



## PREPARATION OF A COMMON HERBAL MEDICINE WITH CULINARY PLANTS FOR SKIN INFECTIONS CAUSED BY *Candida albicans* AND *Propionibacterium acnes*

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### ABSTRACT

The current study was done to find a natural medicine based upon the activity of three easily available herbs, namely, *Plectranthus amboinicus*, *Mentha piperita* and *Ocimum basilicum* along with *Aloe vera* gel against bacterial and fungal skin infections. The organisms were isolated from skin infections like acne (whiteheads and black heads), papules, pustules, skin rashes etc. The main pathogens isolated were *Propionibacterium acnes* and *Candida albicans*. A decoction of the chosen herbs was prepared at a concentration of 100 mg/ml and was tested against the isolated pathogens using agar well diffusion method. Highest inhibitory activity was observed with the methanol extracts. *Aloe vera* gel was extracted and a mixture of the herbs and the gel was made and tested against the isolated pathogens. Synergism was observed indicating that it is a potential formulation to treat common skin ailments, especially acne that bothers most of the teenage population across the world.

**KEY WORDS:** *Plectranthus amboinicus*, *Mentha piperita*, *Ocimum basilicum*, *Aloe vera*, skin infections, acne, antimicrobial activity, antifungal activity, yeast infections, *Candida albicans*, *Propionibacterium acnes*.

### INTRODUCTION

The earth is a treasure casket of medicines that can cure several diseases. However, due to the increased incidence of new infections and the increasing incidence of bacterial resistance to antibiotics, there is a need to discover the diverse natural compounds that are easily available amongst plants and animals. The use of synthetic drugs containing microbes that is biochemically and genetically modified as a treatment of common infectious disease are not reliable due to many controversies. Synthetic drugs are not only expensive and inadequate but also often had issues with adulterations and side effects. Moreover, different plant parts or plant species are used in combination to achieve the same goal with great efficacy. In fact, it is thought that herbal remedies have the advantage in combining their active components to obtain synergistic or additive effects which give to the plants an efficiency superior to some of their isolated components. Dermal or epidermal infections are skin infections that are associated with soft tissues. The pathogen involved is usually a bacterial or fungal species that causes superficial infections. Such infections often require treatment by antibiotics. Viral and parasitic skin infections also occur but are less common. Common skin infections include moles, skin tags, ringworm infections, acne, cellulitis, hives, chicken pox, eczema, rosacea, seborrheic dermatitis, psoriasis, vitiligo, impetigo, warts, skin cancer and other dermatophyte infections that can be cured by several traditional medicines such as *Azadirachta indica* (Pratibha Nand *et al.*, 2016), *Zizyphus mucronata*, *Aloe vera*, turmeric, by using a combination of different natural substances like clay (Preeti *et al.*, 2016), other therapeutic drugs (Priyam Sinha *et al.*, 2014) etc. Recurrently, *Acne vulgaris* is a common dermatological condition allied with

depression, anxiety, and other psychological sequelae. Acne is one of the most common multifactorial chronic inflammatory diseases of the pilosebaceous follicles that can be treated by a combination of several medicines (Elin Julianti *et al.*, 2017). *Propionibacterium acnes* is one of the most common pathogens of acne (Amrita *et al.*, 2012). Rashes caused by *Candida albicans* are very common amongst women.

A family of flowering plants commonly known as the mint or deadnettle family, Lamiaceae has several medicinal culinary herbs. Indian borage (*Plectranthus amboinicus*), is a medicinal plant used widely in Indian medicine. It is a succulent, aromatic, perennial herb belonging to the family Lamiaceae, and decoction of its leaves is used for several medicinal purposes (Greetha *et al.*, 2016). The antimutagenic, antitumorigenic and antigenotoxic effects of Indian borage leaves are well documented, and recently its antimicrobial properties have also been reported Arunkumar *et al.* (2011). *Ocimum basilicum*, also known as Sweet Tulsi is a culinary herb due to the characteristic flavors it imparts. Basil seeds have been used in traditional medicine for a long time to treat colic ulcer, dyspepsia, diarrhea and inflammations, among other ailments (Ilhan Kaya *et al.*, 2008)). *Mentha piperita*, is a medicinally important plant and commonly known as peppermint is a hybrid of *Mentha spicata* (spearmint) and *Mentha aquatica* (watermint). In Eastern and Western traditional medicine peppermint and its oil have been used as an antispasmodic, aromatic, antiseptic and also in the treatment of cancers, colds, cramps, indigestion, nausea, sore throat and toothaches (Bupesh *et al.*, 2007). *Aloe vera* gel is widely known for its anti-inflammatory properties and is used for the treatment of various skin infections. It is well known for its soothing and healing nature.

## MATERIALS AND METHODS

### Sample collection and processing

Infected skin samples (about 27 samples) from the face and rashes were collected from people suffering from skin infections by using sterile cotton swabs. These swabs were then inoculated into a basal medium such as Fluid thioglycollate medium and Sabouraud dextrose broth for fungi. The pathogens were isolated and identified using biochemical tests and preliminary tests. Germ tube test was performed to identify *C. albicans*. *P. acnes* was grown on a special anaerobic medium containing 3% trypticase soy, 2% agar, 1% yeast extract and 0.5% Tween 80 grown in anaerobic jar containing GasPak system at 37°C (Charles Evans -1979) and *C. albicans* was grown on Sabouraud dextrose agar.

### Plant material collection and processing

Mature, fresh leaves of *Plectranthus amboinicus* were picked from home-grown plants and *Mentha piperita* leaves were collected from a local market. Plant species were identified by verifying the colour pictures followed by description and identification characters. The plant leaves were thoroughly washed with water to remove dust. The leaves were then allowed to dry under shade for 15-20 days and were powdered by using electric blender. Finally, fine powder was collected from the powdered leaves by sieving through a strainer and used for extraction. Matured seeds of *Ocimum basilicum* were collected from a local market and the seeds were powdered into a fine dry powder using an electric blender and stored in air tight bottles for extraction, protected from moisture.

### Preparation of plant extracts

There are various extraction methods proposed by several researchers for medicinal and aromatic plants. Extraction methods were discussed by Sukhdev Swami *et al.* (2008). FT-IR analysis was done to find the different functional groups present in the plants.

#### Preparation of aqueous plant extract

About 10g of each powdered plant material was added to 100 mL of distilled water (Wan Nordini *et al.*, 2013). The mouth of the conical flask was covered with aluminium foil and agitated manually for 5-10 minutes for thorough mixing and also for complete elucidation of active materials to dissolve in water. The solvent was evaporated in a boiling water bath for 1 hour. Then, the extract was filtered by using a clean muslin cloth followed by filtration with Whatman no. 1 filter paper. The filtrate was then centrifuged for 5 minutes at 3500 rpm at room temperature. The supernatant was collected and stored for further use (Azwanida *et al.*, 2015).

#### Preparation of methanol extract

Methanol extract was prepared by taking about 10 g of the powdered sample was extracted with 100 mL of methanol by mixing well in a flask followed by filtration through Whatman No. 1 filter paper. The filtrate was centrifuged at 5000 rpm for 15 minutes at room temperature. The supernatant was collected and concentrated using a water bath, and the crude extract collected stored in tubes until further use.

#### Preparation of acetone extract

For acetone extract, about 10 g of dried powder was weighed and homogenized with 100 ml of acetone at a concentration of 100mg/ml (N. Lall *et al.*, 2000). The mixture was filtered, and filtrate was collected and

centrifuged at 5000 rpm for 15 min at room temperature. Supernatant was collected and stored until further use.

## STUDY OF ANTIMICROBIAL ACTIVITY

### Preparation of isolated organisms for antimicrobial activity

The isolated organisms were separately inoculated into basal broth media like Fluid thioglycollate medium and Sabouraud dextrose broth and incubated for 24-48 hours until turbidity was observed, and it was adjusted according to Mac Farland's standard of 0.5.

### Agar well diffusion method

Different isolates were made into a lawn culture on Mueller- Hinton agar plates. Agar plates were punched with a sterile cork borer of 4 mm size and 100 µL of each extract was poured with micropipette in the wells and positive and negative controls were also employed. Both individual and combined extracts were poured in different wells to study individual and combined activity of the extracts. The plates were allowed to standby for 30 min. The plates were then incubated at 37°C for 24- 48 hours.

### Preparation of medicinal extract

Fresh plant medicinal formulation was prepared using *Aloe vera* gel as a base. *Aloe vera* leaves were plucked and the gel was scooped out. The liquid viscous gel was then heated for clarification (Chandegara *et al.*, 2015). All the three plant powders were taken and mixed with fresh Aloe gel at a concentration of 100mg/ml of gel in the ratio 1:1:1:1. The prepared gel was then stored in a tube and refrigerated until use. No preservatives were added, and the efficiency of this formulation was tested against the isolated pathogens. The results were then compared with the activity of pure *Aloe vera* gel extract. No preservatives were added to the gel and the efficiency of the gel was tested after a week and after a month.

## RESULTS AND DISCUSSION

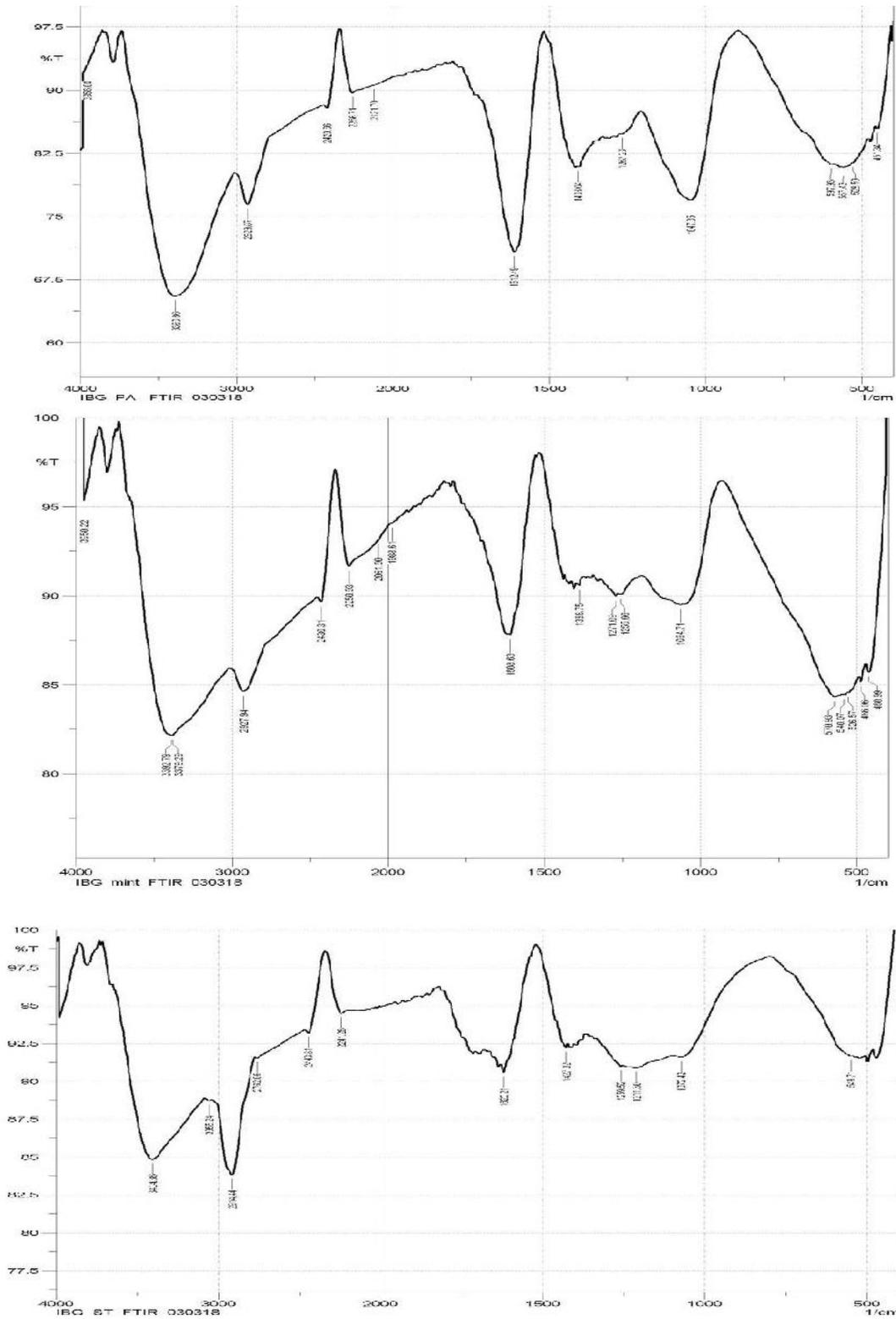
The pathogens were found to be *Propionibacterium acnes* and *Candida albicans*. Bacteria were found to be more prevalent on the surface of the skin than fungi. *Candida albicans* was common in thigh rashes. FT-IR analysis was done to find the functional groups present in the crude extract. FT-IR analysis of *Plectranthus amboinicus* showed peaks at 597.93 cm<sup>-1</sup>, 1047.35 cm<sup>-1</sup>, 1267.23 cm<sup>-1</sup>, 2929.87 cm<sup>-1</sup> and 3390.86 cm<sup>-1</sup> indicating the presence of C-N, C-H and O-H functional groups. *Mentha piperita* extract showed peaks at 570.93 cm<sup>-1</sup>, 1388.75 cm<sup>-1</sup>, 2250.93 cm<sup>-1</sup>, 2927.94 cm<sup>-1</sup>, 3379.29 cm<sup>-1</sup> and 3392.79 cm<sup>-1</sup>. This indicated the presence of functional groups such as CN, C-H and N-H groups. *Ocimum basilicum* seed extract showed the peaks at 1072.42 cm<sup>-1</sup>, 1427.32 cm<sup>-1</sup>, 1620.21 cm<sup>-1</sup>, 2241.28 cm<sup>-1</sup> and 2762.06 cm<sup>-1</sup> indicating functional groups such as C-H, C=C, C C and O-H groups. This could belong to alkenes, alkaloids, glycosides, flavonoids or tannins.

### Antimicrobial assay

Antimicrobial assay was performed, and it was found that the effectiveness of the extract was found to be more in methanol extracts followed by aqueous extracts as shown in the Table 4 and 2. Acetone extracts were least effective. *Staphylococcus epidermidis* was found to be more sensitive to these plant extracts. The pure *Aloe vera* extract was found to be less effective against the isolated pathogens. However, the formulated extract containing

mixture of *Aloe* and the other culinary herbs showed the highest activity against all the pathogens, thus showing a

synergistic effect. The formulated gel was found to be effective for a month without any preservatives.



**FIGURE 1.** FT-IR Analysis of *Mentha piperita*, *Plectranthus amboinicus* and *Ocimum basilicum* crude extracts respectively

**TABLE 1:** Confirmatory biochemical and sugar fermentation tests

Sl.No.	Biochemical tests	<i>P. acnes</i>
1.	Catalase test	+
2.	Oxidase test	-
3.	Indole test	+
4.	Nitrate test	+
6.	Gelatinase test	+
Sugar fermentation		<i>C. albicans</i>
7.	Dextrose	+

**TABLE 2:** Diameter of zone of inhibition for crude extract

Sl. no.	Name of the plant extract	Zone diameter (mm)	
		<i>P. acnes</i>	<i>C. albicans</i>
	Crude Extract	<i>P. acnes</i>	<i>C. albicans</i>
1	<i>Plectranthus amboinicus</i>	16	8
2	<i>Mentha piperita</i>	10	6
3	<i>Ocimum basilicum</i>	8	-

**TABLE 3:** Diameter of zone of inhibition for Acetone extract

Sl. No.	Name of the plant extract	Zone diameter (mm)	
	Acetone Extract	<i>P. acnes</i>	<i>C. albicans</i>
1	<i>Plectranthus amboinicus</i>	10	12
2	<i>Mentha piperita</i>	14	-
3	<i>Ocimum basilicum</i>	4	-

**TABLE 4:** Diameter of zone of inhibition for Methanol extract

Sl. No.	Name of the plant extract	Zone diameter (mm)	
	Methanol Extract	<i>P. acnes</i>	<i>C. albicans</i>
1	<i>Plectranthus amboinicus</i>	16	30
2	<i>Mentha piperita</i>	16	20
3	<i>Ocimum basilicum</i>	2	12

**TABLE 5:** Comparison of Diameter of zone of inhibition for extracts and control

Zone in mm	<i>P. acnes</i>	<i>C. albicans</i>
<i>Aloe vera</i>	-	2
Formulated extract	20	10
Positive control	Penicillin G	Amphotericin B
	16	10
Negative control	Erythromycin	Clotrimazole
	4	2

**Antifungal activity of acetone, methanol extracts against *C. albicans***      **Antifungal activity of crude, formulated extracts against *C. albicans***



**Antibacterial activity of different extracts against *P. acnes***



The current study investigated the skin infections caused by *Candida albicans*, and acne-causing organism

*Propionibacterium acnes*. Plant extraction methods vary and maceration and Soxhlet extraction is one of the widely

used methods (Amita Pandey *et al.*, 2014). A comparative study of different solvents for plant extraction by Dixon Dhawan and Jeena Gupta (2017) showed maximum yield with methanol, water and acetone followed by ethanol and chloroform. The results from the studies of K. Padmalochana and M.S. Dhana Rajan (2014) have shown that every phytochemical could be extracted with different solvents. This might differ according to the polarity of the solvent. Methanolic leaf extract has shown that it has extracted most of the compounds and this is confirming that methanol is being used as a solvent in Ayurveda centers for extracting bioactive compounds. Hence, the polarity of the solvent is the major characteristic of them to be used as a basic for extraction. The present study used methanol, acetone and crude extracts to obtain maximum yield of plant drugs. Synergistic activity of *Aloe vera* and *Curcuma longa* extracts were studied by Vanka Kanth Swaroop *et al.* (2014). The synergistic effect may be due to certain complex formation which becomes more effective in the inhibition of a particular species of microorganisms either by inhibiting the cell wall synthesis or by causing its lyses or death.

## CONCLUSION

Thus, it can be concluded that to control a particular disease *in vitro* experiments should be carried out with various antibiotics and their combination as well as antibiotics and plant extracts, so that a right combination may be administered to the patient for early and safe recovery from a specific ailment. All the combinations do not produce synergistic effect and therefore a number of combinations are required to be tested. As there are few clinical evidences about the effectiveness and safety of these plants in the treatment of acne and other skin infections, chemical drugs seem to still be the first choice in the treatment of acne and skin infections. Some plants reviewed in this paper have shown promising results. Hence, they might possibly be used alone or as adjuvant with other therapeutic measures or in mild to moderate situations. Possible contact sensitization especially in topical or oral use should be considered. Some plants, especially the roots of mountain grapes, tea tree oil, *Saccharomyces*, and perhaps *Ocimum basilicum* due to their effectiveness and safety can be compared to alternative treatments with synthetic drugs for mild to moderate acne and other skin infections (Hamid Nasri *et al.*, 2015). *Plectranthus amboinicus* has shown a wide range of biological properties and proved to be effective in curing respiratory, cardiovascular, oral, skin, digestive and urinary diseases and its antimicrobial properties have been reported by Sandeep Kumar Gupta *et al.* (2016) and R. Yuthistran *et al.* (2015). The biological properties are attributed to the occurrence of a wide range of bioactive compounds in the plant extracts as well as an essential oil. Basil seeds are traditionally believed to be used for therapeutic purpose to improve blood circulation, reduce inflammation, reduce the oxidation of cholesterol, and increase immune function and to control blood sugar level. The strongest inhibitory activity of basil seeds extract was observed against *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae* and *Klebsiella pneumoniae* (Muafia Shafique *et al.*, 2011)

Peppermint is a perennial glabrous and strongly scented herb belonging to family Lamiaceae. The plant is

aromatic, stimulant and used for allaying nausea, headache and vomiting. Its oil is one of the most popular widely used essential oils in food products, cosmetics, pharmaceuticals, dental preparations, mouthwashes, soaps and alcoholic liquors. The antibacterial potential of six extracts from leaf, stem and root of *Mentha piperita* against pathogenic bacteria such as *Bacillus subtilis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae* with the organic (ethanol, methanol, ethyl acetate, chloroform, hexane and petroleum ether) extracts of the leaves are said to possess strong antibacterial activity against a range of pathogenic bacteria and its pharmacological effects have been reported by Punit Shah *et al.* (2004).

This study was done to evaluate the antibacterial activity of these three extracts against the different organisms isolated from skin infections. The organisms were found to be sensitive to all the extracts. Methanol extracts were more inhibitory towards the organisms. The acetone extracts had less effect compared to the other extracts. The formulated extract combined with *Aloe vera* gel showed significant increase in the antimicrobial activity indicating synergism of the extracts.

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