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FORMULATION AND EVALUATION OF HERBAL COUGH SYRUP OF ECHINOPS ECHINATUS ROXB ROOTS

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Abstract:

The objective of the present study was to formulate and evaluate antitussive herbal cough syrup because designing of oral herbal formulation is still a challenge in modern pharmaceutics and the syrup formulation presents many technical problems to the industrial pharmacist. Potential Antitussive herb was used for developing herbal syrup. Aqueous extract of roots of *Echinops Echinatus* Roxb was used in the formulations. Syrup containing Mulethi extract (*Glycyrrhiza glabra* LINN.), Lindi pepper extract (*Piper longum*), Haldi (*Curcuma longa* Linn.), Menthol (*Mentha piperita*), Clove oil (*Eugenia caryophyllus*), Ajwan oil (*Trachyspermum ammi*), Ginger oil (*Zingiber officinale*) which was prepared by Solution with heat method. The formulations were subject to several evaluation parameters like Density, Viscosity, Specific Gravity, P^H, Microbial Contamination, Acute Toxicity study etc. Syrup was also subjected for the accelerated stability for the period of three months at accelerated temperature conditions. No marked changes were noticed in all the evaluated parameters during three months of accelerated stability study. The laboratory scale preparation of herbal Syrup may be used as a stable, liquid dosage form and the work done in stability testing may help in the progress of shelf-life determination studies.

Keywords: Antitussive, herbal formulation, Syrup, *Echinops Echinatus* Roxb.

Introduction ^[1-14]

The oral use of liquid pharmaceuticals has generally been justified on the basis of ease of administration to those individuals who have difficulty in swallowing solid dosage forms. With rare exceptions, a drug must be in solution in order to be absorbed. A drug administered in solution is immediately available for absorption, and in most cases, is more

rapidly and efficiently absorbed than the same amount of drug administered in a tablet or capsule. Designing of oral herbal formulations (solutions) is a challenge in modern pharmaceuticals till date. However the final preparation must satisfy the requirements of pharmaceutical elegance with regard to taste, appearance and viscosity. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations. The protection of knowledge, innovations and practices of traditional and indigenous medicine has been in the forefront of international developments.

Formulation for a combination of herbal medicine in new dosage form with higher therapeutic efficacy are patent eligible subject matter. Further, new indications, extraction and separation of active ingredients from herbal drugs used in the production of pharmaceutical substances, preparation and processing techniques of standardized extracts are also patentable. A wide range of products such as herbal preparations, homeopathic remedies, and nutritional supplement of natural origin constitutes complementary medicine.

Herbal products contain aerial or underground parts of plants or other plant materials or combinations of these. Health professionals generally face the problem of assessing the effectiveness and safety of natural remedies. Some of these products have not been as extensively studied as allopathic drugs and are usually sold without much knowledge of their mechanisms of action or side effects. Though the use of complementary medicines is sometimes beneficial and the evidence for the efficacy of some of these medicines in literature is limited, they are often sold with allopathic drugs in medical shops.

A cough is a sudden and often repetitively occurring reflex which helps to clear the large breathing passages from secretions, irritants, foreign particles and microbes. The cough reflex consists of three phases: an inhalation, a forced exhalation against a closed glottis, and a violent release of air from the lungs following opening of the glottis, usually accompanied by a distinctive sound.

Coughing is either voluntary or involuntary. Frequent coughing usually indicates the presence of a disease. Many viruses and bacteria benefit evolutionarily by causing the host to cough, which helps to spread the disease to new hosts. Most of the time, irregular coughing is caused by a respiratory tract infection but can also be triggered by choking, smoking, air pollution, asthma, gastroesophageal reflux disease, post-nasal drip, chronic bronchitis, lung tumours, heart failure and medications such as ACE (Angiotensin Converting Enzyme) inhibitors.

Advantages of Herbal Medicine:

Cost:

Herbs cost much less than prescription medications. Research, testing, and marketing add considerably to the cost of prescription medicines. Herbs tend to be inexpensive compared to drugs.

Availability: Because the FDA tightly regulates the pharmaceutical industry, there are simply far more herbal medicine products available for consumption. These products are also available without prescription.

Safety:

Herbal medicines tend to derive themselves from relatively harmless plant matter that the human body can easily digest. Prescription drugs, on the other hand, are composed of a great variety of products that supplement the lead compound.

Usually herbs grow in very common places, but they do not require complicated preparation procedures and are also easily available.

Natural:

The lead compound of pharmaceutical drugs tend to be natural, they are often times mixed with synthetic and artificial variables which can cause adverse side effects.

Disadvantages of Herbal Medicines:

Lack of Regulation:

Though herbal medicines contain an inherent positive due to their lack of regulation, they can, at the same time, occasionally cause issues.

Patience Needed:

They have not been optimized in a laboratory for effectiveness, and, due to this naturalness, it takes time to implement effectively.

Adverse Interaction with Prescription Drugs:

If you are taking prescription drugs on a regular basis, such as antidepressants, herbal medicines can mix with these chemicals to create adverse effects. Another disadvantage of herbal medicine is the very real risks of doing yourself harm through self-dosing with herbs.

While you can argue that the same thing can happen with medications, such as accidentally overdosing on cold remedies, many herbs do not come with instructions or package inserts. There's a very real risk of overdose. Many herbal remedies have negative side effects.

Most of these side effects take months to appear. Since governments do not regulate the industry of herbal medicine, there is no chance of any quality control during the manufacturing of the herbal supplements.

Materials and Methods:

Table 1 List of Material.

Sr.No.	Material	Source
1.	Brahmdandi Extract (Brahmdandi plant)	Sangola Area
2.	Lindi Pepper Extract(Lindi pepper)	Local Sangola Market
3.	Ginger Oil(Ginger)	Local Sangola Market
4.	Mulethi Extract(Mulethi)	Local Sangola Market
5.	Menthol(Mentha)	Local Sangola Market
6.	Ajwan oil(Ajwan)	Local Sangola Market
7.	Clove oil(Clove)	Local Sangola Market
8.	Haldi Extract(Haldi)	Local Sangola Market
9.	Flavoured Syrup(Sugar)	Local Sangola Market

Formulation Development:

Herbal cough syrup of *Echinopsechinatus* Roxb. was prepared for F1 to F5 batches by using different quantities of excipients mentioned in the Table 5.1.keeping the total volume (100 ml) of the syrup constant in all the formulations.

Step 1.Preparation of simple syrup:

The simple sugar syrup was prepared by using Solution with heat method. In this method the sugar syrup having the concentration 66.67% (as per I.P.) was prepared.

Step 2.Preparation of solution of extracts:

The powdered extracts i.e. Brahmdandi extract, Mulethiextract, Lindi Pepper extract and Haldi were dissolved in the purified water to prepare a uniform solution.

Step 3.Preparation of oil mixture:

Menthol is dissolved into oils i.e. Ginger oil, Ajwan oil, Clove oil, with continuous stirring to obtain homogeneous mixture of oils.

Step 4.Preparation of final syrup:

The solution of extracts and oil mixture was added into the sugar syrup by simple stirring to obtain uniform and consistent syrup. The final volume was adjusted by using simple sugar syrup.

❖ Composition of *Echinopsechinatus* Roxb. Herbal Cough Syrup:

Table-2: Composition of Batches of Syrup.

Sr.No.	Ingredients	F1	F2	F3	F4	F5
1.	Brahmdandi powder	13.6 gm	13.6 gm	13.6 gm	13.6 gm	13.6 gm
2.	Lindi Pepper extract	1 gm	0.5 gm	0.5 gm	0.1 gm	0.1 gm
3.	Ginger oil	1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
4.	Mulethi extract	2 gm	2 gm	2 gm	2 gm	2 gm
5.	Menthol	1 gm	0.75 gm	0.5 gm	0.1 gm	0.1 gm
6.	Ajawan oil	1 ml	0.5 ml	0.1 ml	0.1 ml	0.1 ml
7.	Clove oil	1 ml	0.75 ml	0.5 ml	0.5 ml	0.1 ml
8.	Haldi	4 gm	3 gm	2 gm	1 gm	1 gm
9.	Sugar Syrup	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
10.	Total volume	100 ml	100 ml	100 ml	100 ml	100 ml

In this formulation from Batch no.F1 to F5 the quantity of Lindi pepper extract and Menthol was decreased from 1gm to 0.1 gm .The quantity of Ginger oil ,Ajawan oil, and Clove oil was decreased from 1ml to 0.1 ml. The quantity of Mulethi extract (2 gm) was kept constant for all 5 batches. The quantity of Haldi was decreased from 4 gm to 1 gm.



Fig. 1. Formulated Batches Cough Syrup.

5.4. Experimental Data:

5.4.1. Plant collection and authentication:

The root of *Echinopsechinatus* Roxb. was collected from the Sangola region of Solapur District, Maharashtra, India.

The plant was identified and authenticated by Botanical Survey of India, Pune.

5.4.2. Preparation of extracts:

There are four extracts are used in the formulation. These extracts were prepared by Maceration method. The prepared extracts involves following

- Brahmdandi extract
- Lindi pepper extract
- Liquorice extract
- Haldi extract

• **Maceration:**^[15-19]

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved.

The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing. The maceration is performed at room temperature and liquids that are most frequently used are water and alcohol or both combination. In this formulation the extracts were prepared by using water only. The maceration in water should not be prolonged for too long as this can present fungal contamination, which does occur in alcohol or hydroalcoholic solutions. So the period of maceration is reduced to 1 day in this formulation as per the requirement of plants.

Total maceration time depends on the type of plant or the part thereof or the active ingredient to extract. The most commonly used ratio is 1:20(Herb/Liquid).

Preparation of Oils:^[20]

There are three oils are used in the formulation. These oils were prepared by using cleengers apparatus i.e. distillation method. The prepared oils are

- Clove oil
- Ajwan oil

- Ginger oil

✓ **Extraction of oil by clavengers distillation:**

The specific amount of drug to be extracted were placed into the necked round extraction flask and soaked with water. Water and drugs were mixed and allowed to boil. Water and extracted oil evaporate.

The vapour mixture condensed using reflux condenser. From condenser distillate water and oil flow into gradated tube.

As the oil is not miscible with water it may be easily separated. The oil was allowed to stand for sufficient time to be clear and then it was collected and stored.



Fig.2 Clevengers apparatus



Fig.3 Apparatus showing oil layer

Preformulation Studies:

Preformulation studies were performed on the drug, which included:

- ✓ Determination of total ash.
- ✓ Determination of acid insoluble ash.
- ✓ Determination of water soluble ash.
- ✓ Determination of microbial load.
- ✓ Water soluble extractive:
- ✓ Moisture content
- ✓ GLC study of oils
- ✓ Accute toxicity study

Determination of total ash:^[27]

Incinerated about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450°C until free from carbon, cooled and weighed. A carbon free ash not obtained in this way so exhausted the charred mass with hot water and collected the residue on an ashless filter paper, incinerated the residue and filter paper, added the filtrate, evaporated to dryness, and ignited at a temperature not exceeding 450°C. Calculated the percentage of ash with reference to the air-dried drug.

$$\frac{100}{Y} \times (Z - X)$$

Where,

X=Weight of empty dish

Y=Weight of sample

Z=Weight of ash +dish

Determination of acid insoluble ash:^[27]

Boiled the ash obtained in total ash for 5 minutes with 25 ml of dilute hydrochloric acid; collected the insoluble matter in a Gooch crucible, washed with hot water and ignited to constant weight. Calculated the percentage of acid-insoluble ash with reference to the air dried drug.

Determination of water soluble ash:^[27]

Boiled the ash for 5 minutes with 25 ml of water; collected insoluble matter in a Gooch crucible, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°C. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash. Calculated the percentage of water-soluble ash with reference to the air-dried drug.

Determination of microbial load:-^[21]

- Labeled the bottom of six petri plates 1-6. Labeled four tubes of saline 10^{-2} , 10^{-4} , 10^{-6} , and 10^{-8} .
- Using aseptic technique, the initial dilution is made by transferring 1 ml of E.Coli sample to a 99ml sterile saline blank. This is a 1/100 or 10^{-2} dilution.
- Immediately after the 10^{-2} dilution has been shaken, uncapped it and aseptically transferred 1ml to a second 99ml saline blank. Since this is a 10^{-2} dilution, this second blank represents a 10^{-4} dilution of the original sample.

- Shaked the 10^{-4} dilution vigorously and transfered 1ml to the third 99ml blank. This third dilution represents a 10^{-6} dilution of the original sample. Repeated the process once more to produce a 10^{-8} dilution.
- Shaked the 10^{-4} dilution again and aseptically transfered 1.0 ml to one petri plate and 0.1 ml to another petri plate. Done the same for the 10^{-6} and the 10^{-8} dilutions.
- Removed one agar pour tube from the 48°C to 50°C water bath. Carefully remove the cover from the 10^{-4} petri plate and aseptically pour the agar into it. The agar and sample are immediately mixed gently moving the plate in a figure-eight motion or a circular motion while it rests on the tabletop. Repeat this process for the remaining five plates.
- After the pour plates have cooled and the agar has hardened, they are inverted and incubated at 25°C for 48 hours or 37°C for 24 hours.
- At the end of the incubation period, select all of the petri plates containing between 30 and 300 colonies. Plates with more than 300 colonies cannot be counted and are designated too many to count (TMTTC). Plates with fewer than 30 colonies are designated too few to count (TFTC). Count the colonies on each plate. A Quebec colony counter should be used.
- Calculate the number of bacteria (CFU) per milliliter or gram of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar.

$$\text{No. of Bacteria per ml} = \frac{\text{No. of colonies (CFU)}}{\text{Dilution} \times \text{Amount plated}}$$

- Recorded the results.

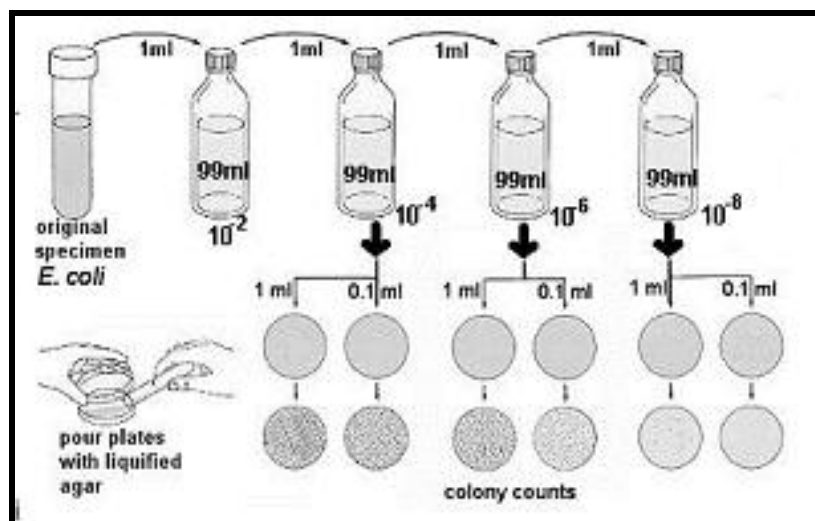


Fig 4. Experimentation of Microbial load test.

Water soluble extractive:

About 5 grams of the powder is macerated with 100 ml of distilled water in a closed flask for 24 hrs. Shake frequently during 6 hrs and allow the same for standing for 18 hrs. It is filtered rapidly and 25 ml of the filtrate is evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive is calculated with reference to the air dried powder.

Moisture content:

Moisture is an inevitable component of the formulation. The presence of moisture results in the active growth of common moulds and bacteria.

Procedure:

Weigh about 1 gm of the powdered crude drug in to a weighed flat and thin porcelain dish. Dry in the oven at 100°C-105°C for half an hour. Cool and weigh the contents. Keep back the contents in oven and repeat the drying at 100°C-105°C for half an hour. Cool the contents and check its weight. Repeated the process to get the concordant value and same process was done for marketed sample also.

$$\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

GLC study of oils:-

Gas chromatographic analysis of absolute oils were carried out at the Nirmiti Labs Pvt.ltd. Mumbai. The percentage of active constituents present in the oils were estimated by using GLC (Gas Liquid Chromatography).



Fig. 5 Oil samples Used for GLC Study.

Post formulation Evaluation Parameters:-^[28-31]

The herbal syrup was evaluated for various physicochemical parameters such as physical appearance (colour, odour, and taste), pH, Density, Viscosity and Specific Gravity.

- ✓ Colour
- ✓ Odour
- ✓ Taste
- ✓ Density
- ✓ Specific Gravity
- ✓ Viscosity
- ✓ pH
- ✓ Microbial Contamination

Bacterial count

- ✓ Stability Study

Color examination:

5 ml final syrup was taken into watch glasses and placed against white back ground in white tube light. It was observed for its color by naked eye.

Odor examination:

2 ml of final syrup was smelled individually. The time interval among 2 smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste examination:

A pinch of final syrup was taken and examined for its taste on taste buds of the tongue.

Determination of pH:

Placed an accurately measured amount 10 ml of the final syrup in a 100 ml volumetric flask and made up the volume up to 100 ml with distilled water. The solution was sonicated for about 10 minutes. pH was measured with the help of digital pH meter.

Determination of Density:

Density can be determined with the help of density bottle (25ml). The density bottle (25ml capacity) was taken and weight of empty bottle was noted. The liquid whose density will be determined is filled in bottle and weighed. Then

both the weight of syrup is calculated by subtract the weight of empty bottle from the filled bottle. Then the density of syrup is calculated by using the formula

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}}$$

Determination of Viscosity:

The viscosity of each formulation was determined by using the Ostwald's U-tube Viscometer.

$$\eta_2 = \frac{t_2 \times \rho_2}{t_1 \times \rho_1} \times \eta_1$$

Where

- ✓ η_2 :-Viscosity of sample
- ✓ η_1 :-Viscosity of water
- ✓ ρ_2 :-Density of sample
- ✓ ρ_1 :-Density of water
- ✓ t_2 :-Time required to flow sample
- ✓ t_1 :-Time required to flow water

Determination of Specific Gravity:

A thoroughly clean and dry Pycnometer was selected and calibrated by filling it with recently boiled and cooled water at 25⁰C and weighing the contents. Assuming that the weight of 1 ml of water at 25⁰C when weighed in air of density 0.0012 g/ml was 0.99602g. The capacity of the Pycnometer was calculated. Adjusting the temperature of the final syrup to about 20⁰C and the Pycnometer was filled with it. Then the temperature of the filled Pycnometer was adjusted to 25⁰C, any excess syrup was removed and weight was taken. The tareweight of the Pycnometer was subtracted from the filled weight. The weight per milliliter was determined by dividing the weight in air, expressed in gram, of the quantity of syrup which fills the Pycnometer at the specified temperature, by the capacity expressed in ml, of the Pycnometer at the same temperature. Specific gravity of the final syrup was obtained by dividing the weight of the syrup contained in the Pycnometer by the weight of water contained, both determined at 25⁰C.

Determination of microbial load:-^[21]

- Labeled the bottom of six petri plates 1-6. Labeled four tubes of saline 10⁻², 10⁻⁴, 10⁻⁶, and 10⁻⁸.

- Using aseptic technique, the initial dilution is made by transferring 1 ml of E.Coli sample to a 99ml sterile saline blank . This is a 1/100 or 10^{-2} dilution.
- Immediately after the 10^{-2} dilution has been shaken, uncapped it and aseptically transferred 1ml to a second 99ml saline blank. Since this is a 10^{-2} dilution, this second blank represents a 10^{-4} dilution of the original sample.
- Shaked the 10^{-4} dilution vigorously and transferred 1ml to the third 99ml blank. This third dilution represents a 10^{-6} dilution of the original sample. Repeated the process once more to produce a 10^{-8} dilution.
- Shaked the 10^{-4} dilution again and aseptically transferred 1.0 ml to one petri plate and 0.1 ml to another petri plate. Done the same for the 10^{-6} and the 10^{-8} dilutions.
- Removed one agar pour tube from the 48°C to 50°C water bath. Carefully remove the cover from the 10^{-4} petri plate and aseptically pour the agar into it. The agar and sample are immediately mixed gently moving the plate in a figure-eight motion or a circular motion while it rests on the tabletop. Repeat this process for the remaining five plates.
- After the pour plates have cooled and the agar has hardened, they are inverted and incubated at 25°C for 48 hours or 37°C for 24 hours.
- At the end of the incubation period, select all of the petri plates containing between 30 and 300 colonies. Plates with more than 300 colonies cannot be counted and are designated too many to count (TMTC). Plates with fewer than 30 colonies are designated too few to count (TFTC). Count the colonies on each plate. A Quebec colony counter should be used.
- Calculate the number of bacteria (CFU) per milliliter or gram of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar.

$$\text{No. of Bacteria per ml} = \frac{\text{No. of colonies (CFU)}}{\text{Dilution} \times \text{Amount plated}}$$

- Recorded the results.

Stability Testing:

➤ Short Term Stability Testing (72 Hrs.):

Stability testing of the prepared poly herbal syrup was performed on keeping the samples at accelerated temperature conditions. Nine portions of the final syrup (1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B and 3C), were taken in amber

colored glass bottles and were kept at accelerated temperature at 4⁰C, Room temperature and 47⁰C respectively. The samples were tested for all the physicochemical parameters, turbidity and homogeneity at the interval of 24 hr, 48 hr and 72 hr to observe any change.

Long Term Stability Testing (3 Months):^[26]

The purpose of the stability testing is to provide proof of how the quality of a finished product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. The studies are conducted at 40°C±2°C /75 % ± 5% RH noted for significant changes occurs during 3 months at an interval of 0,1,2,and 3 months .The post evaluation parameters are checked during this interval.

Results:

Preformulation studies:

Table.3.Physicochemical constituents of root extract of *Echinopsechinatus* Roxb.

Sr.No.	Physical constant	Average % of physicochemical constant
1	Total ash	11
2	Acid insoluble ash	4
3	Water soluble ash	7
4	Water soluble extractive	12.4
5	Moisture content	3.25

Microbial count:

Table No.4.Microbial count of root extract of *Echinopsechinatus* Roxb.roots.

Sr.No.	Parameters	Result	Limit as per WHO
1	Total microbial count	Nil	NMT 1000 CFU /ml
2	E.coli	Absent	Should be absent

(NMT-Not more than, CFU-Colony forming unit)

GLC study of oils:

Table 5: Retention time and area % of active constituents of oils.

Sr.No.	Name of the oil	Retention Time	Area of the peak in %	Active constituent
1	Clove oil	5.57	85.3583	Eugenol
2	Ajwan oil	18.95	47.9135	Thymol
3.	Ginger oil	10.09	30.4133	Zingiberenol

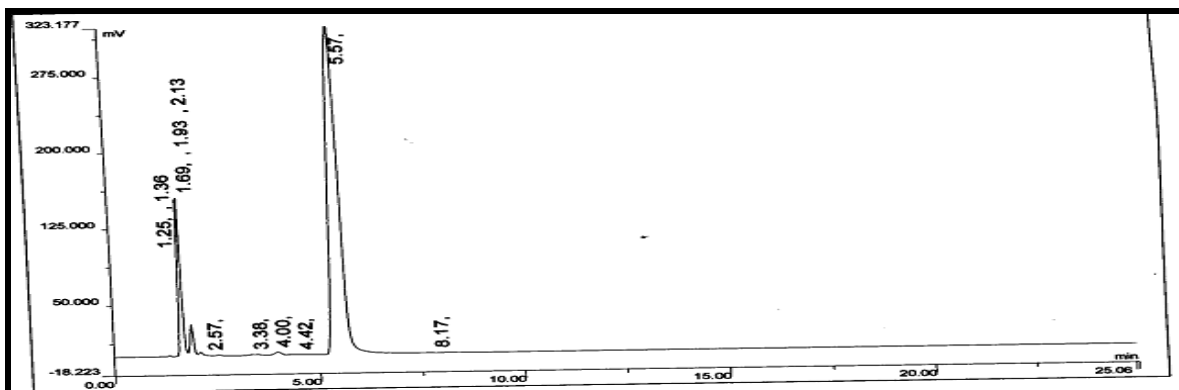


Fig 6. Chromatogram of Clove Oil.

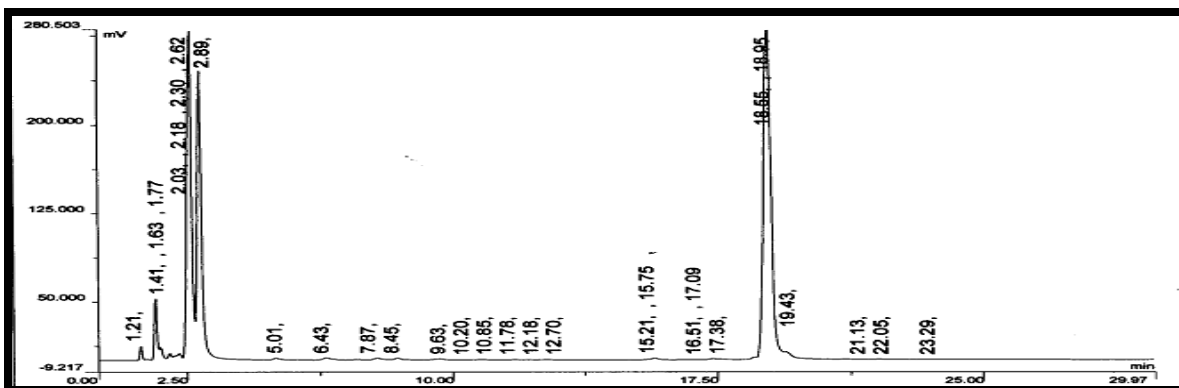


Fig.7 Chromatogram of Ajwan oil.

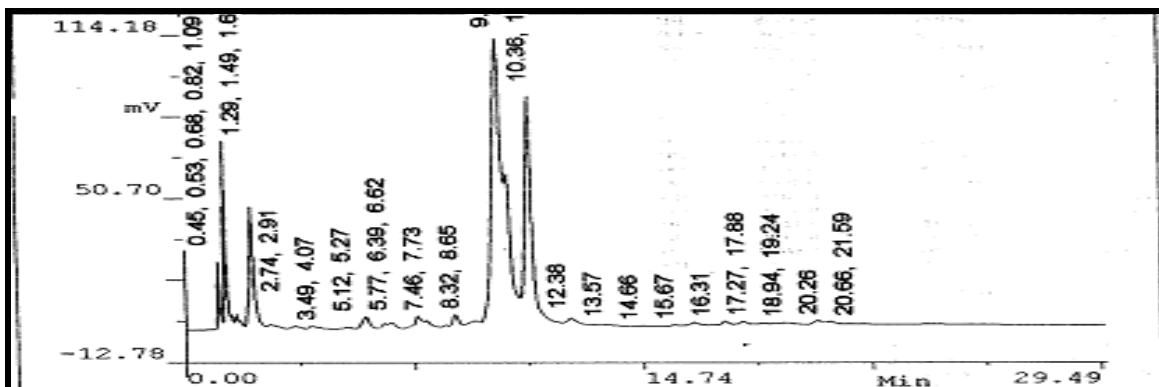


Fig 8. Chromatogram of Ginger oil.

Post Formulation studies

Table no.6: Result of Physicochemical parameters of developed poly herbal syrup.

Batch	Color	Odour	Taste
F1	Yellowish -Brown	Aromatic	Pungent
F2	Yellowish –Brown	Aromatic	Pungent
F3	Reddish Brown	Pleasant	Slightly pungent
F4	Brown	Pleasant	Sweet
F5	Dark Brown	Pleasant	Sweet

Table No. 7: Quantitative Evaluation of Developed Herbal Syrup Dosage Form.

Sr.No.	Parameter	F1	F2	F3	F4	F5
1	Density(gm/ml)	1.16±0.01	1.13±0.02	1.19±0.02	1.14±0.01	1.20±0.02
2	Viscosity(poise)	0.46±0.02	0.41±0.03	0.899±0.02	0.43±0.03	1.01±0.03
3	Specific Gravity	1.16±0.01	1.13±0.02	1.19±0.02	1.14±0.01	1.20±0.02
4	pH	6.6	6.13	6.6	6.2	6.7

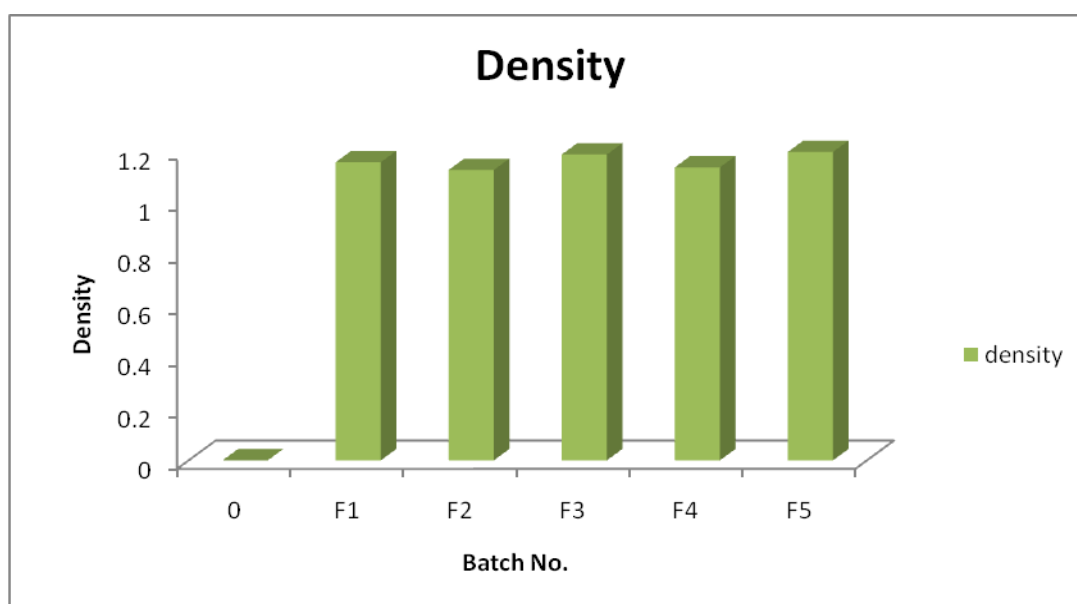


Fig. no. 9 Comparative Study of Density of all 5 Batches.

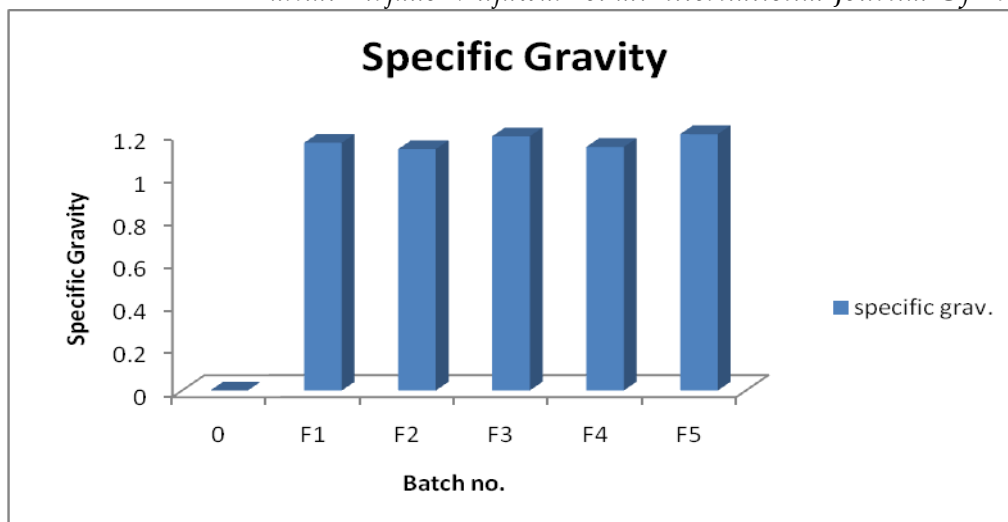


Fig.no. 10.Comparative study of Specific gravity of all 5 batches.

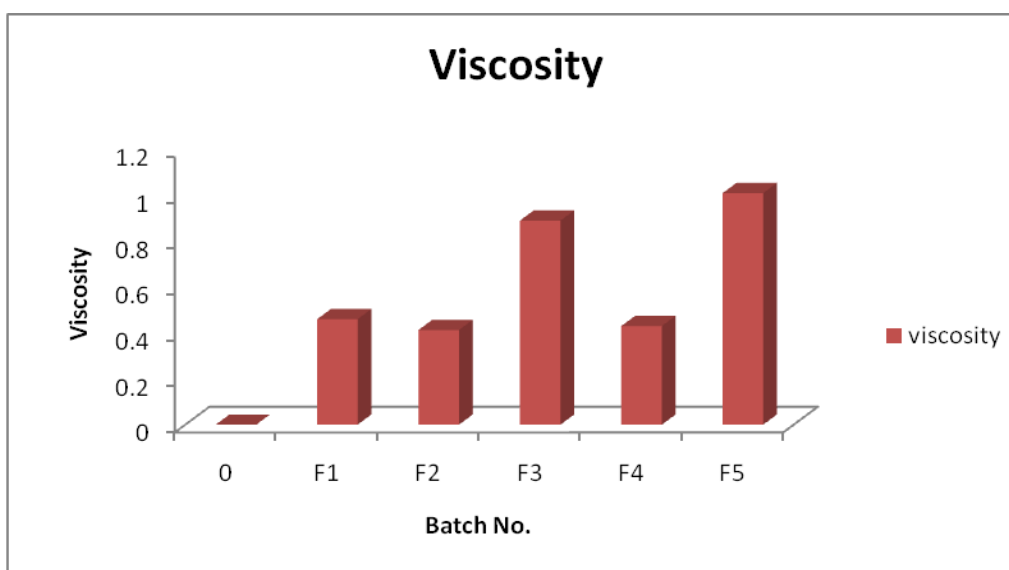


Fig.no.11.Comparative study of viscosity of all 5 batches

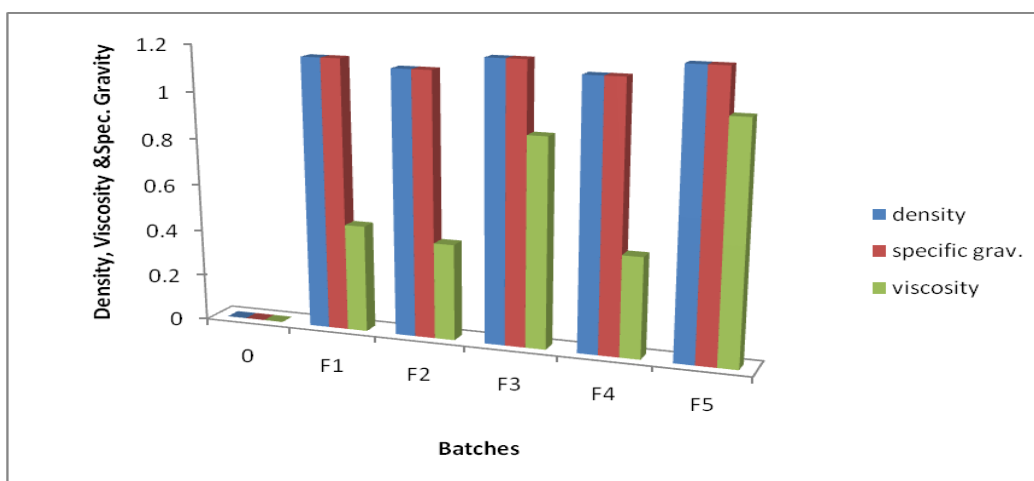


Fig. 12. ComparosionBetween Density, Specific gravity, Viscosity.

Microbial count study of Formulated Syrup:**Table No.8. Microbial count of Formulated syrup of *Echinopsechinatus* Roxb.roots.**

Sr.No.	Parameters	Result	Limit as per WHO
1	Total microbial count	Nil	NMT 1000 CFU /ml
2	E.coli	Absent	Should be absent

(NMT-Not more than, CFU-Colony forming unit)

Stability studies of developed herbal Syrup (Short term 72 Hrs):**Table . 9. Short term Stability study of developed polyherbal syrup, Long Term satability studies (3 Months):**

Sample Code	Time Duration (in hour)	Temperature (°C)	Physicochemical parameters						
			Colour	Odour	Taste	pH	Density	Specific Gravity	Turbidity/ Homogeneity
1A	24 hr	4 ⁰ C	NC	NC	NC	6.7	1.20	1.20	No Turbidity
1B		Room Temp.	NC	NC	NC	6.7	1.20	1.20	-
1C		48 ⁰ C	NC	NC	NC	6.7	1.20	1.20	Homogeneity
2A	48 hr	4 ⁰ C	NC	NC	NC	6.7	1.20	1.20	No Turbidity
2B		Room Temp.	NC	NC	NC	6.7	1.20	1.20	-
2C		48 ⁰ C	NC	NC	NC	6.7	1.20	1.20	Homogeneity
3A	72 hr	4 ⁰ C	NC	NC	NC	6.7	1.20	1.20	No Turbidity
3B		Room Temp.	NC	NC	NC	6.7	1.20	1.20	-
3C		48 ⁰ C	NC	NC	NC	6.7	1.20	1.20	Homogeneity

Table.10.Data Showing the Stability Studies of “0” Month at 40°C±2°C and 75 %±5% Rh.

Sr.No	Parameter	Formulation Nature
1	Colour	Dark Brown
2	Odour	Pleasant
3	Taste	Sweet
4	pH	6.7
5	Density	1.20±0.02
6	Specific Gravity	1.20±0.02
7	Viscosity	1.01±0.03

Table 11. Data Showing the Stability Studies of “1” Month at 40°C±2°C and 75 %±5% Rh.

Sr.No	Parameter	Formulation Nature
1	Colour	Dark Brown
2	Odour	Pleasant
3	Taste	Sweet
4	pH	6.7
5	Density	1.20±0.02
6	Specific Gravity	1.20±0.02
7	Viscosity	1.01±0.03

Table 12: Data Showing the Stability Studies of “2” Month at 40°C±2°C and 75 %±5% Rh

Sr.No	Parameter	Formulation Nature
1	Colour	Dark Brown
2	Odour	Pleasant
3	Taste	Sweet
4	pH	6.8
5	Density	1.20±0.02
6	Specific Gravity	1.20±0.02
7	Viscosity	1.01±0.03

Table 13 Data Showing the Stability Studies of “3” Month at 40°C±2°C and 75 %±5% Rh

Sr.No	Parameter	Formulation Nature
1	Colour	Dark Brown
2	Odour	Pleasant
3	Taste	Sweet
4	pH	6.8
5	Density	1.20±0.02
6	Specific Gravity	1.20±0.02
7	Viscosity	1.01±0.03

Discussion:**Preformulation Studies:**

Total ash: Table 3 shows the results obtained for total ash value of *Echinopsechinatus* Roxb. root extract. The value was found to be in the range of 10% to 15% and it was 11%.

Acid insoluble ash: Table 3 shows the results obtained for Acid insoluble ash value of *Echinopsechinatus* Roxb. root extract. The value was found to be in the range of 1% to 5% and it was 4%.

Water soluble ash: Table 3 shows the results obtained for water soluble ash value of *Echinopsechinatus* Roxb. root extract. The value was found to be in the range of 5% to 10% and it was 7%.

Water soluble extractive: Table 3 shows the results obtained for water soluble extractive value of *Echinopsechinatus* Roxb. root extract. The value was found to be in the range of 10% to 15% and it was 12.4%.

Moisture content: Table 3 shows the results obtained for Moisture content of *Echinopsechinatus* Roxb. Root extract. The value was found to be in the range of 1% to 5% and it was 3.25%.

Microbial count

Table 4 shows the results obtained for Microbial count of *Echinopsechinatus* Roxb. Root extract. The total microbial count was found to be nil which is not more than 1000 cfu/ml & E. coli was absent as per the WHO.

GLC study of oils

Table 5 shows the results obtained for GLC study of oils (Clove oil, Ajwan oil, Ginger oil.). The chemical constituents present in the clove oil, Ajwan oil, Ginger oil was found to be Eugenol 85.35%, Thymol 47.91%, Zinziberenol 30.41% respectively.

Post formulation Studies:-

Color: Table 7 shows the results obtained for colour of formulated batches of syrup. The colour of formulation was found to be Dark Brown for the optimized batch. The colour of the formulation ranges from Yellowish-brown to Dark brown for F1 to F5 batches.

Odour: Table 7 shows the results obtained for odour of formulated batches of syrup. The odour of formulation was Aromatic for the F1- F2 Batches and it was pleasant for F3 –F5 batches.

Taste: Table 7 shows the results obtained for odour of formulated batches of syrup. The odour of formulation was Aromatic for the F1- F2 Batches and it was pleasant for F3 –F5 batches.

Density: Table 8 shows the results obtained for Density of formulated batches of syrup. The Density of formulation was found to be 1.20 gm/ml for the optimized F5 batch. The value was found to be in the range of 1.13 gm/ml – 1.20 gm/ml for all five batches.

Viscosity:

Table 8 shows the results obtained for Viscosity of formulated batches of syrup. The Viscosity of formulation was found to be 1.01 poise for the optimized F5 batch. The value was found to be in the range of 0.41-1.01 poise for all five batches.

Specific Gravity:

Table 8 shows the results obtained for Specific Gravity of formulated batches of syrup. The Specific Gravity of formulation was found to be 1.20 for the optimized F5 batch. The value was found to be in the range of 1.13 -1.20 for all five batches.

pH:

Table 8 shows the results obtained for Specific Gravity of formulated batches of syrup. The Specific Gravity of formulation was found to be 1.20 for the optimized F5 batch. The value was found to be in the range of 1.13 -1.20 for all five batches.

Microbial count study of Formulated Syrup:

Table 9 shows the results obtained for Microbial count of *Echinopsechinatus* Roxb. Root herbal cough syrup. The total microbial count was found to be nil which is not more than 1000 cfu/ ml & E. coli was absent as per the WHO.

Stability Study:

Short Term Stability Study (72 hrs.):

Table 10 shows the results obtained for Short Term Stability Study of optimized batch of syrup. All the evaluation parameters of the syrup was found to be constant for the optimized F5 batch. The values were found to be remain stable after 72 hrs stability study. The turbidity or phase separation was not found after exposing the formulation to the different temperature conditions.

Long Term Stability Study (3 Months):

➤ After 1 month:

Table 11 shows the results obtained for 1 months Stability Study of optimized batch of syrup. All the evaluation parameters of the syrup was found to be constant for the optimized F5 batch. The values were found to be remain stable after 1 month stability study. The turbidity or phase separation was not found after exposing the formulation to the 40°C±2°C temp. and 75 %±5% Rh.

➤ After 2 month:

Table 12 shows the results obtained for 2 months Stability Study of optimized batch of syrup. All the evaluation parameters of the syrup was found to be constant for the optimized F5 batch. The values were found to be remain stable after 2 month stability study. There is only slightly change in the pH observed. The turbidity or phase separation was not found after exposing the formulation to the $40^{\circ}\text{C}\pm 2^{\circ}\text{C}$ temp. and $75\% \pm 5\%$ Rh.

➤ After 3 month:

Table 13 shows the results obtained for 3 months Stability Study of optimized batch of syrup. All the evaluation parameters of the syrup was found to be constant for the optimized F5 batch. The values were found to be remain stable after 3 month stability study. There is only slightly change in the pH observed. The turbidity or phase separation was not found after exposing the formulation to the $40^{\circ}\text{C}\pm 2^{\circ}\text{C}$ temp. and $75\% \pm 5\%$ Rh.

Conclusion:

- ✓ The result obtained in this study suggests that the herbal formulation prepared possesses significant “Antitussive” activity. The component of the herbal formulation Echinopsechinatus Roxb. was selected due to its reported action that play a preventative & curative role in the prevention of cough.
- ✓ Preformulation studies of EchinopsEchinatusRoxb. were performed the Acute toxicity study revealed that the drug is non toxic and safe .
- ✓ Herbal cough syrup Echinopsechinatus Roxb. can be prepared by solution with heat method using different extracts such as Mulethi, Lindi pepper, Haldi, and oils such as Ginger oil, Ajwan oil and Clove oil.
- ✓ Amongst all the formulations, containing quantity of oil 0.1 ml and having the viscosity value 1.01 poiseis fulfilling all the parameters satisfactorily.
- ✓ From the above data it can be concluded that there were no appreciable change in physicalcharacteristic were observed in optimized batch (F5) & after stability testing. Therefore theformulation was stable at 40°C and 75% RH.& it was concluded that herbal syrup prepared in the form of cost effective syrup to minimize consumer compliance in regarding suppressing side effects and enhancing positive effects on the body.

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