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FORMULATION AND EVALUATION OF HERBAL ANTIDANDRUFF GEL OF *KAEMPFERIA GALANGA*

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ABSTRACT

Herbs have been used from antiquity and were the first medicine used by man. Even in cosmetics, herbs are first choice. Dendruff is the most common hair problem which is caused by the fungus ***Malasezzia furfur*'.** The plant drug obtained from ***K.galanga***, have good antidandruff activity against the ***M.furfur***. The plant extract was formulated as gels using the polymers Sodium CMC, HEC and Carbopol 934. Optimizations of gels were carried out in terms of appearance, pH, viscosity and spreadability. Though the physical properties of gels formulated with Carbopol 934 were better than Sodium CMC, it was observed that there was maximum release of marker content in Sodium CMC gels. **Conclusion:** Thus from the present study it can be concluded that topical application of ***K.galanga*** (5%), in the Sodium CMC gel base can be of potential use for local antifungal activity against dandruff causing fungus ***M.furfur***

KEYWORDS*:* Malasezzia furfur, SodiumCMC, HEC, Carbopol.

INTRODUCTION

Herbs have been used as a medicine since the start of human civilization. The use of herbs in the written record actually dates back for several thousand of years B.C. Traditional systems of medicine which have deep rooted societal acceptance in different countries include: **Ayureveda** in India, **Kampo medicine** in Japan, **Chinese Herbal Medicine** in China etc.[1]

The Indian subcontinent is enriched by variety of flora. Hence our country occupies top position in the use of herbal drugs.[2,3]

The concept of beauty and cosmetics is as ancient as mankind and civilization. The ancient science of cosmetology is believed to have originated in Egypt & India, but the earliest records of cosmetic substances and their application dates back to 1550 B.C. to the Indus valley civilization. According to Global Cosmetics Industry Business Magazine, the global market for natural beauty products will be worth more $429.8 billion by 2022.[4,5]

Dandruff[6]

Dandruff is a condition of scalp characterized by excessive clinically non-inflammatory scaling i.e. flaking and shedding (desquamation) of stratum corneum (i.e. dead cell) from the scalp . Dandruff is also called as scurf and historically termed as Pityriasis capitis.

Etiology[7,8]

The causation of dandruff is still debatable. There are many possible causes so far put forth by the scientists.

The skin or the scalp is made up of three layers. Starting from top

1. Epidermis
2. Dermis
3. Hypodermis (subcutaneous)

Epidermis is a stratified squamous epithelium.

Function of epidermis is to produce keratin (fibrous protein) that helps skin giving protection. The most commonly and widely accepted full-proof theory is that, the dandruff is caused by an overgrowth of yeast like fungus called as '***Malasezzia furfur'*** commonly found on the scalp.

Culture characteristics

Optimum requirements for the growth of ***Malassezia furfur*** are as follows

a) pH : 7 to 9

b) Temperature : 30±2˚C

c) Growth condition : Aerobic

d) Growth medium : Sabouraud's Dextrose Agar base (Himedia M286). This medium has to be supplemented with 2% butter as ***Malassezia furfur*** is a lipophilic fungus.

Growth was detected in 48 to 72 hours. A subcultured lab strain grows rapidly. Colonies appear as smooth to wrinkle.

Age group commonly affected[7]

The secretion of sebum from sebaceous gland is stimulated by hormones, especially androgens. These glands are relatively inactive during childhood but are activated in both sexes during puberty, when androgen production begins to rise. Thus dandruff mostly occurs after puberty (between ages of 20 and 30 years) and affects males more than females.

Treatment for dandruff[10]

As dandruff is a natural process, it cannot be eliminated. Dandruff is chronic, despite treatment. Treatment can prevent or minimize its occurrence. Currently available treatments include therapeutic use of zinc pyrithione, salicyclic acid, imidazole derivatives, glycolic acid, steroids, sulphur and tar derivatives.

Herbs having traditional claim and potential to be used in dandruff treatment according to literature[11]

Benincasa hispida, *Cicer arietinum, Hibiscus rosa sinensis, Acacia concinna, Urtica dioica*, *Humulus lupulus, Santalum album, Citrus aurantinum,* ***Kaempferia galanga***, *Petroselium cripsum, Achillea millefolium, Datura metel, Sapindus trifoliatus etc.*

Kaempferia galanga

Fig 1.1 Dried rhizomes of *Kaempferia galanga L.*

Taxonomical Classification[15]

Kingdom : Plantae

Sub-kingdom : Phanerogamae

Division : Spermatophyta

Subdivision	: Angiospermae
Class	: Monocotyledonae
Series	: Epigynae
Order	: Scitaminales
Family	: Zingiberaceae
Subfamily	: Zingiberoideae
Genus	: Kaempferia
Species	: *Kaempferia galanga L*

Origin and distribution[17]

The plant is supposed to have been originated in East Asia, most popularly in Burma. It is widely distributed in Asia, Africa, Australia, China, Nigeria and Mexico. In India it is cultivated mainly in Karnataka. It is also found in mountainous region of Nepal**.**

Description[18]

It is a stem less herb with tuberous, aromatic rootstock and having fleshy cylindrical non-aromatic root fibres.

Propagation[19]

Kaempferia galanga requires warm, humid climate and rainfall of 1500-2500 mm. It thrives well up to an elevation of 1500m. It requires rich loamy soil with good drainage and cannot stand water logging. It is a shade loving plant.

Chemical constituents[21]

The volatile oil content obtained from the rhizomes of ***K.galanga*** *was* 1.11*% v/w* and it exhibited yellow color with a characteristic odor. The components of ***K.galanga*** oil were found to be ά-pinene (1.28%), camphene (2.47%), carvone (11.13%), benzene (2.87%), eucalyptol (9.59%), borneol (2.87%), methyl cinnamate (23.23%), pentadecane (6.42%) and ethyl-p-methoxycinnamate (31.77%).

Review of pharmacological activities

1. Anti microbial activity[22]

Volatile oil of dried rhizome of ***Kaempferia galanga*** obtained by water distillation was tested against Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus faecalis and Bacillus subtilis*); Gram-negative bacteria (*Salmonella typhi*, *Shigella flexneri*, *Escerichia coli*) &

fungi *Candida albicans*. The results revealed that oil possessed marked anti microbial activity against Gram-positive bacteria with the inhibition zones from 12.0-16.0 mm and 8.0-12.0 mm against Gram-negative bacteria whereas it potently inhibited *Candida albicans* with an inhibition zone of 31.0mm which was stronger than that of standard anti fungal **clotrimazole (diameter-25.0mm).**

2. Anti nociceptive and anti inflammatory activity[21]

Aqueous extract of ***Kaempferia galanga*** leaves, in the doses of 30, 100 and 300 mg/kg was administered subcutaneously in mice/rats 30 minutes prior to the test. The extract exhibited significant ($P<0.05$) anti ociceptive activity when assessed using the abdominal constriction, hot plate and formalin tests, with activity observed in all tests occurring in a dose-dependent manner. The extract also produced a significantly ($P<0.05$) dose-dependent anti inflammatory activity when assessed using the carrageenan-induced paw-edema test.

3. Sedative activity[21]

Hexane extract of ***Kempferia galanga*** and two active aromatic compounds (compound 1. ethyl trans-p-methoxycinnamate and compound 2. ethyl cinnamate) from the extract were investigated for sedative effects by means of inhalation in mice. Inhalation of hexane extract at the doses of 1.5 and 10mg showed significant reduction of locomotor activity, indicating sedative and relaxant effects. Compound 1 and 2 were proved to possess sedative effects at 0.0014mg and 0.0012mg respectively.

4. Anticancer activity [22]

Ethyl p-methoxy trans-cinnamate was isolated from the methanolic extract of rhizomes of *K.galanga* and correlated to the cytotoxic activity by measuring the inhibitory activity on the colony-forming ability of Hela cells. Concentration giving 50% inhibition of colony-forming ability of Hela cells (IC_{50}) was found to be 100µg/ml.

Screening of Antidandruff Activity of *Kaempferia Galanga*

Extraction of plant materials

Powdered crude drug was extracted using two solvents namely methanol and water. Methanolic extraction was carried out in Soxhlet assembly till exhaustion of the constituents. Water extraction was carried out by boiling the powdered crude drugs for half an hour and then filtered and cooled. The obtained extracts were concentrated by rotary flash evaporator and then dried at 45˚C in vacuum oven. The dried extract was stored at 2-8˚C in refrigerator.

Experimental

Collection and processing of plant materials

Rhizomes of ***Kaempferia galanga***, was purchased from drug market, Masjid Bandar, Mumbai. Crude drugs were dried in an oven at temperature not exceeding 60˚C. After drying all the crude drugs were coarsely powdered and stored separately in an air tight container at room temperature.

Extraction of plant materials.

Screening of plant extracts for their potential to act as antidandruff agents.

One of the causes for dandruff is due to yeast like fungus ***Malassezia furfur***. Thus the plant extracts effective against yeast ***Malassezia furfur*** can act as a good antidandruff agents. Literature search revealed that inflorescence of *A.millefolium*, mixture of Citrus peels, rhizomes of ***Kaempferia galanga***, seeds of *Cicer arietinum*, fruits of *Sapindus triafoliatus* are traditionally used to control dandruff. Hence it was thought worthwhile to screen ***Kaempferia galanga*** for their antifungal activity against ***Malassezia furfur***.

A) Agar diffusion method for screening of the plant extracts for their potential to act against *Malassezia furur*

It is the technique in which the potency or concentration of compound is assayed by determining its effect on microorganisms. This involves inoculating the surface of an agar plate with the organism to be tested, creating tiny wells with a borer, and placing solution of the anti-microbial agent into these wells. The antimicrobial agent diffuses into the medium in a circle around the wells and inhibits the growth of microorganism. The width of the zone, which depends on the concentration of active agent used, forms the quantification basis of the test.

B) Preparation of test solutions

1. Sabouraud's dextrose agar solution: 4.7gm of Sabouraud's dextrose agar was dissolved with aid of heat in 100ml of distilled water.

2. 0.9% NaCl (Saline) Solution: 0.9gm of NaCl was dissolved in distilled water and volume made up to 100ml with distilled water.

3. Different extract solutions: Accurately weighed methanolic extracts (3g) the plant drug was dissolved in DMSO (Dimethyl sulphoxide) and transferred to volumetric flask and

volume made up to 10ml to obtain stock solution of 30% w/v. Further dilutions were made from the stock solution to obtain 1% w/v, 2% w/v, 4% w/v, 5% w/v, 10% w/v, 15% w/v, 20% w/v and 25% w/v. Accurately weighed water extracts (3g) the plant drugs were dissolved in double distilled water and transferred to volumetric flask and volume made up to 10ml to obtain stock solution of 30% w/v. Further dilutions were made from the stock solution to obtain 1% w/v, 2% w/v, 4% w/v, 5% w/v, 10% w/v, 15% w/v, 20% w/v and 25% w/v.

C) Sterilization of glasswares and media.

The glasswares required were sterilized by dry heat in an oven at 160°C for 2 hrs. The Sabouraud's Dextrose agar media was sterilized by autoclave at a temperature of 121°C for 15min at 15psi.

D) Maintenance of pure culture.

Standard cultures of dandruff causing fungus, ***Malassezia furfur*** (**MTCC 1374**) were obtained from the **Institute of Microbial Technology, Chandigarh.**

Pure culture of microorganism was maintained on Sabouraud's Dextrose agar slant. The Sabouraud's Dextrose agar slant was prepared by placing 5 to 7ml of media in test tube. It was then sterilized and allowed to set at an angle to give a slope. Pure culture was subcultured every month on the agar slopes to maintain its purity.

E) Preparation of standard inoculums.

A standard inoculum of fungus containing 1x10^{6}organism/ml was prepared. The number of fungus in liquid medium was adjusted by visually comparing the visibility of the liquid medium to standard that represented a known number of fungus in suspension. Turbidity standard was developed by McFarland using barium sulphate. For the purpose of this study McFarland std.no.1 was prepared by mixing 0.1ml of 1% aqueous barium chloride and 9.9ml 1% Sulphuric acid. This standard gives an approximate cell density of 3x10^{8} organism/ml.

For standard inoculum, a 24 hour old culture was washed with saline and the turbidity of the suspension was made equal in level to McFarland std.no.1. A 1:500 dilution of this suspension was prepared which gave an approximate cell density of 1x10^{6} organisms/ml.

F) Preparation of agar plates

About 15-17 ml of sterilized Sabouraud's Dextrose agar media was added aseptically to sterile plates to prepare a basal layer. The plates were incubated at 37°C $\pm$ 0.5^{o}C for 24 hrs.

The basal layer was seeded the next day with 5-6ml of sterile Sabouraud's Dextrose agar media containing 1ml suspension of standard inoculum. The plates were allowed to set and four tiny wells of 0.8cm in diameter were then bored in the solidified medium.

RESULTS AND DISCUSSION

A) Extraction of plant materials

Powdered crude drugs were extracted using two solvents namely methanol and water. The extractive values are given in the table 1.1 powdered crude drugs exhibited higher extractive values for water than methanol.

Table. 1.1 Methanol and Water Extractive values of the crude drugs.

Sr.no.	Name of the drug	Part Used	Extractive Value (% w/w) (Mean $\pm$ S.D.	
			Methanol	Water
(1)	***Kaempferia galanga***	Rhizomes	6.96 $\pm$ 0.00	15.66 $\pm$ 1.14

B) Screening of plant extracts for their potential to act as antidandruff agent.

Water and methanolic extracts of plant species ***Kaempferia galanga***, were screened against the growth of the fungus, ***Malassezia furfur*** by agar diffusion method. The antifungal activity was measured as a function of diameter of zone of inhibition. The water extracts of the plants did not show any activity whereas their methanolic extracts exhibited antifungal activity. The zone of inhibition exhibited by each of the extracts in the concentration range of 1% to 30% w/v is given in the table 1.2 and 1.3. Photographs of the same are given in the fig 1.2 – 1.3 it can be observed that increasing the concentration of the extract showed better antifungal activity. The zone of inhibition was compared with a standard **(Clotrimazole 1%, Selenium sulphide 2.5%).** The concentration that showed zone of inhibition similar to that of standard was taken for further studies. It was also necessary to limit the concentration below 10% so as to facilitate easy incorporation of the extract in gel formulation. With this view the following drug and concentration was selected.

1. *K.galanga* (5%w/w)

Thus phytochemical studies and formulation development were carried out with ***Kaempferia galanga*** exreact.

Table. 1.2: Zones of inhibition observed for Methanolic extracts of five plant species in concentration range of 1 – 10%w/v.

Sr. No.	Name of Drug	Part used	Zone of inhibition in mm (Mean ± S.D.)				
			1%w/w	2%w/w	4%w/w	5%w/w	10%w/w
	K.galanga	Rhizome	17.33±0.57	19.00± 0.00	20.36± 0.5	22.33± 0.4	23.33± 0.5

Fig 1.2 Antimicrobial activity of methanolic extract of *K.galanga.*

TLC & HPTLC studies.

The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the "chemical integrities" of the herbal medicines and therefore be used for authentication and identification of the herbal products.[21]

TLC is used as an easier method of initial screening with a semiquantitative evaluation together with other chromatographic techniques. The advantages of using TLC to construct the fingerprints of herbal medicines are its simplicity, versatility, high velocity, specific sensitivity and sample preparation.

A) TLC studies

Methanolic extracts of the plant drug was subjected to TLC studies. The stationary phase used was Silica gel 60 F254 plates (thickness 0.2mm).Various solvents of analytical grade were tried as mobile phase and the solvent system which gave maximum resolution of the spots are as follows.

1. ***K.galanga*:-** **Chloroform:toluene** (7.5:2.5)

Schematic representations of developed TLC plates are given in figs 1.3

B) HPTLC fingerprinting

HPTLC fingerprints were established for methanolic extracts of the above mentioned plant drug using the optimized chromatographic conditions as mentioned below.

a) Preparation of the extract solutions

- 50 mg of the methanolic extract of ***K.galanga*** was accurately weighed, dissolved in methanol and transferred to a volumetric flask and the volume made up to 10ml with methanol to obtain a concentration of 5000µg/ml.

b) Conditions of sample application

1. Instrument used : Camag Linomat 5 “Manually set to executed”
2. Test plates : TLC Silica gel 60 F_{254}
3. Format : 10.0x10.0 cm
4. Spotting volume : ***K.galanga*-10µl,**
5. Band length : 4.0mm
6. Interband space : 10mm

c) Conditions of chromatography

1. Solvent system : As given in the TLC studies
2. Separation technique : Ascending
3. Developing chamber : Camag twin- trough chamber
4. Distance of migration: 80.0mm
5. Slit dimensions : 3.00 x 0.30mm

d) Conditions of scanning

1. Instrument : Camag TLC Scanner3
2. Mode : Absorption and Fluorescence
3. Lamp : D_2 (Absorption) and Hg (Fluorescence)
4. Wavelength : 254nm, 366nm
5. Slit dimensions : 3.00 x 0.30mm

e) Procedure

The HPTLC analysis was performed on aluminium sheet pre-coated with silica gel 60 F_{254}. Before use, plates were pre-washed with methanol and dried in an oven at 105˚C for 1hour. Methanolic extract of ***K.galanga*** (10µl), was spotted on the plates as bands of 4mm width

with the help of linomat-5 sample applicator. The plates were developed in Camag twin-trough chamber previously equilibrated with suitable solvent system. After development, plates were allowed to air dry and then scanned at 254 and 366nm using Camag TLC scanner3 for the development of chromatograms.

HPTLC chromatograms are given in the figs 1.4 & 1.5.

RESULTS AND DISCUSSION

A) Phytochemical evaluations of the extracts

Preliminary phytochemical analysis was carried out on the methanolic extracts of ***K.galanga***, for the presence of phytoconstituents. The results are given in table 1.3 *K.galanga* showed the presence of Carbohydrates, tannins, alkaloids and amino acids.

Table 1.3 Preliminary phytochemical evaluation of methanolic extract of *K.galanga*

Tesst of carbohydrate	**KG**
a) Millon's test	-
b) Biuret test	+
c) Ninhydrin test	-
3) Test for steroids	
a) Salkowski test	-
b) Libermann test	-
c) Libermann-Barchard test	-
4) Test for Cardiac glycosides	
a) Keller-killani test	-
b) Legal's test	-
5) Test for Saponin glycosides	
a) Foam test	-
6) Test for flavonoids	
a) Shinoda test	-
7) Test for tannins	
a) Lead acetate solution	+
b) Diliete $KMnO_4$	+
c) 5% $FeCl_3$ solution	+
8) Test for alkaloids	
a) Dragendraff's test	+
b) Mayer's test	+
c) Hager's test	+
d) Wager's test	+

KG: *K.galanga*,

'+' : Presence of phytoconstituents

'–' : Absence of phytoconstituents

Table 1.4 R_f values of spots resolved at 254 nm and 366 nm

Methanolic extract	No. of spot		R_f value	
	254 nm	366 nm	254 nm	366 nm
K.galanga	5	5	0.12, 0.22, 0.37, 0.46, 0.58	0.1, 0.17, 0.22, 0.37, 0.45

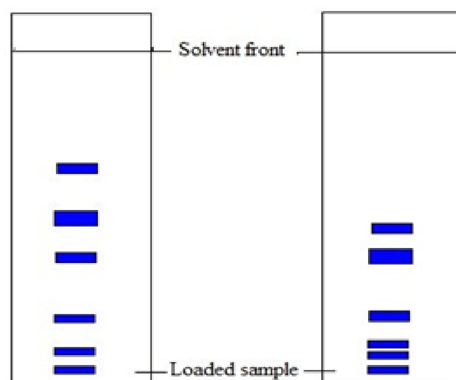
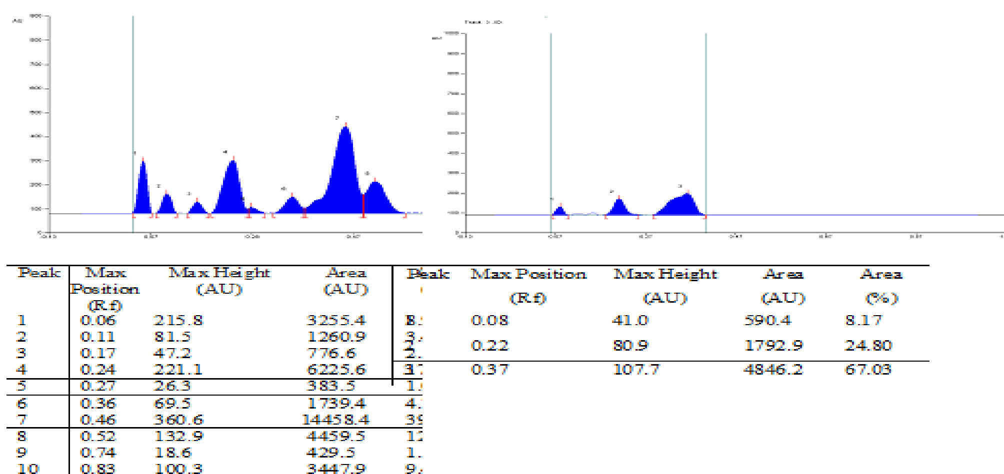


Fig. 1.3: Schematic representation of developed TLC plates of methanolic extracts *K. galangal.*



Peak	Max Position (Rf)	Max Height (AU)	Area (AU)
1	0.06	215.8	3255.4
2	0.11	81.5	1260.9
3	0.17	47.2	776.6
4	0.24	221.1	6225.6
5	0.27	26.3	383.5
6	0.36	69.5	1739.4
7	0.46	360.6	14458.4
8	0.52	132.9	4459.5
9	0.74	18.6	429.5
10	0.83	100.3	3447.9

Peak	Max Position (Rf)	Max Height (AU)	Area (AU)	Area (%)
	0.08	41.0	590.4	8.17
	0.22	80.9	1792.9	24.80
	0.37	107.7	4846.2	67.03

Fig 1.4 HPTLC fingerprinting of methanolic extract of *K.galanga* at 254nm

Fig 1.5 HPTLC fingerprinting of methanolic extract of *K.galanga* at 366nm

Standardisation of methanolic extract of *Kaempferia galangal L.*

Literature search showed the presence of ethyl cinnamate as one of the major constituent of *K.galanga*.[21,22] λ_{max} of ethyl cinnamate was determined by scanning with UV-Vis spectrophotometer (Elico SL 159) and was found to be 276 nm. The methanolic extract of *K.galanga* also showed a λ_{max} of 276nm which confirmed the presence of ethyl cinnamate in it.

- **Quantification of ethyl cinnamate in methanolic extract of *K.galanga* by UV method**

A) Preparation of extract solution

10 mg of the methanolic extract of ***K.galanga*** was accurately weighed, dissolved in methanol and transferred to volumetric flask and volume made to 100 ml to obtain a concentration of 100µg/ml. 1ml of this solution was diluted to 10ml to give a concentration of 10µg/ml.

B) Preparation of standard ethyl cinnamate solution

Accurately weighed 1gm of ethyl cinnamate (weight in volume) was made to 1000ml in volumetric flask with methanol to obtain a concentration of 1000µg/ml. 1ml of this solution was diluted to 100ml to get secondary stock solution of concentration 10µg/ml.

C) Preparation of standard curve of ethyl cinnamate

From the secondary stock solution of ethyl cinnamate (10µg/ml) 1, 2, 3,4,5,6,7,8 and 9ml of the solution was pipetted separately, transferred to volume made up to 10 ml with methanol to obtain a concentration range of 1-9µg/ml. Absorbance of each of the above prepared dilutions was measured at 276 nm against methanol. Five replicate analysis were carried out and the calibration curve was plotted as concentration (µg/ml) v/s absorbance. Methanol was used as reagent blank.

D) Quantification of ethyl cinnamate

The absorbance of extract solution (10µg/ml) was measured at λ_{max} of 276 nm against reagent blank as that of standard by UV-Visible spectrophotometer. The amount of ethyl cinnamate (%w/w) in the extract was determined by extrapolating from the standard curve. The assay was performed in triplicate and the results are reported as mean±SD.

D) Standardisation of the methanolic extract of *Kaempferia galanga*

The λ_{max} of ethyl cinnamate on scanning with UV-Vis spectrophotometer (Elico SL 159) showed maxima at 276nm. Also the methanolic extract of *K.galanga* showed λ_{max} of 276 nm confirming the presence of ethyl cinnamate in it. Spectra comparision also confirmed the presence of ethyl cinnamate in extract. Therefore the methanolic extract was quantified for content of ethyl cinnamate. A linear plot was obtained for ethyl cinnamate in the concentration range of 1-9µg/ml with a 'r' value of 0.9913. The ethyl cinnamate content was calculated from the standard regression equation and was found 50.5%w/w in the extract.

The total flavonol content and ethyl cinnamate content was used as marker compounds in formulation studies and evaluation.

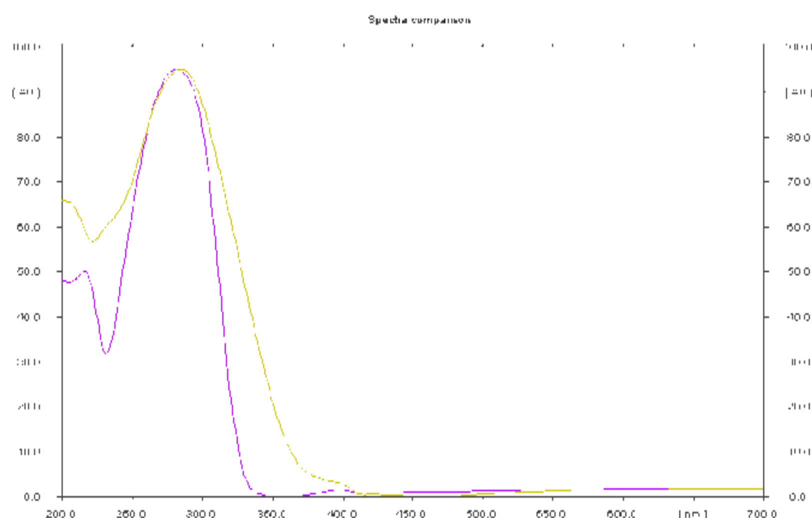


Fig. 1.6 Spectra comparison of methanolic extract of *K.galanga* and ethyl cinnamate.

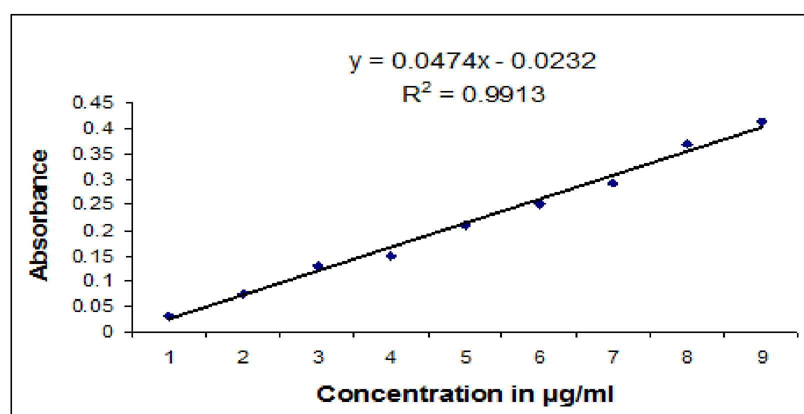


Fig. 1.7 Standard curve for ethyl cinnamate.

Formulation of Antidandruff Gel

Plant drugs namely ***K.galanga***, was found to be effective against ***Malassezia furfur*** with zone of inhibition of 22.33±0.4, mm approximately.

Preparation of gels of *K.galanga* extract

The preparation of gels comprised of two phases:-

Phase 1: Polymer dispersion

Phase 2: Extract solution

Phase 1: Polymer dispersion

1) Weighed quantities of methyl paraben was added to sufficient amount of distilled water and heated to 70ºC to affect dissolution. After dissolution of methyl paraben.

2) Accurately weighed amount of polymer as described in table 1.5 was dispersed in the aqueous medium. The polymers Sodium CMC and HEC were soaked for two hours and polymer dispersion of carbopol 934 was kept for overnight soaking.

3) After two hours/overnight soaking propylene glycol was added to polymer dispersion and mixed well to form uniform gel base.

4) Phase 2: **Extract solution**

5) Methanolic extract of ***K.galanga*** was completely soluble in ethanol. Hence accurately weighed amount of extract was dissolved in ethanol. Propyl paraben was added to this solution and stirred well.

6) Phase 2 was incorporated in phase 1 by slow trituration in glass mortar.

7) In the formulation containing carbopol 934 triethanolamine was added dropwise until gel was formed.

8) The volume was made up to 25gms with respect to all formulation by addition of distilled water.

Table. 1.5 Compositions of *K.galanga* extract gels (%w/w).

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carbopol 934	0.5	1	2	_	_	_	_	_	_
Sodium CMC	_	_	_	3	4	5	_	_	_
HEC	_	_	_	_	_	_	2	3	4
Ethanol	30	30	30	30	30	30	30	30	30
Propylene glycol	15	15	15	15	15	15	15	15	15
Triethanolamine	q. s	q. s	q. s	_	_	_	_	_	_
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Distilled water	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s
Extract	5	5	5	5	5	5	5	5	5

Physical evaluation of formulated gels to optimize the polymer concentration.

Polymer concentration determines the viscosity, spreadability and release of the active principles from the gels. As the polymer concentration increases the network structure of gel becomes more compact increasing the viscosity and lowering the release of active ingredients.[20] Hence gels were formulated using different polymers like Carbopol 934, Sodium CMC and HEC with varying concentrations and evaluated to optimize the polymer

concentration. Evaluation was done on the basis of its physical characteristics like appearance, pH, viscosity and spreadability etc.

A) Appearance: Gels were evaluated for its appearance considering its color, uniform dispersion, pliability and elegance.

B) pH measurements: 1gm of gel was accurately weighed and dispersed in 30ml of distilled water. The pH of dispersion was measured using pH meter (Elico LI 127). pH measurements of each gel were done in triplicate and reported as the mean ±S.D.

C) Viscosity: A Brookfield viscometer (RV model) was used to measure the viscosity of the gels. In 50 ml beaker 20 gm of gel was taken and spindle # 7 was rotated at 10 rpm for 5 minutes. The dial readings were recorded and multiplied by conversion factor to convert it in centipoise. Viscosity measurements of each gel were done in triplicate and reported as the mean ±S.D.

D) Spreadability

Spreadability of the gel formulations were determined with a modified apparatus consisting of wooden block provided with two glass slides. Lower slide was fixed on wooden block and upper slide was tied to weight pan. 2.5 gm of the gel was placed between two glass slides and 1000 gm weight was placed over it for 5 min to press the sample to a uniform thickness. 50 gm weight was added to a pan. The time (in seconds) required to separate the two slides was taken as a measure of spreadability (S). Shorter the time interval to cover the distance of 7.5cm indicates better spreadability. Spreadability readings of each gel were done in triplicate and reported as the mean ±S.D.

$S = M \times L / T$

Where M = weight tide to upper slide in gm

L = length moved on the glass slide (7.5 cm)

T = time taken in seconds

The results of physical evaluation of the gels are given in table 1.6.

Gels were formulated using different polymers like Carbopol 934, Sodium CMC and HEC with varying concentrations. Evaluation was done on the basis of its physical characteristics like appearance, viscosity and spreadability. This was required because; at higher polymer concentrations the active substance is trapped in smaller polymer cells and it is structured by

its close proximity to the polymer molecules. This increases the diffusional resistance more than expected. Also high polymer concentration increases the gel viscosity and decreases its spreadability and vise a versa. Hence a need to optimize the polymer concentration in formulation of gels was essential. The gels were formulated with varying concentration of Carbopol 934 (0.5, 1, 2%), Sodium CMC (3, 4, 5%) and HEC (2, 3, 4%). Gels were optimized for polymer concentration on the basis of their smoothness, homogeneity and elegance. It was observed that gels formulated with 4% Sodium CMC, 1% Carbopol 934 and 3% HEC were smooth, pliable, homogenous and elegant in appearance when applied on the skin with finger as compared to other concentrations of the polymers.

Stability Study: All selected formulations were subjected to stability testing as per ICH guideline. Prepared gels were stored in glass containers (well stoppered) for three months at a temperature of 25ºC±2ºC and 60%±5 relative humidity, 40ºC±2ºC and 75%±5 relative humidity.

Experimental: The selected formulations were analyzed on 0th day and monthly throughout three-month period for the change in appearance, pH, viscosity, spreadability, total flavonol content, ethyl cinnamate content, HPTLC fingerprinting etc. The results of which are presented in tables 1.6 for gels stored at 25ºC±2 ºC and table 1.6 for gels stored at 40ºC±2ºC.

RESULTS AND DISCUSSION

The table 1.6 indicates that spreadability remained constant at 36.80 gm.cm/sec on 0th day to 36.76 gm.cm/sec after three months for Sodium CMC gels prepared using ***K.galanga*** extracts with an average ethyl cinnamate content equivalent to 49.4%w/w. The zone of inhibitions exhibited by ***K.galanga*** gels formulated using Sodium CMC remained constant at 20.7 mm on 0th day to 20.6 mm after three months. These results were observed at 25ºC and 60% relative humidity. Similar results could be seen in the table 1.6 wherein the evaluation parameters were checked at 40ºC and 75% relative humidity. The gels formulated with other two polymers also did not show any significant change with respect to pH, viscosity, spreadability and chemical content release of drug. The antidandruff activity also remained constant throughout the three month study. Also no changes were observed in the HPTLC fingerprinting of the gels during the stability studies. The combination gels remained to give synergistic effect at both the temperature and humidity conditions throughout the studies. The results of the stability studies are given in table 1.6.

Table. 1.6 Stability studies data of *K.galanga* gels at 25ºC and 60% relative humidity.

Code	Polymer	Month	pH	Viscosity 10^3 centipoise	Spreadability gm.cm/sec	Ethyl cinnamate content (%w/w)	Release (%)	Diameter of zone of inhibition (mm±S.D)
F5	Sodium CMC (4%)	0	5.71±0.01	8.33±0.57	36.80±0.01	49.8±0.30	98.61±0.03	20.7±0.00
		1	5.70±0.00	8.33±0.00	36.83±0.00	49.5±0.20	98.02±0.03	20.66±0.00
		2	5.70±0.03	8.66±1.15	36.77±0.01	49.4±0.30	97.82±0.02	20.33±1.15
		3	5.72±0.01	8.33±1.15	36.76±0.03	48.9±0.10	96.83±0.03	20.6±1.7
F8	HEC (3%)	0	5.84±0.02	3.66±1.15	12.72±0.00	49.7±0.20	98.42±0.00	20.00±0.00
		1	5.83±0.02	13.00±0.00	12.70±0.00	48.6±0.20	96.24±0.01	20.5±0.5
		2	5.84±0.01	13.33±1.15	12.68±0.01	48.5±0.00	96.04±0.00	20.3±1.2
		3	5.82±0.03	13.40±1.15	12.71±0.01	48.5±0.01	96.03±0.04	20.66±1.15
F2	Carbopol 934 (1%)	0	6.00±0.00	6.00±0.00	71.59±0.03	47.3±0.20	93.66±0.03	16.66±1.15
		1	6.00±0.00	5.33±1.15	70.00±0.02	47.1±0.10	93.27±0.01	16.33±1.15
		2	5.99±0.02	5.66±1.15	72.11±0.01	46.0±0.00	91.08±0.01	16.00±0.00
		3	5.84±0.01	6.33±0.57	70.23±0.03	47.2±0.20	93.47±0.00	16.66±0.00

CONCLUSION

The plant drug obtained from ***K.galanga***, had good antidandruff activity against the ***M.furfur*.** The plant extract was formulated as gels using the polymers Sodium CMC, HEC and Carbopol 934. Optimizations of gels were carried out in terms of appearance, pH, viscosity and spreadability. Though the physical properties of gels formulated with Carbopol 934 were better than Sodium CMC, it was observed that there was maximum release of marker content in Sodium CMC gels. Thus from the present study it can be concluded that topical application of ***K.galanga*** (5%), in the Sodium CMC gel base can be of potential use for local antifungal activity against dandruff causing fungus ***M.furfur*.**

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