



## Preformulation and Formulation Studies of the Poly Herbal Syrup of Hydroalcoholic Extracts of *Zingiber officinale* and *Piper nigrum*

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### ABSTRACT

The present work carried out for the development of polyherbal formulation in the form of syrup containing ginger and black pepper which is most commonly used as spices and these two herbal drugs serves as health foods possessing multiple beneficial effects. In the Preformulation studies of hydro alcoholic extracts of these two herbs showed promising results of antibacterial and anthelmintic activities at lower concentrations and the inhibitory action against pathogenic organism's increases with increasing in the concentration of the extracts. The antibacterial activity was tested against seven bacteria by cup plate agar diffusion method and the anthelmintic activity tested by using *Pheretima posthuma*. Both the extracts showed maximum antibacterial activity against *Staphylococcus aureus* at tested concentrations of 2.5 mg/ml, 5.0 mg/ml and 10 mg/ml. The ginger extract showed maximum anthelmintic activity at concentration lower than the standard drug Albendazole when compared to black pepper extract. Hydro alcoholic extracts of ginger and black pepper was prepared in the form of polyherbal oral syrup met the standards of Indian Pharmacopoeia 1966, second edition. This polyherbal syrup may act as health tonic possess multiple of therapeutic benefits even when administered as preventive measure for various diseases. These formulations need to be further standardizing for accelerated stability studies as per prescribed standards.

**Keywords:** *Piper nigrum*, Polyherbal syrup, *Zingiber officinale*.

### INTRODUCTION

Recently, the World Health Organization estimated that about 70 – 80 % of people worldwide rely on herbal medicines for some part of their primary health care.<sup>1,2</sup> Ancient physicians including Hippocrates and Galen cemented the bricks for herbal medicine.<sup>3</sup> In western medical herbalism, traditional Chinese medicine and Ayurveda, combinations of herbs are fundamental to their philosophy as well as being due to empirical observations and historical usage. This attitude to their formulation and use differentiates herbal products from conventional medicines, even those which contain an isolated plant constituent. We have considerable pharmacological and clinical evidence to support synergistic pharmacological actions of phytomedicinals when they were used in combinations instead of single herb or isolated constituent.

The present work is carried out for emergence of antibiotic resistant bacterial strains and the gastrointestinal helminthes diseases which are a growing problem in nowadays. The present work is based on the development of herbal medicine made in the form of orally administrable herbal syrups. Even though there are several thousands of new drugs or new chemical entities which are discovered from natural sources, they may lack sufficient effort to be made in the dosage forms which are meant for easy administration.

#### Ginger

It consists of scraped or unscraped rhizomes of *Zingiber officinale* Roscoe belonging to the family of

Zingiberaceae. In India it has a long history of medicinal use, for conditions such as headaches, nausea, antiemetic,<sup>4,5</sup> anti-inflammatory and anti platelet aggregation properties are to potent inhibitory actions of the gingerols against prostaglandin synthetase, cholesterol biosynthesis inhibitory activity by HMG COA reductase inhibition,<sup>6</sup> rheumatism, cold, flu, stomachic, carminative, general stimulant, effective in motion sickness, antiulcer and strong antibacterial and antifungal activities.<sup>7</sup> It is commonly used as digestive aid and potential source of food antioxidant.<sup>8</sup> It also possesses positive ionotropic, spasmolytic actions and also used in sore throat and loss of voice benefited by chewing a piece of ginger.<sup>9</sup>

#### Black pepper

Pepper consists of dried unripe fruits of perennial climbing vine *Piper nigrum* Linn, belonging to the family of Piperaceae. It has been traditionally used in India to treat diarrhea. Black pepper possesses anti tumourigenic, immune stimulatory, stomachic, and carminative and anti cholestrolemic activities. It is also used in treating digestive disorders, gonorrhoea particularly to eradicate parasitic worms and as an appetite stimulant and to treat coughs, colds, breathing and heart problems.<sup>10</sup>

Oral solutions, syrups, elixirs etc., are prepared and used for specific effects of the medicinal agents present in the herbal drugs. Liquid forms of drugs have certain limitations but public demand or expectations are very high for such formulations. Moreover, some products are



more effective in a liquid form and are used commonly by children or the elderly to overcome problem of swallowing the solid dosage forms.<sup>11</sup> Designing of oral herbal formulations is a challenge till now in modern pharmaceuticals.

Ginger contains chemically unstable range of similar active compounds and probably acting synergistically, hence its use is preferred in the form of whole extract than the isolated individual compounds.<sup>12, 13</sup>

## MATERIALS AND METHODS

Ginger is commonly called as "sonti" purchased from the local spice market in required quantity (500gm) and dried ginger is coarsely powdered by us.

Black Pepper was purchased (500gm) from local market for spices and coarsely powdered in a mortar with pestle.

### Extraction of plant materials

The extraction of ginger and pepper for exhausting the crude drugs involves initial maceration of 250gms of individual powdered materials (sieve no. 20) with 700ml of alcohol and allowed to macerate for 7 days with occasional shaking. These alcoholic extracts subjected to filtration with the help of a muslin cloth and the marks were left over. The alcoholic extracts were subjected again to filtration to remove any pulpy matter before distillation and alcohol was separated from the extracts and the concentrated extracts were kept in refrigerator. To the marks left over were subjected to soxhlet extraction for 6 cycles with alcohol as menstrum. The soxhlet extracts were concentrated by distilling off the solvent and the concentrated extracts of 20ml were obtained. To the marks that were obtained in soxhlet extraction, water was added and boiled for 2 hours at temperature of 80° C. The water extracts and exhausted marks were separated by vacuum filtration and water extracts were concentrated to 20ml. All the 3 extracts of ginger and pepper that were obtained from maceration, soxhlet extraction and water extraction were mixed together separately. These were liquid in consistency, so the extracts were further concentrated with the help of a rectangular hot plate for 1 hour at 40° C. The semi liquid viscous extracts of ginger (17.5gms, 7.0 %) and black pepper (9.3gms, 3.7%) were obtained.

### Antibacterial activity of ginger and pepper extracts

#### Cup Plate Method<sup>14, 15</sup>

Standard drug

Vial of Ampicillin and Cloxacillin for injection, AMPOXIN-500, 500mg (manufactured by Unichem laboratories Pvt. Ltd.).

Test organisms

A total of seven bacteria were selected for this study.

### Gram positive bacteria

*Micrococcus luteus* MTCC 106

*Bacillus subtilis* MTCC 121

*Staphylococcus aureus* MTCC 3160

*Bacillus pumalis* MTCC 2808

### Gram negative bacteria

*Klebsiella pneumonia* MTCC 3384

*Escherichia coli* MTCC 443

*Proteus vulgaris* MTCC 426

### Method

#### Preparation of standard drug solution

Stock and sub stock solutions

The vial containing 500mg of sterile drug powder was reconstituted with 5ml sterile water for injection. The resulted solution was taken as stock solution (100mg/ml). From this stock solution, standard concentration (10µg/ml) was prepared by using sterile water for injection in a volumetric flask.

#### Preparation of test extracts solutions

The same procedure for both extracts of ginger and black pepper was followed.

Stock solution

100mg of accurately weighed extract was dissolved in 20% DMSO and made up to 10 ml in a volumetric flask. The resulted solution was taken as stock solution (10mg/ml).

Test concentrations

2.5mg/ml and 5mg/ml concentrations were prepared by diluting each of 2.5ml and 5ml of stock solutions to 10ml with 20% DMSO respectively.

#### Preparation of inoculum

Inoculum was prepared under aseptic conditions in the laminar air flow chamber from recently grown stock cultures of above test organisms. Each of the test organisms were subcultured on the surface of a 15ml volume of sterile nutrient agar medium by simple streaking method. The culture tubes were incubated at 37°C for 24 hours using sterile water. The bacterial strains were harvested and bacterial suspensions were prepared. These suspensions were used as inoculums.

#### Preparation of agar plates

Each of the test organisms were inoculated into 25 ml of the warm sterile nutrient agar medium under aseptic conditions in the laminar air flow chamber. The inoculated medium was immediately poured into sterile Petri dishes and uniformly spread over the plates and allowed to solidify.

### Procedure for Antibacterial activity

The Antibacterial activity of the crude extracts was investigated against 7 bacterial strains by the cup plate agar diffusion method. The inoculated solidified nutrient agar medium in petridishes was made in five wells (cups /holes) of uniform diameter (6mm) using sterile aluminium borer and then using micro pipette, 30 $\mu$ l of each of test extract concentrations, standard drug concentration and vehicle control (sterile 20 % DMSO) was added directly into the respective wells in the laminar

air flow chamber. The Petri dishes were carefully placed in refrigerator for 15min to allow the diffusion of the solution in the wells into the medium. All the Petri dishes were then incubated at 37 $\pm$ 1 $^{\circ}$ C for 24 hrs. The diameter of inhibition zone around each cup was measured in millimeters [Including the diameter of the well (6mm)]. The experiment was performed 3 times and the average values were represented in the table 1 and table 2 for ginger and black pepper extracts respectively.

**Table 1:** Antibacterial activity of ginger extract

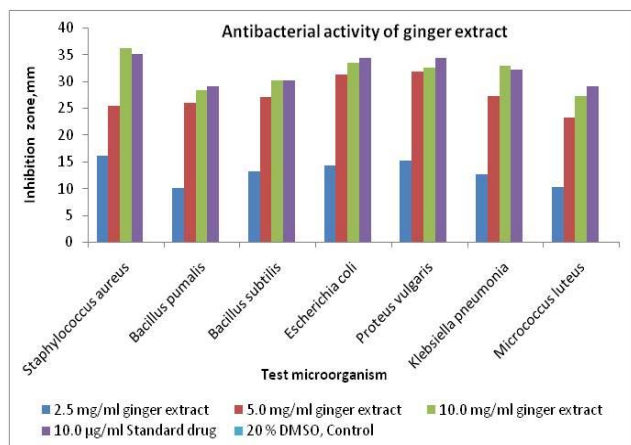
Bacterial strains used	Diameter of inhibition zone (mm)				
	Hydro alcoholic extract of ginger (mg/ml)				Standard drug 10 $\mu$ g/ml
	2.5	5.0	10.0	Control	
<i>Staphylococcus aureus</i>	16.12 $\pm$ 2.43	25.52 $\pm$ 4.21	36.28 $\pm$ 2.38	0.00	35.19 $\pm$ 1.14
<i>Bacillus pumalis</i>	10.14 $\pm$ 3.52	26.13 $\pm$ 2.85	28.52 $\pm$ 1.94	0.00	29.16 $\pm$ 2.84
<i>Bacillus subtilis</i>	13.18 $\pm$ 2.81	27.19 $\pm$ 3.47	30.28 $\pm$ 3.56	0.00	30.28 $\pm$ 3.91
<i>Escherichia coli</i>	14.34 $\pm$ 2.61	31.46 $\pm$ 2.18	33.56 $\pm$ 4.23	0.00	34.46 $\pm$ 2.95
<i>Proteus vulgaris</i>	15.32 $\pm$ 3.53	31.93 $\pm$ 2.53	32.62 $\pm$ 4.61	0.00	34.52 $\pm$ 3.43
<i>Klebsiella pneumonia</i>	12.67 $\pm$ 2.91	27.32 $\pm$ 2.83	32.96 $\pm$ 3.86	0.00	32.32 $\pm$ 2.73
<i>Micrococcus luteus</i>	10.35 $\pm$ 1.93	23.28 $\pm$ 3.42	27.38 $\pm$ 4.16	0.00	29.25 $\pm$ 4.13

All values were represented as the mean  $\pm$  standard deviation of three experiments

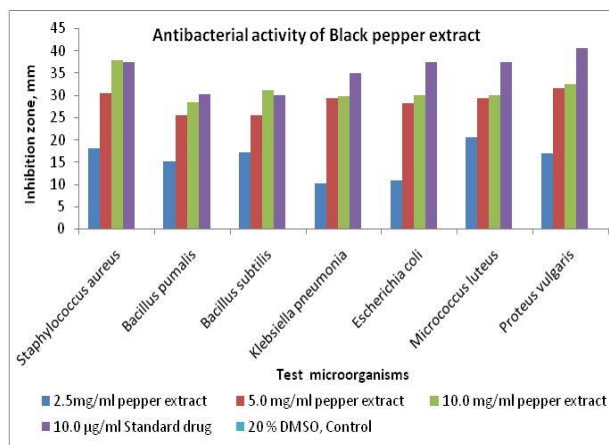
**Table 2:** Antibacterial activity of black pepper extract

Bacterial strains used	Diameter of inhibition zone (mm)				
	Hydro alcoholic extract of black pepper (mg/ml)				Standard drug 10 $\mu$ g/ml
	2.5	5.0	10.0	Control	
<i>Staphylococcus aureus</i>	18.23 $\pm$ 2.18	30.62 $\pm$ 3.43	37.96 $\pm$ 2.94	0	37.59 $\pm$ 2.76
<i>Bacillus pumalis</i>	15.35 $\pm$ 1.96	25.62 $\pm$ 4.16	28.68 $\pm$ 2.87	0	30.36 $\pm$ 2.83
<i>Bacillus subtilis</i>	17.32 $\pm$ 2.54	25.68 $\pm$ 3.81	31.37 $\pm$ 3.48	0	30.24 $\pm$ 3.12
<i>Klebsiella pneumonia</i>	10.36 $\pm$ 2.84	29.45 $\pm$ 4.15	29.82 $\pm$ 2.53	0	35.18 $\pm$ 2.18
<i>Escherichia coli</i>	10.92 $\pm$ 5.16	28.43 $\pm$ 2.93	30.26 $\pm$ 3.19	0	37.62 $\pm$ 1.52
<i>Micrococcus luteus</i>	20.64 $\pm$ 3.48	29.39 $\pm$ 2.57	30.16 $\pm$ 2.87	0	37.58 $\pm$ 2.41
<i>Proteus vulgaris</i>	17.12 $\pm$ 3.48	31.67 $\pm$ 3.49	32.67 $\pm$ 3.73	0	40.69 $\pm$ 1.97

All values were represented as the mean  $\pm$  standard deviation of three experiments



**Figure 1:** Graphical representation of antibacterial activity of ginger extract



**Figure 2:** Graphical representation of antibacterial activity of black pepper extract

**Anthelmintic activity of ginger and pepper extracts****Standard Drug**

Albendazole suspension (200mg/5ml, Manufactured by Ranbaxy laboratories ltd)

**Test Organisms**

Indian adult earthworms (*Pheretima posthuma*, Annelida, Megascoclecidae) collected from moist soil near to college campus and washed them with normal saline to remove soil matter and kept in normal saline. The earthworms 6-8cm in length and 0.2-0.3cm in width were selected for the study. *Pheretima posthuma* was used for present experiment because of their anatomical and physiological resemblance with intestinal roundworms present in the human body.<sup>16</sup>

**Method****Standard drug concentration (20mg/ml)**

5ml of suspension was withdrawn from Albendazole suspension (200mg/5ml, Manufactured by Ranbaxy laboratories ltd) and was diluted with 5ml of 20% DMSO was prepared in normal saline.

**Test extracts concentrations**

Same procedure was followed for both ginger and pepper extracts. 5mg/ml, 10mg/ml, 15mg/ml concentrations

were prepared by dissolving 50mg, 100mg, 150mg quantities of extracts in 10 ml volumetric flask separately with 20% DMSO was prepared in normal saline.

**Procedure for Anthelmintic Activity<sup>17, 18</sup>**

Anthelmintic activity of extracts was determined by exposing the adult *Pheretima posthuma* to different concentrations of test extracts and standard drug. 10ml of test extract concentrations and standard concentration were taken in separate Petri dishes. 20% DMSO was prepared in normal saline taken as control. Near equal sized earthworms were selected and each one was placed in all Petri dishes. All Petri dishes were kept at room temperature.

Observations and time taken to complete paralysis and death for individual worms were recorded. Each worm was frequently applied with external stimuli which stimulates and induces movement in earthworms, if alive. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor dipped in warm water (50°C). Experiment was carried out three times and average values were taken. The results of the Anthelmintic activity are given in the below table 3.

**Table 3:** Anthelmintic activity of ginger and pepper extracts

Substance Tested	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control (normal saline)	----	----	----
Albendazole suspension (standard)	20mg/ml	11.07±2.84	16.05±4.81
Test pepper Extract	5mg/ml	19.00±3.73	33.03±4.57
	10mg/ml	18.21±2.83	25.21±3.45
	15mg/ml	12.06±3.18	20.08±3.18
Test ginger Extract	5mg/ml	8.54±2.98	12.53±1.83
	10mg/ml	6.31±3.17	11.03±2.13
	15mg/ml	5.53±2.67	10.14±3.17

All values were represented as the mean± standard deviation of three experiments

**Preparation procedure for formulation 1 and formulation 2<sup>19, 20</sup>****Preparation of syrup**

66.7gms of sugar was dissolved in 70 ml of boiled distilled water with continuous stirring on a water bath at 80°C for few minutes. Final volume was adjusted to 100ml with boiled distilled water.

**Preparation of strong ginger tincture**

1.04 g of ginger extract was dissolved in 40 ml of 90 % v/v of alcohol. The resulting ginger tincture was showed weight/ml of 0.836 g.

**Preparation of strong pepper tincture**

0.62 g of black pepper extract was dissolved in 44 ml of 90 % v/v of alcohol. The resulting ginger tincture was showed weight/ml of 0.824 g.

**Preparation of ginger and pepper herbal syrups**

The procedure involved in the preparation of herbal syrups here was **co solvency** techniques because of extracts were composed of active constituents which belongs to oleoresins mainly.

**Formulation 1 (Ginger syrup)**

5ml of strong ginger tincture was added to 95ml of simple syrup solution gradually with continues stirring to made upto100ml.

**Wt per mL:** 1.302 g



**Formulation 2 (Black pepper syrup)**

5ml of strong pepper tincture was added to 95ml of simple syrup solution gradually with continues stirring to made up to 100ml.

Wt per mL: 1.286 g

**Formulation 3 (Polyherbal syrup)****Preparation procedure of polyherbal syrup**

2.5ml of strong ginger tincture was added to 47.5ml of simple syrup solution made up to 50ml (formulation 1). 2.5ml of strong black pepper tincture was added to 47.5ml of simple syrup solution made up to 50ml (formulation 2). Mixing of formulation 1 and formulation 2 we obtained the polyherbal formulation 3, possessing mixed medicinal uses of both the crude drugs, ginger and black pepper .

Wt per mL: 1.296 g



**Black pepper syrup**

(1.302 gm/ ml)

**Ginger syrup**

(1.286 gm / ml)

**Polyherbal syrup having ginger and Pepper** (1.296 gm / ml)

**RESULTS AND DISCUSSION**

From the results shown in table 1 and table 2 the antibacterial screening of extracts of ginger and pepper revealed that there was a significant antibacterial activity against almost all the tested bacterial strains. The maximum antibacterial activity of ginger and black pepper extracts was exhibited against *Staphylococcus aureus* when compared with standard drug at tested concentrations. The hydro alcoholic extract of black pepper was also exhibited in similar significant inhibition against remaining pathogenic organisms. The hydro alcoholic extract of ginger was also exhibited in similar significant inhibition against *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumonia*.

From the results shown in table 3, the predominant effect of Ginger extract on the worm is to cause a flaccid paralysis that result in expulsion of the worms by peristalsis. It was observed that both extracts showed a remarkable dose dependent on Anthelmintic activity against *Pheritima posthuma*. The Ginger extract exhibited the least time to cause paralysis and death of the worms than the standard drug Albendazole [20mg/ml] below the standard drug concentration. The observation of the result show that the Anthelmintic activity of ginger extract was more potent when compared to black pepper extract. The results obtained in the experimental study of Anthelmintic activity suggest that the ginger & pepper extract has beneficial Anthelmintic effect against the Indian earth worm, *Pheritima posthuma*.

**CONCLUSION**

In the pre formulation studies of hydro alcoholic extracts of these two herbs were prepared by maceration, soxhlation and decoction for exhausting the plant materials showed promising results of antibacterial and Anthelmintic activities. Hydro alcoholic extracts of ginger and black pepper was prepared in the form of polyherbal oral syrup would meet the standards of Indian Pharmacopoeia 1966, second edition, 1966 and these two herbal drugs possess multiple of therapeutically active agents which are combined for greater therapeutic outcomes in the polyherbal syrup formulation. Moreover the combination of ginger extract with black pepper extract in the polyherbal syrup results in the enhancement of bioavailability due to presence of alkaloid piperine is a potent inhibitor of drug metabolism.<sup>21</sup> This novel polyherbal syrup may act as health tonic possess multiple of therapeutic benefits even when administered as preventive measure for various diseases. These formulations need further standardize for accelerated stability studies as per prescribed standards.

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