

Formulation Development And Evaluation From Garlic Oil Macerate

R Patil, R Ravindra

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Abstract

Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases such as, atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes. It is also effective against bacterial as well as fungal infections. Garlic oil obtained by two methods steam distillation and cold maceration. Garlic has different active constituents among which ajoene shows antibacterial and antifungal activity. Ajoene is highly unstable it remains stable only in oil macerate form. It is a great challenge for scientists all over the world to make a proper use of garlic and enjoy its maximum beneficial effect as it is the cheapest way to prevent bacterial as well as fungal infection so, an attempt to form topical gel from garlic oil macerate for its antibacterial as well as antifungal activity and evaluation.

INTRODUCTION

Garlic is used for different purposes such as a palliative for the heat of the sun in field labor, preventing heart disease (including atherosclerosis, high cholesterol, and high blood pressure), cancer and cardiovascular benefits of garlic. It is also alleged to help regulate blood sugar blood sugar levels and also shows antimicrobial activity. In modern naturopathy, garlic is used as a treatment for intestinal worms and other intestinal parasites, both orally and as an anal suppository. Garlic cloves are used as a remedy for infectious (especially chest problems), digestive disorders, and fungal infections such as thrush, candidiasis, aspergillosis and cryptococcosis. (1),(2),(3) The garlic is mention as remedy for skin diseases in Ayurveda & it is chosen for antibacterial activity because every antibiotic there is development of resistance by bacteria but in case of garlic there is no resistance developed against garlic. When garlic chopped or crushed, allinase enzyme, present in garlic, is activated and acts on alliin (present in intact garlic) to produce allicin. The enzyme allinase responsible for converting alliin (s-allyl cysteine sulphoxide) to allicin is inactivated by heat.(4) Allicin is a volatile and short lived (hours or days) compound, which will break down in to compounds, such as diallyl disulphide. In a matter of hours it will further degrade in to an oily withes brew of bisulphides, trisulphides such as allyl methyl trisulphide and vinyl dithiins and polysulphides and many others. Allicin is powerful natural antibiotic (about one-fiftieth as powerful as penicillin

and one-tenth as powerful as tetracycline) that kill many kind of bacteria (including bacillus, escherischia (e coli), mycobacterium, pseudomonas, staphylococcus an streptococcus). It also has anti-fungal and anti-viral properties.(4),(5) There are different garlic formulations in the market such as oil macerates of fresh garlic formulated commonly in soft gelatin capsules, the dry powder products of fresh garlic formulated either as sugar or film coated tablets in European market and dry powder products of aged garlic formulated either as sugar or film coated tablets in American market. In India not much work done on garlic oil macerate so it is used for its combined effects against all bacteria. The macerated garlic oil contains the vinyl-dithiins and ajoenes. The antifungal activity of ajoenes was reported against the Candida albicans and Aspergillus niger.(6),(7) Ajoene remain stable for long period only in form of garlic oil macerate. First garlic oil macerate obtained by Garlic is crushed first and then remain for half an hour exposed to air, so that there is formation of the allicin from alliin by enzyme allilcinase(8),(9) and then allow to macerate for seven to eight days. Maceration is done with two different oils ie sesame and sunflower oil. The two different strains of garlic are used for maceration first single clove and multiple clove garlic. After maceration separate the macerated oil from crushed garlic by filtration through suction pump. After filtration the crushed garlic taken on piece of cloth and all oil content is removed by applying pressure. The concentrated part is also removed along with

oil so, filter this oil through suction pump to obtain clear oil macerate. The topical gel using different gelling agents are prepared along with garlic oil macerate.

EXPERIMENTAL PROCEDURE

CHEMICALS AND REAGENTS

The chemicals and reagents along with grade are as listed in table 1.

INSTRUMENTS

Instruments used are as follows electronic balance from Shimadzu, Autoclave from Medica Instrument Pvt. Ltd, Magnetic stirrer from Bio lab, pH meter of Lab India and suction pump from Besto Pvt. Ltd

MACERATION PROCEDURE

Maceration was done with two different oils i.e. sesame oil and sunflower oil. The two different strains of garlic are used for maceration first single clove and multiple clove garlic. Maceration is the process of extracting drug constituent in to particular solvent. In case of garlic, 50 g was weighed and then crushed with the help of mortar pestle or grinder. The crushed garlic was exposed to environment for 10 to 15 minutes so that there is conversion of alliin to allicin by enzyme allinase. Then 25 ml of oil was poured in crushed garlic and mixed with help of stirrer. Then the container was wrapped with aluminum foil and kept for seven days to macerate. After seven days, the oil was separated from crushed garlic by filtration using suction pump. The crushed garlic was placed on the piece of cloth and oil traces were removed by applying pressure. The concentrated part is also removed along with oil so as to obtain a clear oil macerate. The same procedure was repeated for both types of oils and both varieties of garlic.

The choice of oils was based on the fact that sesame oil is the most common form of oil used in Ayurveda (including garlic products) as it is considered to have the best medicinal properties. Sunflower oil was selected on the basis of availability, cost and also because physicochemical stability.

DIFFERENT TRIALS TRIED WITH GARLIC OIL MACERATE

DIFFERENT TRIALS FOR OINTMENT

The trials are listed in table number 3 and method of preparation for each trial as follows

Ointments are prepared by different methods like trituration as well as by fusion method. We preferred fusion method to

make ointment. First take required amount of hard paraffin and melt it with heating in porcelain dish. After complete melting hard paraffin, add cetosteryl alcohol and white soft paraffin. Add oil macerate finally along with eucalyptus oil to mask odor of garlic oil macerate. Allow it to cool and solidify.

Heating is required for melting hard paraffin, cetosteryl alcohol and white soft paraffin. The temperature required up to 70 to 80°C for melting of hard paraffin and Cetosteryl alcohol. GOM is added in the end at a temperature below 60°C to minimize the loss of active moieties.

These trials for ointment were conducted. In first trial, hard paraffin and white soft taken and melted together and then remaining ingredients were added. In trial 2, concentration of white soft paraffin was increased and that of hard paraffin was decreased.

DIFFERENT TRIALS FOR GELS

The trials are listed in table number 4 and method of preparation for each trial as follows Method for Trial 1

First weigh the 2g of CMC then mix it with help of magnetic stirrer in 100ml of water. After this break the clumps that are formed during mixing with the help of glass rod. Then add triethanolamine to adjust pH to neutralization point. Check the pH with help of pH paper. Add garlic oil macerate (2.0ml), preservative methyl paraben 0.2, propyl paraben 0.02g and eucalyptus oil to mask the odor of GOM. Kept it for overnight stability.

Method for Trial 2 and 3

Method for Trial 2 and Trial 3

First weigh the 2g of CMC then mix it with help of magnetic stirrer in 50ml of water. After this break the clumps that are formed during mixing with the help of glass rod. Then add triethanolamine to adjust pH to neutralization point. Check the pH with help of pH paper. Add tween 80 as a surfactant then GOM and preservatives i.e methyl paraben and propyl paraben. Mask the odor by adding sufficient quantity of camphor to formulation.

Method for Trial 4

First weigh 3.5gm of sodium alginate which is gelling agent then mix it with magnetic stirrer to 50ml of water. Add 10 ml of GOM, 3.5 ml of glycerol and 0.2 gm of methyl paraben & propyl paraben 0.02 gm as preservative. Mask the smell of GOM by addition of eucalyptus oil.

Method for Trial 5

Take xanthan gum as gelling agent 1.0gm and then mix it with help of magnetic stirrer in 50ml of water. After this break the clumps that are formed during mixing with the help of glass rod. Then add triethanolamine to adjust pH to neutralization point. Check the pH with help of pH paper. Add tween 80 as a surfactant then GOM and preservatives i.e methyl paraben and propyl paraben. Mask the odor by adding sufficient quantity of camphor to formulation.

Method for Trial 6

First weigh 1 gm of carbopol 940 then mix it with help of magnetic stirrer in 50ml of water. After this break the clumps that are formed during mixing with the help of glass rod. Then add triethanolamine to adjust pH to neutralization point. Check the pH with help of pH paper. Add tween 80 as a surfactant then GOM and preservatives i.e methyl paraben and propyl paraben. Mask the odor by adding sufficient quantity of camphor to formulation.

RESULTS

DIFFERENT TRIALS FOR OINTMENT

TRIAL 1

Observation: ointment formed was too hard, so we moved to next trial.

TRIAL 2

Observation: ointment formed was hard, so we moved to next trial with increase in concentration of white soft paraffin and reducing concentration of hard paraffin.

TRIAL 3

Observation: ointment formed was soft smooth and easily spreadable.

RESULT: This Ointment form was found stable and it was carried forward for the evaluation.

INFERENCE: Although this formulation was found to be physically stable and acceptable, it was rejected in the final analysis due to heating during preparation of ointment activity of allicinase enzyme lost which directly affect the activity of the drug, so we moved to next semisolid formulation i.e. Gels.

DIFFERENT TRIALS FOR GELS

TRIAL 1

Observation: In trial first the gel formed with 1.0% CMC

was found very low in viscosity and consistency, so we moved to next trial by lowering the water quantity.

TRIAL 2

Observation: In second trial, gel was formed with 2% CMC, TEA and tween 80 as co- surfactant. Gel formed had good consistency and viscosity but we have concentration of drug about 20% for gel so we moved to next trial.

TRIAL 3

Observation: The gel formed in third trial with 20% drug having low viscosity and good consistency, We moved to next trial with different gelling agent. (sodium alginate)

TRIAL 4

Observation: Sodium alginate used as gelling agent for this trial. The gel formed was found unstable. The oil phase was separated from water, so we moved next trial with different gelling agent. (i.e. xanthan gum)

TRIAL 5

Observation: Gel formed with xanthan gum was found with good viscosity and consistency, The next trial with different gelling agent. (i.e. Carbopol 940)

TRIAL 6

Observation: gel formed with carbopol 940 was found to be of the best viscosity and consistency among all the gelling agents. The gel formed was also found to be stable.

Result: Among all the trials, gel formed with 2% carbopol 940 was found to be stable and with good consistency, so further evaluation of this gel was performed using different evaluation parameters such as pH, Viscosity and antifungal assay.

EVALUATIONS OF OINTMENTS AND GELS

Evaluation of ointments was performed for trial 3 and for gel for trial nos. 3, 5 and 6 because all these batches were found to be stable.

Evaluations results are listed in table number 5.

PH

The pH was determined using digital pH meter of Lab India. pH values for ointment and gels are shown in table number 5

HOMOGENEITY

Homogeneity was calculated by visual inspection. Results are shown in table no.5

SKIN IRRITATION TASTE

Skin irritation test was performed on five healthy volunteers by applying gel on back side of the hand and covering it with a suitable material and retaining it for 24 hours. After 24 hours, the reports about skin irritation were collected from the concerned volunteers; the same are exhibited in table no.5

SPREADABILITY

Spreadability was performed using two glass slides, one of which is fixed and the other is movable. The results obtained for spreadability are shown in table no.5

ANTIFUNGAL ASSAY

Antifungal assay was performed only for ointment trial 3 and gel trial 6 because from above results, it was concluded that only these products comply with all tests. The culture used for antifungal assay was candida albicans 3471 obtained from NCIM Pune. The medium used for culture growth was potato dextrose agar from Himedia Laboratories. Agar diffusion method was used for antifungal assay.

A. Cultures used:

Fungi:

Candida Albicans 3471 NCIM Pune

B. Media used:

For Fungi: Potato Dextrose Agar (Hi-Media)

C. Inoculum size:

Fungi: 1 X10⁶ Cells Per ml

D. Concentration of Compound:

100 µg/Disc (Prepared In DMSO)

E. Method used:

Agar diffusion assay (well method, well size 6 mm)

F. Dilution of drug:

Stock prepared 1mg per ml prepared in DMSO

[100 µg/disc]

G. Results:

Results are as shown in table number 6

The zone of inhibition of gel (trial no. 6) was found to be

10.12 and for ointment, no zone of inhibition observed, so ointment is not showing any anti-fungal activity. Activity in ointment might have been lost due to heating during preparation of ointment which causes loss of allcinase enzyme. However, the gel was shown to exhibit anti-fungal activity, comparable to a standard anti-fungal drug- Nystatin.

Figure 1

Table 1

Sr.No	Drug/Excipients/Chemicals	Source
1.	Garlic (single clove)	Local market
2.	Garlic (multiple clove)	Local market
3.	Sunflower Oil	Fortune House
4.	Sesame Oil	Raj oil mills
5.	Carboxy methyl cellulose (A.R.)	Pure Chem Laboratories
6.	Xanthan Gum (A.R.)	Loba Chemie Pvt.Ltd
7.	White soft Paraffin (A.R.)	S. d. fine chemie Pvt. Ltd
8.	Hard Paraffin	Pure Chem Laboratories
9.	Cetosteryl Alcohol (A.R.)	Loba Chemie Pvt.Ltd
10.	Tween 80 (L.R.)	Fine-Chem Ltd Mumbai
11.	TEA (L.R.)	Merck Pvt.Ltd Mumbai
12.	Camphor	Pure Chem Laboratories
13.	Carbopol 934 (A.R.)	Vishal-chem Mumbai
14.	Dextrose (A.R.)	Loba Chemie Pvt.Ltd
15.	Starch (A.R.)	Central Drug Laboratory
16.	Agar-Agar	Himedia Laboratories
17.	Ascorbic acid (A.R.)	Merck Pvt. Ltd Mumbai
18.	Poly ethylene glycol (L.R.)	Qualigens Fine Chemie.
19.	Span 80 (L.R.)	Loba Chemie Pvt.Ltd
20.	Ecalyptus oil.	R. K. Pharma Mumbai

Figure 2

Table 2

Sr. No	Equipment	Make
1.	Electronic balance	Shimadzu
2.	Autoclave	Medica Instrument Pvt Ltd.
3.	Magnetic Stirrer	Bio Lab
4.	Hot Air Oven	Electron
5.	pH meter	Labindia
6.	Viscometer	Electron
7.	Suction pump	Besto Pvt. Ltd

Figure 3

Table 3

Sr. No	Ingredients	Trial 1	Trial 2	Trial 3
1.	Oil macerate (ml)	11.2	10.4	11.4
2.	White soft paraffin (gm)	2.8	5.2	6.8
3.	Cetosteryl alcohol (gm)	2.8	3.1	2.3
4.	Hard paraffin (gm)	8.2	3.0	4.5
5.	Eucalyptus oil	q.s	q.s	q.s

Figure 4

Table 4

Sr. No	Ingredients	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
1.	GOM (ml)	1.0	2.0	10	10	10	10
2.	CMC (gm)	0.5	1.0	1.0	-	-	-
3.	TEA (ml)	0.25	2.0	2.0	-	1.0	1.2
4.	Tween 80 (ml)	-	1.0	1.0	-	1.0	1.0
5.	Carbopol (gm)	-	-	-	-	-	1.0
6.	Xanthan gum	-	-	-	-	1.0	-
7.	Sodium Al.	-	-	-	3.5	-	-
8.	Glycerol (ml)	-	-	-	3.5	-	-
9.	Methyl p.	0.1	0.2	0.2	0.2	0.2	0.2
10.	Propyl p.	0.01	0.02	0.02	0.02	0.02	0.02
11.	Camphor	-	q.s	q.s	-	q.s	q.s
12.	Eucalyptus oil	q.s	-	-	q.s	-	-
13.	Water (q.s gm)	50	50	50	50	50	50

GOM- garlic oil macerate, CMC- carboxy methyl cellulose, TEA- Triethanolamine
Methyl P.- Methyl paraben, Propyl p.- Propyl paraben

Figure 5

Table 5

Trial No.	pH	Homogeneity	Skin irritation taste	Spreadability
Oint trial 3	5.3	Good	Nil	1 min
Gel trial 3.	6.5	Fair	Nil	60 sec
Gel trial 5.	5.8	Fair	Nil	45 sec
Gel trial 6.	7.1	Good	Nil	30 sec

Figure 6

Table 6

Compound	Candida albicans NCIM 3471
Control (DMSO)	-
R1 (Gel)	10.12
R2 (Ointment)	NA
Nystatin(100U/disc)	22.1

Diameter in mm calculated by Vernier Caliper, '-' means no zone of inhibition, NA Not applicable.

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Author Information

R. Patil

School of Pharmacy and Technology Management, NMIMS University, Mumbai Shirpur Campus 425405 . Maharashtra, India

R.P. Ravindra

School of Pharmacy and Technology Management, NMIMS University, Mumbai Shirpur Campus 425405 . Maharashtra, India