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# Evaluation of antimicrobial activity of selected indigenous medicinal plants

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**Abstract**
Herbal medicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Along with other dosage forms, herbal drugs are also formulated in the form of ointment. An ointment is a viscous semisolid preparation used topically on a variety of body surfaces. The objective of the study was to evaluate the Pharmacognostical, Phytochemical and evaluation of the antimicrobial herbal ointment from the different extracts of dried leaves of selected medicinal plants like *Aerva Lanata, Bauhinia variegata, Acmella uliginosa.* The anti-Microbial properties of extracts were evaluated by Agar well diffusion method using gram positive bacteria like *Staphylococus aureus, Bacillus subtilius,* gram negative bacteria like *Escherichia coli, Klebseilia pneumonia.* Amongst the test extracts, the results suggested that, Methonolic extracts showed significant anti-Microbial activity compared with standard drug. Plants show high efficiency of antimicrobial activity due to the presence of various phytochemicals, antimicrobial tests of the combinations were carried out. The most effective combination was then determined by comparing the results of the zone of inhibition. Then the minimum inhibitory concentration of the effective combination was found out.

**Keywords:** Poly Herbal ointment, antimicrobial activity, minimum inhibitory concentration, irritancy, spreadability, diffusion, stability

## Introduction
Plants are used as medicine since time immemorial. India is a rich source of medicinal plants. They are widely used in ancient systems of medicine. It is reported that, two or three antibiotics that are launched every year are derived from microorganisms. Antimicrobial activity is the ability of a substance to inhibit or kill bacterial and fungal growth. Different types of antimicrobial and chemotherapeutic agents are being used in the treatment of one form of disease or the other. Herbal medicine is the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. In this present study an attempt was made to evaluate the Antimicrobial properties of selected indigenous medicinal plants for polyherbal formulation based on the literature studies.

## Plant material
Leafs of *Aerva lanata* Plant collected from Urlugonda (village), Suryapet District, and Telangana. Leafs of Bauhinia *variegata* collected from Herbal Garden Chilkur Balaji College of Pharmacy, *Acmella uliginosa* obtained from Herbal garden, Himayath Sagar, Ranga Reddy district, Hyderabad, all the three plants ere authenticated and Botanical identification of the plants was done by Botanical Survey of India, Deccan Regional Centre, Hyderabad, Specimens of *Aerva lanata, Bauhinia variegata, Acmella uliginosa* (Voucher number:BSI / DRC / 16-17 / Tech/1005).On 22/03/2017.

## Drying of plant material
Plant materials were shade dried about for 15-20 days. The shade dried plant material was further coarsely powdered and the powder was passed through the mesh and stored in airtight container for further analysis.

**Fig 1:** Powders of *Aerva lanata, Bauhinia variegata, Acmella uliginosa*

**Chemicals and instruments:** Compound microscope, glass slides, cover slips, watch glass and other common glasswares and the basic apparatus and instruments, Soxhlate apparatus used for the study. Solvents *viz*. Methanol, petroleum ether, N-hexane, Ethanol, and reagents *viz*. phloroglucinol, glycerin, hydrochloric acid, chloral hydrate, Safranin and Fast green. Different strains of microorganism included for study were gram positive bacteria like *Staphylococus aureus*, *Bacillus subtilius*, gram negative bacteria like *Escherichia coli*, *Klebseilia pneumonia.*

**Collection of Bacterial strains**

The lyophilized form of different strains of microorganism included for study were gram positive bacteria like *Staphylococus aureus*, *Bacillus subtilius*, gram negative bacteria like *Escherichia coli*, *Klebseilia pneumoniae*. The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C. All the bacterial strains were procured from Osmania University, Hyderabad, Telangana. After receiving strains were sub-cultured by using nutrient agar media for evaluation of antimicrobial activity at Microbiology lab at Chilkur Balaji College of Pharmacy.

**Phyto-chemical screening**

The different extracts were subjected to preliminary Phyto-chemical screening techniques provided in literature

***In vitro* anti-Microbial activity**

The *in vitro* antibacterial study was performed by measuring the diameter of the zone of inhibition on the inoculum agar plate. The zone of inhibition can be defined as the clear region around the susceptible disc or well with an antimicrobial agent on the agar surface. It is designated to test the ability of the antimicrobial agent to inhibit the growth of microorganisms [150]. The larger the zone of inhibition is, the better the inhibition of the particular antimicrobial agent.

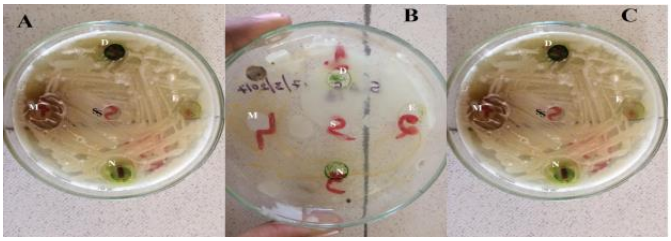


**Fig 2:** Inhibition zones of A) *Aerva lanata*, B) *Bauhinia variegata*, C) *Acmella uliginosa*

**Preparation of Nutrient agar plates**

28 g of nutrient agar was dissolved in 1000 ml of distilled water and boiled to dissolve the agar completely and then sterilized by autoclaving at 15 lbs pressure at 121 ºC for 15 minutes (pH 7.4±0.2). 20-25 ml of cooled agar medium was poured onto the sterile prepared 15 × 100 mm sterile Petri dishes. (The agar plates were allowed to cool to room temperature and stored in a refrigerator (2℃ - 8℃) until used) Ealuation of anti-Microbial activity by Agar Well Diffusion

**Evaluation of Antibacterial activity**

Leaf extracts of Aerva Lanata, Bauhinia variegata, Acmella uliginosa at a concentration of 500µg/ml, 750µg/ml, 1000µg/ml were tested against the gram positive bacteria like Staphylococus aureus, Bacillus subtilius, gram negative bacteria like Escherichia coli, Klebseilia pneumoniae, by Well Diffusion Method, the same method employed for evaluation of antifungal activity with some modifications in temperatures and standard drug according to litarutre survey and standard methods employed previously.

Antibacterial activity of the plants extract was tested using Well diffusion method.31 The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method. Wells were made on the agar surface with 6mm cork borer.

The dried extracts were dissolved in 95% of DMSO for preparation of different concentration ranges of extracts. The extracts were poured into the well using sterile syringe. The plates were incubated at 37 °C±2°C for 24 hours for bacterial activity. The plates were observed for the zone clearance around the wells. The extracts of the dried scale leaves of three plants were used for the study. The extracts were dissolved in DMSO to form dilution such as 500µg/ml, 750µg/ml and 1000µg/ml. Each concentration of the extract was tested against different bacterial pathogens. Ciprofloxacin, at a concentration of 5µg/ml and 10µg/ml was used as standard antibacterial drug. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all three replicates 32 and the average values were tabulated.

Triplicates were performed and the experiments were repeated thrice and the average values were recorded. The results were compared with the zone of inhibition produced by standard anti microbial drug.

From the above results came to know that the selected plants shown significant antibacterial activity in methonolic extract, in individual, then we made an attempt for evaluation of synergistic effect of selected medicinal plants leafy extracts of Aerva Lanata, Bauhinia variegata, Acmella uliginosa, and determined the study of antimicrobial activities against same species of bacterial strains with same procedure employed above i, ie Agar Well Diffusion and results were reported at.

**Results**

**Table 1:** Morphological Evaluation

| Parameter | *Aerva lanata* | *Bauhinia Variegata* | *Acmella Ulignosa* |
|---|---|---|---|
| Color | Pale green | Greenish | Dark green |
| Odor | Characteristic | Weak | Strong |
| Taste | Bitter to acrid | Slightly bitter | Burning, pungency, numbness |
| Shape | Ovate | Cordate, nerved | ovate, narrowed at base |
| Texture | Smooth | Rough | Smooth |
| Size | 0.5 to 1.5 in wide (13 to 38 mm) long | 13 to 15 cm length 2-14 cm wide | 2.5 to 5 cm long 1.5 to 2 cm wide |

**Table 2:** Phytochemical evaluations of *Aerva lanata* leaves

| Sl. No. | Name of extract | Alkaloids | Sapo nins | Pro teins | Glyco sides | Flava noids | Carbo hydates | Phyto sterols | Tannis |
|---|---|---|---|---|---|---|---|---|---|
| 1 | N-hexane | + | + | - | - | + | + | + | - |
| 2 | Petroleum um ether | + | + | - | - | + | + | - | - |
| 3 | Ethanol | + | + | + | - | + | + | + | |
| 4 | Methanol | + | - | - | - | + | + | - | + |

**Table 3:** Phytochemical evaluation of *Bauhinia variegata*

| Sl. No. | Name of extract | Alkaloids | Sapo nins | Pro teins | Glyco sides | Flava noids | Carbo hydates | Phyto sterols | Tannis |
|---|---|---|---|---|---|---|---|---|---|
| 1 | N-hexane | - | + | + | - | + | + | + | - |
| 2 | Petroleum um ether | - | + | - | - | + | + | + | + |
| 3 | Ethanol | - | + | + | - | + | + | + | |
| 4 | Methanol | - | + | - | + | + | + | + | + |

**Table 4:** Phytochemical evaluation of *Acmella uliginosa*

| Sl. No. | Name of extract | Alkaloids | Sapo nins | Pro teins | Glyco sides | Flava noids | Carbo hydates | Phyto sterols | Tannis |
|---|---|---|---|---|---|---|---|---|---|
| 1 | N-hexane | + | + | + | - | + | + | - | + |
| 2 | Petroleum um ether | + | + | - | - | + | + | - | + |
| 3 | Ethanol | + | + | + | - | + | + | - | + |
| 4 | Methanol | + | - | - | - | + | + | - | + |

***Where, + indicates the presence of secondary metabolites, - indicates the absence of secondary metabolite Anti microbial evaluation

**Table 5:** Results for Anti-Bacterial activity of *Aerva lanata* leaves

| Solvent extracts 1000µg/ml | Ciprofloxacin | | Zone of Inhibition | | | |
|---|---|---|---|---|---|---|
| | 5µg/ml | 10µg/ml | Staphylococus aureus, | Bacillus subtilius | Escherichia coli, | Klebseilia pneumoniae |
| N-hexane | 7.5 mm | 9 mm | 5 mm | 6 mm | 7 mm | 5 mm |
| Petroleum um ether | 7 mm | 9 mm | 6 mm | 7 mm | 6 mm | 5 mm |
| Ethanol | 7 mm | 9 mm | 7 mm | 6 mm | 7 mm | 8 mm |
| Methanol | 7.5 mm | 9.5 mm | 8 mm | 6.5 mm | 8 mm | 8 mm |

**Table 6:** Results for Anti-Bacterial activity *Bauhinia variegata*

| Solvent extracts 1000µg/ml | Ciprofloxacin | | Zone of Inhibition | | | |
|---|---|---|---|---|---|---|
| | 5µg/ml | 10µg/ml | Staphylococus aureus, | Bacillus subtilius | Escherichia coli, | Klebseilia pneumoniae |
| N-hexane | 7.5 mm | 9 mm | 3 mm | 4mm | 5 mm | 4 mm |
| Petroleum um ether | 7 mm | 6 mm | 4 mm | 3 mm | 4 mm | 3 mm |
| Ethanol | 7 mm | 7 mm | 5 mm | 4 mm | 5 mm | 5 mm |
| Methanol | 7.5 mm | 9.5 mm | 9 mm | 8mm | 7 mm | 9 mm |

**Table 7:** Results for Anti-Bacterial activity of *Acmella uliginosa*

| Solvent extracts 1000µg/ml | Ciprofloxacin | | Zone of Inhibition | | | |
|---|---|---|---|---|---|---|
| | 5µg/ml | 10µg/ml | Staphylococus aureus, | Bacillus subtilius | Escherichia coli, | Klebseilia pneumoniae |
| N-hexane | 7.5 mm | 9 mm | 4 mm | 4mm | 6 mm | 4 mm |
| Petroleum um ether | 7 mm | 6 mm | 5 mm | 4 mm | 5 mm | 6 mm |
| Ethanol | 7 mm | 7 mm | 6 mm | 5 mm | 6 mm | 7 mm |
| Methanol | 7.5 mm | 9.5 mm | 10 mm | 9 mm | 9 mm | 8 mm |

**Formula for Polyherbal Ointments**

| Ingredients | F 1 (2%) | F 2 (4%) | F3 (6%) |
|---|---|---|---|
| *Aerva lanata* Methonolic extract | 2gm | 4gm | 6gm |
| *Bauhinia variegata* Methonolic extract | 2gm | 4gm | 6gm |
| *Acmella uliginosa* Methonolic extract | 2gm | 4gm | 6gm |
| Emulsifying ointment | q.s to 100 gm | q.s to 100 gm | q.s to 100 gm |

**Table 8:** Physicochemical evaluation of formulated Poly-Herbal Ointment

| Physicochemical parameter | F 1 (2%) | F 2 (4%) | F3 (6%) |
|---|---|---|---|
| Color | Green | Green | Green |
| Odour | Characteristic | Characteristic | Characteristic |
| Loss on drying | 39% w/w | 41% w/w | 45% w/w |
| $P^H$ | 6.90 | 6.84 | 6.81 |
| Spreadability | 12 sec | 12 sec | 14 sec |
| Extrudability | 170g | 170g | 172g |
| Diffusion study (After 60min) | 0.7 cm | 0.8 cm | 08 cm |

**Table 9:** Antimicrobial activity of formulated ointment

| Ointments | Zone of inhibition in cm | | | |
|---|---|---|---|---|
| | Staphylococus aureus | Bacillus subtilius | Escherichia coli, | Klebseilia pneumoniae |
| F 1 (2%) | - | - | - | - |
| F 2 (4%) | 1.2 | 0.9 | 1 | 1 |
| F3 (6%) | 1.3 | 1.2 | 1.2 | 1.2 |
| Standard Betadine(5%w/w) | 1.2 | 1.2 | 1 | 1.3 |

## Discussion

Literatures revealed that the selected three herbs *Aerva lanata, Bauhinia variegata, Acmella uliginosa* have antibacterial activity. Extraction and the phytochemical screening was done using Methanol, petroleum ether, N-hexane, and Ethanol as solvents. Phytochemical screening confirmed the presence of various phytoconstituent like carbohydrate, glycosides, flavonoids, Alkaloids, Volatile oils and tannins. In the present study, polyherbal ointments were prepared by fusion method using emulsifying ointment as the base. Antimicrobial study shows that the prepared ointments has better and equal activity against gram positive bacteria like *Staphylococus aureus*, *Bacillus subtilius*, gram negative bacteria like *Escherichia coli*, *Klebseilia pneumonia*, compared to standard 5% Betadine ointment. Hence the study concludes that an efficient antimicrobial ointment can be formulated from the methonolic plant extracts of *Aerva lanata, Bauhinia variegata, Acmella uliginosa* which can also be used for various bacterial skin infections.

## Conclusion

In the present experimental study showed that it is possible to develop and evaluate the anti-microbial polyherbal ointment with methonolic extract of plant materials of *Aerva lanata, Bauhinia variegata, Acmella uliginosa*, and the results were compared with those of different formulations. Compared to all three formulations F2 and F3 formulation shows good activity against selected bacterial strains. From this study concluding that poly herbal formulations are safe and effective and show synergetic actions. There is a much research is in need for future prospects in drug development and dosage design from natural sources.

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