

Antacid Studies of Newly Developed Polyherbal Formulation

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ABSTRACT

A lot of herbal plants are used across the globe to remedy various diseases. Herbal treatment is an alternative form for medicine where natural herbs and their extracts are used to cure a situation. Some drugs are proved to contain pharmaceutical ingredients suitable for treatment of stomach acidity or ulcers. The present work deals with the claim that a polyherbal formulation can be formulated which can be used as an alternative to already existing antacid formulations in the market. Therefore, some selected herbs with the tendency to neutralize acid in the stomach are selected and a formulation is formed. 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: Rosette-rise test, antacid, polyherbal, microemulsion

INTRODUCTION

The present system of popular medicine also involves a lot of essential oils and their components for treatment. Cardamom, Ajowan, Fennel, Caraway, Coriander and Peppermint are used in Indian and Chinese system of medicines from hundreds of years. These are also claimed to be useful in stomach ailments which qualifies them as candidate for present investigation²⁻⁸. Steam distillation is commonly used technique for extraction of essential oil from their dried seed or leaf. The approaches taken for extraction of Cardamom, Fennel, Coriander, Caraway, ajowan and peppermint oil from their dried seed using Clevenger apparatus steam distillation apparatus. 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 spectroscopy⁹⁻¹³. This is the second study within the process to verify the accuracy of results¹. 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.

MATERIALS AND METHODS

Dried seed of Cardamom, Coriander, Fennel, Caraway, Ajowan and peppermint oil was procured from trade market. Marker 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 Na₂SO₄ and kept in the freezer. The same process has been repeated to get desired volume.

Preparation of blend of sample

20ml Volumetric flasks were filled with 1g of each six oils. To add soothing effect, 500mg of peppermint oil was added. The base used is of sunflower oil. The sample was then stored in amber colored bottle.

Preparation of Microemulsions

Tween 20 was used as surfactant for preparation of microemulsion. Ethanol was chosen as co-surfactant 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

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

Table 1: Rosettee-rise Test Results

Sample No.	Quantity of blend	Essential oil blend	Maintenance time of pH 3 (min)
1	5ml	12.5mg	4.11±0.10
2	10ml	25mg	10±0.10
3	20ml	50mg	10±0.15

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±1oC. 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 headspace the oven temperature was kept at 85 0C , Needle temperature 95 0C, 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 RossettRice method. The in vitro acid neutralization capacity of the drug product was evaluate against standard NaHCO3 and Rossett-Rice time i.e. the time during which the pH is 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°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. 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.

RESULTS AND DISCUSSION

The quantity of each essential oil was obtained by steam distillation. The extraction was repeated to obtain required volume of essential oil. The Physical characterizations of

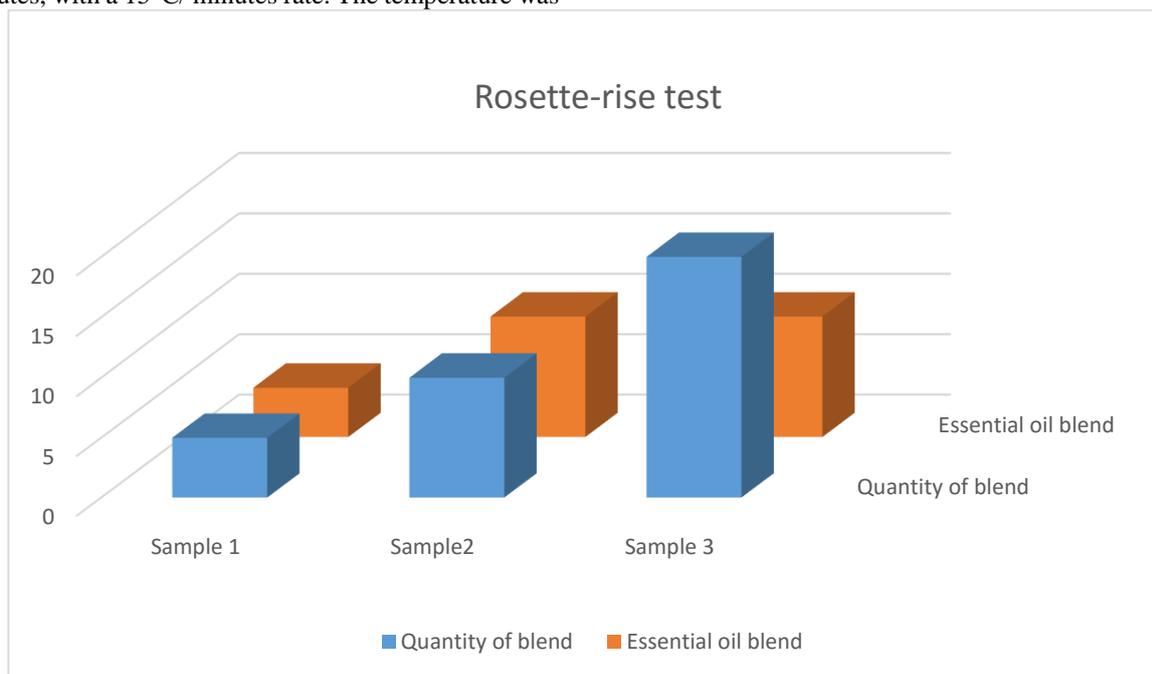


Figure 1: Graphical Representation of Rosette-rise test

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. The purity was established of blend of essential oil using marker compound of each oil as standard

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 which 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 NaHCO₃ which maintained the pH for 10.08 ± 0.10 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 cosurfactant 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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