Effect of Plant Extracts Formulated in Different Ointment Bases on MDR Strains

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Pawar and Nabar: Plant Extracts Ointments and MDR Strains

Extracts of Aloe vera whole plant, Eucalyptus globulus leaves, Ficus infectoria bark, Ficus religiosa bark and Piper betel leaves were studied for antibacterial activity on resistant and sensitive strains, isolated from skin and soft tissue infections. A combination of hot alcoholic extracts of Ficus infectoria, Ficus religiosa and Piper betel were found to be more effective against all the isolates. The combined extract was formulated in different ointment bases such as polyethylene glycol, gelatin, sodium alginate, carbopol, cream base and honey. These were then evaluated to find a suitable base for preparation of an ointment. In vitro study of the release of antimicrobials and kill-time studies of the herbal ointments was carried out against multi-drug resistant isolate of Pseudomonas. The ointment showed bactericidal activity within 2 h against the resistant strain of Pseudomonas spp.

Key words: Aloe vera, Eucalyptus globulus, Ficus infectoria, Ficus religiosa, multi-drug resistant strains, Piper betel, Pseudomonas

Multi-drug resistance among many bacterial species has been reported to be on the increase due to inappropriate or widespread use of antimicrobials[1]. Resistance to antimicrobials has been observed in various parts of the world. During 1997-1998, Staphylococcus aureus was found to be the causative agent in most of the skin and soft tissue infections, and among these infections methicillin resistant Staphylococcus aureus (MRSA) was the major causative agent[2]. Vancomycin resistant Enterococcus faecium has also shown a progressive increase in various infections[3]. Drug resistant strains are causing severe problems in many infections including skin infections such as carbuncles, folliculitis, impetigo, and burn wound sepsis. Antimicrobial resistance prolongs the duration of hospitalization, thereby increasing the cost of patient care.

Globally medicinal plants have been used since ancient times in one form or the other. Aloe vera gel[4], neem[5] and eucalyptus oil[6] have been reported to show antimicrobial activity[6]. The aim of the project was to develop an herbal ointment for controlling skin and soft tissue infections due to multi-drug resistant strains.

Five commonly available medicinal plants were selected for the study; they were procured from Kashele Forest Academy, Karjat, India. They were authenticated by the Department of botany, Smt. CHM College for the identification of the plant species before employing them for the study. The plants collected were whole plant of Aloe vera, Eucalyptus globulus (Nilgiri) leaves, Ficus infectoria (Pakar) bark, Ficus religiosa (Pipal) bark, and Piper betel (Betel) leaves. The extracts were prepared with ethyl alcohol of analytical grade and distilled water for aqueous extract preparation.

The plant parts were extracted using ethanol and water. Hot alcoholic extract (HAE) was prepared using a Soxhlet extractor. For hot water extract (HWE) 30 g of powder with 300 ml of water was heated in a water bath maintained at 100° till the volume was reduced to one fourth of the original volume[7]. Cold alcoholic extract (CAE) was prepared with 300 ml of ethyl alcohol added to 30 g of powder and shaken for 72 h at room temperatures. For the preparation of 50% cold alcoholic extract (50% CAE) 30 g of powder was taken and to that 150 ml alcohol and 150 ml water was added. This was kept under shaker conditions for 72 h. All the extracts were dried at 50°[7].

Swabs were collected from patients suffering from skin infections like burn wound sepsis, carbuncles, cellulitis, folliculitis, furuncles and impetigo infections. In case of hospital samples transport media

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TABLE 1: SAMPLE COLLECTION DATA

<table>
<thead>
<tr>
<th>Types of samples</th>
<th>Burn wound sepsis</th>
<th>Acne</th>
<th>Furuncles</th>
<th>Impetigo</th>
<th>Folliculitis</th>
<th>Footsores</th>
<th>Infective eczema</th>
<th>Wounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>20</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>4</td>
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<td>4</td>
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<tr>
<td>Total isolates</td>
<td>20</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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found to be resistant to major antibiotics. Nine of the *Staphylococcus* was resistant to β-lactams, aminoglycosides, glycopeptides, quinolones, tetracycline and chloramphenicol. From *Klebsiella* spp and *Enterobacter* spp four were resistant. All the three *Escherichia coli* isolates were resistant to major antibiotics.

Initial screening of the antibacterial properties of the selected plant extracts was carried out by agar ditch method using 5% concentration of extract. Hot alcoholic extracts of *pakar*, *pipal*, *betel*, cold alcoholic extract of *Aloe vera*, *pakar*, *betel*, *nilgiri* and hot aqueous extract of *betel* were found to possess antibacterial activity against both the resistant and sensitive isolates and also against standard ATCC strains of *Staphylococcus aureus* ATCC no. 2901 and *Pseudomonas aeruginosa* ATCC no. 2862.

MIC of the above extracts (Table 2) was carried out by plate dilution method in the range between 0.5% and 2%. *Betel* HAE and CAE inhibited the sensitive strains at 0.5% and all the resistant isolates at 1.5%. While *pakar* HAE and CAE inhibited all the isolates at 2% concentration, *Pipal* HAE and *nilgiri* CAE showed activity against all the isolates at 2%. On the basis of MIC results, a combination of hot alcoholic extracts of *pakar* (bark), *pipal* (bark) and *betel* (leaves) was prepared. The *in vitro* antibacterial activity and MIC showed that most of the isolates were inhibited at 0.5% concentration and the rest of the isolates were inhibited at 1.5% concentration.

The individual plant extracts and the combination extract were evaluated for its toxicity on topical application. The extracts exhibited no toxic effect i.e., no erythema, edema and necrosis were observed on topical application. Hence all the extracts were found to be non-toxic in nature and could be used as a topical agent to control infection.

The combination of extracts was further formulated into different bases selected, to prepare a topical ointment. The antibacterial activity of the ointment was checked by agar cup method using resistant strains.

![Fig. 1: Percentage of different organisms isolated from skin and soft tissue samples.](image)

![Fig. 2: Number of resistant and sensitive strains isolated from the samples. The graph depicts the comparative study of the resistant (●) and sensitive (□) strains isolated from skin and soft tissue samples from patients.](image)

| TABLE 2: DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION OF THE PLANT EXTRACTS |
|-----------------------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| **Isolates**                      | **Extracts**    | **E**          | **K**          | **K**          | **E**          | **E**          | **P**          | **P**          | **P. a**        | **S**          | **S**          |
|                               |                 | **R**          | **R**          | **R**          | **R**          | **R**          | **R**          | **R**          | **R**           | **R**          | **R**          |
| *Nilgiri*                      | CAE             | 2%             | 2%             | 2%             | 2%             | 2%             | 2%             | 1.5%           | 2%             | 2%             | 1.5%           |
|                               | HAE             | 2%             | 2%             | 1.5%           | 2%             | 1.5%           | 2%             | 2%             | 1.5%           | 1%             | 1.5%           |
|                               | 50% CAE         | 2%             | 2%             | 2%             | 2%             | 2%             | 2%             | 2%             | 2%             | 2%             | 1.5%           |
| *Pakar*                       | HAE             | 2%             | 2%             | 1.5%           | 2%             | 1.5%           | 2%             | 2%             | 1%             | 1.5%           | 1%             |
|                               | 50% CAE         | 2%             | 2%             | 2%             | 2%             | 2%             | 2%             | 2%             | 2%             | 2%             | 1.5%           |
| *Pipal*                       | HAE             | 1.5%           | 1.5%           | 1%             | 1.5%           | 1.5%           | 0.5%           | 1%             | 1.5%           | 0.5%           | 1%             |
|                               | 50% CAE         | 2%             | 2%             | 1.5%           | 2%             | 1.5%           | 0.5%           | 1%             | 1.5%           | 0.5%           | 1%             |
| *Betel*                       | HAE             | 1.5%           | 1.5%           | 1%             | 1.5%           | 1.5%           | 0.5%           | 1%             | 1.5%           | 0.5%           | 1%             |
|                               | 50% CAE         | 2%             | 2%             | 1.5%           | 2%             | 1.5%           | 0.5%           | 1%             | 1.5%           | 0.5%           | 1%             |
| *Aloe vera*                   | CAE             | 2%             | 2%             | 2%             | 2%             | 2%             | 2%             | 1.5%           | 2%             | 2%             | 1.5%           |

Determination of minimum inhibitory concentration of the hot alcoholic (HAE) and cold alcoholic (CAE) plant extracts against Gram negative resistant *Klebsiella* strains (Kr), sensitive *Klebsiella* strains (Ks), resistant *Enterobacter* strains (Enr), sensitive *Enterobacter* strains (Ens), resistant *Escherichia coli* strains (Er), resistant *Pseudomonas* strains (Pr), sensitive *Pseudomonas aeruginosa* strains (Ps) and standard *Pseudomonas aeruginosa* ATCC no. 2862. (P. a). The Gram positive resistant *Staphylococcus* strains (Sr), sensitive *Staphylococcus* strains (Ss), resistant *Micrococcus* strain (Mr) and standard *Staphylococcus aureus* ATCC NO.2901(S. a).
strains of *Pseudomonas* spp and *Staphylococcus* spp (Table 3).

Hydrophilic ointment, Aloe gel, petroleum jelly and oil base did not exhibit any zone of inhibition towards the resistant organism, due to reduced diffusible property of the formulated ointment. Ointments prepared in sodium alginate base and gelatin base were water-soluble and exhibited good antibacterial activity. However the drawbacks of the bases were that, they were contaminated easily and had no shelf life. Hence the above bases were not considered for further studies.

Kill time studies showed that all the above selected ointments exhibited antibacterial property within 2 h. Base B was effective but was found to be unstable after 2 w, showing change in pH and cracking of the base. Honey and polyethylene glycol bases themselves possessed antibacterial activity, and was observed that the base inhibited the isolate within 7 h and 6 h, respectively.

Base A, polyethylene glycol base, carbopol base and honey showed good antibacterial property. The bases were non-greasy, water-soluble and water washable. These bases were selected for formulation of the plant extract in the form of an ointment.

The ointments showed bactericidal activity within 2 h against resistant strain of *Pseudomonas* spp. Toxicity test carried out on Sprague Dawley rats showed no erythema, edema and necrosis, proving the extracts to be non toxic topically. Thus ointments can be further studied for its wound healing properties in vivo.

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**REFERENCES**

Rapid Liquid Chromatographic Method for the Determination of Roflumilast in the Presence of Degradation Products

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Barhate and Deosthalee: Rapid Liquid Chromatographic Method of Roflumilast

A forced degradation study on roflumilast drug substance was conducted under the conditions of hydrolysis, oxidation, thermal and photolysis. The method was developed and optimized by analyzing forcefully degraded samples. The best separation was achieved on a Zorbax SB C18 1.8 µm column with 0.005 M ammonium formate buffer pH 3.5 and acetonitrile as mobile phase in a 13 min run time. The proposed method was able to resolve all the possible degradation products formed during stress study. The drug was stable to neutral, thermal and photolytic conditions but unstable to acidic, alkaline and oxidative conditions at 80º for 24 h. The degradation products resulting from stress study did not interfere in assay and related substances of roflumilast and thus the method can be regarded as stability indicating. An alternate method was also developed on a conventional 250×4.6 mm, 5 µm column wherein runtime was 38 min. Thus rapid resolution high throughput column was able to reduce the run time from 38 min to 13 min.

Key words: Forced degradation, liquid chromatography, roflumilast, RRHT

Roflumilast is a novel, potent, selective phosphodiesterase 4 (PDE4) inhibitor for the treatment of chronic obstructive pulmonary disease (COPD) and asthma (fig. 1). Its empirical formula is C17H14Cl2F2N2O3 and its molecular weight is 403.21. There is no reported method for the analysis of roflumilast in the presence of its degradation products. In the absence of an official roflumilast monograph in the pharmacopoeias, including the European Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia, development of such a method would prove useful for the industry. The objective of this work was, to develop a simple, economic and rapid HPLC method for the quantitative analysis of roflumilast.

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Roflumilast was received as a gift sample from MSN laboratories limited. Acetonitrile and methanol both (Merck) were of HPLC grade. GR grade ammonium acetate, monobasic sodium monophosphate monohydrate, dibasic sodium phosphate anhydrous, hydrochloric acid, acetic acid glacial, formic acid, ortho phosphoric acid and sodium hydroxide pellets were used.

Fig. 1: Structure of roflumilast.