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International Journal of Research in Pharma & Pharmaceutical Science
Volume 1 Issue 1

Formulation and Evaluation of Garlic Gel for Tongue Ulcer

Harsha Sagar[1], K. K. Jha[2], Shaweta Sharma[3]
[1,2,3]Teerthanker Mahaveer University Moradabad, (Uttar Pradesh) India

ABSTRACT

The research work "Formulation and Evaluation of garlic gel for tongue ulcer" aimed to design a novel formulation for the treatment of tongue ulcers. In the present work, good attempt was made to establish the potential of herbal gels (of garlic extract). Garlic (Allium sativum) has been exhaustively investigated for its medicinal values over several decades and used against infectious diseases. It has been used as a food ingredient in cooking due to its own characteristic aroma, flavor, and condiment, protective and digestive properties. Besides, it has been used as folk medicine also. In Indian subcontinent, people have been using garlic in various conditions i.e. common cold, hay fever, asthma, as antimicrobial agent and to relieve from traumatic inflammation as well as for wound healing. However, the tongue healing property has not yet been established. Initially, aqueous garlic extract was prepared by using fresh garlic clove. Prepared extract was evaluated for various phytochemical parameters. The extract was lyophilized (at - 60℃) to get dried powder extract which was further used for preparation of gel formulations. HPLC analysis predicted % amount of Allicin (active constituent) as 0.568% (approximately). Garlic extract was found to be compatible with the different gelling agents used in gel formulation. Three batches of Gel formulations were prepared by using different gelling agents (i.e. HPMC, Carbopol and Methyl cellulose). Menthol crystal (to enhance the penetration and soothing effect), PEG400 & Glycerin (as a plasticizer), Aspartame (as sweetener) and Clove oil (to mask the characteristic odour and taste of the garlic) were used. To preserve the formulation longer Sodium benzoate preservative was used. Prepared gels were evaluated for various physiochemical parameters i.e. clarity, color, odor, taste, pH, viscosity, spreadability and in-vitro drug release. Based on the above results, formulation F_2 was considered as optimized batch pertaining to transparency, good spreadability and extrudability characteristics and maximum drug release. Ex-vivo permeation study, thus conducted on F_2 formulation, revealed that about 84.395 ± 2.115% drug was released (in pH 6.8 phosphate buffers) through goat tongue membrane within 24 hours. The release data were fitted into various kinetics models i.e. Zero and first order, Higuchi and Koresemeyer-peppas that confirmed zero order kinetics. No significant changes were seen in optimized formulation upon storage under accelerated conditions as it showed 79.93% release at the end of 90 days which indicated that the formulation was stable. The present study suggested that such novel formulation design could be more acceptable by the patients due to masked taste, characteristic odor, rapid soothing effect and ease of application.

INTRODUCTION

Herbal therapy has got significance all across the globe and seems to have set for miraculous comeback reflecting the age of ancestors who were dependent upon herbal resources for the treatment of various ailments. Herbal products are supposed to be safer for human as well as the environment comparable to the synthetic counter parts.

Garlic (*Allium sativum* L.) and its Medicinal Background

- The medicinal potency of garlic was established and is well known to the people since 1500 BC.
- Greek physicians, Hippocrates and Galen had mentioned its use in the treatment of GIT disorders while ancient Japanese and Chinese had been utilizing it for headache, flu, sore throat and fever treatments.
- Nigerian peoples have been using garlic for the treatment of diarrhea abdominal disorders and infections of respiratory tract.
- In Indian subcontinent, people have been using garlic in various conditions i.e. common cold, asthma, as antimicrobial agent as well as for wound healing.

Oral Drug Delivery Systems

Oral drug delivery is the most ancient and utmost utilized practice since the time unmemorable to the public of present arena. It surpasses all other routes of drug administration. The wide acceptability and higher popularity is pertaining to the ease of administration and patient compliance.

Anatomical and Physiological features of Oral Mucosa

The buccal surface constitutes one third of total area of the oral cavity (100 cm^2). It is lined with layer of epithelial cells (0.5 mm thickness).Oral epithelium has two distinct regions i.e. keratinized and non-keratinized which differ from each other with reference to cellular lipid composition.

Oral Permeability

- The permeability of buccal mucosa is around 4000 times higher than the skin itself.
- Sublingual permeability is the highest while the palatal permeability lies at the lowest magnitude intermediating the buccal type.

Characteristics of Topical Oral Formulations

The formulations, intended for local action at any specific area of the oral cavity, must be designed to overcome the following limitations:

- Salivary secretion and mechanical stress bringing about rapid loss of the applied drug (dosage form) from the site of absorption.
- Non-uniform distribution of drug released by the delivery system in the saliva resulting in some of the areas of the oral cavity receiving least or no concentration of drug.
- Non-agreeable taste and consequent oral sensation, associated with the incorporated drug leading to reduced patient compliance.
- Comparative permeability of mucosal tissue (oral) and obstacle offered by certain regions of the mucosa retarding the drug absorption rate.

Mucoadhesive Dosage Forms

Mucoadhesion is commonly defined as the adhesion between two materials, at least one of which is a mucosal surface. Mucoadhesive dosage forms also prolong the residence time of the dosage form at the site of application.

Types of Mucoadhesive Dosage Forms

- Monolithic/matrix dosage form
- Membrane control/reservoir dosage form

Advantages of Oral Mucoadhesive Drug Delivery System

- Enhances the residence time.
- Enhances absorption determining drug efficacy.
- Excellent accessibility.
- Faster absorption attributed to rich blood supply and circulation rates.
- Increased drug bioavailability.
- Surpassing GIT degradation.
- Easy drug administration.
- Rapid onset of action.
- Apparent patient compliance.

Tongue Ulcers

Oral ulcers are sores or open lesions in the mouth or tongue which are caused by various disorders. Lesions are less common on the heavily keratinized palate or gingiva. In mild recurrent aphthous ulcers (tongue ulcers), the lesions reach a size of 0.3 to 1 cm and begin healing within a week.

Fig. 1: Tongue ulcer

Types of Oral Ulcers

- Minor apthous ulcers
- Major apthous ulcers
- Herpetiform ulcers

Causes of Tongue Ulcers

The reasons for development of oral ulcers have not been established and may be attributed to one or more causative factors such as:

- Disturbed immune system
- Injury
- Hormonal changes
- A lack of iron
- Certain vitamins deficiency
- Food hypersensitivity
- Allergy
- Genetic factors
- Stress/anxiety
- Tobacco consumption
- Medications

MANAGEMENT & TREATMENT OF RAS

Table no. 1: Management & Treatment of RAS (Recurrent Aphthous Stomatitis)

S. No.	Category	Drugs & Formulations
1	Antiseptc, anti-inflammatory and analgesic drugs	Chlorhexidine mouth rinse or gel, triclosan gel, topical diclofenac; Amlexanox
2	Antibiotics	Tetracycline, Cephalexin, Azithromycin suspension
3	Topical corticosteroids	Triamcinolone acetonide, fluocinolone acetonide, Clobetasol Propionate
4	Hyaluronic acid	0.2% gel, two weeks
5	Topical anesthetics	Topical lidocaine, mepivacaine,Tetracaine spray or gel, mouth wash solution containing Benzocaine and Cetylpyridinium chloride
6	Others	Sucralfate suspension, Laser, Cauterization, quercetin, myrtle, rosa damascene.

Preferred Local Pharmaceutical Treatments

Table no. 2: Preferred Local Pharmaceutical Treatments

S. No	Category	Drugs & Formulation
1	Antibiotics	Penicillin G Potasium 50 mg tb, 4 days
2	Corticosteroids	Prednisolone or Prednison equivalents 10-30 mg / day 1-2 months
3	Others	Colchicine 0.5-2 mg/ day, 7-14 days Dapsone 25-100 mg / day, 3 days Clofazimin 100 mg/ day, 6 months Pentoxifylline 300-400 mg 1-3x1,1 month
4	Essential elements	Zinc sulphate (150 mg / day), vitamin B12, iron, folic acid replacement
5	Immunomodulators	Thalidomide 50-100 mg / day, Levamisole 150 mg 3 times a week, 6 months
6	Homeopathic materials	Phosphorus, Sulphuric acid, Nitric acid.

NUTRITIONAL SUPPLEMENTS

- Vitamins: B1, B2, B6 and B complex on daily basis.
- Aloe (*Aloe vera*): *Aloe vera* juice (1–3 tsp) as mouthwash.
- Licorice (DGL) (from *Glycyrrhiza glabra*): mixture of powdered DGL (200 mg) and warm water (200 ml).
- Chamomile (*Matricaria recutita*): A diluted tincture or strong tea made from chamomile flowers.
- Echinacea (Echinacea purpurea, E. angustifolia, E. pallida): liquid echinacea (4 ml) can be mixed with warm water.
- Myrrh (Commiphora molmol): herbal extract (200–300 mg) of myrrh with warm water.
- Other herbs: Neem, Curcumin etc.

LITERATURE REVIEW

1. Literature Review based on Tongue Ulcer

S.No.	Author's Name	Inference	Journal
1	Sakly A. *et al*. (2016)	Evaluated the in vitro biocompatibility of cream Aphto Fix which was developed for the treatment of mouth ulcer.	BMC Oral Health, 1-7.
2	Thorat Y. S. *et al*. (2015)	Prepared curcumin loaded thermo-reversible mucoadhesive gel to treat the mouth ulcer using Pluronic F68 and Pluronic F127 as thermo-reversible agent along with carbomers and xanthan gum as mucoadhesive polymers	International Journal of Pharmacy and Pharmaceutical Sciences, 7(10); 399-402.
3	Subiksha P. S. (2014)	Reported that recurrent aphthous stomatitis (painful oral ulcers) could be cured with 5% Amlexanox.	J. Pharm. Sci. & Res. 6(6); 251-253.
4	Zaher. R. A. *et al.* (2014)	Studied and evaluated the effect of curcumin, derived from curcuma longa, on tongue ulcer in rats.	Mansoura Journal of Dentistry; 1(3), 85-89.
5	Belenguer-Guallar I. *et al.* (2014)	Reported the strategic treatments of recurrent aphthous stomatitis (RAS).	Pubmed J ClinExp Dent. 6(2); e168-74.
6	Khidir Agab mohd. H. *et al.* (2013)	Investigated liquorice mouth wash intended for the treatment for oral ulcers	Elsevier www.pharmanest.net 4 (3); 335-340.
7	Sheikh S. *et al.* (2013)	Developed and validated HPTLC methods for the determination of curcuminoids contained in the polyherbal gel formulation intended for application in the treatment of mouth ulcer.	IOSR Journal of Pharmac, 3(1); 29-34.
8	Hegde Mithra N. *et al.* (2012)	Investigated differential diagnostic techniques for long term tongue viz; common ulcerative disorders like aphthous and traumatic ulcers and squamous cell carcinoma	IRJP 3 (8); 145-148.
9	Karavana Y. S. *et al.* (2012)	Formulated a solid lipid nanoparticulate bioadhesive gel of cyclosporine-A intended for the treatment of recurrent aphthous stomatitis.	International Journal of Nanomedicine, 7, 5693–5704.
10	Carina-Magalhaes E. D. *et al*. (2011)	Evaluated the oral ulcer healing potential of Chamomilla recutita on the tongue of the experimental rats.	Med Oral Patol Oral Cir Bucal. 16 (6); 716-721.
11	Dosani M. A. *et al.* (2011)	Formulated and evaluated semisolid herbal jelly for the treatment of mouth ulcer.	International Journal of PharmTech Research, 3 (3); 1705-1713.

12	Yilmaz. N. *et al.* (2009)	Concluded honey to possess more therapeutic effectiveness than TGO in oral mucosal ulcers.	Pubmed 9 (4); 290-295.

2. Literature Review based on Formulation Technology

S.No.	Author's Name	Inference	Journal
1	D. Jyothi *et al.* (2016)	Developed topical gel containing *Trigonella foenumgreacum* (fenugreek) seed extract using carbopol-934 and Hydroxypropyl methyl cellulose (HPMC K4M) as gelling agents. The anti-inflammatory activity of suitable gel formulation was investigated.	International Journal of Pharmacy and Pharmaceutical Sciences, 8 (1); 41-44.
2	Sarkar B., *et al.* (2015)	Formulated and evaluated the herbal gel containing Cedrus Deodara extract.	www.ijpcsonline.com 4 (1); 67-70.
3	Bodhankar. M. M. *et al.* (2011)	Developed & characterized topical formulations (cream, ointment, gel) containing bioactive volatile oils of Allium sativum and Zingiber officinale. The results showed that the percentage release of oils through micro porous membrane was significantly higher with carbopol gel as compared to ointments and creams.	International Journal of Pharmacy & Technology,3 (3), 3165-3174.
4	Das. S. *et al.* (2011)	Formulated and evaluated the herbal gel containing Clerodendroninfortunatum extract. The gel was prepared by using various polymeric bases i.e. Sodium CMC and Carbopol 934 besides PEG 400, methyl and propyl paraben with sufficient distilled water.	International Journal of PharmTech Research 3 (1); 140-143.

Literature Review Based on Plant

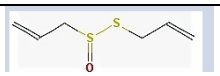
S.No.	Author's Name	Inference	Journal
1.	A. M. Q. Shaqra *et al.* (2015)	Studied *in-vitro* inhibitory activity against various microorganisms *i.e.* candida species, applying aqueous garlic extract gel (AGE) and lotion.	International Journal of Pharmaceutical Sciences and Drug Research, www.researchgate.net 7(3); 304-307.
2.	Z. Wencui. *et al.* (2015)	Prepared garlic oil loaded SLN (solid lipid nanoparticles) by high	Eur Rev Med Pharmacol Sci, 19 (19); 3742-3750.

		pressure homogenization technique. SLNs were administered orally into the rats and pharmacokinetic parameters were studied by using LC/MS/MS method.	
3.	Gebreyohannes G. *et al.* (2013)	Reviewed and reported garlic products for their therapeutic efficacy and safety parameters	International Journal of Medicine and Medical Sciences. 5 (9); 401-408.
4.	Johnson O. O. *et al. (*2013)	Investigated the synergistic effects of volatile oil blend containing Garlic clove (Allium sativum) and tangerine fruits (Citrus reticulata) for antimicrobial effects comparable to the individual volatile oil applications.	IJPSDR 5 (4); 187-193.
5.	Rajam K. *et al.* (2013)	Studied aqueous garlic extract as nontoxic corrosion inhibitor by mass-loss method.	Hindawi Publishing Corporation Journal of Chemistry, 1-4.
6.	Rahman, M. M. *et al.* (2012)	Used 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging technique to evaluate antioxidant potential of garlic extract which confirmed the clinical utility in protecting from cellular injuries caused by reactive oxygen species (RAS).	International Food Research Journal, 19(2); 589-591.
7.	Kudva. S. *et al.* 2012)	Determined the effect of garlic incorporated in mouthwashes.	Archives of Orofacial Sciences, 7 (1); 1-8.
8.	Bhaskar R. *et al.* (2011)	Reviewed different possible therapeutic mechanisms of garlic in their investigation reports.	International Journal of Drug Formulation & Research. 2 (2) 94-110.
9.	H. N. Wanyika, *et al.* (2011)	Developed and validated for UV spectrophotometric method for the quantitative determination of allicin in aqueous garlic extract.	Journal of Agriculture, Science and Technology, 74-82.
10.	P. Bocchini. *et al.* (2001)	Utilized reversed phase HPLC including UV and electrochemical detector for the development of analytical method to determine allicin content in garlic samples.	www.elsevier.com 37 –43.
11.	Serge Ankri *et al.*	Reported Allicin (from garlic) to possess antibacterial potential towards Gram-	Microbes and Infection © Elsevier,

	(1999)	negative as well as Gram-positive bacteria. Moreover, antifungal (against *Candida albicans*), antiparasitic (*Entamoebahistolytica* and Giardia lamblia) and antiviral potentials were also reckoned.	Paris 2, 125−129.

PLANT PROFILE: **GARLIC**

Garlic- occurs as fresh or dried bulbs of *Allium Sativum* possessing composite nature. It belongs to the family Lilliaceae. Official standards prescribed the medicinal value when allicin content is not less than 0.2% calculated with reference to the dried form.

Synonyms	Rasona, Yavanesta, Garlic, Lasan, Lassun, Lahasun, Lahsan, Seer, Ajo
Chemical constituents	Allin, allicin, 2 mercapto-Lcysteins, anthocyanins, glycosides of kaempferol and quercetin, polysaccharides, allinase, sterols, hydrocarbons, sativin I and II, scordinines A and B; Essential oil *etc*. Allicin is reported as the most important biologically active compound, responsible for the characteristic odor and flavor of fresh garlic.
Molecular & Structural formula of Allicin	$C_6H_{10}S_2O$ 
Uses	Garlic has been reported to possess antioxidant, antihyperlipidemic, antiatherosclerotic, fibrinolytic, platelet inhibiting, anticancer, hypoglycemic, antimicrobial, antirheumatic and antispasmodic potentials.

Nutritional Values and Properties of Garlic

Table 3: Properties of garlic. (Values expressed per 100 g of raw garlic)

Properties	Values
Energy	119 kcal
Moisture	70 %
Protein	4.3 g
Carbohydrate	24.3 g
Fiber	1.2 g
Fat	0.23 g
pH	6.05
Acidity	0.172 %

Table 4: Minerals of Garlic.(Values expressed per 100g of raw garlic)

Minerals	Values
Potassium	446 mg
Phosphorus	134 mg
Magnesium	24.1 mg
Sodium	19 mg
Calcium	17.8 mg
Zinc	1.1 mg
Iron	1.2 mg
Iodine	4.7 µg

Table 5: Vitamins Present in Garlic. (Values expressed per 100 g of raw garlic)

Vitamins	Values

Thiamin (Vit.B1)	0.16 mg
Riboflavin (Vit. B2)	0.02 mg
Niacin (Vit. B3)	1.02 mg
Pyridoxine (Vit.B6)	0.32 mg
Folic acid	4.8 µg
Ascorbic acid (Vit. C)	14mg
Carotenoids (β -carotenes)	5µg
Vitamin A	Traces
Vitamin E (Tocopherols)	0.011 µg

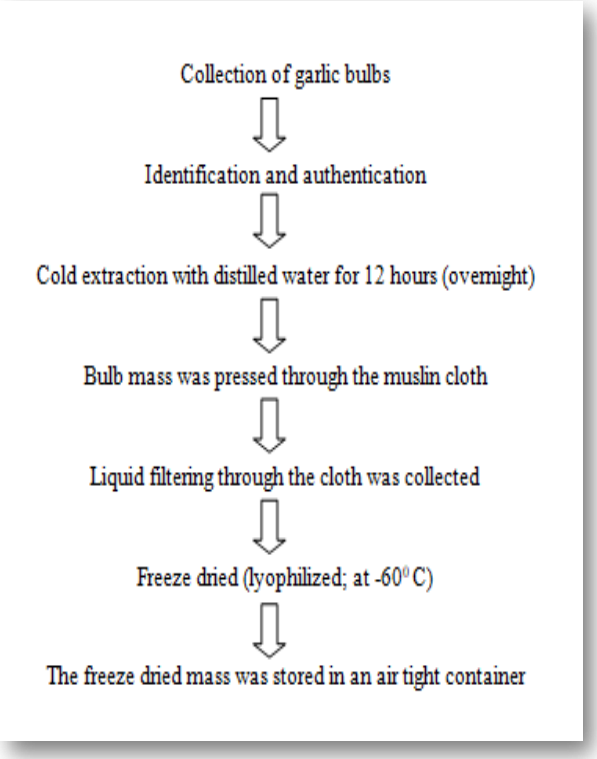
Excipients Used

- CARBOPOL 934
- HPMC K100M
- METHYL CELLULOSE
- MENTHOL CRYSTAL
- GLYCEROL
- POLYETHYLENE GLYCOL (PEG) 400
- ASPARTAME
- SODIUM BENZOATE
- CLOVE OIL

Adopted Methodology

- Collection of Plant Material
- Organoleptic Evaluation
- Macroscopic & Microscopic Evaluation
- Pharmacognostical Test for detection of organic constituents
- Formulation development and evaluation of garlic gel

Extraction Method



Chemical Tests for Detection of Organic Constituents

A. Test for Sulphur containing compounds

- Few drops of Sodium nitroprusside sol. was added to the garlic sample.
- Appearance of red & orange color might indicate the presence of sulphur containing compounds.

B. Test for Alkaloids

- **Dragendroff's Test:** The aq. extract was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate might indicate the presence of alkaloids.
- **Wagner's Test:** The aq. extract was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate might indicate the presence of alkaloids.

- **Hager's Test:** The aq. extract was treated with Picric acid. Formation of yellow precipitate might indicate the presence of alkaloids.
- **Mayer's Test:** The aq. extract was treated with Mayer's reagent. Formation of white precipitate indicates the presence of alkaloids.

C. Test for Flavonoids

- **Lead acetate Test:** The aq.extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate might indicate the presence of flavonoids.
- **NaOH Test:** The aq. extract treated with NaOH. Formation of yellow precipitate which de-colorized on addition of mineral acid.

D. Test for Phenolic and Tannins compounds

- **Ferric Chloride Test:** The collected bulb extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour might indicate the presence of phenols.
- **Gelatin Test:** 1% gelatin solution consisting of sodium chloride was added to the collected extract. Formation of white precipitate might indicate the presence of Tannins.

E. Test for Steroids

- **Salkowski's Test:** The garlic bulb extract was treated with chloroform and filtered. The filtrate was treated with few drops of Conc. H_2SO_4 solution, shaken and allowed to stand. Appearance of golden yellow colour might indicate the presence of Triterpenes

F. Test for Glycoside

- **Keller-Kiliani Test:** 2 ml of bulb extract, treated with glacial acetic acid, was added with one drop of $FeCl_3$ (5%) and conc.H_2SO_4. Reddish brown color appeared at the liquid interface. Upper layer appeared bluish green indicating the presence of glycosides.
- **Brontrager's Test:** The extract solution and Dil. H_2SO_4. (1:1) were boiled, filtered followed by the treatment of equal volumes of filtrate with chloroform. The organic layer was separated and treated with ammonium solution. Pinkish red color of the ammonia layer indicated the presence of glycoside.

G. Test for Carbohydrates

- **Benedict's Test:** A predetermined amount of garlic bulb extract was treated with Benedict's reagent and heated gently. Orange red precipitate might indicate the presence of reducing sugars.
- **Fehling's Test:** Fehling's A & Fehling's B solutions, each 1 ml were mixed and boiled, 2 ml extract was added, heated on water bath for 10 min. Appearance of yellow and then brick red precipitate.
- **Molish's Test:** It was performed as per official procedure as mentioned in book of standard. The appearance of deep violet colour (at the junction of liquids) indentified carbohydrate.

H. Test for Fatty acids & oil

- **Spot test:** Small amount of the bulb extract was pressed between two filter papers. Appearance of oily stain might indicate the presence of fixed oil.

FORMULATION DEVELOPMENT AND EVALUATION OF GARLIC GEL

- **Standard Preparation-**
  To prepare Allicin standard solution, this compound (5 mg) was dissolved in methanol (10 mL) for analysis. Standard solutions were injected (2, 5,

10, 15 and 20 ppm respectively) and run for calibration curves.

- **Sample Preparation-**
  Samples were dissolved in methanol and volume was made upto 10 mL. The afforded solution was filtered through 0.45 µm syringe filter prior to HPLC use.
- **Chromatographic analysis-**
  Standard & Sample products were measured and determined by ion-pair reversed-phase liquid chromatography (RP-LC) with UV detection at 210 nm. Chromatographed on octadecyl silane column [ODS C18 (250 x 4.6 mm id)] with gradient elution from 0.01M phosphate buffer (pH 2.5) with 5M heptanslfonic acid (mobile phase A) to 0.01M phosphate buffer (pH 2.5) acetonitrile (1:1) (mobile phase B).

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CHROMATOGRAPHIC CONDITIONS

Table 6: Various Chromatographic Conditions

S. No.	Parameters	Specification
1.	Column	octadecyl silane column [ODS C18 (250 x 4.6 mm id)]
2.	Mobile phase	0.01M phosphate buffer (pH=2.5) with 5M heptanslfonic acid (mobile phase A) to 0.01M phosphate buffer (pH=2.5) acetonitrile (1:1) (mobile phase B)
3.	Volume of injection	20 µl
4.	Detection	Ion-pair reversed-phase liquid chromatography (RP-LC) with UV detector
5.	Detected wave length	210 nm
6.	Retention time	5.105 min

DRUG-EXCIPIENT COMPATIBILITY STUDY

TLC Method

- TLC (thin layer chromatography) was performed by dissolving the dried extract of garlic bulb in methanol. The extract was spotted on silica gel GF254 plate.

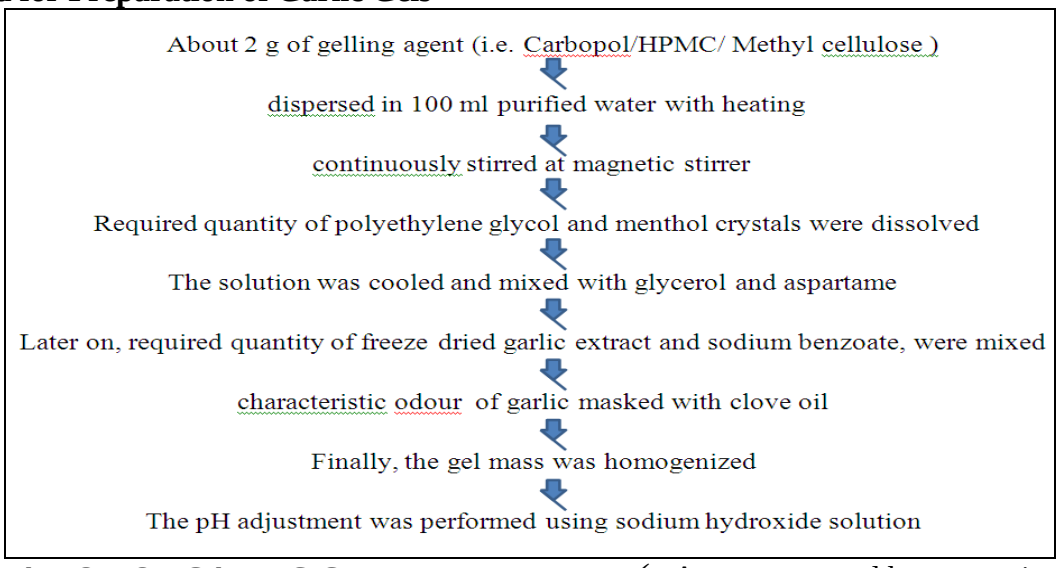
- **Mobile phase**: - A mixture of four organic solvents i.e. butyl alcohol, n-propyl alcohol, glacial acetic acid and distilled water (3:1:1:1) was used as mobile phase. TLC plate was prepared with the spots of garlic bulb extract alone and in combination with incorporated ingredients. After wards, the plate was dried and spots were detected by exposure to iodine vapors. The Rf values of various spots were calculated .

Formulation Design of Garlic Gels

Table 7: Formulation Design of Garlic Gels

S.No	Ingredients	Quantities		
		F_1	F_2	F_3
1.	Garlic freezed powder (extract)	1.5 gm	1.5 gm	1.5 gm
2.	HPMC	2 gm	-	-
3.	Carbopol	-	2 gm	-
4.	Methyl cellulose	-	-	2 gm
5.	Glycerol	1 gm	1 gm	1 gm
6.	Polyethylene glycol (PEG)	1.5 gm	1.5 gm	1.5 gm
7.	Menthol crystal	2 gm	2 gm	2gm
8.	Aspartame	1%	1%	1%
9.	Clove oil	5 drops	5 drops	5 drops
10.	Sodium benzoate	1%(w/w)	1%(w/w)	1%(w/w)
11.	Purified water	100 gm	100 gm	100 gm

Method for Preparation of Garlic Gels



EVALUATION OF GARLIC GEL FORMULATION

- Physical Evaluation
  - ✓ Clarity
  - ✓ Appearance and homogeneity
  - ✓ Grittiness
- Measurement of pH
- Spreadability

- Viscosity
- Extrudability
- In-vitro diffusion study
- Ex-vivo diffusion study
- Release kinetics
- Accelerated stability studies

RESULTS AND DISCUSSION

Organoleptic Evaluation

Table no. 8: Organoleptic Evaluation of Garlic Cloves

S.No.	Parameters	Garlic clove	
1.	Measurement and shape of garlic cloves	Length	3-4 cm
		Breadth	2-3 cm
		Shape	Partially kidney shaped
2.	Color		White
3.	External surface	Texture	Soft and shinning
		Arrangement of cloves	Macroscopically, each bulb has several cloves which arranged in concentric rings & enclosed in shinning white or pinkish Wrapper.
		Smell	Strong characteristic
		Taste	Characteristic Pungent

Fig. 2 Arrangement of garlic cloves in a bulb

Fig. 3 Cloves of garlic

Microscopic study: A light yellowish garlic powder studied microscopically. The powder showed numerous fragments of parenchyma and groups of spiral or annular vessels accompanied by thin-walled parenchyma.

Chemical Test for Detection of Organic Constituents

Table no. 9: Phytochemical Tests and Their Result

S. N0.	Chemical class	Tests	Inference
1.	Test for sulphar containing compounds	Sod. Nitroprusside test	+
2.	Alkaloids	Dragenderoff reagent	_
		Mayer's Test	_
		Wagner's Test	_
		Hager's Test	_
3.	Carbohydrates	Benedict's Test	+
		Molish's Test	+
		Fehling's Test	+
4.	Flavonoids	Lead acetate Test	+
		NaOH Test	+
5.	Test for phenolic and Tannins compounds	Ferric Chloride Test	+
		Gelatin Test	+
6.	Steroids	Salkowski's Test	+
7.	Glycoside	Keller-Kiliani Test	+
		Brontrager Test	+
8.	Test for fatty acids & oil	Spot test	+

HPLC analysis

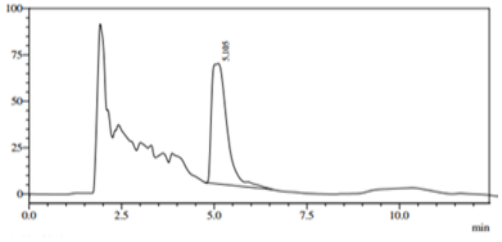


Fig 4: Chromatogram of pure Allicin (Standard)

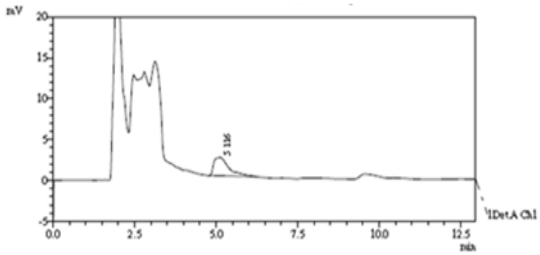


Fig 5: Chromatogram of Garlic extract (sample)

Detector A Ch1 210nm

Peak#	Name	Ret. Time	Area	Area %
1	Std_100ppm	5.105	1871222	100.000
Total			1871222	100.000

Fig 6: HPLC analysis of pure Allicin (Standard)

Detector A Ch1 210nm

Peak#	Name	Ret. Time	Area	Area %
1	Sample	5.116	72113	100.000
Total			72113	100.000

Fig 7: HPLC analysis of garlic extract (sample)

Standard Calibration curve of Allicin

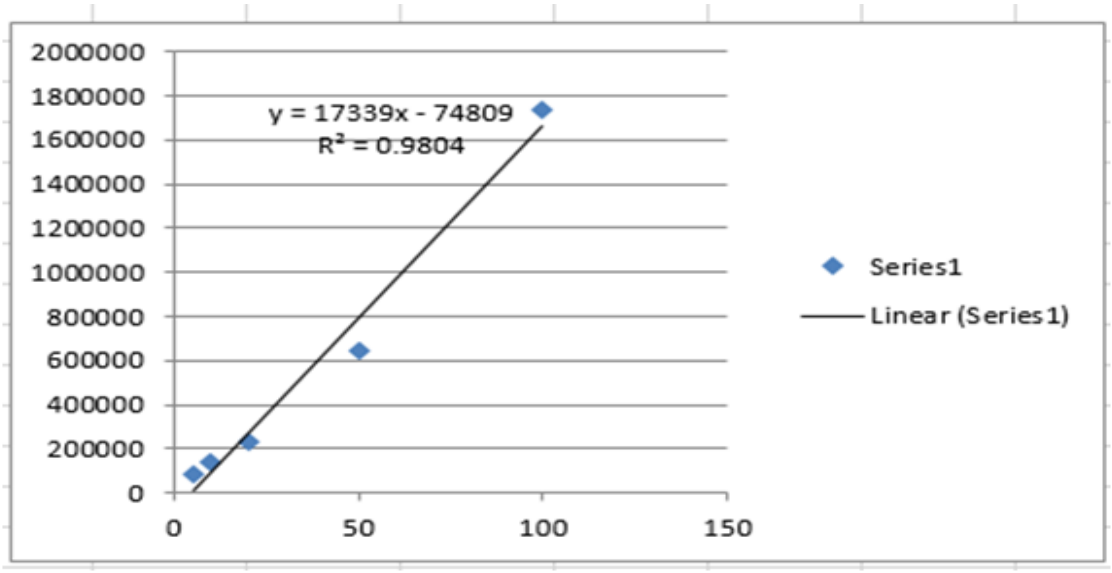


Fig. 8. Standard Curve of Allicin (Pure Compound)

The HPLC study thus conducted on prepared sample revealed that the Allicin content in garlic extract was found to be 0.5680 %.

Thin Layer Chromatography (TLC)

Table no. 10: Rf value of Different Combinations of Extract with Excipients

Spots	Samples	Rf value
A	Garlic (Extract)	0.8229
B	Extract : Carbopol	0.7941
C	Extract : HPMC	0.7647
D	Extract : Methyl cellulose	0.7411

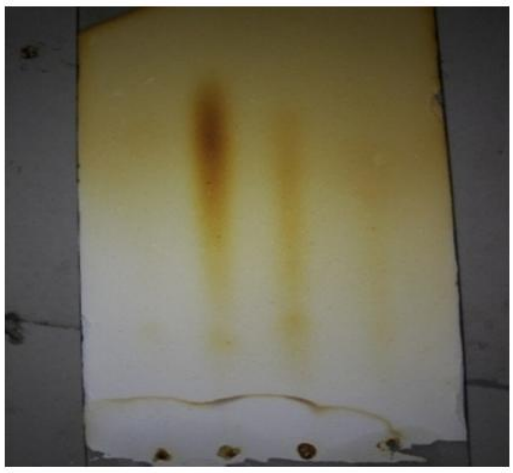


Fig. 9: Photographic representation of TLC of Garlic extract with different gelling agents

EVALUATION OF GARLIC GELS

Physical Evaluation

Physical Parameters	F_1	F_2	F_3
Clarity/color	Clear	Clear	Clear

Homogeneity	+++	+++	++
Grittiness	-	-	+

Spreadability	8.74 gm. cm /sec	8.73 gm. cm / sec	8.64 gm. Cm /sec

Odor & Taste

Parameters	**F_1**	**F_2**	**F_3**
Odor	+++	+++	+
Taste	+++	+++	++

Viscosity

Parameter	**F_1**	**F_2**	**F_3**
Viscosity	5428 cps	6649 cps	5764 cps

Measurement of pH

Parameter	**F_1**	**F_2**	**F_3**
pH	7.2	6.8	7.0

Extrudability

Parameter	**F_1**	**F_2**	**F_3**
Extrudability	++	+++	++

Note: (++ good, +++ very good, + presence, - absence)

Spreadability

Parameter	**F_1**	**F_2**	**F_3**

In-vitro Diffusion Study of the Garlic Gels

S.no.	Time (hrs.)	% drug release		
		F_1	F_2	F_3
1	0	0	0	0
2	2	6.48	7.14	6.35
3	4	11.34	12.4	10.24
4	6	18.45	19.57	16.22
5	8	23.86	24.95	22.58
6	12	38.69	40.49	39.56
7	14	47.98	52.02	46.20
8	16	56.55	59.14	56.64
9	20	70.67	73.25	68.82
10	24	79.49	81.89	77.98

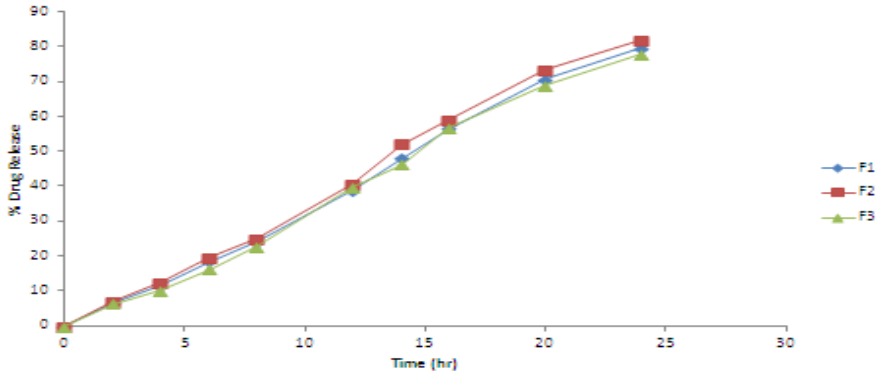


Fig 10: Percentage drug release profile of prepared garlic gels (F_1-F_3) in pH 6.8 phosphate buffer

On the basis of above results, an optimized batch (F_2) was selected which was further evaluated for ex-vivo diffusion, release kinetics and stability studies.

Ex-vivo Diffusion Study

S.no.	Time (hrs.)	%drug release of optimized batch (F_2) through goat tongue membrane (mean ± S.D)
1	0	0.000 ± 0.000
2	2	7.361 ± 0.113
3	4	13.905 ± 0.176
4	6	19.913 ± 0.171
5	8	25.808 ± 1.208
6	12	41.543 ± 1.945
7	14	53.100 ± 1.681
8	16	60.725 ± 1.830
9	20	75.174 ± 2.133
10	24	84.395 ± 2.115

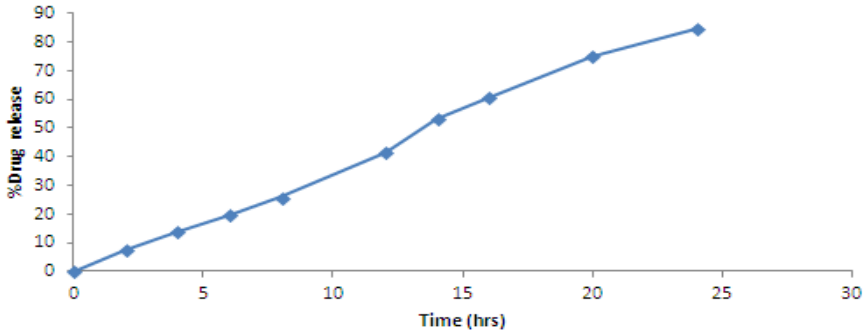


Fig no.11: Percentage drug release profile of optimized batch (F_2) through goat tongue membrane in pH 6.8 phosphate buffer (n=3)

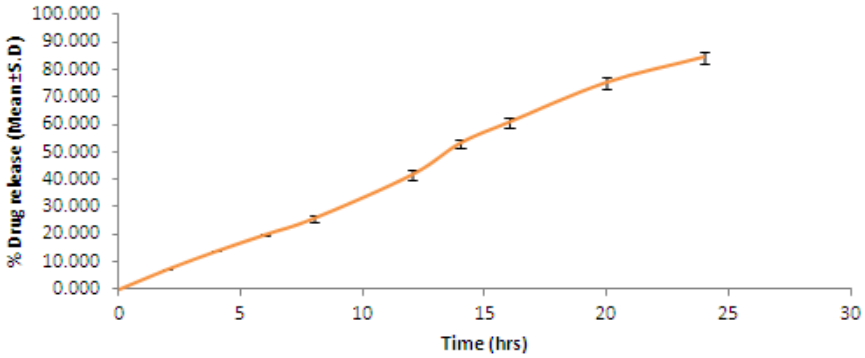


Fig no.12: Percentage drug release profile of optimized batch (F_2) through goat tongue membrane in pH 6.8 phosphate buffer (mean ± S.D values)

Release Kinetic Studies

Optimized Batch	Zero order		First order		Higuchi		Korsemeyer-peppas		Hixon Crowel	
F_2	Y	R^2	Y	R^2	Y	R^2	Y	R^2	Y	R^2
	3.661	0.990	0.064	0.748	1.576	0.909	1.328	0.944	0.016	0.962

Fig no. 13: Zero order drug release of optimized gel (F_2) in pH 6.8 phosphate buffer

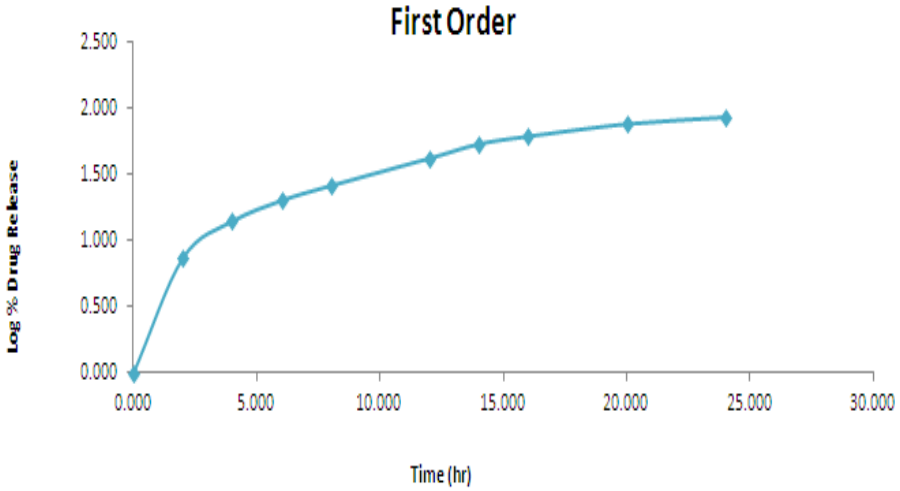


Fig no. 14: First order drug release of optimized gel (F_2) in pH 6.8 phosphate buffer

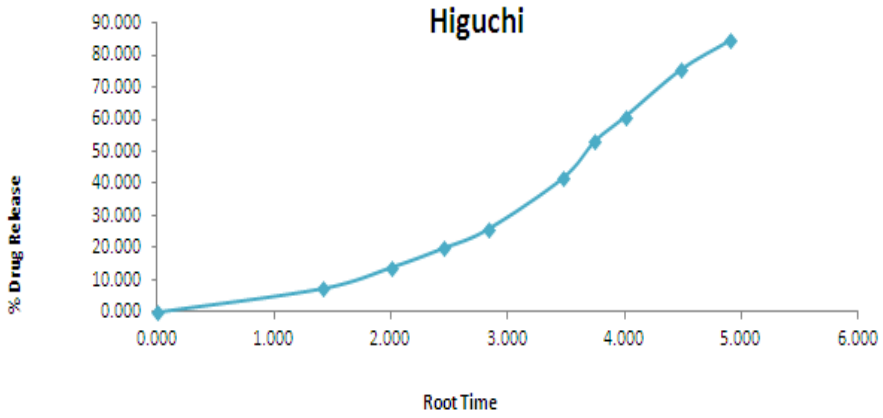


Fig no. 15: Higuchi drug release of optimized gel (F_2) in pH 6.8 phosphate buffer

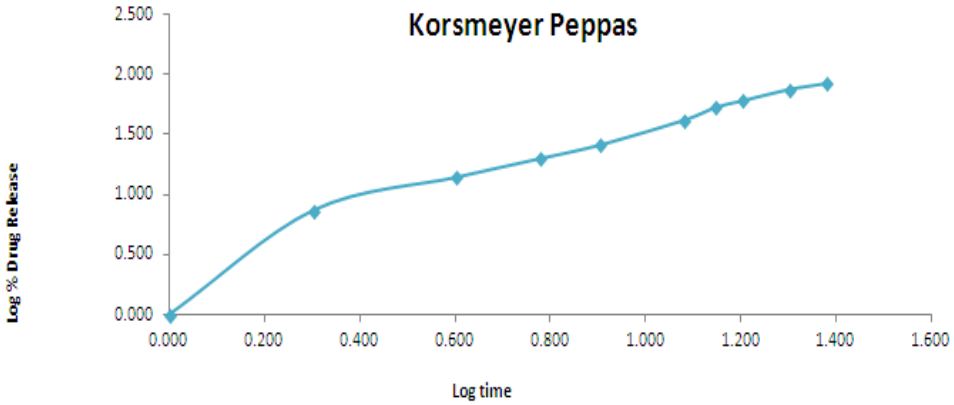


Fig no. 16: Koresemeyer peppas drug release of optimized gel (F_2) in pH 6.8 phosphate buffer

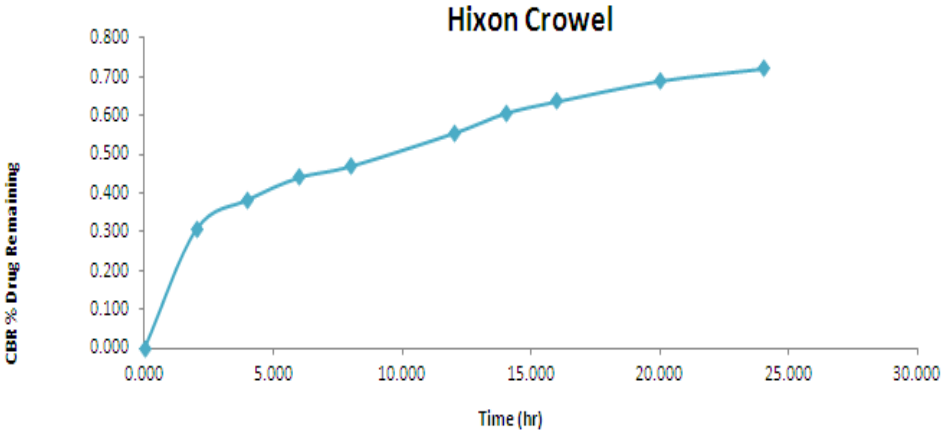


Fig no. 17: Hixon Crowel drug release of optimized gel (F_2) in pH 6.8 phosphate buffer

Stability Studies of Optimized Garlic Gel

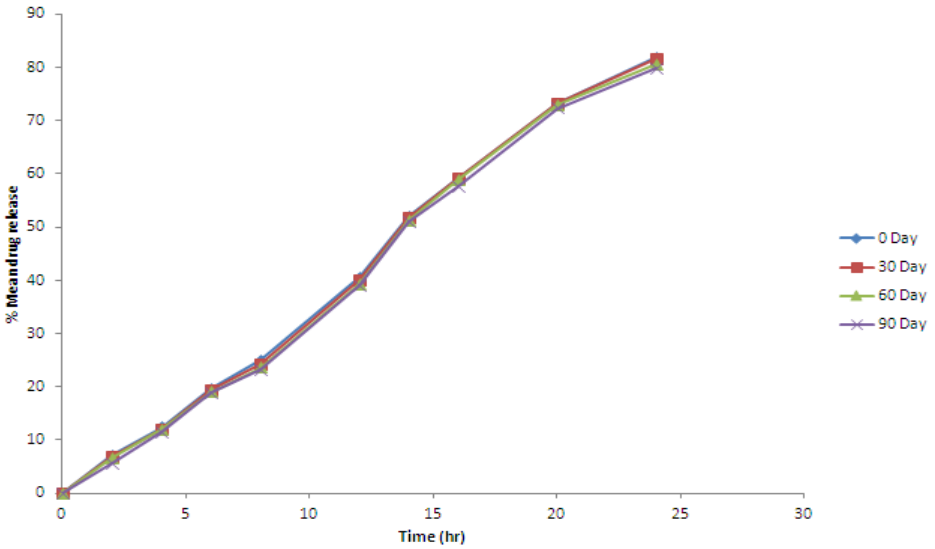


Fig no. 18: Comparative Release Profile of Optimized Batch (F_2) on Stability

CONCLUSION

- The research work was carried out aiming at side effects concerned with chemical entities, increasing the local healing and designing an inexpensive formulation for the treatment of tongue ulcers. Herbal formulations possess growing demands in the world market. In the present work, an attempt has been made to establish the antiulcer potential of Garlic extract presented in the form of herbal gel. Initially, aqueous garlic extract was obtained and evaluated for various phytochemical parameters. The extract was lyophilized (at -60°C) to get powder extract of garlic. Three batches of gel formulations were prepared by using different gelling agents *i.e.* HPMC, Carbopol and Methyl cellulose. HPLC analysis reflected % Allicin (active constituent) content (in garlic extract) as 0.568%. Prepared gels were evaluated for various test *i.e.* color & odor, pH, viscosity, texture and spreadability. The pH of prepared gels ranged between 6.8 - 7.2. The viscosity of prepared formulations was ranged between 5428 - 6649 cps. Percentage drug release from prepared gels was: 79.49%, 81.89% & 77.98% from F_1, F_2 and F_3 batches respectively (in pH 6.8 phosphate buffer) at the end of 24 hours, based on results.

Conclusively, the optimized batch formulation (F_2) prepared with Carbopol, was further subjected to release kinetic, ex-vivo and stability studies. Ex-vivo permeation study thus conducted on F_2 formulation revealed that about 84.395 ± 2.115% drug was released in pH 6.8 phosphate buffer through goat tongue membrane within 24 hours. The release data was fitted into various kinetics models i.e. Zero & First order, Higuchi and Koresemeyer-peppas which confirmed zero order kinetics. No significant changes were seen in optimized formulation upon storage under accelerated conditions (40°±2°C &

75%±5%RH) as it showed 79.93% release at the end of 90 days which indicated that the formulation was stable. Finally, it was concluded that such novel preparation would be beneficial in the treatment of tongue ulcer and could be applied to other ailments pertaining to diverse pharmacological spectrum widening the realm of numerous application venues.

REFERENCES

1. Joy P.P. et al. (1998) "*Book of Medicinal plants",* Aromatic and Medicinal Plants Research Station, Kerala. 3-5.
2. Onyeagba R, Ugbogu OC, Okeke CU, Iroakasi O. (2004) "*Studies on the antimicrobial effects of garlic (Allium sativum L.), ginger (Zingiber officinale Roscoe) and lime (Citrus aurantifolia L.)",* Afr. J. Biotechnol, 3 (10); 552-554.
3. Villamial M et al. (2010). *"A comprehensive survey on a garlic functionality*", Nova science publishers, 1-60.
4. Gebreyohannes G., Gebreyohannes M. (2013) *"Medicinal values of garlic: A review",* International Journal of Medicine and Medical Sciences. 5 (9); 401-408.
5. Hooda R., Tripathi M., Kapoor K. (2012) *"A review on oral mucosal drug delivery system",* The Pharma Innovation, 1, 14-22.
6. Paderni C., Compilato D., Giannola L. (2012) *"Oral local drug delivery and new perspectives in oral drug Formulation",* Elsevier Inc., 114, 25-34.
7. Santanu R., Singh D., Gupta R., Masih D. (2012) *"A Review on Phramaceutical Gel",* IJPRBS 1(5); 21-36.
8. Tangri P., Khurana S., Madhav S. (2011) *"Mucoadhesive Drug Delivery: Mechanism And Methods Of Evaluation",* International Journal of Pharma and Bio Sciences, 2, 458-467.
9. Mangold Aaron R., Torgerson Rochelle R., Rogers III Roy S. (2016), "*Diseases of the Tongue, Clinics in Dermatology",* www.researchgate.net
10. Zaher. R. A., Elsabaa H. M., Abou Elkhier T. M., Elhindawy M. M. (2014) *"Impact of Curcumin on Tongue Ulcer Healing in Albino Rats",* Mansoura Journal of Dentistry; 1(3), 85-89.
11. Maheswari T. N. U., Gnanasundaram N. (2010) *"Stress related oral diseases- A Research Study",* International Journal of Pharma and Bio Sciences, 6(6); 251-253.
12. arakji B., Gazal G., Al-Maweri S., Azzeghaiby S., Alaizari N. (2015) "*Guideline for the Diagnosis and Treatment of Recurrent Aphthous Stomatitis for Dental Practitioners*", Journal of International Oral Health, 7(5);74-80.
13. P. S. Subiksha (2014). *"Various Remedies for Recurrent Aphthous Ulcer- A Review",* J. Pharm. Sci. & Res. 6(6); 251-253.
14. Yalcin M. B., Unal M., Ozturk O. (2014) *"A Patient Having Recurrent Aphtous Stomatitis After Three Years of Smoking Cessation; A Case Report and Review of Literature",* J Addict Res Ther, 5(4); 202: 1-5.
15. Belenguer-Guallar. I., Jiménez-Soriano Y., Claramunt-Lozano A. (2014) *"Treatment of recurrent aphthous stomatitis. A literature review",* Pubmed J ClinExp Dent.6(2); e168-74.
16. Sakly A., De Wever B., Jutla B., Satia M., Pierre Bogaert J. (2016) *"The safety and efficacy of*

AphtoFix® mouth ulcer cream in the management of recurrent aphthous stomatitis", BMC Oral Health, 1-7.

17. D. Jyothi, Konald M. (2016) *"Formulation And Evaluation Of An Herbal Anti- Inflammatory Gel Containing Trigonella Foenum Greacum Seed Extract",* International Journal of Pharmacy and Pharmaceutical Sciences, 8 (1); 41-44.
18. Thorat S. Y., Sarvagod A. M., Kulkarni V. S., Hosmani H. A. (2015) *"Treatment of mouth ulcer by curcumin loaded thermoreversible mucoadhesive gel: A technical note",* International Journal of Pharmacy and Pharmaceutical Sciences, 7(10); 399-402.
19. Sarkar B., Devgan M., Chowdary Y. A., Ramaiah M. (2015) *"Formulation and Evaluation of Herbal Gel Containing Extract of Cedrus deodara"* www.ijpcsonline.com 4(1); 67-70.
20. Abu Shaqra Q. M, Abu Shaqra Q. A. (2015) *"In vitro Inhibition of Candida Species by Aqueous Garlic Extract in Gel and Lotion: Problem of Stability"* International Journal of Pharmaceutical Sciences and Drug Research, www.researchgate.net 7(3); 304-307.
21. Z. Wencui. Qi Z., Wing Y., Di W. (2015) *"Preparation of solid lipid nanoparticles loaded with garlic oil and evaluation of their in vitro and in vivo characteristics",* Eur Rev Med Pharmacol Sci, 19 (19); 3742-3750.
22. Fernandes R., Tuckey T., Lam P., Allidina S., Sharifi S., Nia D., "*The Best Treatment For Aphthous Ulcers. An evidence-based study of the literature*", 1-7.
23. Khidir Agab mohd. H., Khalil S. (2013) *"Liquorice mouth wash as treatment for mouth ulcer",* Elsevier www.pharmanest.net 4 (3); 335-340.
24. Johnson O. O., Ayoola G A., Adenipekun T. (2013) *"Antimicrobial Activity and the Chemical Composition of the Volatile oil Blend from Allium sativum (Garlic clove) and Citrus reticulate (Tangerine fruit)",* IJPSDR 5 (4); 187-193.
25. Sheikh S., Asghar S., Ahmad S. (2013) *"Development of HPTLC Method and Its Validation for the Estimation of Curcuminoids from Polyherbal Mouth Ulcer Gel Formulation",* IOSR Journal of Pharmac, 3(1); 29-34.
26. Rajam K., Rajendran S., Saranya R. (2013) *"Research Article: Allium Sativum (Garlic) Extract as Nontoxic* Chemistry, 1-4.
27. Sampath Kumar. K. P., Yadav A., Srivastava S., Paswan S., Dutta Sankar A., Bhowmik
28. D. (2012) *"Recent Trends in Indian Traditional Herbs Syzygiumaromaticum and its Health Benefits",* Journal of Pharmacognosy and Phytochemistry. 1; 13-22.
29. Rahman, M. M., Fazlic, V., Saad, N. W. (2012) *"Antioxidant properties of raw garlic (Allium sativum) extract",* International Food Research Journal, 19(2); 589-591.
30. Hegde Mithra N., Hegde N. D., Puri A., Bhat R. (2012)*"Differential Diagnostic Techniques For Long Term Tongue",* IRJP 3 (8); 145-148.
31. Kudva S., Prabhakar S., Pai V., Tegginamani A. (2012) *"Effects of garlic extract on salivary pH: a clinical study",* Archives of Orofacial Sciences, 7 (1); 1-8.
32. Karavana Y. S., Gokçe H. E., Rençber S., Ozbal S., Pekçetin C., Guneri P., Ertan G. (2012) *"A new approach to the treatment of recurrent aphthous stomatitis with bioadhesive gels*

containing cyclosporine A solid lipid nanoparticles: in vivo/in vitro examinations", International Journal of Nanomedicine, 7, 5693–5704.
33. Bhaskr Radhika, Singla V., Bhaskar R. (2011) *"Garlic: A review"* International Journal of Drug Formulation & Research. 2 (2) 94-110.
34. Carina-Magalhaes E. D., Quirino M., Patrocínio M., Anbinder A. (2011) *"Effects of Chamomilla recutita (L.) on oral wound healing in rats",* Med Oral Patol Oral Cir Bucal. 16 (6); 716-721.
35. Wanyika N. H., Gachanja N. A., Kenji M. G., Keriko M. J., Mwangi N. A. (2011) *"A Rapid Method Based On UV Spectrophotometry For Quantitative Determination Of Allicin In Aqueous Garlic Extracts",* Journal of Agriculture, Science and Technology, 74-82.
36. Bodhankar. M. M., Dr. Patil A. T., Agnihotri V. (2011) *"Development of topical formulations containing bioactive volatile oils: relative characterization of drug release for cream, ointment and gel",* International Journal of Pharmacy & Technology, 3 (3), 3165-3174.
37. Das. S., Haldar K. P., Pramanik G. (2011) *"Formulation and Evaluation of Herbal Gel Containing Clerodendron infortunatum Leaves Extract"* International Journal of PharmTech Research 3 (1); 140-143.
38. Dosani M. A., Sakarkar D.M, Kosalge S.B, Sheikh S. (2011) *"Formulation Development and Evaluation of Unit Moulded Herbal Semisolid Jelly useful in treatment of Mouth Ulcer",* International Journal of PharmTech Research, 3 (3); 1705-1713.
39. Yilmaz. N., Nisbet O., Nisbet C., Ceylan G., Hoşgör F., Dede O. (2009) *"Biochemical Evaluation Of The Therapeuti Effectiveness Of Honey In Oral Mucosal Ulcers",* Pubmed 9 (4); 290-295.
40. P. Bocchini, Andalò C., Pozzi R., Galletti C. G., Antonelli A. (2001) *"Determination of diallyl thiosulfinate (allicin) in garlic (Allium sativum L.) by high-performance liquid chromatography with a post-column photochemical reactor",* www.elsevier.com 37–43.
41. Serge A., Mirelman D. (1999) *"Antimicrobial properties of allicin from garlic",* Microbes and Infection © Elsevier, Paris 2, 125−129.
42. Villamiel M.,Cardelle-CobasA.,CristinaSoriaA., Corzo-Martínez M (2010)*"A Comprehensive Survey of Garlic Functionality", www.researchgate.net* Nova Science Publishers, 1-60.Pubchem.com/Carbopol/HPMC
43. Wikkipidea.com/clove oil/sodium benzoate/aspartame/PEG 400/menthol crystal
44. Drugbank, Accession Number (D00554)/ methyl cellulose
45. www.fao.org/ag/agn/jecfa-additives/ clove oil/methyl cellulose
46. Raymond C Rowe, Paul J Sheskey, Marian E Quinn, *"Handbook of Pharmaceutics experience",* 6th edition, 627-629.
47. Kokate C K., Purohit A P. and Gokhale S B. (2012) *"Pharmacognosy",* Nirali Prakashan, 20th edition, Pune, 4.01-4.08.
48. *Indian Pharmacopoeia* (2014) Published by The Indian Pharmacopoeia Commission Ghaziabad 7 edition (III); 3236-3237.
49. Khandelwal K. R. [2008] *"Practical Pharmacognosy Technique and Experiments",* Nirali Prakashan, 9th edition, March.

50. Abdul Ghani J. Mohd. (2010) *"Determination of Alliin and Allicin in different types Garlic using High Performance Liquid Chromatography",* J. of University of Anbar for Pure Science, 4, (2), 1-8.
51. Grace X F., Vijetha R., Raj S R, Latha S, Shanmuganathan S. (2015) "*Formulation and Evaluation of Polyherbal Anti-Acne Gel",* Adv J Pharm Life Sci Res, 3;1:5-8.
52. Aiyalu A., Govindarjan A., Ramasamy A. (2016) "*Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal mode",* Braz. J. Pharm. Sci. 52 (3); 493-507.
53. Misal G, Dixit G, Gulkari V. (2012) *"Formulation and Evaluation of Herbal gel",* IJNPR 3(4); 501-505.
54. Haneefa KP MD, Hanan K. S, Mohanta G, Nayar C. (2010) *"Formulation and evaluation of herbal gel of Pothos scandens Linn",* Asian Pacific Journal of Tropical Medicine, ScienceDirect 988-992.
55. Patil S.C., Gadade D.D, Rathi P.B. (2015) *"Design, Development and Evaluation of Herbal Gel for Treatment of Psoriasis",* JIPBS, Vol 2 (1); 72-87.