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FORMULATION AND EVALUATION OF BETA-MYRCENE EMULSION FOR PEPTIC ULCER DISEASE

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ABSTRACT

Peptic ulcer disease (PUD) is a common gastrointestinal illness comprised of mucosal ulcers in the duodenum and stomach, most often caused by Helicobacter pylori infection, hyperacidity, or chronic NSAID consumption. The traditional drugs are proton pump inhibitors, antacids, and antibiotics, but long-term consumption induces side effects and resistance. Over the last decade, phytochemicals possessing anti-inflammatory and antioxidant activities have been researched as a safer alternative. β-Myrcene, a naturally occurring monoterpene in essential oils, has been reported to possess significant anti-inflammatory, analgesic, gastroprotective, and antioxidant activities. Although rich in therapeutic potential, β-myrcene is characterised by poor aqueous solubility and instability, which restricts its bioavailability and clinical use. In a bid to surmount these handicaps, the current study was designed to formulate a stable oil-in-water (O/W) emulsion of β-myrcene and investigate its potential for oral treatment of peptic ulcer disease. Oil-in-water (O/W) emulsions were prepared by high-shear homogenization and the utilisation of non-ionic surfactants like Tween 80 and Span 20. The formulations were then optimised for the size of the droplets, concentration of the surfactant, and oil-to-water ratio. The following tests were assessed after formulation, i.e., physical appearance tests, pH, viscosity, phase separation, and creaming index. In conclusion, the synthesised β-myrcene emulsion possessed desirable physicochemical and biological properties and therefore, can be a potentially effective gastroprotective delivery system for the management of peptic ulcer disease. The results of

the present work highlight the effectiveness of emulsion-based systems in improving the solubility, stability, and therapeutic efficacy of hydrophobic phytoconstituents like β-myrcene. Clinical confirmation is also recommended to establish its efficacy and safety in human subjects.

KEYWORDS: Peptic ulcer disease, Diagnosis, Treatment, Beta-myrcene, Monoterpenes, Emulsion.

1. INTRODUCTION

The persistent rupture of the mucosa or skin's covering epithelium as a result of molecular death is known as an ulcer. Peptic ulcer disease is brought on by imbalance between the lining of the gastrointestinal tract protectives and destructive components. PUD (duodenum), H pylori infection, NSAIDs use, having a first-degree relative with PUD, immigrating from industrialised nations and being African American or Hispanic are risk factors for PUD. Peptic ulcer frequently results in a mucosal defect that extends to the muscularis mucosa. When the mucous membrane that shields the surface is compromised, the interior layers become vulnerable to acid. Additionally, the mucosal cells' capacity to secrete bicarbonates is compromised. The stomach mucosa is known to become localised and inflamed by H. pylori, which raises stomach acidity, encourages metabolism, and suppresses the release of bicarbonate.[1,2] A gastrointestinal tract (GIT) wound that penetrates the inner, submucosal, and potentially outer muscular layers and results in perforation and death is referred to as a peptic ulcer (PUD). Organ wall leads to the illness, raising public health expenses and affecting a sizable section of the global population. Ulcers are a prevalent ailment that affects individuals globally. Because of the unpleasant side effects, treating ulcers with symptoms is bad for your health.[3] The primary mechanism of NSAIDs-induced damage to the gastroduodenal mucosa is the systemic inhibition of constitutively expressed cyclooxygenase-1 (COX-1), which is linked to mucus and bicarbonate secretion, decreased mucosal blood flow, and inhibited cell proliferation. COX-1 is responsible for prostaglandin synthesis. The enzymes are reversibly and concentration-dependently inhibited by NSAIDs. When exogenous prostaglandin and COX-2 selective NSAIDs are used together, mucosal damage and ulcer risk are decreased.[4] Up until the early 1900s, the majority of diagnoses for peptic ulcer were made based on clinical indication and symptoms. The first step is to determine whether the symptoms being described have nothing to do with functional dyspepsia, and the second is to pinpoint the exact origin of the ulcer.[5] The World Health

Organisation (WHO) states that because of their accessibility, effectiveness, and maybe lower side effects, plant bioactive compounds are a safe substitute for developing novel treatments for stomach ulcers. Several mono-terpen compounds, including carvacrol, citronellol, α-pinene, myrtenol, α-terpineol, eucalyptol, limonene, thymol and β-myrcene, have been shown to exhibit preventive and curative properties in experimental models of stomach ulcers.[6] The bioactive chemicals having potential therapeutic roles of a wide range of phytochemicals, including alkaloids, glycosides, flavonoids, tannins, terpenoids, polysaccharides, and saponins, are important for managing ulcers since they have shown remarkable anti-ulcerogenic activities in preclinical and clinical investigations.[7]

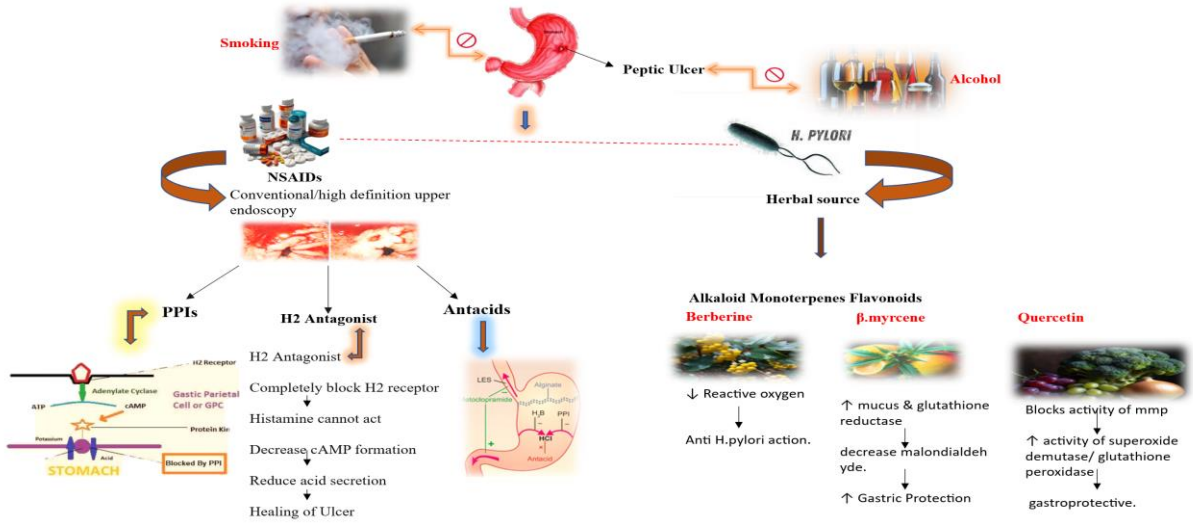

Figure 1: Mechanism of action of different drugs in the treatment of PUD.

Beta-myrcene, chemically known as 7-methyl-3-methylene-1,6-octadiene, the compound is an important flavouring agent widely used in the food and beverages sector. It is also widely used in products like detergents and cosmetics. It is used commercially as an important ingredient in flavour production, like linalool and geraniol.[8] β-myrcene is available from various sources like oranges, hop essential oil, the cannabis plant, lemongrass, and mangoes. The compound has a wide range of pharmacological activities. Specifically, it has sedative and anticonvulsant effects on the nervous system.[9,10,11] Additionally, it has antioxidant and anti-inflammatory effects. Additionally, it is usable in pain relief and peptic ulcer disease management. β-myrcene has ulcer-healing and antioxidant properties; it can be used to manage oxidative stress-induced disease conditions. In the Ethanol-induced ulcer model, β-myrcene inhibited ulcer formation by 60% (dose 7.5 mg/kg) via an increase in glutathione,

glutathione reductase and glutathione peroxidase. It also reduced the levels of superoxide dismutase. Also, in the indomethacin-induced model, β-myrcene reduced ulcers by 74% via a decrease in myeloperoxidase in the gastric mucosa. In the ischaemia-reperfusion model, β-myrcene reduced ulcers by 86% through the increase in glutathione level and limiting myeloperoxidase. Pretreating animals with $_{L}$-NAME reversed the ulcer protective effect shown by β-myrcene. It uncovered that the nitric oxide pathway is vital for the component of activity for ulcer protection by β-myrcene. It also increased the level of adhered mucosa by 50% in pylorus-ligated rats. The minimum inhibitory concentration for causing hindrance in the growth of H. pylori was found to be 500 µg/ml via serial dilution.[12]

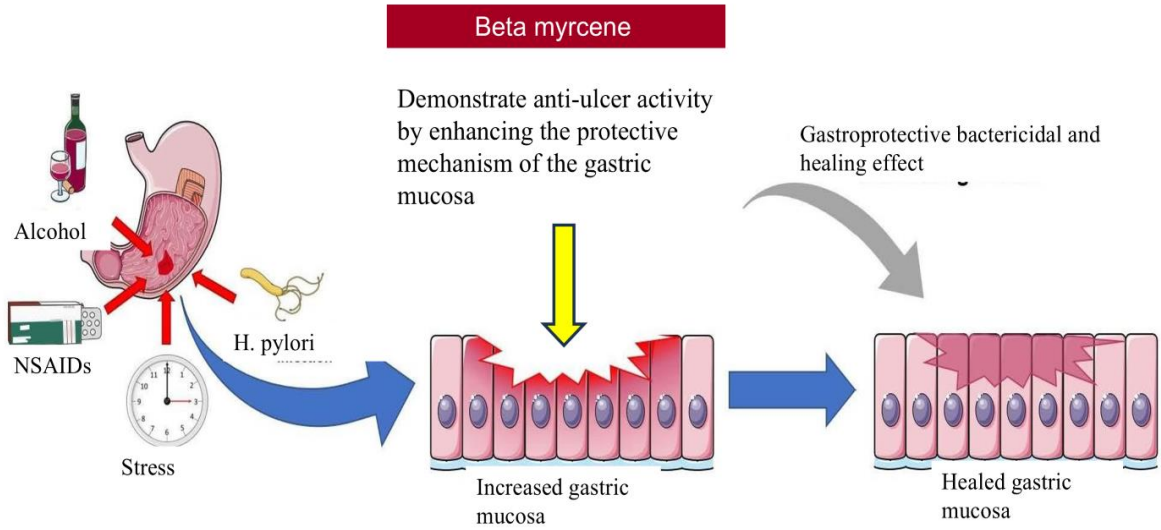

Figure 2: Mechanism of action of Beta-myrcene in the treatment of PUD.

Beneficial Role of Emulsion and Nano Emulsion in Peptic Ulcer

Most cells can be readily penetrated by nanoscale devices with a diameter of less than 50 nanometers, and as the blood arteries move through the body, objects with a diameter of less than 20 nanometers can readily leave them. Nanoscale devices can readily interact with biomolecules both inside and outside of cells because of their small size. It is therefore composed of high molecular weight nanoparticles that have ulcer-treating potential. Using a high-speed homogeniser and ultrasonic techniques to create high molecular weight nanoparticles improves the drug's solubility and absorption, boosting its availability at the site

of action and therapeutic index.[13] The particles may penetrate the mucus and reach the colon cells more quickly if their diameter is smaller than 14 nm, penetrating in 2 min, while a 415 nm diameter dose so in 4 min, particles take 30 min, while particles at 1000 nm are unable to move this barrier.[14]

2. MATERIAL AND METHOD

Table 1: Ingredients.

S. NO	Ingredients	Properties
1	Beta-Myrcene	Anti-Inflammatory, Antinociceptive, Antioxidant, Sedative, Antibacterial.
2	Poloxamer 144	Copolymer surfactant / Emulsifier
3	Soy. Lecithin	Emulsifier / Stabiliser
4	Glycerol	Co-Solvent / Stabiliser
5	SMPB	Preservative
6	Tween 80	Surfactant / Emulsifying agent
7	Span 20	Surfactant / Emulsifying agent
8	Span 80	Surfactant / Emulsifying agent
9	Tocopherol	Antioxidant

Formulation Table: Table 2: Formulation Table

S. NO	INGREDIENTS	F1 (gm)	F2 (gm)	F3 (gm)	F4 (gm)	F5 (gm)	F6(gm)
1	Beta-Myrcene	7.5	7.5	7.5	7.5	7.5	7.5
2	Poloxamer 144	10	-	2.5	7.5	-	3.5
3	Soy. Lecithin	-	-	1	3	-	1
4	Glycerol	10	10	5	10	7	10
5	SMPB	0.1	0.1	0.1	0.1	0.1	0.1
6	Tween 80	-	10	-	-	3.5	-
7	Span 20	-	-	-	-	7	3
8	Span 80	-	10	-	-	-	-
9	Tocopherol	0.1	-	0.1	0.1	-	0.1

Method

1: Dry gum method

2: Wet gum method

3: Hot Process

1. Selection of Ingredients

- Oil Phase: Beta-myrcene.
- Aqueous Phase: Purified water.
- Emulsifying Agent: Tween 80, Span 20, Span 80, lecithin, poloxamer 144.
- Preservatives: Sodium methyl parahydroxybenzoate.
- Stabilisers/Viscosity Enhancers: Glycerol.

2. Pre-formulation evaluations:

- **Viscosity:** The viscosity of β-myrcene (a monoterpene hydrocarbon) is relatively low, as it is a light, non-polar, oily liquid at room temperature. It was measured by using an Ostwald viscometer and a Brookfield viscometer.
- **Partition coefficient:** The partition coefficient (commonly expressed as log P) of β-myrcene describes how it distributes between a hydrophobic phase (usually octanol) and a hydrophilic phase (usually water). This value is important for understanding its lipophilicity and predicting absorption, distribution, and bioavailability.

- **Density:** Density is a physical property that describes how much mass is contained in a given volume of a substance. It is commonly used in physics, chemistry, and engineering to compare materials and understand their behaviour under different conditions. The density of myrcene was calculated by using a density bottle.

3. Preparation methods

All the preparations were made as per the standard procedure. In F1, poloxamer was dissolved in water and glycerol with other additives such as preservatives and an antioxidant to form a mucilage. Then, myrcene oil was incorporated slowly until a clicking sound was heard. The primary emulsification was achieved using a mortar and pestle. Then, it was placed under a laboratory stirrer for 15 minutes.

F2 and F5 were made by the dry gum method, where the surfactant was mixed with the myrcene oil with the help of a mortar and pestle, then mixed with the water phase containing glycerol, water and sodium methyl parahydroxybenzoate to obtain a primary emulsion, then placed under a laboratory stirrer for 20 minutes.

F3 and F4 contain soy. Lecithin as a co-surfactant were made by mixing the myrcene oil with lecithin and heating to the optimum temperature to get a stable oil phase, followed by formulation of an aqueous phase containing glycerol, poloxamer, Sodium parahydroxybenzoate, and tocopherol. The temperature of both phases was maintained at 40 °C using a heating mantle. Then mixed using a magnetic stirrer at a constant temperature. Followed by homogenization for 15 minutes and sonication for 20 minutes.

F6 was made by:

Oil phase preparation- 7.5 gm myrcene and 1 g of lecithin were taken in a beaker and kept in a Water phase- 10 gm glycerol, 3.5 gm poloxamer, 3 gm Span 20 and 0.1 gm Sodium methyl parahydroxybenzoate were mixed properly, and the temperature was maintained similar to the oil phase.

Primary emulsification- Mixed both oil and water phases with the help of a magnetic stirrer, and volume was made upto 100 ml with distilled water.

Final formulation: Then kept under a homogeniser for 25 minutes.

Evaluation

- **Zeta potential:** Zeta potential is the electrical potential at the slipping plane of particles in a colloidal system. It indicates the degree of electrostatic repulsion or attraction between particles and is a critical indicator of colloidal stability. The zeta potential of formulation 8 was performed by Saif Chandigarh using Litesizer 500.

- **Viscosity:** Viscosity is a measure of a fluid's resistance to flow. It describes how "thick" or "thin" a liquid is. In formulations, it affects flow, spreadability, and stability. The viscosity was measured with the help of a Brookfield viscometer.

- **Particle size distribution:** Particle Size Distribution (PSD) refers to the range and proportion of different particle sizes present in a sample. It is a critical parameter in fields like pharmaceuticals, cosmetics, food, and materials science, affecting stability, bioavailability, texture, and performance. The particle size distribution of formulation 8 was performed by Saif Chandigarh using Litesizer 500.

- **Stability:** Emulsion stability refers to the ability of an emulsion to resist changes over time, such as separation, creaming, coalescence, or phase inversion. A stable emulsion maintains a uniform distribution of droplets without significant aggregation or separation. It depends upon particle size distribution, zeta potential and viscosity. Accelerated stability testing was performed by using centrifugation.

- **pH:** The pH of an emulsion refers to the hydrogen ion concentration in its aqueous (continuous) phase. It is an important parameter that affects the stability, preservation,

efficacy, and safety of the emulsion, especially for pharmaceutical and cosmetic applications. pH was measured using pH paper.

- **Physical appearance:** The physical appearance of an emulsion gives important clues about its type, stability, and quality. Physical appearance is characterised by appearance, colour, odour, consistency and phase separation.

3. RESULT

Formulation 6 showed suitable characteristics for controlled-release applications, with a viscosity of 1143 cp and stable physical properties under stress and over time. It had a neutral pH (7.0), making it compatible with biological systems, and an electrical conductivity of 0.518 mS/cm, indicating ionic content. Particle size analysis showed a uniform nanoscale distribution (~191–218 nm) with a zeta potential of -32 mV, suggesting strong colloidal stability. The formulation was white with a citral-like or unripe mango odour. Evaluations of other formulations are shown in Table 3.

Table 3: Post-formulation evaluation table.

S. No	Parameter	F1	F2	F3	F4	F5	F6
1	Viscosity	1326 cp	5327 cp	1258 cp	5477 cp	4325 cp	1143 cp
2	ph	7	8	6	7	7	7
3	Colour	white	Pale yellow	white	white	White	white
4	Odour	characteristic	characteristic	characteristic	characteristic	characteristic	characteristic
5	Phase separation	+	-	+	-	+	-
6	Stability	Phase separation	Highly viscous	Phase separation	Creaming	Sedimentation	good

4. DISCUSSION

- **Pre-formulation studies**

Pre-formulation studies for emulsions assess the physicochemical properties of ingredients to ensure stability and effectiveness. Key evaluations include solubility, pKa, partition coefficient, and compatibility with emulsifiers and phase components. Myrcene showed a low viscosity (1.2903 mPa/s) and low density (0.80 g/mL), indicating it is a light, low-viscosity liquid that floats on water. Its high partition coefficient (Log P= 4.17) indicates strong lipophilicity, low water solubility, and high membrane permeability, which are important for drug delivery and potential bioaccumulation.

Evaluation parameters for pre-formulation studies:

Table 4: beta-myrcene characteristics

S. No	Parameter	Result
1	Viscosity	1.2903 mPa
2	Density	0.80 gm/ml
3	Partition coefficient	4.17

- **Post-formulation Studies**

Post-formulation analyses of emulsions are necessary to assess their stability, safety, and performance under storage conditions. Such analyses involve tests of physical appearance, viscosity, droplet size, pH, and zeta potential to check for any changes concerning time.

Evaluation parameters for formulations

Physicochemical characterisation and stability testing of the five emulsion formulations (F1–F5) disclosed significant performance and structural integrity differences. Viscosity measurement showed considerable variation, with F2 (5327 cp) and F4 (5477 cp) showing notably higher viscosities, pointing to more robust internal structural networks and possible resistance to gravitational separation. Conversely, F1 (1326 cp), F3 (1258 cp), and F5 (4325 cp) exhibited relatively lower viscosities, possibly indicating weaker intermolecular interaction of the dispersed phase. All of the formulations had pH values in the dermally acceptable range (pH 6–8), of which F3 had the lowest (pH 6) and F2 had the highest (pH 8), indicating topical application viability without considerable risk of irritation or acid-base instability.

Visual inspection revealed that all preparations, except for F2, had a white colouration, with F2 appearing pale yellow, possibly suggesting either compositional variation or differential oxidation of formulation components. All samples retained a characteristic odour pattern, suggesting a lack of microbial contamination or breakdown of fragrance-containing components throughout the test duration.

Stability measurements showed clear differences in emulsion performance. Phase separation was seen in F1, F3, and F5, indicating low emulsifier concentration or inappropriate oil-to-water ratios. F4 showed creaming, a reversible instability characteristic commonly linked to the density gradient between phases and a lack of sufficient viscosity to suspend the droplets. F5 additionally showed sedimentation, which indicates gravitational settling of denser dispersed particles caused by flocculation or failure of the emulsifying system. Interestingly,

F2 was physically stable during the test period. Its high viscosity could have helped in increased kinetic stability by reducing droplet motion and coalescence.

Table 5: Evaluation table for formulation 6.

S. No	Parameter	Result
1	Viscosity	1143 Cp
2	Stability	Centrifugation (20 min, 100 RPM) No Phase separation.
3	Ph	7
4	Seperation	Nil
5	Conductivity	+ve (0.518 mS/cm)
6	Particle size distribution	218.6 nm
7	Colour	White
8	Odour	Characteristic (Citral or Unripen mango-like)
9	Zeta- Potential	-32 mV
10	Hydrodynamic diameter	191.36 nm

Figure 7: Viscosity of emulsion using Brookfield viscometer.

With a viscosity of 1143 centipoise (cp) demonstrated in Figure 7, the produced formulation (6) demonstrated a fairly viscous system appropriate for controlled-release applications. Centrifugation at 100 RPM for 20 minutes was used to test the formulation's stability; no phase separation was seen during this time, indicating that the formulation was physically stable under stress. The pH of the formulation was 7.0, which indicated a neutral atmosphere compatible with biological systems and usable for dermal or mucosal applications. There was no visible phase separation or precipitation upon standing, further indicating the stability of the formulation.

A reading of 0.518 mS/cm for electrical conductivity validated the formula's capability of interacting with the biological system using electrochemical reactions and indicated the presence of ionic species within the aqueous phase.

Dynamic light scattering identified the hydrodynamic diameter to be 191.36 nm, while the particle size distribution study determined an average of 218.6 nm. A relatively narrow size distribution and uniform size in the nanoparticulate system are indicated by the high degree of agreement in size measurements. With a zeta potential of -32 mV, the nanoparticles were found to be highly clustered under electrostatic stability. High particle repulsion is usually indicated by a zeta potential above ±30 mV, which hinders aggregation and ensures long-term colloidal stability. The formulation had a white colour and a strong odour that was likened to the aroma of citral or unripe mango.

5. CONCLUSION

The current study aimed to formulate and evaluate a beta-myrcene-based emulsion as a novel gastroprotective system for the management of peptic ulcer disease. Beta-myrcene, a monoterpene natural product from plants, is extensively reported for its anti-inflammatory, antioxidant, and antibacterial activities but suffers from poor water solubility and low bioavailability for therapeutic use. To address these shortcomings, eight emulsion formulations (F1-F8) were formulated with different combinations of surfactants, co-surfactants, stabilisers, and methodologies of emulsification, including dry gum method, heating-homogenization, and sonication-assisted emulsification.

All the formulations were optimised systematically and analysed for important physicochemical properties. Among these, Formulation 8 was the best choice, showing higher stability, homogeneous particle size distribution, and good rheological characteristics. Zeta potential of -32 mV established remarkable colloidal stability, whereas hydrodynamic diameter of 191.36 nm and an average particle size distribution of 218.6 nm established the formation of a highly dispersed emulsion. The emulsion had a neutral pH of 7.0 and a viscosity of 1200 cp, both suitable for use in gastrointestinal applications. Moreover, under stress testing conditions, no phase separation was found, favouring the physical stability of the formulation.

The choice of stabilisers and surfactants, including poloxamer 144, lecithin, Tween 80, Span 20, and glycerol, was key to the emulsification stability and efficiency of the formulations.

Pre-formulation studies also facilitated the choice of beta-myrcene, log P of 4.17 and density of 0.80 g/ml, ascertaining its lipophilic nature and tendency towards inclusion in the oil phase of emulsions.

Though in vivo pharmacological investigations were not within the scope of this project, the in vitro results provide a solid basis for future development. The last optimised emulsion is significant in that it possesses significant potential as a natural, stable, and biocompatible drug delivery system for beta-myrcene in treating peptic ulcers.

In summary, the feasibility of employing emulsion-based technology to improve the delivery, solubility, and stability of beta-myrcene has been effectively demonstrated by this research. It is recommended that further research involve in vivo efficacy testing, pharmacokinetic characterisation, and toxicological evaluation in order to ascertain its full clinical use and open doors towards potential commercialisation.

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