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DESIGN, DEVELOPMENT AND EVALUATION OF POLYHERBAL FLOATING FORMULATIONS FOR PEPTIC ULCER

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ABSTRACT

Peptic ulcers, characterized by erosions in the gastrointestinal mucosa, present a significant health challenge worldwide. While conventional pharmaceutical approaches have been effective in treating these ulcers, concerns regarding side effects and drug resistance persist. In light of this, the development and optimization of alternative, natural therapies, such as herbal formulations, have gained considerable attention. This research endeavors to create a novel herbal formulation tailored for the treatment of peptic ulcers. A systematic approach was followed, encompassing the selection and preparation of herbal ingredients renowned for their anti-ulcer properties, rooted in both traditional knowledge and contemporary scientific evidence. The formulation underwent rigorous optimization employing Design of Experiments (DoE) techniques to determine the ideal ratios and extraction methods for its constituents. This study signifies a crucial step towards the advancement of natural therapies for peptic ulcer treatment. By marrying traditional wisdom with contemporary optimization techniques, a novel herbal formulation has emerged as a potential alternative or adjunct to conventional pharmaceutical treatments. As peptic ulcers continue to pose significant clinical challenges, the development of effective and safe herbal formulations represents a promising avenue for improved patient care and underscores the importance of bridging traditional knowledge with modern scientific methodologies. Further clinical investigations are warranted to validate these findings and explore the practical applications of this herbal formulation in clinical settings.

INTRODUCTION

Peptic ulcer is one of the world's major gastrointestinal disorders and affecting 10% of the world population. Many herbal medicines have been used globally for the treatment of Peptic Ulcer disease. About 279 plants from 89 families are identified that may be used in the treatment of ulcers. Herbal drugs have certain advantages over traditional medicines such as lower risk of side effects, widespread availability and low cost. But, most of the plant actives such as glycosides, tannins, flavonoids etc, are polar in nature and poorly absorbed due to large molecular size limiting the absorption via passive diffusion, poor lipid solubility hence preventing their ability to cross the lipid rich biological membranes. These limitations lead to reduced bioavailability and hence, low therapeutic index of plant actives. To minimize these problems, various novel drug delivery systems such as phytosomes, ethosomes, transferosomes, transdermal patches, microspheres are used now a days by which protection from physical and chemical degradation, enhancement in stability and improved

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bioavailability can be achieved. Thus, a carrier system is required for successful targeted delivery of the drug at the site of action. The successful treatment of gastric ulcers also requires the enhanced gastric residence time so that the drug can be infringed to the sub-mucosa region of stomach for better action.

In the present work, the extract of leaves of *Adina cordifolia*. was used to prepare floating microspheres by using chitosan, glutaraldehyde and span 80 as polymer, cross-linking agent and emulsifying agent respectively. The prepared microspheres were further optimized by Box-behnken design. The floating microspheres were evaluated for various *in-vitro* and *in-vivo* parameters. The prepared microspheres can effectively enhance the gastric residence time and *in-vitro* release studies revealed the prolong release of phenolic compounds like rutin and quercetin till 24 h. The results obtained from the *in-vivo* study showed that the prepared gastroprotective floating microspheres have good anti-ulcer activity. Histopathology of tissue sections also confirmed the protection of gastric mucosa on pre-treatment with microspheres at 500 mg/kg p.o. On the basis of findings, we can conclude that prepared microspheres can be used to develop the sustained release formulation of extract for the management of gastric ulcers.

Chronic peptic ulcer disease is caused by an imbalance between the gastric mucosa's innate protective factors—such as mucus and bicarbonate-secretion , sufficient blood- flow , prostaglandin E2 , nitric oxide, sulfhydryl compounds, antioxidant enzymes and aggressive forces (acid and pepsin secretions). Additionally, the aetiology ofstomach ulcers has been connected to behavioural and environmental factors likesmoking, a poor diet, drinking alcohol, using non-steroidal anti-inflammatorymedicines, and *Helicobacter pylori* infection (Lemos *et al.*, 2012). A mucosal rupturein the stomach or duodenum that is more than 3-5 mm and has a visible depth issometimes referred to as having peptic ulcer disease. In contrast to dyspepsia, which isa clinical diagnosis based solely on symptoms, it is thus an endoscopic diagnostic. Animbalance between factors that protect the stomach and duodenal mucosa and thosethat harm it leads to peptic ulcer disease . Duodenal and stomach ulcer patients bothexhibit comparable symptoms. They might experience pain in the retrosternum or theepigastrium, early satiety, nausea, bloating , burp, or postprandial misery . Thesesymptoms are vague, making it challenging to diagnose them as functional dyspepsia(Jaiswal *et al.*, 2021). An open sore on the skin or mucous membrane known as anulcer is characterised by the shedding of inflammatory dead tissue. Lesions on the skin's or mucous membrane's surface known as ulcers are characterised by a superficialloss of tissue. Although they can occur practically everywhere, ulcers are most frequently discovered in the digestive system and on the skin of the lower limbs.

PLANTS USED FOR MANAGEMENT OF GASTRIC ULCERS

It has been discovered that a variety of plants, minerals, and herbs can help prevent or treat stomach and peptic ulcers. Although there aren't many human trials, many have positive results in animal or in vitro research. Numerous herbal remedies have been claimed to have antiulcer properties, although the published literature has focused mostly on pharmacological effects in test animals (Ustün *et al.*, 2006).

The use of herbal remedies along with conventional anti-gastric ulcer medications may have a synergistic effect in the fight against *H. pylori* and the condition that causes stomach ulcers, as well as improving the prognosis for those who already have them. It is

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advised to carry out additional clinical research with bigger sample sizes on the effectiveness and safety of medicinal herbs with antiulcer activity because there are so few human studies available. Additionally, arranging studies to investigate and clarify the mechanisms of action of medicinal plants used for the prevention or treatment of peptic ulcers would be beneficial (Roy *et al.*, 2013).

RESEARCH METHODOLOGY

Collection and Authentication of selected plant material

Before the study began, a botanist authenticated and identified the leaves of the chosen plant by collecting them from a reputable source. For future use, a sample was retained at the department as a specimen.

Preparation of extract of *Adina cordifolia* leaf

Adina cordifolia leaf extract was made using the technique given by Yang Zo *et al.*, 2013 with small modifications. The collected leaves were cleaned by washing thoroughly three times with water followed by temperature-controlled shade drying. Ina grinder, the dried leaves were reduced in size, sieved (40 mesh) and then kept in an airtight glass jar. Leaf powder was pretreated with petroleum ether to remove the pigments and fatty compounds. The defatted powder (50 g) of dried leaves was extracted with ethanol using Soxhlet apparatus. Afterwards, ethanol was evaporated to dryness of the extract (Ou-Yang *et al.*, 2013).

Adina cordifolia (Cheksum, 1988)

Plant name: Adina cordifolia

Family: Rubiaceae

Common name

Bengali - Keli-kadam

Hindi - Haldu

Sanskrit - Dharakadanba

Tamil - Manje-kadame

Synonyms: Haldina cordifolia, Adina ledermanii (hallealedermannii), Adina pilulifera (Cephalanthus), Adina rubella, Nauclea cordifolia.

Habitat: In the lowlands and lower hills, you can find Adina cordifolia growing in deciduous forest. Teak (Tectonagrandis L.f.) is a wood species commonly associated with Burma (Myanmar) and Thailand. Occasionally seen in Peninsular Malaysia, Thailand, and the Indian subcontinent (India, Sri Lanka, and Burma/Myanmar)..

Uses:- Febrifuge, Antiseptic, Anti-fertility, Anti-inflammatory, Anti-rheumatoid, Bitter tonic, Anti-cancer, Anti-microbial.

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Preliminary Screening of *Adina cordifolia* leaf Extract

The preliminary screening of the prepared extract was done by the following methods (Faiz & Faiz, 2021):

Test for Alkaloids

0.5g of plant extract was taken, 8 mL of 1 percent HCl was added to it, then heated and filtered. Separate treatments with Mayer's and Dragendorff‟s reagents were performed on 2mL of the filtrate. A pale-yellow precipitate with a reddish precipitate indicates the presence of alkaloids.

Test for Flavonoids (Shinado‟s Test)

The extract was heated for five minutes with some magnesium turnings and a fewdrops of strong hydrochloric acid. Flavonoids are identified by their red colouring.

Test for Terpenoids (Salkowki‟s test)

2mL of the extract, 1mL of chloroform, and a few drops of strong sulfuric acid were combined. Terpenoids were found because a reddish-brown precipitate was created.

Test of Tannins

In a test tube, 0.5 g of the material was cooked in 20 mL of distilled water before filtering. Simple filter paper was used for the filtration process, and after adding 0.1 percent $FeCl_3$ to the filtrate, it was checked for blue-black colouring, which indicated the presence of tannins.

Test for Phenols (Ferric chloride test)

A portion of the extract was subjected to aqueous (5%) ferric chloride treatment, andthe development of a deep blue or black colour was monitored.

Test for Saponins

In a test tube, 2 mL of the extract was mixed with 6 mL of distilled water. The combination was forcefully agitated, and the presence of saponins was confirmed after 15 minutes of watching for the development of persistent froth.

Test for Quinones

Concentrated hydrochloric acid was used to treat a tiny amount of extract, and the development

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of yellow precipitation was monitored (or coloration).

Test for Cardiac Glycosides (Keller Kelliani‟s test)

The extract was combined with two millilitres of glacial acetic acid and one drop of ferric chloride solution, totaling five millilitres. This was minimised using 1 cc of "concentrated" sulfuric acid. At the interface, a brown ring representing the deoxysugar characteristics of cardenolides may be seen. A violet ring may appear below the ring, and a greenish ring may form in the acetic acid layer

Test for Carotenoids

In a test tube with vigorous shaking, 1g of the material was extracted with 10mL of chloroform. After filtering the mixture, 85 percent sulfuric acid was added. The presence of carotenoids was shown by a blue tint at the interface.

Test for Carbohydrate (Fehling‟s test)

After diluting the extract in 5mL of distilled water, then it was filtered. After being treated with 1mL of Fehling's reagent A and B, the filtrate was boiled in a boiling waterbath for five to ten minutes. The presence of carbohydrates is indicated by the precipitate's reddish orange colour.

Test for Protein and Amino acids

2mL of extract was mixed with 2-3 drops of 1% ninhydrin in acetone solution, and the mixture was then submerged in a water bath for one to two minutes. The presence of protein and amino acids is shown by the presence of the colour purple.

RESULT AND DISCUSSION

Table 1: Summary of the validated protocol for analysis of Rutin and Quercetin

Validation Parameter	Value
Linearity range	**1-6 µg/ml**
Correlation coefficient (R^2)	**0.999 (Rutin), 0.998 (Quercetin)**
Regression equation	**y = 38434x +40353 (Rutin), y = 73603x -48074 (Quercetin)**
Specificity	**Specific**
LOD (µg /ml)	**0.06 (Rutin), 0.10 (Quercetin)**

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LOQ (µg /ml)	**0.18 (Rutin), 0.31 (Quercetin)**
Precision	**Precise**
Accuracy	**Accurate**
Robustness	**Robust**
Ruggedness	**Rugged**

To confirm the presence of polyphenolic constituents (rutin and quercetin) of therapeutic significance, the HPLC analysis of the extract was carried out by a validated RP-HPLC method in our laboratory. The concentration of rutin and quercetin was found to be 0.43±0.12% and 0.63±0.21% respectively. On comparison with past results, it was found that the rutin concentration is almost half whereas the quercetin was about three times the previously reported yield (Polumackanycz *et al.*, 2019). Different geographical source and environmental conditions of the raw material severely affect the concentration of secondary metabolites and could also be the reason for variation in the present case (Pant *et al.*, 2021).

There were phenolic chemicals like rutin and quercetin found in the plant extract after HPLC analysis and these compounds could be responsible for the protective action of *Adina cordifolia* extract. In the past also such compounds were reported to reduce the gastric ulcers by enhancing the prostaglandin content from gastric mucosa, inhibiting the *Helicobacter pylori* and by scavenging the free radicals (Borrelli F, 2000; Marotta *et al.*, 1999; Sharath *et al.*, 2015).

Floating ability of optimized formulation

The floating ability of prepared microspheres, *Optimized formulation*, was analyzed and results indicated the buoyancy capacity of optimized formulation was about 86.19±0.15% for 24 h. The purpose of the floating test was to see if the prepared microspheres couldfloat in gastric fluid or not. Following the microspheres, the percentage of them that settled down over time was calculated after the distribution over the surface of the buffer medium. In present study, Chitosan-based optimized formulation demonstrated good floating ability for about 24 h. The hollow nature of the microspheres is likely to be responsible for their good buoyancy behaviour. Similar results were presented by Noopur *et al.*, 2016 (Ma *et al.*, 2008; Noopur Pandey, Dr. Archana Negi Sah, 2016).

***In-vitro* release study**

The *in-vitro* release of rutin and quercetin from *Adina cordifolia* leaves extract and prepared *Optimized formulation* was analyzed. The results indicated that about 80% of rutin and quercetin was released within two hours from the extract whereas the similar concentration of these compounds was analyzed in the *Optimized formulation* after eight hours (Table 5.17, Figure 5.35). These findings indicated that prepared floating microspheres can effectively enhanced the gastric residence time and would be suitable for the creation of an effective medication delivery system of *Adina cordifolia* extract (*ACE*). Fadhila M. *et al.*, (2019) also reported the release of phytoconstituents from *Adina cordifolia* root extract nanoemulsion ina sustained manner (Fadhila *et al.*, 2019).

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Table 2: *In-vitro* Release of Rutin and Quercetin from optimized Microspheres (*Optimized formulation*) and *Adina cordifolia* Extract (*ACE*) in 0.1N HCl Buffer

Time (hours)	% Rutin release from *ACE*	% Quercetin release from *ACE*	% Rutin release from *Optimized formulation*	% Quercetin release from *Optimized formulation*
0	0	0	0	**0**
0.25	28.03±0.05	26.14±0.02	12.58±0.04	**15.12±0.01**
0.5	47.42±0.11	46.44±0.00	17.69±0.06	**20.45±0.02**
1	62.17±0.08	64.93±0.02	26.53±0.07	**31.51±0.02**
2	81.34±0.02	82.64±0.49	38.78±0.02	**42.52±0.02**
4	87.64±0.09	89.82±0.10	52.07±0.16	**60.04±0.03**
6	88.93±0.18	93.85±0.22	65.08±0.93	**73.07±0.02**
8	89.29±0.57	95.21±0.02	78.47±0.17	**84.54±0.01**
10	90.33±0.28	95.56±0.02	84.91±0.16	**88.48±0.05**
12	91.11±0.44	95.75±0.05	84.98±0.05	**89.55±0.17**
24	**92.22±0.04**	**96.04±0.02**	**86.51±0.09**	**90.09±0.01**

Stability Study

The chosen optimised formulation (*Optimized formulation*) was assessed for a number of factors, including physical appearance, % yield, Flow property (Repose angle, tapped density, Hausner's ratio, bulk density, and Carr's index), % Drug Entrapment & drug loading and floating ability for 6 months (0, 30, 60, 90 and 180 days) of storage. The drug content and other characteristics didn't vary noticeably in any way after 180 days of storage at accelerated stability conditions. Summary of the stability study is given in table 5.19. Based on the

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results it was concluded that the chitosan microspheres (*Optimized formulation*) were stable despite being stored for 6 months under accelerated stability conditions (Puthli & Vavia, 2009).

Table 3: Summary of Stability study of optimized formulation (*Optimized formulation*)

Parameters	Storage conditions			
	Control (RT)	**Refrigerator 4±2°C**	**25±2°C**	40±2°C
Physical appearance	Light yellow colour microsphere andno change on storage	Light yellow colour microsphere andno change on storage	Light yellow colour microsphere andno change on storage	**Light yellow colour microsphere andno change on storage**
% Yield	No significant changes	No significant changes	No significant changes	**No significant changes**
Flow Property	No significant changes	No significant changes	No significant changes	**No significant changes**
% Entrapment Efficiency andDrug loading	No significant changes	No significant changes	No significant changes	**No significant changes**
Floating ability	**No significant changes**	**No significant changes**	**No significant changes**	**No significant changes**

CONCLUSION

The development and optimization of a novel herbal formulation for peptic ulcer treatment represent a significant stride towards enhancing therapeutic options for individuals suffering from these debilitating gastrointestinal conditions. This comprehensive research endeavor amalgamated traditional herbal wisdom with modern scientific methodologies, aiming to create a safe, effective, and alternative approach to peptic ulcer management. In conclusion, the research journey embarked upon in the development and optimization of this novel herbal formulation signifies a profound advancement in peptic ulcer treatment. By synergizing traditional healing practices with modern scientific methodologies, a promising alternative or complementary approach to conventional pharmaceutical therapies has emerged. This novel

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herbal formulation holds the potential to address the limitations and concerns associated with existing treatments, including side effects and drug resistance.

As peptic ulcers continue to exert a significant clinical burden, the development of this herbal formulation signifies hope for improved patient care and quality of life. The journey, however, is far from over. Further clinical investigations, involving human subjects, are warranted to validate the efficacy and safety of this herbal formulation in real-world settings. Moreover, as we continue to bridge the gap between traditional knowledge and contemporary research, this study underscores the enduring value of nature-derived therapies in addressing complex medical conditions.

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