Research Article

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# FORMULATION AND EVALUATION OF ANTHELMINTIC HERBAL FORMULATIONS

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**ABSTRACT**

The current investigation was carried out with the aim to evaluate the anthelmintic activity of an herbal formulation. The ethanolic extracts of the resin of *Ferula asafoetida* (Apiaceae), flower buds of *Eugenia caryophyllus* (Myrtaceae), bulb of *Allium sativum* (Liliaceae) and leaves of *Azadirachta indica* (Meliaceae) were screened for anthelmintic activity using the adult earthworm *Pheritima posthuma*. The herbs were coarsely powdered and extracted in ethanol. These extracts were first screened for their anthelmintic activity and then best two of these were selected for formulating herbal syrup which was again evaluated for its anthelmintic potential. The anthelmintic activities of all the four plants were time dependent. Finally, the best two herbs were selected and syrup was made using them, i.e., *Ferula asafoetida* (Apiaceae) and *Eugenia caryophyllus* (Myrtaceae). Again, the anthelmintic activity of this syrup was evaluated, which was also time dependent and caused the paralysis in 9 min & 50 s but did not cause death of the earthworms. Albendazole suspension was used as standard during both the screening and evaluation. The herbal extracts of *Ferula asafoetida* (Apiaceae) and *Eugenia caryophyllus* (Myrtaceae) were as effective as the standard Albendazole suspension while the herbal syrup prepared from these extracts was found not to be as effective as the standard. The herbal syrup can be further explored for its Phytochemistry and its possible mechanism of action.

**Keywords:** *Ferula asafoetida, Eugenia caryophyllus, Allium sativum, Azadirachta indica, Pheritima posthuma,* Anthelmintic activity

## INTRODUCTION

Anthelmintics are a group of antiparasitic drugs that expel parasitic worms (helminths) and other internal parasites from the body. They are used to treat people who are infected by helminths, a condition called helminthiasis. These drugs are also used to treat infected animals. The anthelmintics expel the worms from the host body by either stunning their growth or killing them, without causing significant damage to the host. The anthelmintics can also be called as vermifuges (those that stun) or vermicides (those that kill).

With the use of the synthetic anthelmintics come various side effects. The commonly used synthetic anthelmintics are Mebendazole, Flubendazole, Piperazine, Praziquantel, Pyrantel, etc. The common side effects associated with these synthetic anthelmintics are - abdominal pain, headache, dizziness, vomiting, nausea, diarrhoea & drowsiness. Other effects observed with the use of specific anthelmintic are urticaria (Piperazine), rashes (Piperazine), malaise (Praziquantel), anorexia (Pyrantel), etc.

Since the origin of human civilization, the plants have been a source of food, clothing, shelter and medicine. Primitive men learnt the utilization of several plants as medicine by trial and error method.

The ancient science of healing mentioned the uses of plants for curing several human diseases. Throughout the world newer researches are being carried out to explore the medicinal benefits of the plants like antidiabetic, anti-inflammatory, hepatoprotective, immune booster, antacid, anthelmintic etc.

Hence, anthelmintics from the natural sources may play a key role in the treatment of these parasitic infections. Because of fewer side effects, the importance of herbal drugs as a remedy has tremendously increased in the recent years. Owing to its natural origin and lesser side effects, it is getting popularized in developing and developed countries. In many developing countries, a large portion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Consequently, the need for the herbal formulation has been felt in routine life. There are currently an increasing number of controlled experimental studies that aim to verify, validate and quantify in a scientific manner such plant activity. Numerous herbs like *Azadirachta indica* (Meliaceae), *Allium sativum* (Liliaceae), *Chenopodium album* (Amaranthaceae), *Cucurbita pepo* (Cucurbitaceae), *Capsicum annum* (Solanaceae), *Curcuma longa* (Zingiberaceae), *Mentha piperita* (Lamiaceae), *Saraca asoca* (Fabaceae), *Ferula asafoetida* (Apiaceae) and *Eugenia caryophyllus* (Myrtaceae) are used for their anthelmintic activity.[1]

*Ferula asafoetida* (Umbelliferae) also known as Asafoetida, Devil's dung in English and Heeng in Hindi is found in Eastern Iran and Western Afghanistan. Its phytochemical composition consists of 45-65% resins, 20% gum, 4-15% volatile oil and about 10% ash. Its alliaceous odour is due to presence of some sulphur compounds like isobutyl propenyldisulphide. It is used medicinally as carminative, expectorant, antispasmodic and laxative.[2]

*Eugenia caryophyllus* (Myrtaceae) also known as Caryophylli, Clove buds, Clove flower in English and Laung in Hindi is a native of Molucca Islands (present in Indonesia) and is cultivated mainly in Island of Zanzibar, Pemba, Amboiana and Sumatra. It

contains 16-21% of volatile oil, a phenolic compound, i.e., eugenol, comprising of 72-90% of the total volatile oil content, 10-15% acetyl eugenol. Also contains α and β –Caryophyllene. It contains 10-13% tannins as pyrogallol tannins. It is used as an antiseptic, stimulant and carminative**.** Clove oil is used in toothache as a local anesthetic.[3]

*Allium sativum* also known as Garlic in English and Lehsun in Hindi is cultivated in India, Russia, USA, Italy and Southern Europe. It contains essential oil - alliin, sulphur containing amino acid, allicin- allylsulphide and a polysulphide responsible for the unpleasant smell of the oil. Also contains amino acids: leucine, methionine, S-methyl cysteine and S-allyl cysteine, allyl propyl-adisulphide. It is used as analgesic, stimulant, anticonvulsant, antibacterial, aphrodisiac and oil is used as an insecticide.[4]

*Azadirachta indica* (Meliaceae) also known as Neem, Nimb or Nimba is found in India, Pakistan, Sri Lanka, Malaya, Indonesia, Japan, Tropical region of Australia and Africa**.** Numerous phytoconstituents like nimbin, nimbidin, nimbidinin, 6-desacetylnimbinene, nimbandiol, nimbinene, nimbolide, ascorbic acid, quercetin, β-sitosterol, n-hexacosanol, nonacosane etc. have been isolated from its leaves. Medicinally, the leaves have been used as antiviral, antifungal, in jaundice, in worm infections and in skin diseases.[5]

## MATERIALS AND METHODS

### Collection of Herbs

Latex from roots of *F. asafetida*, dried flower buds of *E. caryophyllus*, bulbs of *A. sativum* were procured from local market of Ghaziabad and leaves of *A. indica* were collected from the medicinal garden of KIET School of Pharmacy, Ghaziabad.

### Extraction

2g of each of the plant materials, i.e. Root latex of *F. asafetida*, flower buds of *E. caryophyllus*, bulbs of *A. sativum* and leaves of *A. indica* were collected and properly dried. All these were then coarsely powdered and then set for extraction in 90% alcohol by placing the drug in closed vessels of 2l. The vessels were allowed to stand for seven days shaking occasionally. The liquid was strained off. The marc was pressed to recover the occluded solution. The strained and occluded solutions were mixed and clarified by filtration. The solution was distilled and concentrated. The extraction process led to 0.0531g (2.655%) of dried light yellowish extract of *F. asafetida,* 0.081g (4.05%) of dried dark reddish-brown extract of *E. caryophyllus,* 0.0352g (1.76%) of dried creamish-yellow extract of *A. sativum and* 0.22g (11%) of dried greenish extract of *A. indica.*

### Screening for Anthelmintic Activity

Adult earthworms (*Pheretima posthuma)* were collected (due to their anatomical and physiological resemblance with the intestinal round worm parasites of human beings) from the local nursery, Muradnagar, Ghaziabad. Earth worms were thoroughly washed with normal saline to remove the adhering material. Six petridishes of equal size were collected, washed and 10ml of normal saline alone was poured in the first petridish. Then, 10 ml of test solutions, that is the ethanolic extracts of dried root latex powder of *F. assafoetida* (4.88 mg/ml), dried flower bud powder of *E. caryophyllus* (2.95 mg/ml), dried bulb powder of *A. sativum* (3.48 mg/ml) and dried leaf powder of *A.indica* (1.07 mg/ml) were placed in the second, third, fourth and fifth petridish, respectively. In sixth petridish, a marketed synthetic preparation, i.e., Albendazole having anthelmintic activity was taken as standard. Then in each petridish, 2 earthworms, of nearly equal size, were placed and time taken to induce paralysis (no visible motion) and/or complete death of earthworms was noted. The confirmation of death of the earthworms was done by slowly pricking pin to their bodies. The results obtained from the test drugs i.e., herbal extracts and that from the market preparation were compared. According to the comparison of the results so obtained, best two herbs were selected and a preparation, using these two herbs, was made, having the optimum anthelmintic activity.[6]

### Formulation of Syrup

*F. assafoetida and E. caryophyllus* were utilized for formulating the simple syrup IP, i.e., 66.67% (w/v) by following the process as given in IP. 10 ml of this simple syrup was prepared. 1-2 ml distilled water was taken in a beaker and 6.667g sugar was added in it. The mixture was heated to dissolve the sugar completely by continuous stirring. This resulted in a super saturated solution of sugar in water. After the sugar had dissolved completely, the extracts were added into the solution, in the amounts as given in table 1. The extracts were added in hot state of the sugar solution and dissolved completely by proper stirring. After the complete dissolution of the extracts, the volume of the syrup was made up to 10ml by adding required amount of distilled water. Finally, the syrup was prepared. The formulation composition of the syrup is given in Table 1.

### Standardization of Herbal Syrup

The standardization of the liquid herbal formulation, i.e., the syrup, was done on the following basis

**(i) Organoleptic Properties**: The taste, colour and odour of the syrup were immediately determined after preparation.[7]

**(ii) pH:** The pH of the syrup was checked by using the pH meter.[8]

**(iii)Viscosity**: The viscosity of the syrup was determined by Brookfield's viscometer.[9]

**(iv)Determination of Crystal Growth**: The crystal growth was determined after 24 hr.[10]

**(v) Thin Layer Chromatography:** The TLC plates were prepared by using Silica gel G slurry. This acted as the stationary phase and the solvent system (Ethanol: Water: Chloroform in the ratio of 7:3:1) was the mobile phase. After the preparation of the plates and the solvent system, the plates were left for air drying and the solvent system was left for saturation. After the plates had dried they were activated in oven at 105℃ for 10-15 min. Then the spot of syrup sample was applied with the help of a capillary at a distance of about 1 cm above the bottom of the plates. The plates were kept in the solvent system after the sample spot dried. The solvent was allowed to run for about 75% of the stationary phase. Then two separate spots of the constituents of the syrup, i.e., asafoetida and clove, were obtained on the plate. The $R_f$ of these spots was calculated by using the formula:

$$R_f = \frac{\text{Distance travelled by the Solute}}{\text{Distance travelled by the Solvent}}$$

### Accelerated Stability Studies

The formulated anthelmintic herbal syrup was stored at 50 °C ± 2 °C and 75 ± 5 % RH for a period of three months. [11] The samples

were withdrawn after a period of 30, 60 and 90 days and analyzed for its colour, taste, odour, pH, viscosity, crystal growth and TLC.

**Screening for Anthelmintic Activity of Herbal Syrup**

Earth worms were thoroughly washed with normal saline to remove the adhering material. Three petridishes of equal size were collected, washed and 10ml of normal saline alone was poured in the first petridish. Then, 10 ml of herbal syrup, and 10ml of marketed synthetic preparation, i.e., Albendazole having anthelmintic activity were placed respectively in second and third petridishes. Then in each petridish, 2 earthworms, of nearly equal size, were placed and time taken to induce paralysis (no visible motion) and/or complete death of earthworms was noted. The confirmation of death of the earthworms was done by slowly pricking pin to their bodies. The results obtained were compared.[6]

## RESULTS AND DISCUSSIONS

**Extraction**

The yields of the extractions are mentioned in Table 2.

**Screening for Anthelmintic Activity of Herbal Extracts**

The individual extracts were subjected to anthelmintic activity. The extract of *Ferula asafoetida* (0.00488g/ml) caused the death of the earthworms within 1min 42 secs, while *Eugenia caryophyllus* extract (0.00295g/ml) induced paralysis after 10 mins and death after 14 mins 36 secs, *Allium sativum* (0.00348g/ml) and *Azadirachta indica* (0.0107g/ml) did not cause any significant death but produced paralysis only after 5 min 15 s and 1 min 58 secs respectively. The results were compared with the standard anthelmintic drug Albendazole (0.4g/ml) which induced paralysis after 1 min. 25 secs. and death after - 4 min 36 s. Thus, best anthelmintic effect was shown by the extract of *Ferula asafoetida* followed by *Eugenia caryophyllus* which were then utilized for preparing the anthelimintic herbal syrup. All these results are compared in Table 3.

**Standardization and Stability Studies of Liquid Herbal Formulation (Syrup)**

The standardization and stability study parameters for colour, odour, taste, pH, viscosity, crystal growth and $R_f$ values for the formulated herbal anthelmintic syrup are mentioned in Table 4. It was observed that all the parameters were almost same throughout the study thus helping us to conclude that the syrup was stable and on ageing also there were no physical and chemical changes.

**Table 1: Composition of Syrup**

| Ingredients | Quantity (g) |
|---|---|
| Sugar | 6.667 |
| Asafoetida | 0.0012 |
| Clove | 0.0007 |
| Distilled water | Q.S up to 10ml |

**Table 2: % Yield of extracts obtained the test herbs**

| Plant | Part taken | Amount of powder taken (g) | Amount of extract obtained (g) | Yield (%) |
|---|---|---|---|---|
| *Ferula asafoetida* | Resins | 2 | 0.0531 | 2.655 |
| *Eugenia caryophyllus* | Flower buds | 2 | 0.081 | 4.05 |
| *Allium sativum* | Bulbs | 2 | 0.0352 | 1.76 |
| *Azadirachta indica* | Leaves | 2 | 0.22 | 11 |

**Table 3: Observations of Screening of Anthelmintic Activity of herbal extracts**

| Drug | Amount Taken (g/ml) | Time taken to induce Paralysis or cause Death |
|---|---|---|
| *Ferula asafoetida* | 0.00488 | Death-1 min. 42 s. |
| *Eugenia caryophyllus* | 0.00295 | Paralysis- 10 min<br>Death- 14 min. 36 s. |
| *Allium sativum* | 0.00348 | Paralysis- 5 min. 15 s.<br>Death- no significant death is caused |
| *Azadirachta indica* | 0.0107 | Paralysis- 1 min. 58 s.<br>Death- no significant death is caused |
| Albendazole | 0.4 | Paralysis- 1 min. 25 s.<br>Death- 4 min. 36 s. |

**Table 4: Standardization Results of Liquid Herbal Formulation (Syrup)**

| Parameters | Day 0 | Day 30 | Day 60 | Day 90 |
|---|---|---|---|---|
| **Colour** | Yellowish Brown | Yellowish Brown | Yellowish Brown | Yellowish Brown |
| **Odour** | Characteristic | Characteristic | Characteristic | Characteristic |
| **Taste** | Bitter | Bitter | Bitter | Bitter |
| **pH** | 7 | 7 | 7 | 7 |
| **Viscosity** | 1210 cps | 1210 cps | 1190 cps | 1190 cps |
| **Crystal Growth** | None | None | None | None |
| **$R_f$ values** | 0.355<br>0.256<br>0.210<br>0.125<br>0.102 | 0.355<br>0.256<br>0.210<br>0.125<br>0.102 | 0.355<br>0.256<br>0.210<br>0.125<br>0.102 | 0.355<br>0.256<br>0.210<br>0.125<br>0.102 |

**Table 5: Observation of the Activity performed with the Herbal Formulation**

| Formulation | Time Taken to induce Paralysis or Cause Death |
|---|---|
| Formulated herbal Syrup (Containing Asafoetida and Clove) | Paralysis- 9 min. 50 s. Death- no significant death is caused |
| Suspension (Containing Albendazole) | Paralysis- 1 min. 25 s. Death- 4 min. 36 s. |

**Anthelmintic Activity of Formulated Syrup**

The formulated herbal syrup containing Asafoetida: 0.0012 g/ml and Clove: 0.0007 g/ exhibited the paralytic effects over the earthworms within 9 min 50 sec but did not result into any significant death while the marketed suspension containing albendazole caused paralysis within 1 min 36 sec and death after 4 min and 36 sec. These results are compiled in Table 5.

## DISCUSSION

The herbal extracts of *Ferula asafoetida* (Apiaceae) and *Eugenia caryophyllus* (Myrtaceae) were as effective as the standard Albendazole suspension in a very low concentration while the herbal syrup prepared from these extracts was found not to be as effective as the standard. The resins of *Ferula asafoetida* and volatile oil composition of *Eugenia caryophyllus* are responsible for the anthelmintic potential of the herbal syrup.

Many mechanisms for action of the anthelmintics have been described. As the samples resulted into the paralysis of the worms, they might have produced a rapid and selective action on their neuromuscular transmission. They might be acting as nicotinic acetylcholine receptor agonist causing spastic paralysis or as GABA agonist causing flaccid paralysis.[12]

The current research investigation provides a golden opportunity for the budding herbal researchers to explore the formulation for its Phytochemistry and its mechanism of action.

## CONCLUSION

*Ferula asafoetida* (Apiaceae) and *Eugenia caryophyllus* (Myrtaceae) have proved to be effective natural remedy for helminthiasis. The herbal syrup prepared from combination of these two herbs was also effective but lesser than the standard Albendazole. As these have exhibited potency in a very low concentration so they provide a safer, effective and easily available anthelmintic remedy.

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