits and ginger were collected randomly from the shops of Hyderabad, India. All these plant parts were procured from the local market of Hyderabad, telangana, india and were authenticated by Dr. Venkata Ramana, Botanist, Osmania University, Hyderabad.

**Phytochemical Extraction:** The powdered peels were shade dried and finely grounded into coarse powder using mechanical blender. The powders were mixed using mixer. The extraction was performed on the poly herbal powdered peels of fruits weighing each about 250gms with 1L of ethanol as extraction solvent loaded in soxhlet apparatus. Approximately 24 cycles were carried out for 3days. The extract obtained was concentrated using rotavapour. The physical nature of the extract was evaluated. The extract was then formulated into a powder and evaluated for antibacterial and antifungal activity.

### Preparation of polyherbal formulation (SRJM-1)

The poly herbal extract obtained (Table-1) was powdered, passed through a 100# sieve to get a uniform mixture. Suitable preservative was added to the herbal formulation to store it for a longer time.

**Table.1:** Parts of Plants used in polyherbal formulation (SRJM-1)

S.No	Plant Name	Family	Peels of Part used	Quantity mg
1	<i>Citrus × sinensis</i>	<i>Rutaceae</i>	Fruits	250
2	<i>Punica granatum,</i>	<i>Lythraceae</i>	Fruits	250
3	<i>Citrus × limon</i>	<i>Rutaceae</i>	Fruits	250
4	<i>Zingiber officinale</i>	<i>Zingiberaceae</i>	Rhizome	250

### Preliminary Phytochemical analysis

Preliminary phytochemical analysis was carried out for the standard poly herbal extract to detect for the presence of different chemical groups of compounds by using the standard conventional protocols [14-16].

### Antimicrobial Activity

#### The Microbial strains

Gram negative bacterial strain *Escherichia coli* (MTCC 443) and fungal strains *Aspergillus niger* (MTCC 282) were chosen based on their pharmacological importance<sup>17</sup>. The strains were obtained from Institute of Microbial Technology, Chandigarh, were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar medium, followed by refrigeration storage at 4°C. The bacterial and fungal strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacterial and fungal strains were grown in the nutrient broth at 37°C and at 28°C respectively, and maintained on nutrient agar slants at 4°C). The inoculums were standardized at 1\* 10<sup>6</sup> CFU/ml

comparing with turbidity standard (0.5 Mac Farland tube). The stock cultures were maintained at 4°C.

### Antimicrobial Activity

#### Determination of zone of inhibition method

*In-vitro* antibacterial and antifungal activities were examined for alcoholic poly herbal formulation (SRJM-1). Antibacterial and antifungal activities of poly herbal formulation (SRJM-1) were investigated by the agar disc diffusion method<sup>18-20</sup>. Antimicrobial activity testing was carried out by using agar cup method. The formulation was dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, Gram-negative, and fungal strains were taken as a standard antibiotic for comparison of the results. The formulation (SRJM-1) was screened for their antibacterial and antifungal activities against the *Escherichia coli* and the fungi *Aspergillus niger*. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains and allowed to stay at 37°C for 3 hours. Control experiments were carried out under similar condition by using ciprofloxacin for antibacterial activity and nystatin for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 18 to 24 hours of incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

## 3. Results and Discussion

### Physical nature of the Poly herbal formulation (SRJM-1)

**Table 2:** Physical Properties of poly herbal formulation (SRJM-1)

Appearance	Colour	Odour	Texture	Particle size
Powder	Cream	Aromatic	Fine	100#

### Preliminary Phytochemical screening:

The qualitative analysis of the poly herbal extract revealed the presence of alkaloids, glycosides, flavonoids, phenols and carbohydrates which may account for the pharmacological action.

**Table.3:** Results for phytochemical preliminary tests.

Constituents	Poly herbal Formulation (SRJM-1)
Alkaloids	+
Glycosides	+++
Alcohol	--
Trace metals	++
Aldehydes	++
Tannins	--
Phenols	+
Flavonoids	++
Amino acids and proteins	+
Carbohydrates	++

- Not present, + present, ++ and +++ significantly present

### Antimicrobial Activity

**Table.4:** zone of inhibition of test sample (SRJM-1) and the standard against gram negative *E. coli*

S.No	Compound Name	Zone of inhibition (mm) (activity index) std
1	Ciprofloxacin	29
2	Test sample (SRJM-1)	18

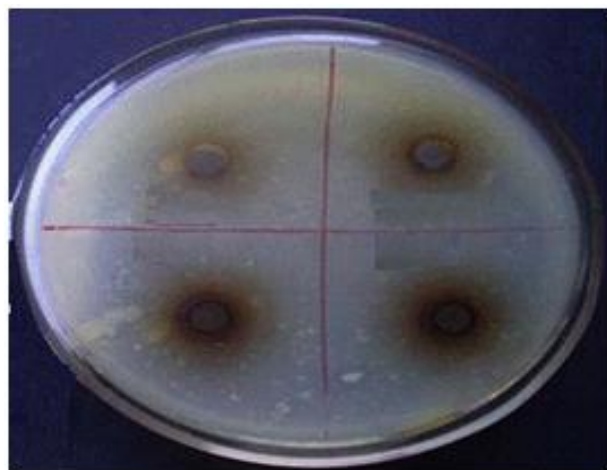
From the above observation by comparison with the standard, it was confirmed that the test sample (SRJM-1) has antibacterial property.

### Antifungal activity:

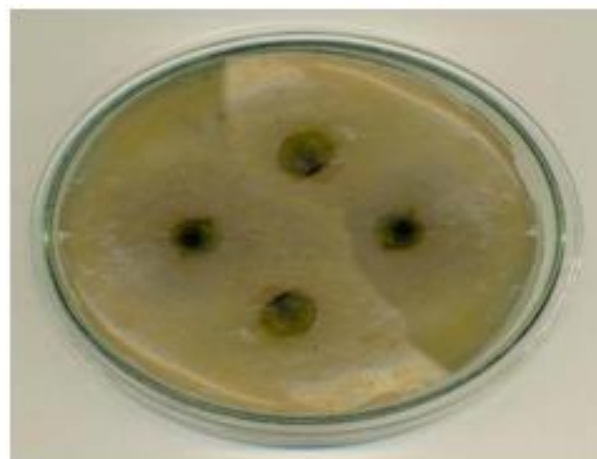
**Table 5:** zone of inhibition of test sample (SRJM-1) and the standard against fungus *A. niger*

S.No	Compound Name	Zone of inhibition (mm) (activity index) std
1	Nystatin	26
2	Test sample (SRJM-1)	10

From the above observation by comparison with the standard, it was confirmed that the test sample (SRJM-1) has antifungal property also.



**Fig.2.** Effect Of methanol poly herbal Extract on *E. coli*



**Fig.3:** Effect of methanol poly herbal Extract on *A.niger*

#### 4. Conclusion

The present study was to investigate the poly herbal powder formulation (SRJM-1) for its antimicrobial activity. The peels of fruits of orange, pomegranate, lemon and rhizome of ginger were selected and grinded into coarse powder. The poly herbal extract was then formulated into a powder using suitable preservative. The poly herbal formulation (SRJM-1) was further evaluated for its antimicrobial activity against gram negative *Escherichia coli* and fungal strain *Aspergillus niger* and the respective zone of inhibition was calculated. The poly herbal powder formulation (SRJM-1) was found to have antimicrobial activity.

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