IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

DESIGN, DEVELOPMENT AND EVALUATION OF GARLIC OIL CREAM FOR IT'S ANTI-BACTERIAL AND ANTI-FUNGAL PROPERTIES

Rajeshwari H Bobade[1*], Sachin B. Dudhe[2], Pruthviraj Meshram[3]

Student[1], Principal[2], Professor[3]

[1,2]Department of Pharmaceutics, Maharashtra Institute of Pharmacy, Betada, Bramhapuri, Maharashtra, India 441206

Abstract

The aim of the present study was to investigate the phytochemical profile as well as the anti-bacterial and anti-fungal properties of herbal garlic extracts. Most traditional natural products are getting more popular. Natural products continue to produce bioactive agents because of the amazing chemical diversity that is readily available. They were assessed as potential therapeutic candidates for the treatment of infectious illnesses in both humans and animals. Our findings indicate that dietary factors contribute to the regional variation of stomach cancer occurrence in Italy, and offer clues for further etiologic and prevention research. Herbal preparations can be formed by combining several plant classes. These preparations are used for the localized effects produced at the site of their application by drug penetration in to the underlying layer of skin or mucous membrane. One possible explanation for the effectiveness of combined medications is that the various mixtures with different mechanisms may add up to produce a more comprehensive therapeutic effect.

Keywords: Anti-Bacterial, Anti-Fungal Property, Garlic, A. sativum; B. subtilis; E. coli.

Introduction

The last few decades have witnessed an increase in fungal infection. Fungal infections are evolvingdiseases in sanatorium institutions. Increase in immunosuppressive diseases and conditions have been influencing the epidemiological pattern of mycoses in hospitalized patients the epidemiologyof invasive fungal infections is currently at a crucial stage. Fungal infection caused by Candida has become more prevalent than Escherichia coli and Pseudomonas sp., Aspergillus sp. and other sp. There are many host factors that predispose patients to fungal infections. These include: immobility; mucositis; use of antibiotics; radiation therapy or certain immunosuppressive agents;intensive care unit (ICU**)** Candida albicans is the most common species in the genus which has been implicated in Candidiasis. The infections range fromsuperficial skin to systemic diseases. C.albicans, C. tropicalis, C. glabrata and C. parapsilosis are part of the normal flora of humans and can be isolated from oral cavity, vaginal and other parts ofbody sites from normal healthy people. Treatment with herbs is an ancient method for curing diseases. Since the Vedic time humans haveused medicinal plant material to cure any disease or to give a satisfactory treatment against that disease. Plants are also known for treating the infectious

and non-infectious skin disorders. The antimicrobial effect of some plants is attributed to the number of phytoconstituents like flavonoid,tannins, triterpenes etc**.** The purpose of the current study is also based on the medicinal property of a plant i.e. Garlic (Allium sativum) Garlic oil shows a wide range antimicrobial activity. Alliin is the main chemical constituent in garlic oil which shows antimicrobial activity. This oil consists of sulfur containing six compounds such as i. Allicin, ii. Alliin, iii. Ajoene, iv. Diallyl disulfide, v. dithiin and vi. Sallylcysteine. These large amounts of sulfur compounds give the smell and taste to the garlic. Diallyl disulfide is an important componentin garlic being a powerful antibiotic and antifungal compound.

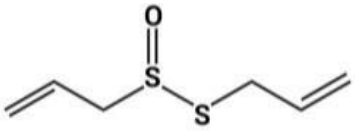


Figure 1. Structure of Dially disulfide

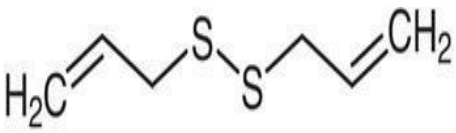


Figure 2. Structure of Allicin

Dietary factors play a key role in the development of various human diseases. Across cultures, there are many different dietary patterns which are believed to promote human health. Despite cultural differences, there are some shared characteristics of healthy dietary patterns. Perceiving plant foods as beneficial diet is advised by the folklore of many cultures over centuries.

Fig. 3 Allium sativum

Medicinal Species: *Allium sativum* L.

Botanical Family: Liliaceae

Common Names (Synonyms): Garlic (Eng.), lasun (Hindi), Ransom & Lahsuna (Sanskrit),Knoblauch

Geographical Source: Central Asia, Southern Europe, USA, India

Chemical Constituents: Allicin is an odorless sulphur containing chemical derived from the amino acid cysteine.

When garlic bulbs are crushed, Alliin is converted into another compound called Allicin.

Allicin is further broken down to a compound called Ajoene, which may be the substance that inhibitsblockage

in blood vessels from clots and atherosclerosis.

Components: Diallyl thiosulfonate (allicin) Diallyl Sulfide, (DAS) Diallyl Disulfide (DADS) Diallyl trisulfide (DATS), E/Z-ajoene, s-allyl-cysteine (SAC) and s-allyl-cysteinesulfoxide (Allin)

Allicin (released when crushed) an amino acid which gives Garlic its strong odor and is responsible forthe powerful pharmacological properties of the plant

- Germanium
- Magnesium
- Selenium
- Vitamin A
- Vitamin C
- Volatile oil of which about 0.5% is composed of sulfur-containing compounds.

Material and Method

Materials

Propylene glycol, Beeswax, Stearyl alcohol, Cetyl alcohol, Triethanolamine, Propyl paraben, Methyl paraben, Liquid Paraffin, Stearic acid, Peppermint oil were purchased from Royal Drug and Pharmaceuticals Mumbai. Garlic Oil is extracted from Garlic respectively by steam distillation in the laboratory.

Biological Source

Garlic is obtained from ripe bulb of Allium sativum Linn. Family: Liliaceae. ChemicalConstituents: Allicin, Alliin, volatile and fatty oils, mucilage and albumin.

Collection of sample

Garlic crude drug is purchased from the market.

Material used

Table 1 various ingredients used in the formulation of garlic oil cream

Sr.no.	Ingredients	Supplier
1	Garlic extract	Amar pharmaceuticals PVD,LID
2	Stearyl alcohol	Saibaba surfactants,PVD,LID, Ahmedabad
3	Cetyl alcohol	Matangi industries,india
4	Liquid paraffin	Swastika , Ahmedabad, Gujarat
5	Stearic acid	Jain acid and chemical , Nagpur
6	White beeswax	Aadra international,gujrat
7	Propylene glycol	Rudraksha Allieed chemical PVD.LID, Nagpur
8	Triethanolamine	A.B.Enterprises ,mumbai

9	Methyl paraben	Anant Pharmaceuticals PVD LID , India
10	Propyl paraben	Chembur East , Mumbai

Table 2 List of Equipment's

Sr.no.	Equipment name	Name of Manufacturer
1	Electronic Balance	AUX 120, Shimadzu, Jaipur
2	Clevenger Apparatus	Kalyan ,badlapur, maharashtra
3	Brook field Viscometer	Thane ,west mumbai , Maharashtra
4	Digital pH meter	Elico model-(I612)
5	Tube Extrudability machine	Ahmadabad, gujrat
6	Spreadability tester	Mumbai, Maharashtra
7	Ultraviolet spectroscopy	Shimadzu 1900,Japan
8	Hot Air Oven	Shalon ,India

Extraction of Oil -

Oil of garlic was extracted by the steam distillation method by using the Clevenger apparatus in the laboratory. Garlic cleaned properly and separately filled in the RBF with solvent arranged the assemblyproperly by attaching Clevenger apparatus and condenser and heat. The obtained oils are separated andfill in an airtight container.

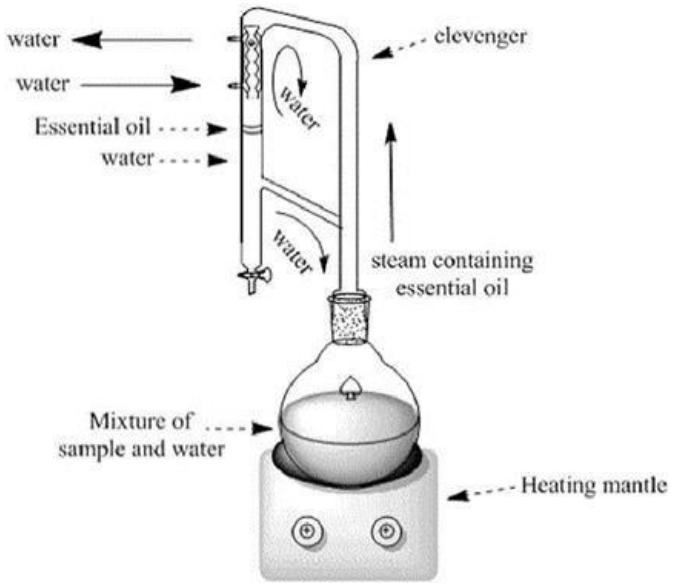


Figure 4 Clevenger Apparatus (steam distillation of Garlic exact)

Formulation

Formulation 1 (F1) table 3 of two phasePart A - Oily Phase

Ingredients	Quantity	Activity
Garlic oil	5 %	Anti- fungal
Stearyl alcohol	5 %	Emollient
Cetyl alcohol	6.5 %	Binding agent
Mineral oil (Liquid paraffin)	5 %	Moisturizer
Stearic acid	2.5 %	Emulsifying agent
White beeswax	1.5 %	Thickening agent

Part B - Aqueous phase

Ingredients	Quantity	Activity
Propylene glycol	5 %	Humectants
Triethanolamine	2 %	Stabilizer
Methyl paraben	0.01 %	Preservation
Propyl paraben	0.04 %	Preservation
Distilled water	Upto 100 %	Solvent based

Formulation 2 (F2) tablet 4 of two phasePart A - Oily Phase

Ingredients	Quantity	Activity
Peppermint oil	5 %	fungal and flavouringagent
Garlic oil	5 %	Anti-fungal
Stearyl alcohol	5 %	Emollient
Cetyl alcohol	6.5 %	Binding agent
Mineral oil (liquid paraffin)	5 %	Moisturizer
Stearic acid	2.5 %	Emulsifying agent
White beeswax	1.5 %	Thickening agent

Part B - Aqueous Phase

Ingredients	Quantity	Activity
Propylene glycol	5 %	Humectants
Triethanolamine	2 %	Stabilizer
Methyl paraben	0.01 %	Preservative
Propyl paraben	0.04 %	Preservative
Distilled water	Upto 100 %	Solvent base

Development

Preparation of oil phase -

All the ingredients like white beeswax, stearic acid, stearyl alcohol, cetyl alcohol were melted in a stainless steel container. To this mixture liquid paraffin was added and allowed to melt. The temperature was then kept between 65 to 70°C.

Preparation of Aqueous Phase –

Water was heated to 65 to 70°C. To this aqueous medium pre weighed all the reagent like propylene glycol, triethanolamine, propyl paraben and methyl paraben were added; Then the temperature of the aqueous phase was maintained at 65 to 70°C

Development of Cream formulation -

Total Oil phase was then slowly pour into the aqueous phase at 65-70°C and mixed for 10 to 15 Minutes. When the temperature of both the medium were at the same temperature, the aqueous phase was slowly added to the oil phase with moderate agitation and was kept stirred until the temperature dropped to 40°C. Garlic oil was added to it. The o/w emulsion was then cool down toroom temperature to change a thick cream base.

In case of Formulation 2(F2), extra reagent peppermint oil was added at the final stage, and immediately transfers in to a container, and closed tightly.

Figure 5 Stages of garlic oil cream formulation

Evaluation parameters

Take about 1 gram of cream in a clean petri dish and observe visually.

Physical examination -

The prepared topical creams were inspected visually for their color, homogeneity, consistency, spreadability and phase separation. The pH was measured in each cream, using a pH meter, whichwas calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrode was inserted in to the sample 10 min priors to taking the reading at room temperature. The pH of a topical preparation should be within the pH range corresponding to the pH of the skin, namely, 4.5-6.5.

Viscosity -

The viscosity of formulated creams was measured by Brook field Viscometer LVD using spindle S94 at varying speed and shear rates. The measurements were done over the range of speed setting from 0.10, 0.20, 0.30, 0.40 and 0.50 rpm in 60 s between two successive speeds as equilibration with shear rate ranging from 0.20 s-1 to 1.0 s-1. Viscosity determinations were performed at roomtemperature.

Tube Extrudability -

In the present study, the method adopted for evaluating cream formulation for Extrudability was based upon the quantity in percentage cream extruded from tube on application of finger pressure.

2. More quantity extruded better was Extrudability. The formulation under study was filled in a clean, lacquered aluminium collapsible 5 gm tube with a nasal tip of 5 mm opening and applied the pressure on the tube by the help of finger. Tube Extrudability was then determined by measuringthe amount of cream extruded through the tip when a pressure was applied on a tube.

Microbiological studies -

Topical formulation with broad, Non-resistance promoting activity against staphylococci, streptococci, dermatophytes or yeast or molds can be of great use in dermatology preparation wereinfections are often mixed. Since formulation containing antimicrobial agents as active moiety, it is likely to protect from microbial growth. To determine the activity of formulation is subject to study the prepared formulation with standard method called Disk diffusion method and the inhibition zone diameters were measured with the help of zone reader.

Results and Discussion

Physical examination (Organoleptic properties)

The prepared herbal antifungal creams were inspected visually for their colour, appearance, odor, and consistency. The pH was measured in each herbal antifungal cream, using a pH meter, whichwas precalibrated with standard buffer solutions at pH 4, 7, 9. The pH meters electrode was insertedin to the cream 10 min before the reading at room temperature. The standard pH of a topical preparation should be within the pH range matching to the pH of the skin, namely 4.5,6.5.

Viscosity

The viscosity of formulated creams was measured by Brook field Viscometer NDJ-8S using spindle S 94 at varying speed and shear rates. The measurements were done over the range of speed setting from 0.15, 0.25, 0.35, 0.45 and 0.55 rpm in 60 s between two successive speeds as equilibration with shear rate ranging from0.25

s-1 to 1.0 s-1. Viscosity determinations were performed at our room temperature.

Spreadability

Spreadability property of a formulation was calculated by an apparatus designed by Muttimer et al.;it made of a wooden block, which was connected by a pulley at a one end. A rectangular shaped ground glass was set on this block. An excess amount of cream (about 3-4 gm) under study was placed on this ground plate. The herbal antifungal cream was then kept in between this plate and aglass plate having the same dimension of fixed ground plate and attached with the hook. A fixed1Kg load was placed on the upper of the plates for about 4-5 minutes to expel all the entrapped air and to provide a uniform film of the cream between the plates. Excess of the cream was scrappedoff from the boundaries. The top plate was then subjected to drag of 80 Gms. With the help out of string attached to the hook and the time (in seconds) required by top plate to cover a distance of 10 cm be noted. A less intervals indicates better Spreadability. Spreadability measured in unit gm.cm/sec Spreadability of the cream may be determined by the following equation,

$$S = M \times L/T$$

Where,

L= length moved by glass slideT= Time in seconds M=Weight in pan

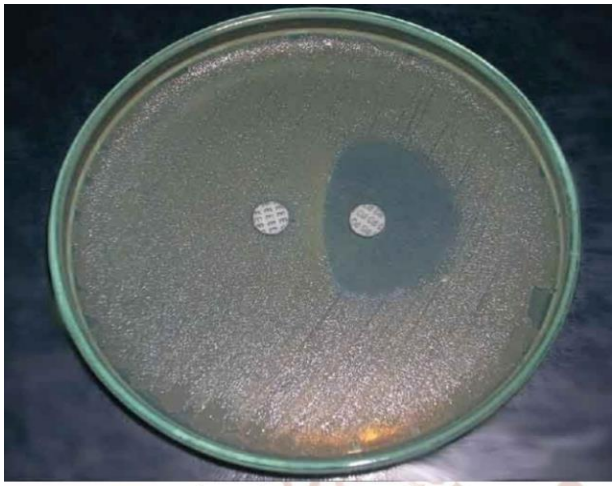
S= Spreadability

Tube Extrudability

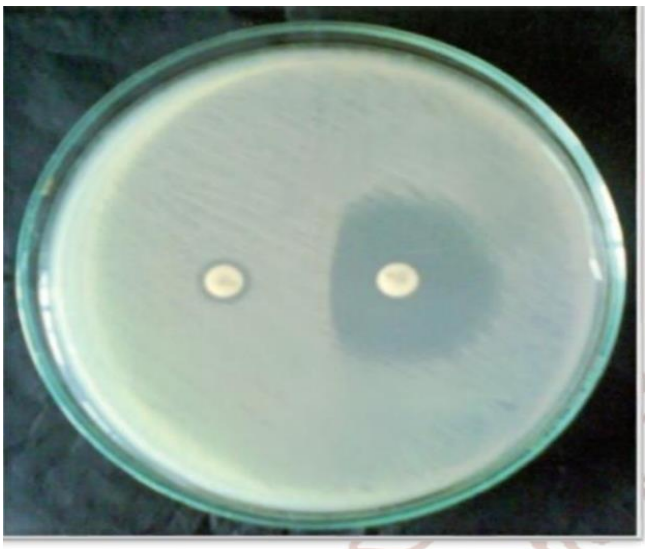
In this present work, the method adopted for evaluating cream formulation for extrudability was based upon the quantity in percentage cream extruded from tube on application of finger pressure7kg. More quantity extruded improved was extrudability. The both the formulation F1 and F2 wasfilled in a clean, lacquered aluminium collapsible tube containing about 5 gm of cream which contains in a nasal tip of 5 mm hole and applied the pressure on the tube by the help of fingertip.The tube extrudability property was determined by, quantity of cream formulations were extrudedfrom the tube tip as when the pressure was applied on the tube body.

Microbiological studies

All types of broad, non-resistance microorganism like staphylococci, streptococci, dermatophytes or yeast or molds can be protected by tropical formulations with anti-microbial agent have enormoususe in dermatology preparation were infections are often mixed. Since herbal anti-fungal cream containing antimicrobial extracts as active constitutent, it is expected to protect from microbial growth. To determination of an anti-microbial activity of herbal antifungal cream Disk diffusion method was followed. For this study standard media was prepared with 65 g Sabouraud Dextrose Agar, and 28 g Nutrient Broth. Both the sample cream formulation was compared with standard Fluconazole. Finally the zone of inhibition diameters was measured with the help of zonereader.



Against E.coli



Against candida albicans

Figure 6 formulated cream showing zone of inhibition

Table 5 Microbial studies

Bacteria	Candida albicans	E.coli
Zone of inhibition	42.32 mm	34.16m

Antifungal Evaluation -

- Materials: Herbal antifungal cream, fungi.
- Media: Sabouraud Dextrose Agar (65 g), and Nutrient Broth (28 g).

- Sample: Herbal antifungal

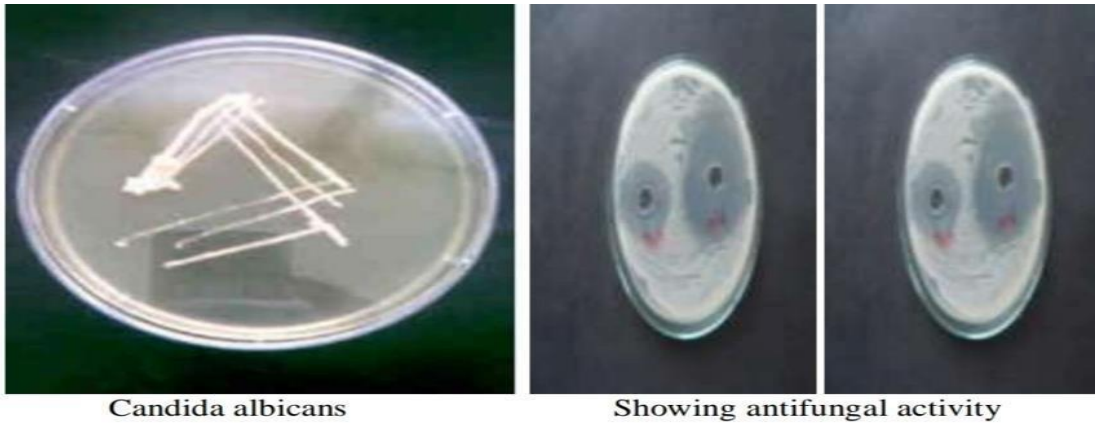


Figure 7 Antifungal activity of Herbal antifungal cream (F2) on organismAntifungal activity of Herbal antifungal cream on organism

Table 6 Effect of antifungal activity

Organism	Extract	Test	Standard
Candida albicans	Ethanol	Susceptible	Susceptible

Table 7 Showing diameters of Inhibition zones

Organism	Plant extract	Zone of Inhibition (mm)		
		Test sample (avgas diameter)		Standard
Candida albicans	Ethanol	F1 - 8	F2 - 10	23

As Herbal antifungal cream shows antifungal activity against Candida albicans it can be formulated as antifungal formulation (cream).

Skin irritancy test

Skin irritancy is determined with that herbal antifungal cream formulations do not affect the humanskin cells or tissues. Irritancy may result in swelling, redness and inflammation on the surface of skin when some particular creams are applied without testing. Hence skin irritancy test was carriedout by marking an area on the left hand dorsal surface. The cream was applied with a spatula to that marked specified area and time was noted. Irritancy, erythema, edema was checked for regularintervals Upto 24 hours. There was no prominent irritation because of the applied herbal antifungalcream hence it was safe to use.

Observation

Table 8 Evaluation Results

Sr. No.	Evaluation parameters	Results	
		F1	F2
1.	Colour	ff yellow tocreamish	Buff yellow
2.	Appearance	Smooth	Smooth
3.	Odour	ngent (stronggarlic oil)	Pleasant peppermint
4.	Consistency	No phase separation	No phase separation
5.	Viscosity	66440 cps	65740
6.	Spreadability (gm.cm/sec)	14.23	18.00
7.	PH	7.5	7.4
8.	Extrudability	96.15 %	89.50 %
9.	Skin irritancy test	No irritancy erythema , edema	No irritancy, erythema ,edema
10.	icrobiological studies(zone of Inhibition)	8 mm	10 mm pH

The prepared both formulations showed good spread ability, no evidence of phase separation and good consistency during the study period. Though stability parameters like visual appearance, is same but the F2 shows better fragrance compare to the formulation F1. And both the formulationsshowed that there was no significant variation during the study period.

Conclusion

The formulated herbal cream was a better alternative to standard therapy, exhibiting promising healing and antimicrobial effects with significant compatibility and safety profile. The use of herbal/bioactive ingredients in cream (cosmetic) influence biological functions of skinsand provide nutrients necessary for the healthy skin against antifungal infection. The prepared formulation (F2) showed good spread ability, no evidence of phase separation and good consistency during the study period. Stability parameters like visual appearance, nature but it hada drawback. In first formulation (F1) the smell was unpleasant as garlic oil was used which givesa very strong unpleasant smell. So we prepared another formulation (F2) to mask this unpleasant smell. In the second formulation peppermint oil was used to enhance the preparation and mask theodor of garlic, which was also acting as a tertiary antifungal agent here.

References

[1] Alemdar S, Agaoglu S. Investigation of In vitro Antimicrobial activity of Aloe vera juice.Journal of Animal and Veterinary Advances 2009: 8(1): 99-102.

[2] Koka S , Pancholi M, Sharma V. Formulation and Evaluation of Topical Antifungal Herbal Gels Containing Hydroalcoholic Extract of Catharanthus roseus and Aloe vera International Journal of Pharmacognosy and Phytochemical Research 2019; 11(3);173-176.

[3] Hugo WB, Russell AD: Pharmaceutical Microbiology: Blackwell Science, 6 Ed, 2003; 35-51

[4] Khan Z K, Gyanchandani A, Candidiasis A Review: PINSA: 1998: B 64 :1-34

[5] Baqars S R, The role of traditional medicine in the real environment, Traditional Medicine inAfrica I ssaq, East African Education Publisher LTD, Nairobi, 1995 p 141-142.

[6] Pimpale A, Formulation and Evaluation of Antibacterial Antifungal Cream of Garlic Oil, International Journal of Trend in Scientific Research and Development (IJTSRD), No: 2456 - 6470 | Volume - 3 | Issue – 1 | Nov – Dec 2018.

[7] Santos A.L., Chierice G. O., Alexander K.S., Riga A., Matthews E. Characterization of theraw essential oil eugenol extracted from Syzgium aromaticum L. J. Therm. Anal. Calorim. 2009;96:821-825

[8] Panizzi L., Falmini G., Cioni P.L., Morelli I. Composition and antimicrobial properties ofessential oil of four Mediterranean Lamiaceae. J. Ethopharm. 1993; 39:167-170.

[9] https://www.researchgate.net/publication/33 3694027 Formulation and Evaluation of Antibacterial Antifungal Cream of Garlic Oil.

[10] Kuda,T.; Iwai, A.; Yano, T. Effect of red pepper Capsicum annuum var. conoides andgarlic Allium sativum on plasma lipid levels and cecal microflora in mice fed beef tallow.
FoodChem. Toxicol. 2004, 42, 1695–1700

[11] Akanksha D, Vikas G, Neetesh KJ, Shailendra S, Neelam B, Dinesh KJ. Formulation and Evaluation of Neomycin Sulphate Ointment containing Natural Wound Healing Agent. Curcuma longa, International Journal of Pharmaceutical Sciences and Drug Research. 2009;1(2):116-18

[12]. Panda P and Ghosh A. Formulation and Evaluation of Topical Dosage Form of Eupatorium Odoratum Linn. And Their Wound Healing Activity. International Journal of Pharma and Bio Sciences. 2010;1(2):201-203

[13]. Purushothamrao K, Khaliq K, Sagare P, Patil SK, Kharat SS, Alpana.K. Formulation and evaluation of Vanishing cream for scalp psoriasis. International Journal of Pharma Sci Tech.
2010; 4(1):33-41.

[14]. Chakole CM, Shende MA, Khadatkar SN. Formulation and evaluation of novel combined halobetasol propionate and fusidic acid ointment. International Journal of ChemTech Research. 2009 Jan-March; 1(1):103-16.

[15] Parmar RB, Baria AH, Faldu SD, Tank HM, Design and Evaluation of Poly-herbal Formulation in Semisolid Dosage Form for its Antibacterial Activity, Journal of Pharmacy Research 2009; 2:1095-1097

[16] Agis F. Kydonieus. Transdermal Delivery of Drugs, Volume 1, CRC Press, Bocaraton,1987;168.

[17] Patel J, Patel B, Banwait H, Parmar K, Patel M, Formulation and evaluation of topical aceclofenac gel

using Different gelling agent, International Journal of Drug Development & Research 2011;3:156 – 164.

[18] More BH, Sakharwade SN, Tembhurne SV, Sakarkar DM, Evaluation for skin irritancy testing of developed formulations containing extract of Buteamonosperma for its topical application, International Journal of Toxicology and Applied Pharmacology 2013; 3:10

[19] Sanna V, Peana AT, Mario D, Moretti L, Development of new topical formulations of diphenhydramine hydrochloride: In vitro Diffusion and In vivo Preliminary studies, International journal of Pharm Tech Research,2010; 2: 863- 889. Biopharmaceutics 2008; 68:380 -389.

[20] Mei X. Chen, Kenneth S. Alexander, and Gabriella Baki, Formulation and Evaluation of Antibacterial Creams and Gels Containing Metal Ions for Topical Application, HindawiPublishing Corporation. Journal of Pharmaceutics, Volume 2016, Article ID 5754349

[21] Hetty Lendora Maha1*, Kasmirul Ramlan Sinaga Masfria,Formulation And Evaluation OfMiconazole Nitrate Nanoemulsion And Cream, Asian J Pharm Clin Res, Vol 11, Issue 3, 2018,319-321.

[22] A. Premkumar, T. Muthukumaran, V. Ganesan , Shanmugam R, Priyanka D.L, Formulation And Evaluation Of Cream Containing Antifungalagents, Antibacterial Agents And Corticosteroids , Hygeia.J.D.Med.6 (2) October 2014; 5-16

[23] M. A. Calvo, E. L. Arosemena, C. Shiva and C. Adelantado, Antimicrobial activity of plantnatural extracts and essential oils, Science against microbial pathogens: communicating current research and technological advances,1179-1185

[24] B. Dethier*, K. Nott*, M.-L. Fauconnier*, (Bio) Synthesis, ExtractionAnd Purification Of Garlic Derivatives Showing Therapeutic Properties, Comm. Appl. Biol. Sci, Xx/X, 2013.

Yilmaz HH, Gormez O, Hastar E, et al. Garlic burn in a patient with trigeminal neuralgia: acase report. Eur J Dent. 2010;4:88–90. [PMC free article] [PubMed

[25] Aviello G, Abenavoli L, Borrelli F, et al. Garlic: empiricism or science? Nat Prod Commun. 2009;4:178517–96. [PubMed] [Google Scholar]

[26] Arnault I, Auger J. Seleno-compounds in garlic and onion. J Chromatogr A. 2006;1112:23–30.[PubMed] [Google Scholar]

[27] Lanzotti V. The analysis of onion and garlic. J Chromatogr A. 2006;1112:3–22. [PubMed][Google Scholar]

[28] Sumiyoshi H. New pharmacological activities of garlic and its constituents. NipponYakurigaku Zasshi. 1997;1101:93P–7P. [PubMed] [Google Scholar]

[29] Jappe U, Bonnekoh B, Hausen BM, Gollnick H. Garlic-related dermatoses: case report andreview of the literature. Am J Contact Dermat. 1999;10:37–9. [PubMed] [Google Scholar]

[30] Borek C. Antioxidant health effects of aged garlic extract. J Nutr. 2001;131:1010S–5S.[PubMed] [Google Scholar]

[31] Imai J, Ide N, Nagae S, et al. Antioxidant and radical scavenging effects of aged garlicextract and its constituents. Planta Med. 1994;60:417–20. [PubMed] [Google Scholar]

[32] Jung EM, Jung F, Mrowietz C, et al. Influence of garlic powder on cutaneous microcirculation. A randomized placebo-controlled double-blind cross-over study in apparently healthy subjects.

Arzneimittelforschung. 1991;41:626–30. [PubMed] [Google Scholar]

[33] Chandrashekar PM, Venkatesh YP. Identification of the protein components displaying immunomodulatory activity in aged garlic extract. J Ethnopharmacol. 2009;124:384–90. [PubMed] [Google Scholar]

[34] Lamm DL, Riggs DR. Enhanced immunocompetence by garlic: role in bladder cancer and other malignancies. J Nutr. 2001;131:1067S–70S. [PubMed] [Google Scholar]

[35] Arnault I, Auger J. Seleno-compounds in garlic and onion. J Chromatogr A. 2006;1112:23–30.[PubMed] [Google Scholar]

[36] Lipke MM. An armamentarium of wart treatments. Clin Med Res. 2006;4:273–93. [PMCfree article] [PubMed] [Google Scholar]

[37] Dehghani F, Merat A, Panjehshahin, Handjani F. Healing effect of garlic extract on wartsand corns. Int J Dermatol. 2005;44:612–5. [PubMed] [Google Scholar]

[38] Yousuf S, Ahmad A, Khan A, et al. Effect of garlic-derived allyl sulphides on morphogenesis and hydrolytic enzyme secretion in Candida albicans. Med Mycol. 2010 Dec 3;[Epub ahead of print] [PubMed] [Google Scholar]

[39] Ledezma E, López JC, Marin P, Romero H, et al. Ajoene in the topical short-term treatmentof tinea cruris and tinea corporis in humans. Randomized comparative study with terbinafine. Arzneimittel-forschung. 1999;49:544–7. [PubMed] [Google Scholar]

[40] Ledezma E, Marcano K, Jorquera A, et al. Efficacy of ajoene in the treatment of tinea pedis:adouble-blind and comparative study with terbinafine. J Am Acad Dermatol. 2000;43:829–32. [PubMed] [Google Scholar]

[41] Ledezma E, DeSousa L, Jorquera A, et al. Efficacy of ajoene, an organosulphur derivedfrom garlic, in the short-term therapy of tinea pedis. Mycoses. 1996;39:393–5. [PubMed] [Google Scholar]

[42] Gamboa-León MR, Aranda-González I, Mut-Martí•n M, et al. In vivo and in vitro control ofLeishmania mexicana due to garlic-induced NO production. Scand J Immunol. 2007;66:508–14. [PubMed] [Google Scholar]