

## ANTIBACTERIAL STUDY OF POLYHERBAL THERAPEUTIC AGENTS USED IN RESPIRATORY DISEASES

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### ABSTRACT

In this study four different crude extracts were prepared from the leaves of *Ocimum sanctum*, *Boswellia serrata*, rhizomes of *Zingiber officinale* and roots of *Glycyrrhiza glabra*. An attempt has also been made to formulate polyherbal syrup and suspension by using these extracts. The anti-microbial activity of the crude extracts and the prepared polyherbal formulations were tested against some bacterial strains which are responsible for generation of various types of respiratory diseases. All the extracts and the polyherbal formulations exhibited antibacterial activity in concentration dependent manner but

the polyherbal formulations displayed better activity than that of their crude extracts. The better activity of the prepared formulations may be due to the combined activity of phytoconstituents present in the extracts. The results from the present work support the incorporation and utilization of herbs in the formulations to give better effect for the management of respiratory diseases by polyherbal formulations.

**KEYWORDS:** Respiratory diseases, extracts, polyherbal, *Ocimum*.

### INTRODUCTION

One of the most important organs of our body is the lungs, whose role is to acquire molecular oxygen and eliminate carbon dioxide. By weight, each day, more than 20 kg of air enters and leaves the human body, a load which exceeds that of food and water ingested during the same time period.<sup>[1]</sup> Depending on the level of air pollution, different types and quantities of foreign particulates and microbial pathogens are inhaled. The inhaled particulates are

deposited on the epithelial lining of the conducting airways and also in the peripheral air spaces where they are retained for various durations before they are removed or destroyed.<sup>[2-4]</sup> Due to these various types of respiratory diseases such as cough, cold, allergies, asthma, bronchitis, pneumoniae and other diseases are developed.<sup>[5]</sup> Some respiratory diseases are caused by bacteria. For instance, *Staphylococcus aureus* causes pneumonia, tonsillitis, pharyngitis, sinusitis<sup>[6]</sup>, *Escherichia coli* causes community-acquired pneumonia<sup>[7]</sup>, *Bacillus subtilis* causes occupational asthma<sup>[8]</sup> which is a reason for allergic reactions of the lungs<sup>[9]</sup>, *Salmonella typhimurium* causes lobar pneumonia<sup>[10]</sup> and *Pseudomonas aeruginosa* typically infects the pulmonary tract and cause pneumonia.<sup>[11]</sup> *P. aeruginosa* is also one of the most common and lethal pathogens responsible for ventilator-associated pneumonia in intubated patients.

In recent years however, renewed interest has grown on traditional herbal remedies because of many side effects observed by using the allopathic medicines. At the same time, WHO also recommends further research on traditional system of medicine. Polyherbal formulations with various active principles and properties have been used from ancient days to treat a wide range of human diseases. Polyherbal formulations are collection of therapeutic entities that are formulated and prepared on the basis of the healing properties of individual ingredients with respect to the condition of sickness. Such herbal constituents with diverse pharmacological activities principally work together in a dynamic way to generate maximum therapeutic benefits with minimum side effects. Currently, polyherbal formulations are employed for the treatment of various types of respiratory diseases such as asthma<sup>[12-15]</sup>, cough<sup>[16-17]</sup>, upper respiratory tract infections<sup>[18]</sup>, bronchitis<sup>[19]</sup>, Severe Acute Respiratory Syndrome<sup>[20]</sup>, tonsillitis<sup>[21]</sup>, pneumonia<sup>[22]</sup> and in other diseases.<sup>[23]</sup> In the present study we prepared four different hydroalcoholic extracts (leaves of *Ocimum sanctum*, *Boswellia serrata*, rhizomes of *Zingiber officinale* and roots of *Glycyrrhiza glabra*) and two polyherbal formulations containing these extracts are evaluated for their antibacterial efficacy against three different bacterial strains which are responsible for various types of respiratory diseases.

## MATERIALS AND METHODS

### Collection of plant materials

The leaves of *Ocimum sanctum* and *Boswellia serrata* were collected in the month of February from nearby area of Berhampur, rhizomes of *Zingiber officinale* were purchased

from local market and the roots of *Glycyrrhiza glabra* were purchased from local chemical and herbal suppliers near Berhampur, India.

### Preparation of extracts

The rhizomes of *Zingiber officinale* and roots of *Glycyrrhiza glabra* were cut into pieces and powdered by using hand mill and passed through sieve 40 # mesh particle size. About 500 g of coarse powder was subjected to extraction by Soxhlet extractor using water and ethanol (2:1) as solvent. After extraction the extracts were concentrated to dryness under reduced pressure and controlled temperature (40°-50°C) using a rotary evaporator. The leaves of the above two plants were dried, crushed to moderately coarse power and stored in airtight container. About 500 g of coarse powder was subjected to extraction by Soxhlet extractor using water and ethanol (2:1) as solvent.<sup>[24]</sup> After extraction the extracts were concentrated to dryness under reduced pressure and controlled temperature (40°-50°C) using a rotary evaporator. These extracts were encoded as HAEBS, HAEZO, HAEGG and HAEOS for hydroalcoholic extracts of *Boswellia serrata*, *Zingiber officinale*, *Glycyrrhiza glabra* and *Ocimum sanctum* respectively during the study.

### Preparation of Poly Herbal formulations

#### Preparation of polyherbal syrup

The simple syrup (66.67% w/v) was prepared as per Indian pharmacopoeia. One gram of each extract was dissolved in simple syrup I.P. (1966) and the volume was made up to 100 mL with simple syrup. About 0.1% of methylparaben was added as preservative. This polyherbal formulation was encoded as PHF-1 during the study.

#### Preparation of polyherbal suspension

Polyherbal suspension was prepared by using the hydro-alcoholic extracts of *Ocimum sanctum*, *Boswellia serrata*, *Zingiber officinale* and *Glycyrrhiza glabra* with a suitable suspending agent. This polyherbal formulation was encoded as PHF-2 during the study. Required quantity of sucrose was taken in a clean mortar and triturates it into powder form and it was dissolved in 50 ml of water. The extracts, tween 80 and sodium CMC in respective quantities were taken and was made into a homogeneous mixture. This mixture was added to 50 ml of sucrose solution. Then the excipients like sorbitol, flavouring agent and preservative were added and it was made into a homogeneous mixture finally the volume was made up to 100ml. The preparation was transferred to a clean and dry amber coloured bottle and stored at room temperature till further study.

**Phytochemical screening**

The different extracts were tested for the presence of steroids, saponins, flavonoids, terpenoids, cardiac glycosides, alkaloids and tannins using standard methods.

**Evaluation of extractive values**

Extractive values estimation are useful for the preliminary evaluation of crude drug. It gives an idea about the nature of the chemical constituents present in a crude drug. It is also useful for the estimation of specific constituents, soluble in that particular solvent used for extraction. Estimation of this values tells us about the justification to whether carrying out the research work further will be beneficial to the community or not. As a part of the preliminary study, the individual yield of each extract of these plant parts was observed and recorded in table-1.

**Determination of ash values**

Determination of ash value is important for determining quality and purity of a crude drug. After incineration the crude drugs leave an inorganic ash which in the case of many drugs varies within fairly wide limits and is therefore of little value for purpose of evaluation. In the determination of the total ash values the carbon and other organic matters must be removed at a temperature of 450°C. If carbon still present after heating at a moderate temperature, the water soluble ash may be separated and the residue again ignited. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. To produce more consistent ash as a sulphated ash, which involves treatment of the drug with dilute sulphuric acid before ignition, is used. In this all oxides and carbonates are converted to sulphonates and the ignition is carried out at higher temperature (600°C). If the total ash was treated with dilute hydrochloric acid, the percentage of acid insoluble ash may be determined. This usually consists mainly of silica and a high acid insoluble ash in drugs indicates contamination with earthy materials. The ash value of the powdered drug was recorded in table-2.

**Moisture content**

To determine the actual moisture content of those powdered drug materials, about 1.5 gm of powdered drug was weighed properly. These drug powders were kept separately in flat and thin porcelain dishes which were dried in oven at 100°C. The process of burning of powder proceeded slowly by taking special care not allowing those materials to catch fire or to give off smoke as dense fumes during the process. After drying, these drug powders were re-

weighed and the loss in weight was recorded as previous content of moisture in the material and the results were shown in table-3.

### Antibacterial study

The three different bacterial strains were used for investigation of the antibacterial potential of different extracts and their polyherbal formulations. The bacterial strains were procured from the Microbial Type Culture Collection, Department of Microbiology, M. K. C. G. Medical College, Berhampur, Odisha, India. The bacterial strains used for the determination of antimicrobial activity are *Escherichia coli* (MTCC 40), *Klebsiella pneumoniae* (MTCC 3296), *Pseudomonas aeruginosa* (MTCC 424).

Well of uniform diameter (6mm) were made on agar plates, after inoculating them separately with the test organisms aseptically. Into these cups the standard drug, control, extracts and the polyherbal formulations were introduced with the help of micropipette. After introduction of the test substances, the plates were placed in the refrigerator at 8-10°C for proper diffusion of drug into the media. After two hours of cold incubation, the petriplates were transferred to incubator and maintained at  $37 \pm 2^\circ\text{C}$  for 18-24 hours. After the incubation period, the petriplates were observed for zone of inhibition. The results were reported by comparing the zone of inhibition shown by the test compounds with standard drug (tetracycline). The results were the mean value of zone of inhibition measured in millimeter of three sets. The results were presented in the figure-1.

## RESULTS

**Table 1: Evaluation of extractive value of different extracts.**

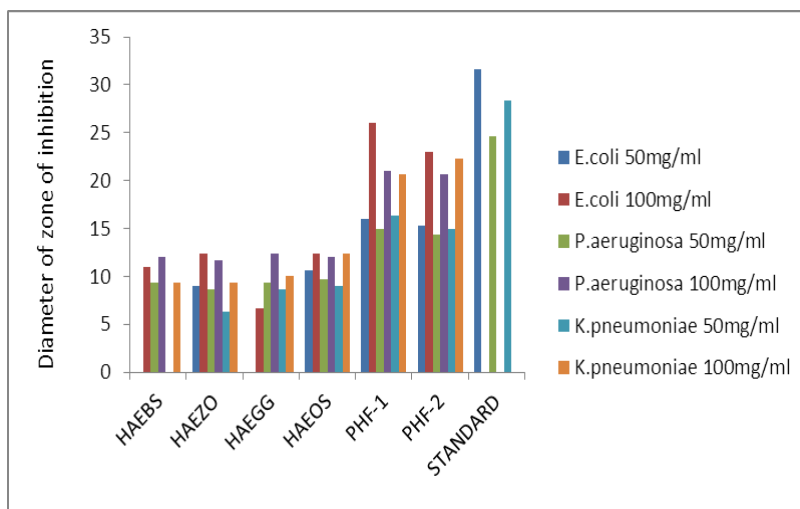
Types of extracts	Extractive Value (%w/w)
HAEBS	2.28
HAEZO	2.17
HAEGG	3.02
HAEOS	2.86

**Table 2: Evaluation of Ash values of the different crude powders.**

Extracts	Total ash	Acid insoluble ash	Water soluble ash	Sulphated ash
	Percentage (w/w)			
HAEBS	6.67	1.28	3.38	2.12
HAEZO	7.62	1.44	3.29	1.64
HAEGG	5.08	1.22	1.03	2.51
HAEOS	7.2	1.34	2.93	0.92

**Table 3: Average percentage of moisture content of the powders of different plants.**

Powders of different plants	Average % of moisture
<i>Boswellia serrata</i>	3.29
<i>Zingiber officinale</i>	5.37
<i>Glycyrrhiza glabra</i>	5.18
<i>Ocimum sanctum</i>	5.73

**Figure 1: Antibacterial activity of the extracts and polyherbal formulations.**

## DISCUSSION

The preliminary qualitative phytochemical screening of the crude powder of the four selected plants in the present study was done to assess the presence of bioactive components. The presence of alkaloids, carbohydrates, glycosides, proteins, tannins and phenolic compounds, steroids, saponins and flavonoids were determined in *Boswellia serrata* and *Zingiber officinale* whereas the crude powder of *Ocimum sanctum* showed the presence of alkaloids, carbohydrates, glycosides, proteins, tannins and phenolic compounds, saponins and flavonoids. In case of the powder of *Glycyrrhiza glabra* except alkaloids and steroids all other secondary metabolites are present. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. From the results obtained in the study it is observed that the extractive value of the crude powder of *Glycyrrhiza glabra* was higher (3.02%), followed by *Ocimum sanctum* (2.86%), *Boswellia serrata* (2.28%) and *Zingiber officinale* (2.17%) (table-1). Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. Total ash of crude powder of *Boswellia serrata* was 6.67%, acid insoluble ash was 1.28%, water soluble ash was

3.38% and sulphated ash was 2.12%. In case of *Zingiber officinale* the ash values were in the order of total ash was (7.62%), acid insoluble ash was (1.14%), water soluble ash was (3.29%) and sulphated ash was (1.64%). The total ash of the crude powder of *Glycyrrhiza glabra* was 5.08%, acid insoluble ash was 1.22%, water soluble ash was 1.03% and sulphated ash was 2.51%. When the ash values of the powder of *Ocimum sanctum* is taken into account it was observed that total ash was 7.2%, acid insoluble ash was 1.34%, water soluble ash was 2.93% and sulphated ash was 0.92 % (table-2). The moisture content of crude powders of *Boswellia serrata*, *Zingiber officinale*, *Glycyrrhiza glabra* and *Ocimum sanctum* were found to be 3.29%, 5.37%, 5.18% and 5.73% respectively (table-3). The result of this study showed that the moisture content of the crude powders was not too high, thus it could discourage bacteria, fungi or yeast growth.

To test the antibacterial activity of the extracts different methods are available in the literature. One of the most commonly followed methods is cup-plate method. This method is based on the diffusion of the test compound from a cavity through the solidified agar layer taken in petriplate, to such an extent that the growth of the added microorganism is prevented entirely in a circular area or zone around the cavity containing the test compound. The main objective of the present study was to investigate the *in-vitro* antimicrobial activity of four different extracts and two polyherbal formulations against three different bacterial strains including both gram negative strains such as *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424) and *Klebsiella pneumoniae* (MTCC 3296) at the concentration of 50 and 100mg/ml by using cup plate method. These bacterial species causes various respiratory diseases. To estimate the comparative effectiveness of these compounds the standard drugs used was Tetracycline. All the test substances, i.e., extracts, polyherbal formulations and standard were exhibited antibacterial activity in a concentration dependent manner. Thus from this antibacterial study it was observed that the polyherbal formulations were exhibited better activity than the extracts from which the polyherbal formulations were prepared fig.1.

## CONCLUSION

These plants which are used in the present study have been used for centuries by native people of India to cure the various types of respiratory diseases such as pneumonia, bronchitis, cough, cold and asthma. The screening of these plants as new anti-infective agents in respiratory diseases will give scientific evidence for the culturally acceptable medicinal



plants for respiratory diseases. It will boost the production of plant-based products by pharmaceutical industries and will prosper the economy of countries like India.

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