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RESEARCH ARTICLE

Formulation and Evaluation of Herbal Gel of *Boswellia Serrata* for the Management of Gout

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ABSTRACT

Herbal medicine has become an item of global importance both medicinal and economical in the modern drug delivery system. Herbal remedies are getting increasing patient compliance as they are devoid of typical side effects of allopathic medicines. Considering these facts present study deals with Topical Drug Delivery system composed of *boswellia serrata* extract which are having Antiinflammatory activity in the form of herbal gel for management of gout were formulated using gelling agent such as aerosil. The drug was evaluated on the basis of solubility, Ultraviolet Spectroscopy, Fourier-transform Infrared Spectroscopy, High Performance Liquid Chromatography, Differential Scanning Colorimetry study. Formulations were prepared by dispersion method with altering the ratio of additives. The prepared herbal gel was subjected for preliminary evaluation such as pH, viscosity, Spreadability, skin irritation study, in vitro drug release. Among the various formulations prepared F2 shows the best results as per the preliminary studies of appearance, viscosity, Spreadability and in vitro release. The drug interaction FT-IR studies, HPLC and DSC indicated that there was no chemical interaction between the drugs and the additives used in gel formulations. The in vitro drug release kinetics study depict that drug release mechanism follows Fickian diffusion.

KEYWORDS

Boswellia Serrata, Aerosil, Dispersion Method, Gel.

INTRODUCTION

Gout is a painful and potentially disabling form of arthritis that has been around since ancient times. The first symptoms usually are intense episodes of painful swelling in single joints, most often in the feet, especially the big toe. The swollen site may be red and warm[1]. Gout describes a group of metabolic disorders where crystals of sodium urate (the sodium salt of uric acid) deposit in tissue. This usually follows a prolonged period where uric acid levels in blood are raised.

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The increase in blood uric acid results from increased purine intake (purines are precursors of uric acid), or increased turnover or production, or from decreased uric acid elimination by the kidneys, or a combination of all these. Though higher purine intake may play a part in high blood uric acid levels, excretion by the kidney should increase to compensate. In most (75%-90%) people with gout, clearance of uric acid by the kidney is significantly reduced. Increased uric acid production or decreased renal clearance can be secondary to other disorders[2].

Herbal drugs are becoming more popular in the modern world for their application to cure

variety of diseases with less toxic effects and better therapeutic effects[3].

Boswellia serrta (family - *Burseraceae*) is selected in the antigout formulation based on its Anti-inflammatory activity due to active compound pentacyclic triterpenic acids namely Boswellic acids (figure1), which possess anti-inflammatory, anti-arthritic, antigout activity. In addition it has hepatoprotective and immunomodulatory activity as well. Boswellic acids are novel, specific, non-redox inhibitor of 5-lipoxygenase, an enzyme involved in arachidonic acid metabolism.[4] Phytochemical profile of Boswellia serrata contain essential oil, gum and resin .its essential oil is the mixture of monoterpene, diterpenes nad sesquiterpenes. Gum portion of the drug consist of pentose and hexose sugar with some oxidizing and digestive enzymes. Resin portion mainly composed of pentacyclic triterpene acid of which boswellic acid is the active moiety[5].

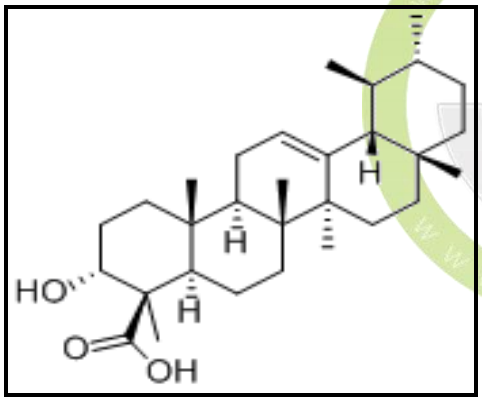

Figure 1: Structure of Boswellic Acid

MATERIALS AND METHOD

- *Boswellia serrta* extract, methyl salicylate, menthol, sesame oil, was obtained as gift sample from Hamdard (Wakf) laboratories, Ghaziabad (U.P.), India.
- Aerosil, BHT, BHA was obtained from Standard chemicals, New Delhi, India.
- All chemicals and reagents used were of analytical grade.

Standardization of *Boswellia serrata*

The evaluation of a crude drug involves the determination of identity, purity and quality. Purity depends upon the absence of foreign matter whether organic or inorganic, while quality refers essentially to the concentration of the active constituents in the drug that makes it valuable to medicine[6]. Various standardization parameters were evaluated to obtain the qualitative information about the purity and quality of *Boswellia serrata* such as total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, foreign matter, melting point, loss on drying[7].

Identification Tests

Identification tests such as Solubility, Ultraviolet (UV) spectroscopy, Fourier Transform Infrared (FT-IR) spectroscopy, and Differential Scanning Colorimetry (DSC) were conducted.

Solubility

Solubility of boswellia serrata was checked by using different solvent like water, methanol, hexane, dichloromethane and acetonitrile.

U.V. Spectroscopy Method

Preparation of Standard Plot of Boswellia serrata Exudates in 6.8 Phosphate Buffer Solution

For making the standard curve of *boswellia serrata* extract serial dilution were made. Initially a solution of the concentration of 1000 mcg/ml was made by weighing 10 mg of drug using digital balance (Shimadzu, AU X 220) and dissolving in 10 ml of 6.8 phosphate buffer solution. From this stock solution different concentration ranging from 25-100 mcg/ml were made in using 6.8 phosphate buffer solution as blank at 260 nm for detection of boswellic acid[8]. A graph was plotted between the concentration (X-axis) and absorbance (Y – axis).

Infrared (IR) Spectroscopy

The infra red spectrum of drug *boswellia serrata* extract was recorded in the range of 400-4000 cm^{-1} using potassium bromide pellet method and was compared for any interaction presents FT-IR was obtained using Shimadzu

FT-IR spectrophotometer, model iraffinity-1CE[8,9].

High Performance Liquid Chromatography

The drug sample was analyzed by using Shimadzu HPLC with an attached UV detector, on a reverse phase column C18 (25 cm × 4.6 mm, 5 µm)

The instrument was adjusted to the following parameters[10]:

a. Mobile phase: Acetonitrile : water (90:10)

b. Flow rate: 1.5 ml/min.

c. Detection: UV, 260 nm

d. Injection: 20 µl

Differential Scanning Calorimetry (DSC)

DSC of the pure drug was taken by using differential scanning calorimeter[11] (Perkin Elmer DSC-7) calibrated with Indium. All samples were run in triplicate. The instrument was adjusted to the following parameters:

a) Atmosphere: Nitrogen inert.

b) Heating rate: 10ºC/min

c) Gas flow rate: 20ml/min

d) Temperature range: 30-200ºC

e) Sample size: 0.5 mg

Formulation of Antigout Herbal Gel of *Boswellia Serrata*

Gel was formulated by dispersion method. Aerosil 200 were dispersed in 50 mL of sesame oil with mechanical stirrer and kept in a dark place for 24 hrs for hydration. Desired quantity of BHT & BHA was added. Further required quantity of *boswellia serrata* plant extract was added to gel with continuous stirring till drug get dispersed in gel completely.

Finally full mixed ingredients were mixed properly to the gel with continuous stirring and tri ethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency[12,13,14]

Table 1: Batch design with varying the concentration of additives

Each 100 g contains

Ingredient	F1 (g)	F2 (g)	F3 (g)
B. serrata extract	26	26	26
Methyl salicylate	19	17	16
Menthol	8.5	7.5	6.5
Aerosil	6.3	5.5	6
BHA	0.095	0.095	0.095
BHT	0.095	0.095	0.095
Sesame oil	q.s.	q.s.	q.s.

Evaluation of Boswellia Gel

Physical Evaluation

The colour, appearance and the feel on application of the prepared herbal gel formulations were noted and the results are shown in Table[15].

pH measurement

The pH of the gel was determined by using a digital pH meter. 5gm gel dissolved in 50 ml water and pH was determined by dipping the glass electrode completely into gel solution system so as to cover the electrode. Then instrument reading in terms of pH was recorded[16].

Spreadability

It was determined by wooden block, which was provided by a pulley at one end. By this method Spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. The upper slide was

then pulled apart horizontally with a string and pulley system. Initially 10 gm weight was tied to the thread and left for 5 minutes, and then the weight was increased by 1 gm at every step. The time required separating the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of Spreadability (S).[17]

Spreadability was calculated using the following formula:

$S = M \times L / T$

Where,

S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide

T = Time (in sec.) taken to separate the slide completely each other.

After feel

Emolliency, slipperiness and amount of residue left after the application of fixed amount of gel was checked.

Removal

The ease of removal of the gel applied was examined by washing the applied part with tap water.

Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Viscosity

Viscosity was measured by Brookfield Viscometer[18] which measures the shearing stress on a spindle rotating at a definite, constant speed while immersed in the sample.

In-vitro Drug Release Study

The dissolution studies were performed in six station dissolution test apparatus (37±0.5^{0}C, 50 rpm) using the USP rotating basket method in 6.8 phosphate buffer solution for 16 hrs by dialysis bag[19].

1 gm of gel was kept in the dialysis bag. The entire surface of membrane was in contact with the receptor compartment containing 900 ml of 6.8 phosphate buffer solution. The sample of 5 ml each was withdrawn at predetermined time interval and were replenished immediately with the same volume of phosphate buffer maintaining sink condition throughout the experiment. The aliquots, following suitable dilution with phosphate buffer were analysed spectrophotometrically at 260 nm. The concentrations of boswellic acid in the test samples were calculated using a regression equation.[8]

Skin Irritation Test

The irritancy of the optimized formulation was determined in the albino Wistar rats (1099/07/CPCSEA, [KSOP, Ghaziabad]). About 1g cream was applied to the left ear of the albino Wistar rat and the right was considered as a control[20,21]. The development of erythema and oedema were monitored for 3 days using the reported method[22].

RESULTS AND DISCUSSION

Ultraviolet Spectroscopy

UV Plot of Boswellic acid in pH 6.8 Phosphate Buffer Solution

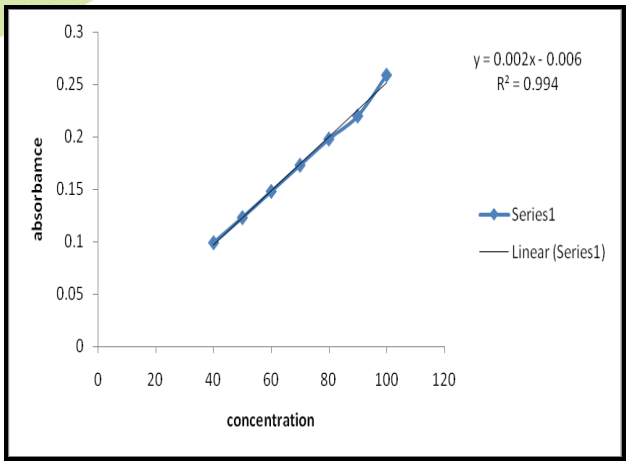

Figure 2: Calibration curve of boswellic acid through U.V.

The calibration curve of U.V. depicts that it obeys Beer's Lambert law where, slope = 0.0026 and R^2 = 0.9947 at 260 nm.

Table 2: Physico-Chemical Properties of *boswellia serrata* exudate

Properties	Observed value	Reported value [according to HAMDARD (Wakf) LAB. monograph]
Appearance	dried gums lumps in granular yellowish brown colour	dried gums lumps in granular yellowish brown colour
Taste and odour	characteristic odour	characteristic odour
Melting point	229.575°C	238.122°C
Loss on drying at 105°C	6.3%	*NMT 12%
Total ash	1.72%	*NMT = 9%
Acid insoluble ash	0.41%	*NMT = 1%
Alcohol soluble extractive	70%	**NMT = 76%
Water soluble extractive	28%	**NMT = 32%
Foreign matter	1.42%	*NMT = 2%

***NMT= Not more than;**

As shown by the Table.2, the physico-chemical properties of the drug was found in accordance with that of standard monograph of Hamdard (Wakf) laboratories.

Solubility

Table 3: Solubility of *boswellia serrata* exudate

S.No.	Solvent	Solubility
1	Water	Slightly soluble
2	Methanol	Soluble
3	Hexane	Soluble
4	Dichloromethane	Soluble
5	Acetonitrile	soluble

Fourier-Transform Infrared Spectroscopy

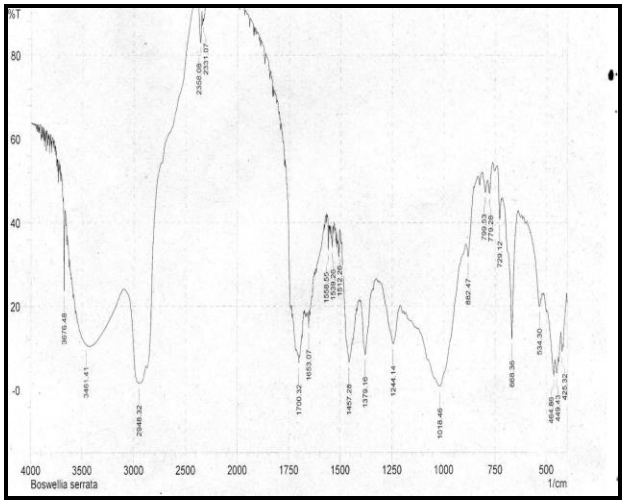

Figure 3: FT-IR spectra of boswellic acid extract

Table 4: Interpretation FT-IR spectra of boswellic acid extract

Wavenumber (Cm^{-1})	Characteristic Functional Group Feasible	Compound Type
2948.32	Ar C-H stretch	Aromatic
799.53	Ar C-H	Aromatic
1653.07	C=C stretch	Cycloalkenes
1700.32	C=0 stretch	Carboxyl
3461.48	O-H stretch	Hydroxyl

High Performance Liquid Chromatography

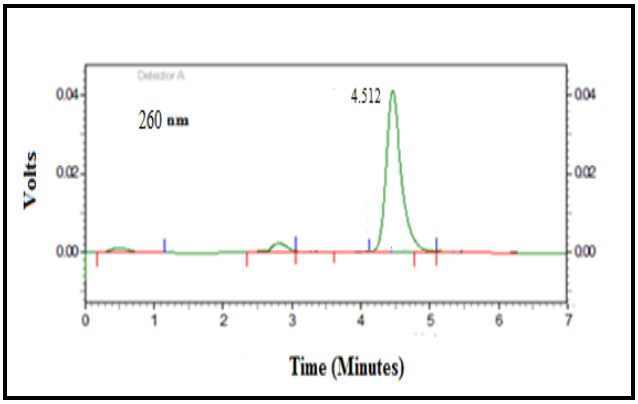

Figure 4: HPLC of *boswellia serrata* extract

The elution time of the extract was found to be 4.512 min. which is in accordance with the standard monograph (4.39 min) of boswellic acid which confirms the presence of Boswellic acid in the extract of *B.serrata*.

Differential Scanning Colorimetry

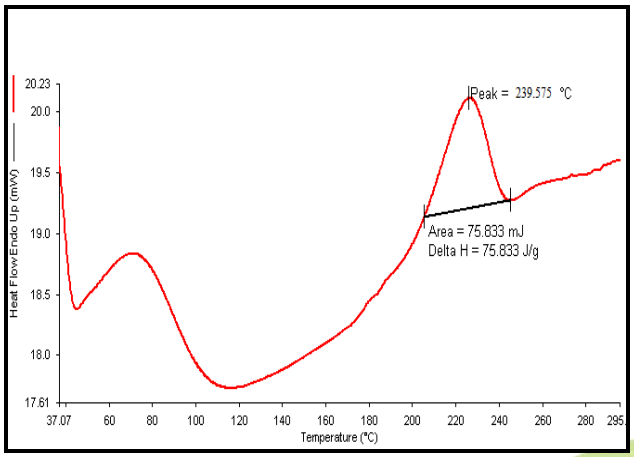

Figure 5: DSC of *Boswellia serrata* extract

The melting point of the extract was found to be 239.575°C which is in accordance with the standard monograph (238.122°C) of boswellic acid which confirms the presence of Boswellic acid in the extract of *B.serrata*.

Table 5: Evaluation data of formulation F1, F2, F3 for various parameters

Parameters	Formulation		
	F1	F2	F3
Color	Yellowish	Yellowish	Yellowish
Appearance	Translucent	Translucent	Translucent
pH	5.80	6.85	5.45
Homogeneity	++	+++	++
Spreadability (g.cm/sec)	27.04	21.73	24.96
Viscosity (cps)	10924 ± 200	9466 ± 200	8251 ± 200
After Feel	E	E	E
Removal	ES	ES	ES

+++: Good; ++: Satisfactory; E: Emollient; NG: Non Greasy; ES: Easy

***In-Vitro* Drug Release Study**

Table 6: Cumulative percent drug release of F1, F2 and F3

Time (hrs)	% CPDR		
	F1	F2	F3
0.5	23.831	29.42	23.831
1	29.42	47.396	33.81
1.5	443.34	53.6	47.39
2	47.39	59.777	51.656
4	51.656	64.43	53.6
6	53.6	67.1	56.58
12	56.58	69.097	58.04

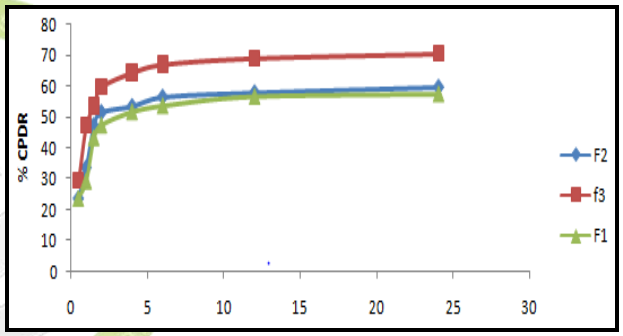

Figure 6: Drug release profile of formulation F1, F2, F3

Drug release from the formulations F1, F2, and F3 shows that F2 formulation has maximum drug release i.e. 69.097 % at 12th hour in comparison with the F1 and F3 in which drug release is 56.58% and 58.04% respectively.

***In Vitro* Release Kinetics Study**

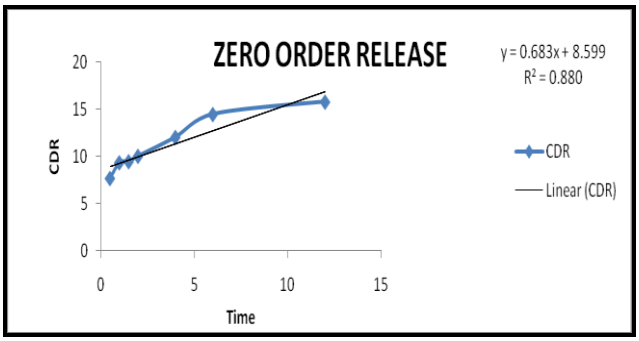

Figure 7: Zero order release kinetics of optimized F2 formulation

Figure 8: First order kinetics of optimized F2 formulation

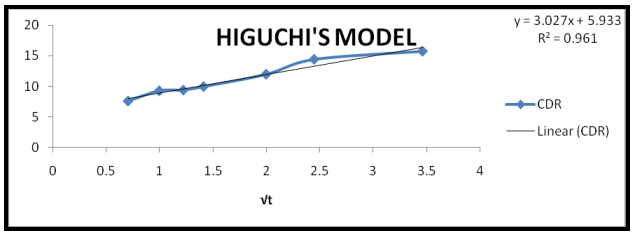

Figure 9: Higuchi's plot of optimized F2 formulation

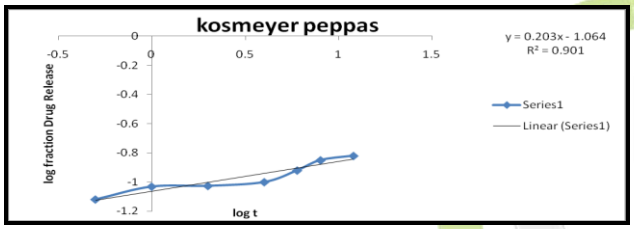

Figure 10: Korsemeyer peppas plot of optimized F12 formulation

Table 7: Model fitting of the drug release profile of formulation F2

Release Model	R^2	SLOPE=K
Zero order model	0.8806	0.6834
First order model	0.9251	-0.0439
Higuchi's square root of time plot	0.9615	3.0271
Korsemeyer peppas plot	0.9017	0.2031

Interpretation of Release Models (*In Vitro*)

The formulation so proposed is following first order release kinetics (first order release model, R^2= 0.9251), where drug is being released through diffusion process (Higuchi model R^2= 0.9615). The gel follows Fickian diffusion (n = 0.2031 from Korsmeyer- Peppas law equation).

Skin Irritation Scores of Formulation (A=Erythema Formation Score; B=Oedema Formation Score)

Rats	Intact skin				Abraded skin			
	24 hours		72 hours		24 hours		72 hours	
	A	B	A	B	A	B	A	B
1	1	0	0	1	0	1	1	0
2	0	1	0	0	0	2	0	1
3	1	1	0	1	1	0	0	2

Final Skin Irritation Scores of Formulation (* =Total Of A And B From Part A.; **=Average Of All Skin Reading Of 24 And 72 Hours)

Rats	Intact skin		Abraded skin		Total Average (i)+(ii)
	24 hours 72 hours		24 hours 72 hours		
	(i)		(ii)		
1	1	1	1	1	1
2	1	0	2	1	1
3	2	1	1	2	1.5
					Combined avg.=1.16

Skin Irritation Test Data

The skin irritation test was performed to confirm the safety of the herbal formulation. Aqil et al.[21] mentioned that a value of skin irritancy score between 0 and 2 indicates that the applied formulation is non-irritant and safe for human skin. The mean value of skin irritancy score for formulation was found to be 1.16 (Table.8). This value indicates that all excipients used in formulation were safe for topical drug delivery.

CONCLUSION

The present study revealed that the optimized herbal formulation F2 consisting of *boswellia serrata* extract shows comparatively better result than other formulations. FT-IR study revealed that there is no possible drug interaction with other components present in extract and DSC study depicts the presence of boswellic acid in the extract. An in vitro drug release study shows F2 releases the drug up to 69.097% in 12 hour. The release of formulation increased when the concentration of aerosil decreased. Release mechanism follows first order kinetics where drug is being released through diffusion process (Higuchi model R^2= 0.9615). The formulation follows Fickian diffusion (n = 0.2031 from Korsmeyer- Peppas law equation). The skin irritancy study shows the optimized formulation F2 is non irritant and can be applied easily. Thus, boswellic acid based anti-gout gel has great potential for the management of gout.

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