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Therapeutic Study of Garlic Gel Formulation for Tongue Ulcer Healing

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ABSTRACT

The medicinal potency of garlic has been were established and practiced by the people since 1500 BC. Greek physicians, Hippocrates and Galen 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 people have been using garlic for the treatment of diarrhea abdominal disorders and infections of respiratory tract. In Indian subcontinent, people have been using it 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, its applicability in tongue ulcer has not yet been mentioned in literature. Thus the present worker prepared the herbal gel formulation of garlic extract for tongue ulcer. Initially, aqueous garlic extract was prepared from fresh garlic cloves. Prepared extract was evaluated for various pharmacognostical parameters. Afterwards, the prepared extract was lyophilized (at -60°) to get dried extract of garlic which was further used for the formulation of herbal gel. The active constituent (i.e. Allicin) in garlic extract was estimated by HPLC and found to be 0.568%. Compatibility of drug with different gelling agents was determined by TLC study. The Rf value of Allicin (pure extract) was 0.8229 while in combinations with different ingredients were 0.7941 (Extract: carbopol), 0.7647 (Extract: HPMC) and 0.7411 (Extract: Methyl cellulose). Conclusively, garlic extract was found compatible with different gelling agents used in gel formulation. The herbal gel of garlic was prepared in triplicate with different gelling agents i.e. HPMC, Carbopol & Methyl cellulose and evaluated for various physicochemical parameters. In vitro diffusion study revealed that the percentage drug release ranged between 79.49 - 81.89% (in pH 6.8). On the basis of above studies and excellent bioadhesiveness of Carbopol, the optimized batch formulation (F2) was selected for ex-vivo & stability studies further.

Keywords*: Garlic Gel, Therapeutic formulation, Tongue Ulcer, Herbal Gel, Gel Formulation.*

INTRODUCTION

Majority of the population feel unaffordable for the products of western pharmaceutical industries and thus exert reliance over the traditional medicines belonging to the botanical origins. Although the developing countries have been estimated to spend 40-50 % of total budget on drugs and health care, 75% of the 3rd world population utilize plant drugs as the modern life saving entities are inaccessible to them. Consequently, strategic developments have been focusing on to reduction in financial burden on developing countries by encouraging the use of herbal remedies which would be creating novel future therapeutic arena. Ayurvedic system of medicines utilize plants possessing biologically potential molecules giving rise to lead structures from which modified effective molecules can be derived to exert significant therapeutic roles with reduce toxic parameters. Vinblastine, vincristine, taxol,

podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine are potentially known compounds obtained from herbal origins. However, crude extracts of many plants also serve as medicaments. Isolation and identification of active constituents, elucidation of their molecular characteristics and establishing the mechanisms of actions, have become relevant in inviting the attention of personnel involved in herbal formulations and researches. When the active molecules are difficult to get synthesized economically, the plant resources become more important to be cultivated from where such effective molecules can be isolated and used therapeutically.

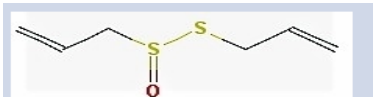
GARLIC AND ITS MEDICINAL BACKGROUND

Garlic (*Allium sativum L.*) is regarded as a plant which was exhaustively investigated for its medicinal values over several decades and being used against infectious diseases. It has been used as food ingredient in cooking due to its own characteristic aroma, enhancing flavor, condiment, protective and digestive properties. Besides, it has been used as folk medicine also. 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, hay fever, asthma, as antimicrobial agent and to relieve from traumatic inflammation as well as for wound healing.

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 & II, scordinines A & 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 1: 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 2: 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

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 cm2). 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:

1. Salivary secretion and mechanical stress bringing about rapid loss of the applied drug (dosage form) from the site of absorption.
2. 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.
3. Non-agreeable taste and consequent oral sensation, associated with the incorporated drug leading to reduced patient compliance.
4. 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.

Advantages of Oral Mucoadhesive Drug Delivery System:

1. Enhances the residence time
2. Enhances absorption determining drug efficacy
3. Excellent accessibility
4. Faster absorption attributed to rich blood supply and circulation rates
5. Increased drug bioavailability
6. Surpassing GIT degradation

7. Easy drug administration
8. Rapid onset of action
9. Apparent patient compliance

Adhesive Semisolid Systems (Gels, Ointments)

According to U.S.P., gels have been defined as a semisolid dispersion system containing inorganic particles (smaller size) or organic molecules (large size) which may either enclosed the liquid molecules or may get interpenetrated by the molecules of continuous phase liquid. Semisolid dosage forms *i.e.* gels or ointments can be easily dispersed throughout the oral mucosal surface and attach intimately to the mucosal membrane inducing faster release of the medicament despite the fact that the drug dosing is not accurate as compared to the other oral dosage forms i.e. tablets, films, patches *etc*.

Bioadhesive formulations have overcome the lower retention time of the applied gels. However the residence time of gels is lesser as the body fluids i.e. saliva wipes them from the functional site. Consequently the drugs with narrow therapeutic window have got limited utility.

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.

Types of Oral Ulcers

1. Minor apthous ulcers
2. Major apthous ulcers
3. 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:

1. Disturbed immune system Injury
2. Hormonal changes
3. A lack of iron
4. Certain vitamins deficiency
5. Food hypersensitivity
6. Allergy
7. Genetic factors
8. Stress/anxiety
9. Tobacco consumption
10. Medications

Table 3. Management and Treatment of Tongue Ulcer

S. No.	Category	Drugs and Formulations
1	Antiseptc, anti-inflammatory and analgesic drugs	Chlorhexidine mouth rinse or gel 3x1, triclosan gel 3x1; topical diclofenac %3 3x1; Amlexanox %5 2-4 x1
2	Antibiotics	Tetracycline, Cephalexin, Azithromycin susp.
3	Topical corticosteroids	Triamcinolone acetonide %0.05-0.5 3-10 x1, fluocinolone acetonide %0.025- 0.05 5-10 x1; Clobetasol Propionate %0.025 3-5 x1
4	Hyaluronic acid	0.2% gel 2 x1
5	Topical anesthetics	Topical lidocaine %2-5 , mepivacaine %1,5, Tetracaine %0,5-1 spray or gel, mouth wash solution containing Benzocaine and Cetylpyridinium chloride
6	Others	Sucralfate suspension, Laser, Cauterization, quercetin, myrtle, rosa damascene.

Table 4. Preferred Local Pharmaceutical Treatments

S. No	Category	Drugs & formulation
1	Antibiotics	Penicillin G Potasium, 50 mg tb 4 x1, 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	Mercuriussolubilis, Phosphorus, Sulphuric acid, Nitric acid.

NUTRITIONAL SUPPLEMENTS

Based on the concept of traditional measures various nutritional supplements have resulted in satisfactory management of oral ulcers. However these have not been proven with scientific supports.

1. Vitamins: B1, B2, B6 and B complex on daily basis.
2. Aloe (Aloe vera): Aloe vera juice (1–3 tsp) as mouthwash.
3. Licorice (DGL) (from Glycyrrhizaglabra): mixture of powdered DGL (200 mg) and warm water (200 ml).
4. Chamomile (Matricariarecutita): A diluted tincture or strong tea made from chamomile flowers.
5. Echinacea (Echinacea purpurea, E. angustifolia, E. pallida): liquid echinacea (4 ml) can be mixed with warm water.
6. Myrrh (Commiphoramolmol): herbal extract (200–300 mg) of myrrh with warm water.
7. Other herbs: Neem, Curcumin, Clove oil etc.

LITERATURE REVIEW

1. Sakly A. *et al*. (2016) evaluated the in vitro biocompatibility of cream AphtoFix which was developed for the treatment of mouth ulcer as per ISO guidelines (10993). Human gingiva was used as an experimental model. 20 subjects were evaluated for the study by the application of this cream into the oral cavity. The safety and efficacy were determined by the application of cream on 19 patients of RAS. The post-marketed clinical efficacy demonstration showed that the size and pain of ulcer reduced after 3 days of the treatment ($p < 0.05$).
2. 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. The study included carrageenan induced rat paw edema model. Conclusively, the prepared gel revealed high anti-inflammatory potential and thus could be effectively used as herbal gel in such treatments.
3. 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. The optimized batches were found to deliver the drug upto the maximum concentration in about 4 hours. Results revealed the residence time and

contact area (at the ulcer) to have significantly increased and also witnessed sustained release of the drug.

4. Sarkar B., *et al.* (2015) formulated and evaluated the herbal gel containing Cedrus Deodara extract. Ethanol extract and other excipients were added to the pre-determined amount of hydrated carbopol dispersion followed by continous stirring. pH, Spreadability, Drug content uniformity, Viscosity and In vitro diffusion studies were carried out. The spectral analysis was also performed to check compatibility and drug integrity throughout the process. The result was found to be promising.
5. 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. The focus was antimicrobial potential and stability parameter investigations of garlic extract in topical formulation. prepared gel and lotions contained 50, 100 and 200 mg garlic/ml. Well diffusion and Muller Hinton agar methods were used to perform anti- candidal evaluation of the prepared formulation. The results concluded that the freshly prepared gel produced promising inhibitory effects but, upon storage, the potential significantly reduced. Moreover, the lotion did not exhibit satisfactory anti candida effects probably due to lost potential in the course of lotion formulation.
6. Z. Wencui. *et al.* (2015) prepared garlic oil loaded SLN (solid lipid nanoparticles) by high pressure homogenization technique. The entrapment & loading efficiency including. SLN recovery were insured by orthogonal experimental method with optimizing concept. SLNs were administered orally into the rats and pharmacokinetic parameters were studied by using LC/MS/MS method.
7. Subiksha P. S. (2014) reported that recurrent aphthous stomatitis (painful oral ulcers) could be cured with 5% Amlexanox. It exhibited triple action viz; reduced pain & healing time and prevented the recurrences.
8. Zaher. R. A. *et al.* (2014) studied and evaluated the effect of curcumin, derived from curcuma longa, on tongue ulcer in rats. 60 male rats were divided into two groups i.e. control (CG) and curcumin treated (CrG) groups (200mg\kg suspended in 0.5% CMC). The tongue ulcer was produced by using round shaped filter paper (5.5 mm in diameter) soaked in 15 ml of 50% acetic acid and pressed on the tongue. Randomly 6 rats, of each group, were sacrificed on the day 3, 6, 9, 12 and 15. Result showed CrG possessed smaller ulcer size clinically, histologically and immune histochemically with faster healing rate. So it could be concluded that curcumin helped in the healing of oral ulcer by increased release of TGF-β and α-SMA.
9. Belenguer-Guallar I. *et al.* (2014) reported the strategic treatments of recurrent aphthous stomatitis (RAS) which occurred due to vitamin deficiencies. Some authors had been found to treat aphthous by vitamin B12 and vitamin C leading to a significant decrease (50%) RAS outbreaks. A broad range of local medications such as antiseptics (chlorhexidine), anti-inflammatory drugs (amlexanox), antibiotics (tetracyclines) and corticosteroids (triamcinolone acetonide) had been used frequently. In the constant intensive painful major aphthae the systemic therapy was recommended in the form of corticosteroids (prednisone), colchicine, dapsone, clofazimine, pentoxifylline, zinc sulfate etc. The immunomodulating agent, thalidomide was found best tolerated.

10. Fernandes. R. *et. al.* several databases, research articles, dental textbooks and investigated personal opinion of oral pathologists for screening the best treatment for aphthous ulcers. It was conclusively inferred that 5% Amlexanox exerted triple action i.e. decreased healing time, preventive recurrences and accelerated pain resolution.
11. Gebreyohannes. G. *et al.* (2013) reviewed and reported garlic products for their therapeutic efficacy and safety parameters. The chemical constituents of garlic were studied with reference to their applications for the treatment of cardiovascular, cancer, diabetes, blood pressure, atherosclerosis and hyperlipidemic cases. The efforts revealed significant performance of such products and the report aimed at apprising the future researchers regarding their medicinal spectra.
12. Khidir Agab mohd. H. *et al.* (2013) investigated liquorice mouth wash intended for the treatment for oral ulcers. Mostly the ulcers recovered from the first day of application and almost healed after the 3rd day. Conclusively, the liquorice mouth wash could be effective remedy for treatment and management of mouth ulcers.
13. 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. Steam distillation process was practiced in the research using Clevenger hydrodistillator. Equal volumes of the extracted volatile oils were blended and evaluated for antimicrobial potential. Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans, Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922) and Candida albicans (ATCC 90028) isolates/cultures were used. GCMS technique was adopted to identify the major components in both the extracted volatile oils. The study revealed higher antimicrobial potential associated with the volatile oil blend which could be exploited for the exploration of new effective antimicrobial entities.
14. 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. The method involve silica gel G. coated aluminium plates as stationary phase and a solvent blend containing Chloroform : methanol : glacial acetic acid (7.5 : 2 : 0.5 v/v) as mobile phase. Bisdemithoxy curcumin, Demethoxy curcumin and curcumin itself resulted Rf values as 0.18, 0.31 and 0.56 respectively.
15. Rajam K. *et al.* (2013) studied aqueous garlic extract as nontoxic corrosion inhibitor by mass-loss method. The formulation contained 2 ml garlic extract and Zn2+ (25 ppm) which offered 70% inhibition efficiency to carbon steel immersed in water. Polarization study revealed that the formulation controlled anodic reaction predominantly. The FTIR results exhibited the formation of protective layer of Fe2+ -allicin complex and $Zn(OH)_2$.
16. Sampath Kumar. K. P. *et al* (2012) illustrated the health benefits of clove (Syzygium aromatic). Since 2000 years, India and China have been using clove as a condiment for the treatment of tooth decay and bad breath. Clove has been mentioned, to possess antiseptic, antibacterial, antifungal and antiviral properties, in German Commission E Monographs. The main

constituents, eugenol, possessed broad antimicrobial activities against Gram-positive, Gram-negative and acid-fact bacteria as well as fungi. It also possessed antiemetic, flatulence and carminative activities. Traditionally it is used for the treatment of diarrhea, most liver, stomach and bowel ailments, malaria, cholera, tuberculosis, worms, viruses, candida, various bacterial and protozoan infections. The volatile oil of cloves (about 85-92% eugenol) was highly bactericidal. Clove is also used for the treatment of premature cancer.

17. 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).
18. Hegde Mithra N. *et al.* (2012) investigated differential diagnostic techniques for long term tongue viz; common ulcerative disorders like aphthous& traumatic ulcers and squamous cell carcinoma. Such cases were treated by lubricating the oral mucosa with CocusNucifera (coconut oil) which possessed Squaline, tocopherol, phytosterols and other sterols. Such constituents reduced the inflammation, eczema and rashes of the tongue/oral mucosa.
19. Kudva. S. *et al.* (2012) determined the effect of garlic incorporated in mouthwashes. There occurred decrease in the salivary pH with caries-resistant effects due to its antimicrobial potential. The test volunteers were categorized as: garlic, chlorhexidine, combination (garlic combined with chlorhexidine) and control (water rinse) groups. The best benefit was observed with combination mouthwash comparable to the garlic and chlorhexidine mouthwashes individually.
20. Karavana Y. S. *et al.* (2012) formulated a solid lipid nanoparticulate bioadhesive gel of cyclosporine-A intended for the treatment of recurrent aphthous stomatitis. CsA-loaded SLNs were prepared by high shear homogenization method using Compritol 888 ATO (C888), CsA, poloxamer 188, Tween 80 and distilled water. The in vivo studies were carried out in rabbits. Histopathological observations were made on days 3, 6, 9, and 12. The results revealed the promising wound healing effects associated with the animals treated with Cs-A SLNs.
21. Bhaskar R. *et al.* (2011) reviewed different possible therapeutic mechanisms of garlic in their investigation reports. Various extraction methods and evaluative parameters i.e. its constituents, stability and dissolution, of garlic tablets were studied.
22. Carina-Magalhaes E. D. *et al*. (2011) evaluated the oral ulcer healing potential of Chamomilla recutita. A lesion (5mm) was created on the tongue of the experimental rats. The test group animals were treated with 0.04 mL/day of the herbal ointment while the control group were left untreated. After 3, 7 and 10 days, rats were sacrificed and studied for the magnitude of wound size, fibroblast count and area of inflammation. The histopathology involved estimation of collagen fibers and re- epithelialization of the created wounds. The results revealed promising wound healing effects.
23. H. N. Wanyika, *et al.* (2011) developed and validated for UV spectrophotometric method for the quantitative determination of allicin in aqueous garlic extract. Allicin was extracted from the garlic bulb using water. The study revealed rapid estimation of allicin from the extract which was extrapolated to determine

allicin contents in marketed pesticides.

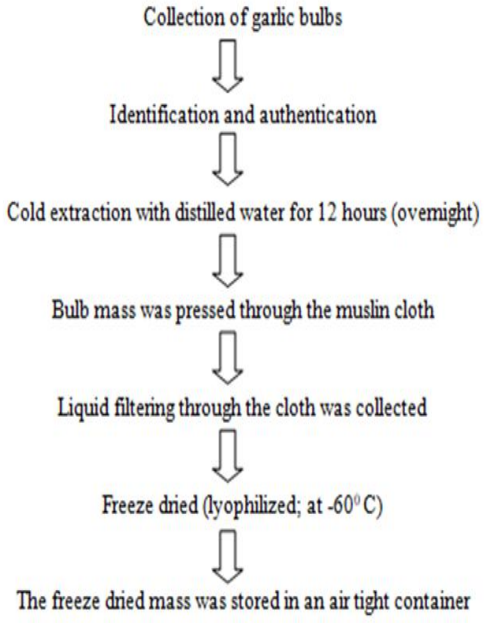
24. Bodhankar. M. M. *et al.* (2011) developed & characterized topical formulations (cream, ointment, gel) containing bioactive volatile oils of allium sativum and zingiber officinale. Drug release studies were compared. The results showed that the percentage release of oils through micro porous membrane was significantly higher with carbopol gel as compared to ointments and creams.
25. Das. S. *et al.* (2011) formulated and evaluated the herbal gel containing Clerodendroninfortunatum extract. The formulation involved aqueous extract of leaves of the chosen plant in variable concentrations (2.5% and 5%) and evaluated. The gel was prepared by using various polymeric bases i.e. Sodium CMC &Carbopol 934 besides PEG 400, methyl & propyl paraben with sufficient distilled water. The formulation containing 2.5% Clerodendroninfortunatum leaf extract gave promising results as far as stability and skin irritancy parameters were concerned.
26. Dosani M. A. *et al.* (2011) formulated and evaluated semisolid herbal jelly for the treatment of mouth ulcer by using glycyrrhizaglabra, jasminum officinale & menthe piperita extracts, pectin, tragacanth gum, gelatin, citric acid, sugar syrup, propylene glycol, sodium benzoate, amaranth and nutrient agar. Altogether eight batches were prepared and evaluated. According to statistical analysis of the obtained results (student's t-test) for unpaired samples in carrageenan induced rat paw edema. The optimized formulation containing glycyrrhizaglabra, jasminum officinale & menthapiperita extracts showed anti-inflammatory activity substantially equivalent to the marketed preparation, as per ICH guidelines. The optimized formulation was found stable for the period of 3 months.
27. Yilmaz. N. *et al.* (2009) evaluated the therapeutic effectiveness of honey in oral mucosal ulcer healing and compared with glyceroloxytriester (TGO) by creating excisional wound on the oral mucosa of thirty wistar rats. Three groups were prepared,the first group was locally treated with honey, second with TGO and third (controlled) group was left untreated and fed with distilled water. From day 7, the surgical process followed. The sample, for biopsy, was taken from left buccal mucosa of rats on day 14. The hydroxypyroline levels were measured and the obtained data were analysed statistically. The result concluded honey to possess more therapeutic effectiveness than TGO in oral mucosal ulcers.
28. Onyeagba R. A. (2004) studied antimicrobial effects of garlic, ginger and lime juices determined Staphylococcus aureus, Bacillus spp., Escherichia coli and Salmonella. The ethanolic and aqueous extracts (garlic and ginger) were found inert to the test organism. The highest inhibition zone (19 mm) was observed with a combined extracts (on Staphylococcus aureus).
29. 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. The one sided post column irradiation (254nm) decreased allicin response to the UV detector while or the other side it was eluted satisfactorily. Conclusively, linearity response was observed in the range of 1–8 mg/l.
30. Serge Ankri *et al.* (1999) reported allicin (from garlic) to possess antibacterial potential towards Gram-negative as well as Gram-positive bacteria. Moreover, antifungal (against Candida albicans), antiparasitic

(Entamoebahistolytica and Giardia lamblia) and antiviral potentials were also reckoned.

ADOPTED METHODOLOGY

1. Collection of Plant Material
2. Organoleptic Evaluation
3. Macroscopic and Microscopic Evaluation
4. Pharmacognostical Test for detection of organic constituents
5. 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

1. **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.
2. **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.
3. **Hager's Test:** The aq. extract was treated with Picric acid. Formation of yellow precipitate might indicate the presence of alkaloids.
4. **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

1. **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.
2. **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

1. **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.
2. **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

1. **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

1. **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.
2. **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

1. **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.
2. **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.
3. **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

1. **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.
2. **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.
3. **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).

Table 5. 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

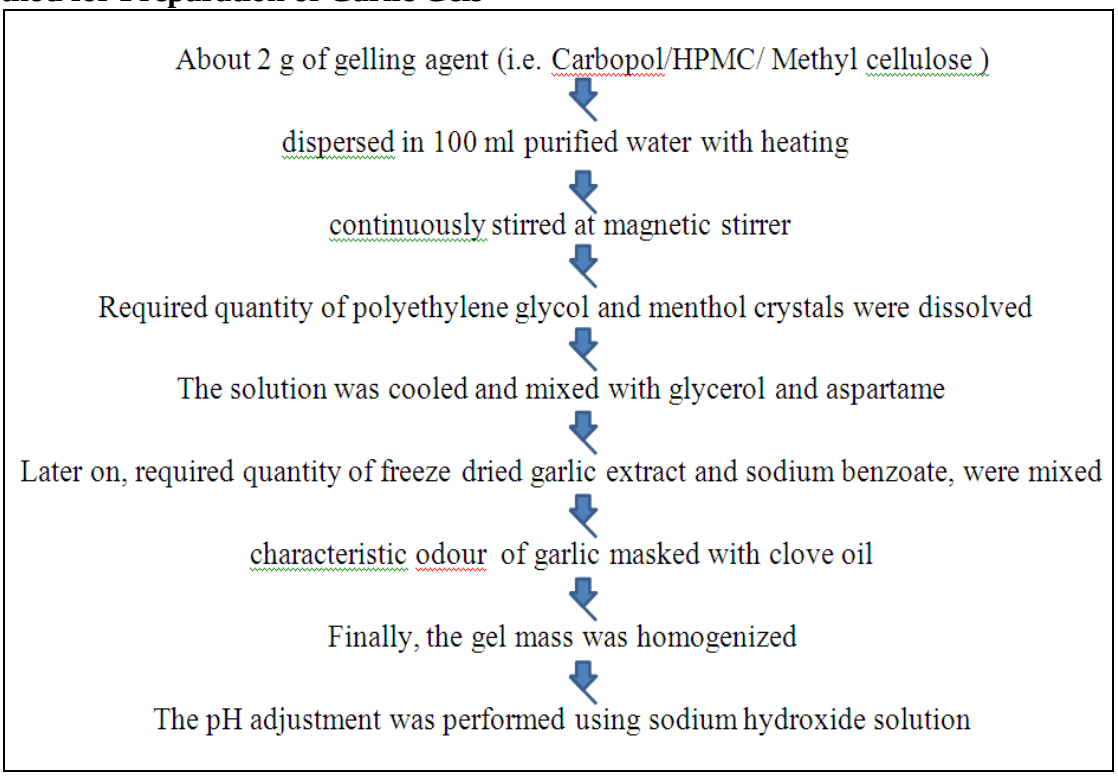
Thin Layer Chromatography: TLC (thin layer chromatography) was performed by dissolving the dried extract of garlic bulb in methanol. The extract was spotted on silica gel GF254 plate.

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.

Table 6. 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 7. 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

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 8. Phytochemical Tests and Their Results

S. No.	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	+

Thin Layer Chromatography (TLC)

Table 9. 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

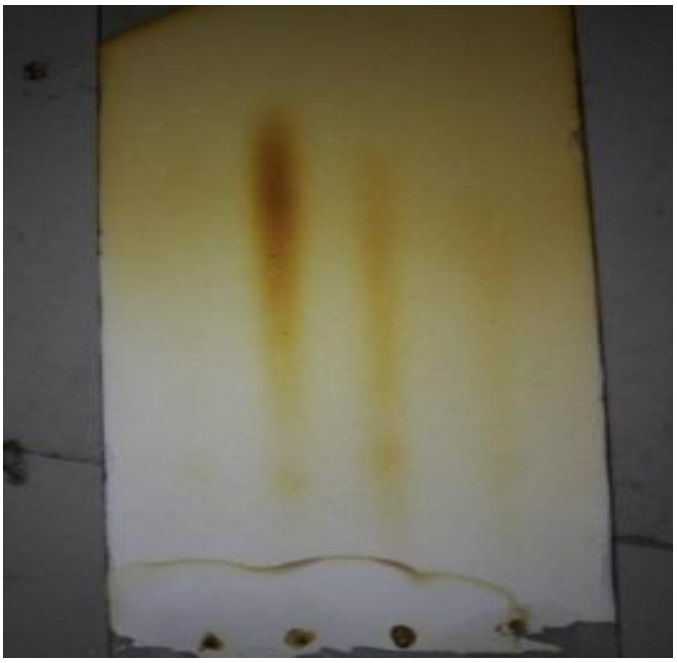


Fig. 1: Photographic representation of TLC of Garlic extract with different gelling agents

HPLC ANALYSIS

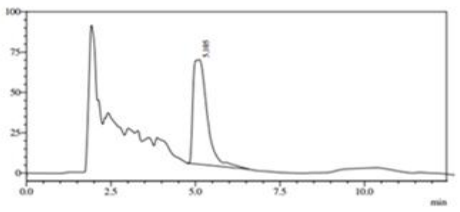


Fig. 2 Chromatogram of Pure Allicin (Standard)

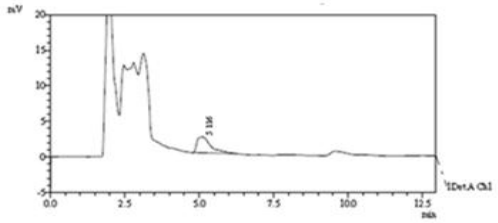


Fig. 3. Chromatogram of Garlic Extract (Sample)

Detector A Ch1 210nm

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

Fig. 4. 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. 5. HPLC Analysis of Garlic Extract (Sample)

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

EVALUATION OF GARLIC GELS

1. Physical Evaluation

Physical Parameters	F_1	F_2	F_3
Clarity/color	Clear	Clear	Clear
Homogeneity	+++	+++	++
Grittiness	-	-	+

2. Odor & Taste

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

3. Measurement of pH

Parameter	F_1	F_2	F_3
pH	7.2	6.8	7.0

4. Spreadability

Parameter	F_1	F_2	F_3
Spreadability	8.74 gm. cm /sec	8.73 gm.cm / sec	8.64 gm. Cm /sec

5. Viscosity

Parameter	F_1	F_2	F_3
Viscosity	5428 cps	6649 cps	5764 cps

6. Extrudability

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

In-vitro Diffusion Study of the Garlic Gels

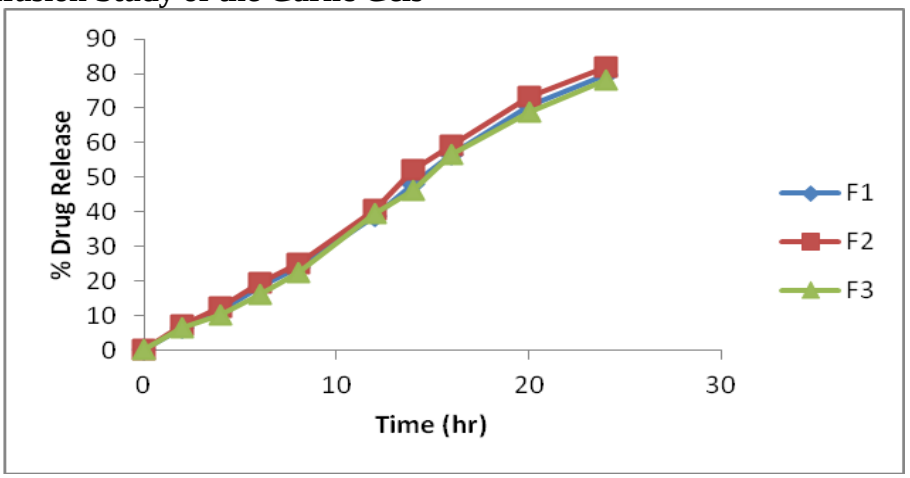


Fig. 6. Percentage Drug Release Profile of Prepared Garlic Gels (F_1-F_3) in pH 6.8 Phosphate Buffer

***Ex-vivo* Diffusion Study of Optimized F2 gel**

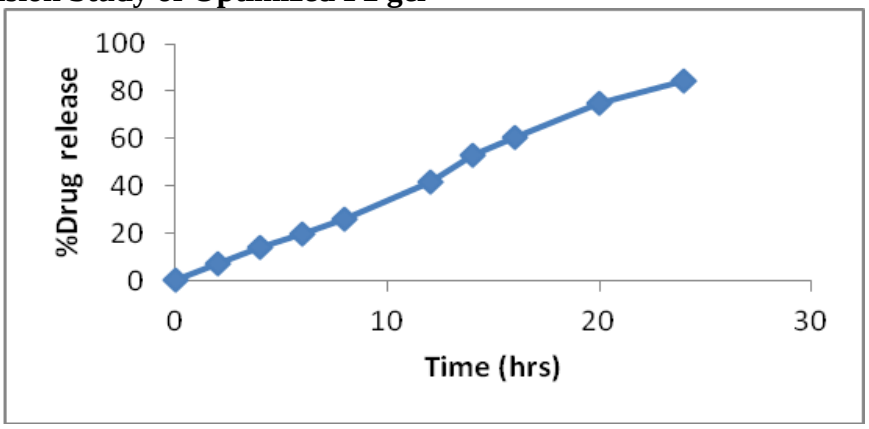


Fig. 7. Percentage drug release profile of optimized batch (F_2) through tongue membrane in pH 6.8 phosphate buffer

CONCLUSION

The medicinal potency of garlic is well established. This plant is very useful in treatment of different types of disease. 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 aq. extract of Garlic 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^{0}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. Menthol crystal (for enhance the penetration and soothing effect), PEG400 & Glycerine (as plasticizers), aspartame (as sweetener), clove oil (to mask the characteristic odour & taste of the garlic) and sodium benzoate (as preservative) were incorporated. HPLC analysis reflected % Alliin (active constituent) content (in garlic extract) as 0.568%. TLC studies were performed including the pure extract alone and in combinations with different excipients to check the compatibility. The Rf value of alliin was found nearly similar with that obtained in combinations with other ingredients. Conclusively, garlic extract was found to be compatible with different gelling agents used in gel formulation. 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 -7.4 and they showed smooth texture with good spreadability (8.64 to 8.74). The viscosities of prepared formulations were 5428, 6649 & 5764 cps. The Extrudability of prepared gel formulations was found to be very good. Percentage drug releases from the gels were: 72.49%, 75.89%, 71.98% from F1, F2 & F3 batches respectively (in pH 6.8 phosphate buffer) at the end of 24 hours. Conclusively, the optimized batch formulation (F_2) prepared with Carbopol, was further subjected to ex-vivo and stability studies. Ex-vivo permeation study thus conducted on F2 formulation which revealed that about 84.395 ± 2.115% drug released in pH 6.8 phosphate buffer through goat tongue membrane within 24 hours. 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 of

garlic extract 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.

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