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Evaluation of Antacid Activity of Microemulsion Formulation of Blend of Essential Oil

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ABSTRACT

Essential oils are having wide range of biological activity is used to achieve therapeutic effects. These are volatile substances sensitive to oxygen, light, moisture and heat. In the present study microemulsion formulation was prepared using a blend of essential oil contains cardamom, coriander, fennel, caraway, ajowan and peppermint oil, water and non ionic surfactant tween 20 and cosurfactant as ethanol. Each essential oil was extracted from dried seed by steam distillation and characterized by Headspace Gas chromatography use of a marker compound which was linalool for coriander oil, cineol for cardamom oil, anethol for fennel oil, carvone for caraway oil, thymol for ajowan oil and menthol for peppermint oil. The marker compound was characterized using mass spectroscopy. Microemulsion of oil showed higher stability with droplet size in the range of 110-410nm. The product then screened for *in vitro* antacid properties which showed significant positive response.

Keywords: Essential oil, Microemulsion, Steam distillation, Headspace gas chromatography, Antacid activity.

INTRODUCTION

Essential oils and their main components have many applications in popular medicine, food, beverages, preservation, cosmetics as well as in the fragrance and pharmaceutical industries. [1-2]

Cardamom, Fennel, Coriander, Caraway, ajown and pippermint is produced from cultivated or wild plants in the mountainous regions of southern India. These has been used in the traditional Chinese medicine and Indian Ayurvedic medicine for thousands of years, mainly for treating respiratory diseases, fevers and digestive complaints. These additionally possess medicinal properties including antibacterial, carminative, antioxidant, digestive etc activity. [3-6]

Steam distillation is commonly use technique for extraction of essential oil from their dried seed or leaf. The approaches taken for extraction of Cardamom, Fennel, Coriander, Caraway, ajown and peppermint oil from their dried seed using Clevenger apparatus steam distillation apparatus. [7]

The physico-chemical properties of each essential oil were verified. Each oil was characterized making use of a marker compound which was linalool for coriander oil, cineol for cardamom oil, anethol for fennel oil, carvone for caraway oil, thymol for ajowan oil and menthol for peppermint oil by a validated Headspace gas chromatographic method. The marker compound was confirmed by mass spectroscopy. [8-12]

Essential oil is unstable, volatile and lipophilic in nature. Microemulsion technology is applied in the pharmaceutical industry to increase drug penetration across the diffusion layers, good appearance, drug solubility and also increase the stability. [13-15]

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Microemulsions are clear, thermodynamically stable, isotropic liquid mixtures of oil, water and surfactant. In contrast to ordinary emulsions, microemulsions form upon simple mixing of the components where two immiscible phases (water and 'oil') are present with a surfactant, the surfactant molecules form a monolayer at the interface between the oil and water, with the hydrophobic tails of the surfactant molecules dissolved in the oil phase and the hydrophilic head groups in the aqueous phase.

The aim of the present study is to formulate microemulsion formulation using a blend of essential oil, water and non toxic, non ionic surfactant Tween 20 with cosurfactant as ethanol. The formulated product then screened for the antacid activity. [16-17]

MATERIALS AND METHODS

Material and Reagent

Dried seed of Cardamom, Coriander, Fennel, Caraway, Ajowan and peppermint oil was procured from trade market. Markers compounds namely linalool, cineol, anethol, carvone, thymol and menthol were procured from Ultra International Limited, Uttar Pradesh, India. Tween 20 (Polyethylene glycol sorbitan monolaurate) was procured from Sigma Aldrich, India. Water was obtained using a Millipore gradient Water System (Millipore Ltd., Bangalore, India) for all the experiments. Methanol, Hydrochloric Acid, Sodium Hydroxide, sodium carbonate and other reagents used for the analysis were of Analytical grade. The purities of all the marker standards were not less than 98%.

Extraction of essential oil

Dried seed of cardamom, coriander, fennel, caraway, ajowan and peppermint oil were collected from the market. 250 g of each seed boiled with 500 ml of distilled water in a Clevenger apparatus up to 6 hours. The volume of essential oils was determined from a calibrated trap. The essential oils in the distillate were dried over anhydrous Na_2SO_4 and kept in the freezer. The same process has been repeated to get desire volume.

Physical characterizations, Identification and estimation of purity of oils

The physico-chemical properties were measured according to Russian Pharmacopoeia (1990). The color of the individual oils was checked by visual observation. Refractive index and optical rotation of the oils has been checked using Abbe's Refractometer and polarimeter. Specific gravity test has been performed using Borosil Specific Gravity Bottle.

A validated Headspace gas chromatography method was used to identify & estimate the purity of individual oil based on marker compound present in each oil where respective marker compound was used as standard.

Characterization of marker compounds by Mass spectroscopy

The individual marker compound was characterized making use of Atmospheric-Pressure Chemical Ionization (APCI) mass spectroscopic method to determine the molecular mass of the marker compound. This study was carried out at Radiant Research Center, Bangalore, India.

Preparation of blend of sample:

A blend of oil was prepared by transferred 1g of each oil in 20 ml volumetric flask. Added approximately 500mg of peppermint oil (used as soothening agent) into it. Made up the volume with sunflower oil used as a base. The sample was stored in a close tight amber color bottle.

Microeuncapslsion Preparation

Microemulsions were formulated using blend of essential oil, Tween 20, ethanol and water. Tween 20 was selected because it is nontoxic and non ionic in nature and it has average HLB (Hydrophilic lipophilic balance) value of 16.7 that is favorable for formulation oil-in-water emulsion. Ethanol was chosen as cosurfactant to get better stability and dispersion of the organic phase into continuous phase.

The surfactant Tween 20 was mixed with cosurfactant ethanol in 3: 1 ratio (S). Different formulation was prepared by mixing blend of essential oil (O) with surfactant-cosurfactant mixer (S) in the ratio of 2:1 (OS1), 2:2 (OS2), 2:3 (OS3) and 2:4 (OS4). The water was added drop wise externally under continuous stirring condition using magnetic stir at 300 rpm.

Characterization of Microemulsion

Stability study

Each formulation was centrifuged at 12,000 rpm for 30 min at room temperature to determine their thermodynamic stability. The physical stability of the microemulsions like phase separation or creaming was assessed by visual inspection of the samples stored in tightly closed tubes at room temperature. The observation was carried out every day in first week followed by every week up to 3 months. The test was performed in triplicate for each sample.

Measurement of pH

Using the Mettler Toledo 320 pH meter, the pH values of the selected formulation samples were measured at 25±1°C. The measurements were carried out in triplicate.

Zeta-potential measurement

Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion/attraction between particles. The zeta potential is a key indicator of the stability of colloidal dispersions. A laser doppler electrophoresis was carried out on the microemulsions with a Zetasizer Nano Series equipment (Malvern, Nanoslight NS500), which is capable of measuring sizes between 10nm to 2000nm. Zeta potential and the dynamic light scattering (DLS) of the microemulsions were analysed in duplicate at 25°C.

Assay of microemulsion formulation

In drug product, 1 mL of emulsion contains 2.5 mg of each oil. A validated Headspace Gas chromatography assay method was used to as determine content of oil present in the emulsion against the standard marker

compound. The gas chromatographic system included a Gas chromatograph with Headspace auto sampler and a flame ionization detector. A DB-624 Capillary column with 30 meters length, 0.32 mm ID and 1.8 µm film thickness was use as a stationary phase. Initial oven temperature was programmed at 60°C with a hold for 2 minutes, with a 15°C/ minutes rate. The temperature was raised to 180°C and hold for 10 minutes. The final temperature was elevated to 240°C at a rate of 15°C/ minutes. Nitrogen was used as a carrier gas at flow of 1.2 ml/min. Detector parameter were programmed as: temperature 270°C, range 1 and attenuation - 4. Split ratio selected 5:1. The total run time is 20 minutes. In the head-space the oven temperature was kept at 85 ^{0}C , Needle temperature 95 ^{0}C, Transfer line temperature 100°C, GC cycle time 22 minutes, thermostat time 30 minutes, pressurization time 2 minutes, injection time 0.5 minutes, withdrawal time 0.5 minutes. Headspace mode was kept constant and Headspace carrier pressure was fixed to 15 psi. Both the product and standard (blend of marker compound) was dissolved in methanol to make the final concentration 0.5 mg/mL.

Antacid activity studies

Antacid activity evaluation was done using Rossett-Rice method. The *in vitro* acid neutralization capacity of the drug product was evaluate against standard $NaHCO_3$ and Rossett-Rice time i.e. the time during which the pH maintained between pH 3.0 and 5.

Three 500 mL glass beaker containing 70 ml 0.1N HCl and 30ml of distill water was kept on magnetic stirrer. The electrode of the pH meter was deep into the solution and temperature of the solution was maintained 37^{0}C. 5 mL of drug product containing 12.5 mg of blend of essential oil added into the solution. A glass burette attached with iron stand filled with 0.1N HCl and kept on the glass beaker shown Fig. 4. A rate of 4 ml/min of 0.1N HCl was added into the solution which is simulates the normal acid secretion rate. The pH was noted & the Rosette-Rice time was determined. The test was repeat with 10mL and 20mL of drug product.

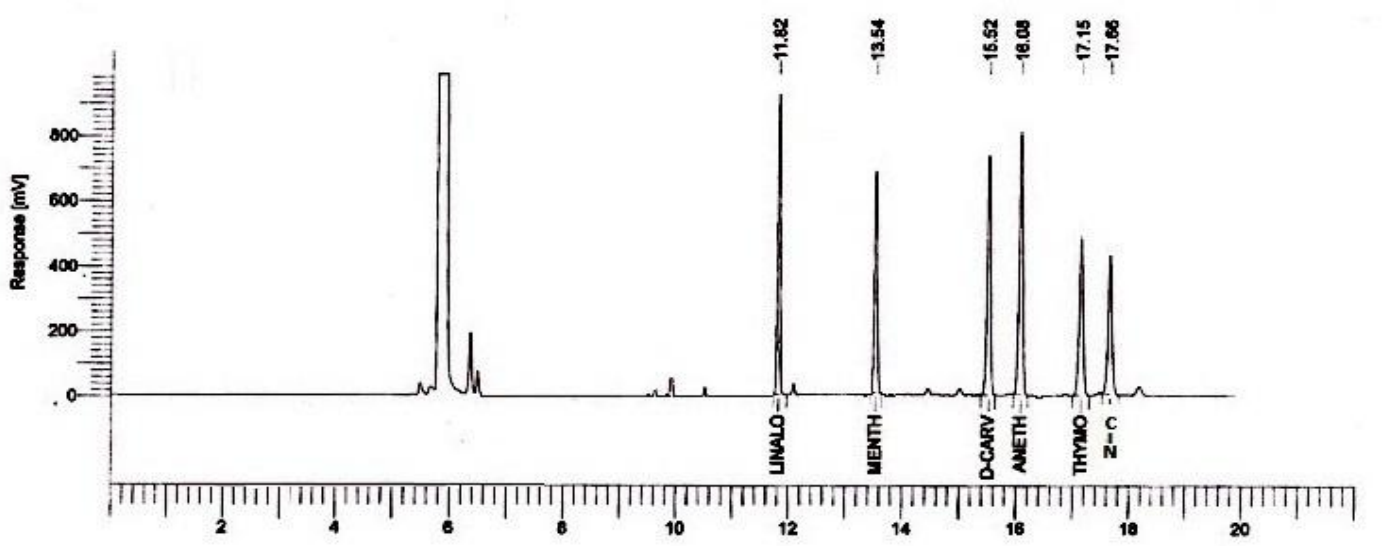

Fig. 1: Typical chromatogram of system suitability solution of standard

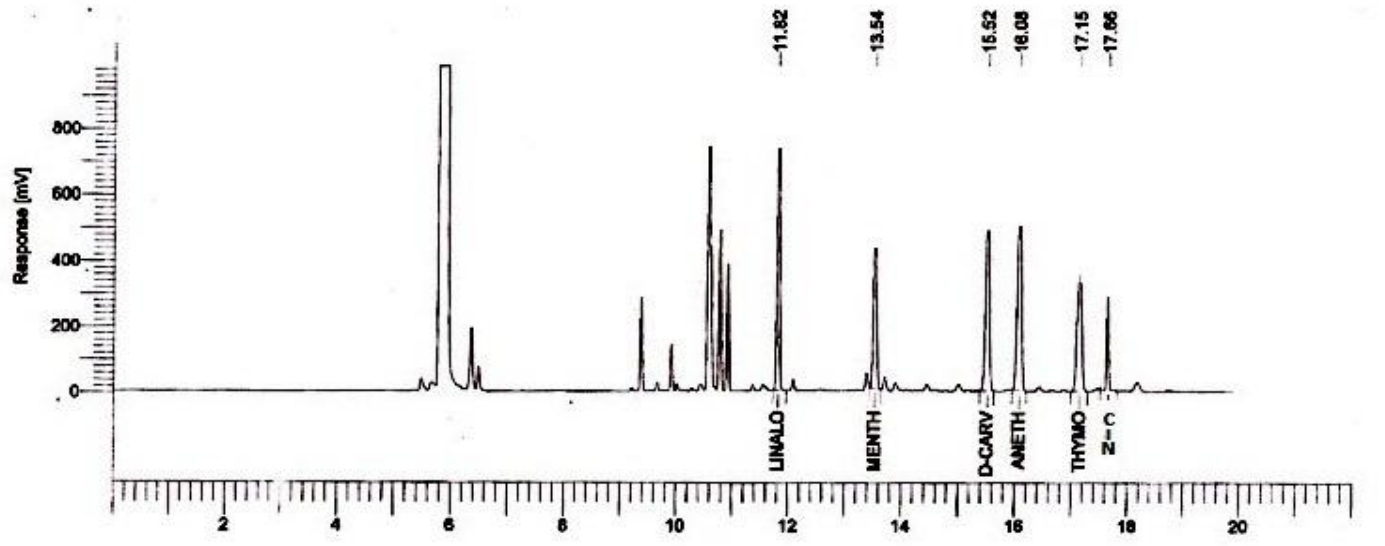

Fig. 2: Typical chromatogram of blend sample solution in precision

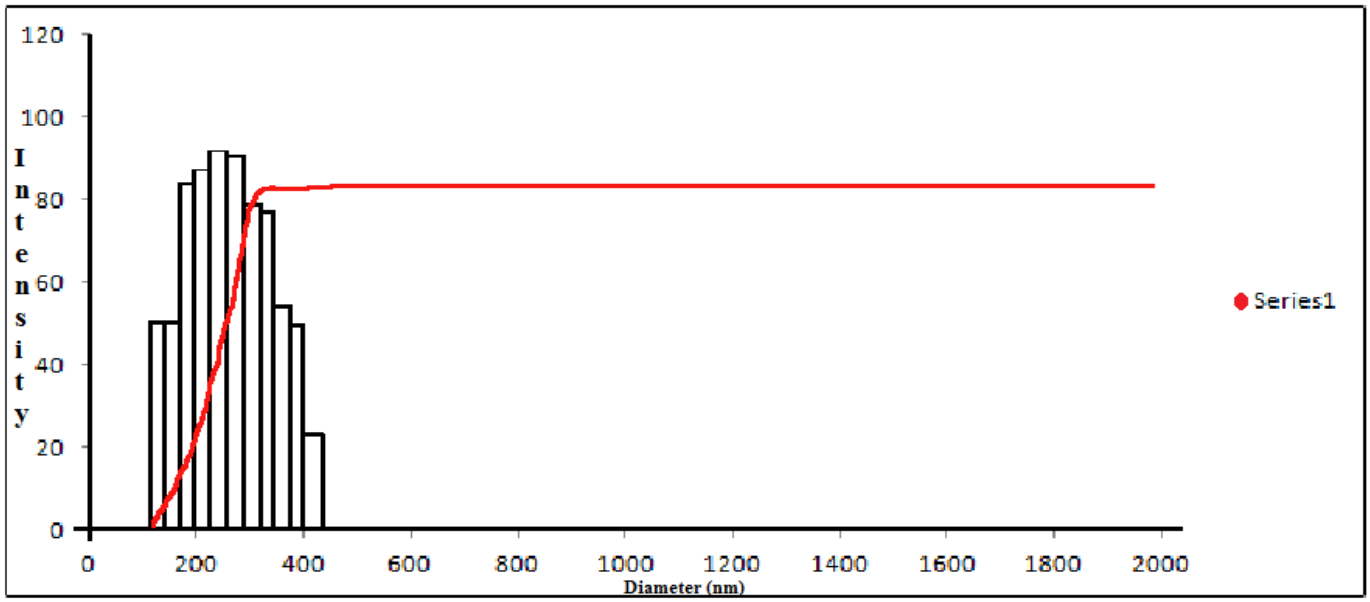

Fig. 3: Droplets size distribution of OS4 formulation

Table 1: The table lists the physical properties of the oils

Plant material	Weight (g)	Volume of distilled water (ml)	Time of heating (hrs.)	Temperature (°C)	Volume of oil obtained (mL)
Cardamom (seeds of the fruit)	250	500	6	80	3.5
Coriander (crushed ripe seeds)	250	500	5	80	2.3
Fennel (crushed seeds)	250	500	5	80	5.0
Caraway (dried ripe seeds)	250	500	5	80	2.0
Ajowan (dried seed)	250	500	5	80	2.5

Table 2: The table lists the physical properties of the oils

Essential oil	Name of Marker compound	Color	Odor	Relative density	Refractive index	Optical rotation (°C)	% of oil extracted by steam distillation (% of Marker present in oil)
Coriander	Linalool	Colorless to pale yellow	Characteristic	0.865	1.463	+ 9.003	60%
Caraway	Anethol	Colorless to pale yellow	Characteristic	0.917	1.485	+ 71.02	61%
Fennel	D-Carvone	Colorless to pale yellow	Characteristic	0.964	1.545	+ 12.185	65%
Ajowan	Thymol	Colorless to pale yellow	Characteristic	0.925	1.503	+ 0.446	51%
Cardamom	Cineol	Colorless to pale yellow	Characteristic	0.922	1.464	+ 26.706	58%

Table 3: Physicochemical characteristics of microemulsions (mean ± SD)

Number of Batch of formulation OS4	pH	Zeta Potential (mV)	Range of Droplet size (nm)	Assay (% of Marker compound present)				
				Linalool	Anethol	D-Carvone	Thymol	Cineol
OS4-1	6.85 ± 0.02	-39±2	139-395	58%	56%	52%	45%	50%
OS4-2	6.82 ± 0.01	-37±2	125-420	52%	55%	57%	45%	51%
OS4-3	6.80 ± 0.01	-35±4	120-405	56%	58%	54%	47%	55%
OS4-4	6.81 ± 0.03	-36±2	110-370	55%	54%	59%	42%	51%
OS4-5	6.82 ± 0.02	37±3	150-355	54%	60%	51%	45%	48%

RESULTS AND DISCUSSION

The quantity of each essential oil was obtained by steam distillation was tabulated in Table 1. The extraction was repeated to obtain required volume of essential oil. The Physical characterizations of the oils that odor, color, refractive index and optical rotation and their purity had done and match with their specification available in the data bank & literature are tabulated in Table 2. The purity was established of blend of essential oil using marker compound of each oil as standard shown in Fig. 1 and Fig. 2.

Selection and physicochemical characterization of Microemulsion formulation

The different microemulsion formulations (OS1, OS2, OS3 and OS4) were prepared were tested for their phase stability after centrifuge of each formulation. After centrifuge, immediate phase separation was observed in OS1 and OS2 formulation but OS3 and OS4 formulation was observed stable and extended their stability at room temperature. Upon storage for two week duration, again phase separation was observed in OS3 but OS4 showed higher stability in entire 3 month

stability period. Hence OS4 was used for further characterization and application of studies.

After getting the stable formulation OS4, the process was optimized by taking 4 different batch formulations with same formula and evaluate their sameness characterization properties.

The pH, refractive index, zeta potential, droplets size and assay for 5 batches of formulation was tested for evaluation of repeatability of the formulation and tabulated in Table 3. and Figure 3 shows the droplet size distribution of OS4 formulation. It is found that all the 5 batches result are consistence and stable.

Result of Antacid activity

The antacid profile was evaluated by *in vitro* test known as Rosette-rise test for 5mL, 10mL and 20mL of drug product contain 12.5 mg, 25 mg and 50 mg of each essential oil. It was found that 5mL dose maintained the pH above 3 for 4.11±0.10 min; 10 mL of dose maintained the pH 10.10±0.10 min. and 20 mL of dose maintained the pH 25.10±0.15 min respectively as compared to standard 2.5 mg $NaHCO_3$ which maintained the pH for 10.08±.010 min. The drug having antacid activity should have an adequate duration of action that can maintain the pH of stomach above 3. 10 mL and 20 mL dose of the product shown *in vitro* similar antacid properties and having significant reactivity towards the acid. Hence the drug product can be considered as good antacid

From the current study it can reasonable to conclude that a stable microemulsion of a blend of essential oil can be prepared by using optimum combination of co-surfactant ethanol and Tween 20 which has a potential as a suitable drug delivery system. The results of the present study recommend that product having significant acid neutralizing capacity and shown resistance against change in pH. Hence this product can be use as antacid to inhibit gastric secretion in the stomach.

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