FORMULATION AND EVALUATION OF HERBAL GEL CONTAINING BOSWELLIA SERRATA, CURCUMA LONGA EXTRACT AND OIL OF WINTERGREEN FOR RHEUMATOID ARTHRITIS.

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ABSTRACT

The present research has been undertaken with the aim to formulate and evaluate the herbal gel containing Boswellia serrata extract, Curcuma longa extract, and Oil of Wintergreen. The gel formulation was designed by using alcoholic extract of Boswellia serrata, Curcuma longa, and evaluated using physiological measurements. The gel was prepared by using various natural and synthetic polymer bases (Pluronic F-127, HPMC, Sodium CMC, and Carbopol 934) and natural polymer bases (Pectin, Sodium alginate, Carrageenan, Xanthum gum, and Guar gum). Among them Carbopol 934 and Carrageenan based gels has given better gel formation. The gel was prepared by using Carbopol 934, Boswellia serrata extract, Curcuma longa extract, Oil of Wintergreen Methyl paraban, Propyl paraben and required amount of distilled water. Then skin pH (6.8-7) was maintained by drop wise addition of tri-ethanolamine. The physiochemical parameters of formulations (pH, viscosity, spreadability, extrudability etc.) were determined. The results showed that the Carbopol 934 has better gel properties than other formulation.

KEY WORDS

Boswellia serrata extract, Curcuma longa extract, and Oil of Wintergreen, Pluronic F-127, HPMC, Sodium CMC, Carbopol 934, Gel, Pectin, Sodium alginate, Carrageenan, Xanthum gum, and Guar gum.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease, which affects 1% of the world's population. Patients with RA not only have a progressive and debilitating disease and severe functional impairment, but can also experience a reduced life expectancy due to frequent involvement of the major organ systems. There have been profound changes in research and patient management of Rheumatoid Arthritis over the last 5 years. Developments in molecular biology have dramatically increased insight into the inflammatory and destructive pathways of this complex disease. Over half a million patients have been successfully treated with biologic therapy and advances in imaging techniques have allowed increasingly early detection of disease onset. Rheumatoid factor is no longer the unchallenged gold standard autoantibody but has competition from the anti-citrulline antibodies. Environmental factors influencing both susceptibility and progression have been sought, and smoking has emerged as a powerful factor. Steady progress in genetics is finally being made and one day it may be possible to predict RA before onset of symptomatic disease.1

The value of DMARDs(Disease-modifying anti-rheumatic drug) for treating OA or RA is also limited by their side effects and the fact that they are more expensive to use than traditional NSAIDs. The major side effects of NSAIDs are stomach ulcers, GI bleeding and perforations. Although a new class of NSAIDs—the specific inhibitors of COX-2—was developed, these drugs have similar efficacy as the general NSAIDs but are safer with respect to gastrointestinal toxicity.2

Because of these and other limitations, the use of complementary and alternative medicine (CAM) therapies, such as acupuncture and extracts of medicinal herbs, is on the rise and according to reports _60–90% of dissatisfied arthritis patients are likely to seek the option of CAM therapy.3,4

MATERIAL AND METHODS

The resins of Boswellia serrata, and rhizomes of Curcuma longa were collected from local market of Delhi (Khari baoli). Plant parts were identified by Dr. H.B. Singh, Head, Raw materials herbarium and museum (RHMD) at National Institute of Science Communication and Information Resources, of Scientific and Industrial Research, New Delhi, India. Reference standard of Boswellia serrata extract, Curcuma longa, extract were obtained from Natural Remedies, Bangalore.

Standardization5

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. The following standardization parameters were evaluated to obtain the qualitative information about the purity and quality of Boswellia serrata and Curcuma longa. The results are shown in Table No. 1
Determination of foreign matter

Foreign matter in herbal drugs consists of either parts of the medicinal plant or it may be any organism, part or product of an organism. It may also include mineral admixtures not adhering to the medicinal plant materials e.g. soil, stones, dust etc. The specified quantity of plant material was spread on a thin layer of paper. By visual inspection and by using a magnifying lens (5X or 10X), the foreign matters were picked out and the percentage was recorded.

Determination of quantitative data

Ash values:

Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.

I. Total ash value

Accurately weighed (about 2 to 3 g) of the powdered drug was taken in a tared silica crucible. Incineration was done at a temperature not exceeding $450^\circ C$ for 4 h, until free from carbon. The crucible was cooled and weighed. The percentage of ash was calculated with reference to air-dried drug.

II. Water soluble ash value

The ash was boiled with 25 ml of water. Insoluble matter was filtered and collected on an ashless filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding $500^\circ C$ for 4 h. cooled in a desiccator and weighed. The weight of insoluble residual matter was subtracted from the total weight of ash. The difference in weight represented weight of water soluble ash. The percentage of water soluble ash with reference to the air-dried drug was calculated.

III. Acid insoluble ash value

The ash was boiled for 5 min with 25 ml of 2 M HCl. The solution was filtered and the insoluble residue collected on an ashless filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding $4500^\circ C$ for 4 h. cooled in a desiccator and weighed. The percentage of acid insoluble ash with reference to the air-dried drug was calculated.
Extractive values

Alcohol soluble extractive value

5 gm accurately weighed, macerated coarse powdered drug was mixed with 100 ml of alcohol (90% v/v) in a stopper flask for 24 h, shaking frequently during first 6 hours. The solution was filtered rapidly through filter paper taking precaution against excessive loss of alcohol. 25 ml of alcoholic extract was evaporated to dryness in a tared dish and weighed. The percentage w/w of alcohol soluble extract with reference to the air-dried drug was calculated.

Extraction of Boswellic Acids

The bark resins of plant *Boswellia serrata* were broken into smaller pieces. 30 gm of the lumps of the gum exudates was extracted in soxhlet apparatus with 250 ml ethyl acetate for 24 hours. The extraction was repeated twice with 250 ml of ethyl acetate and residue discarded. The combined extract after filtration was concentrated by keeping at room temperature for 24 hours. The syrupy mass obtained was treated with 3% solution sodium hydroxide till the pH of 9-10 was attained. The solution was vigorously stirred for 10 hours till a uniform emulsion was formed. The upper solvent layer was separated and discarded. To the lower aqueous layer a dilute hydrochloric acid solution 4% was added till a pH of 3-5 was attained. The acid precipitated and was filtered and washed several times with water till neutral to litmus. The product was dried in an oven at 40-45°C to a creamish yellow powder having the yield of 8.4g.

Thin Layer Chromatography

**Sample Preparation:** 10 mg of *Boswellia serrata* extract obtained above was weighed by electronic balance (Citizen) and dissolved in 10 ml of methanol (Rankem). Spot of above solution was then applied on the TLC plate with the help of thin capillary tube.

**Solvent system:** Chloroform (Rankem) : Methanol (Rankem)(90:10)

**Detection:** After drying, spot of both the samples are identified by spraying vanillin sulphuric acid. (It is prepared by 0.5% vanillin sulphuric acid and ethanol in ratio 4:1) solution on the TLC plate.

Extraction of Curcuminoids

Fresh rhizomes were cleaned, washed with distilled water. They were sliced and dried in the sun for one week and again dried at 50°C in a hot air oven for 6 hours. Dried rhizomes were cut in small pieces, powdered by grinder. Approximately 50gm of sample was taken into a thimble and placed in a soxhlet apparatus. 250 ml of ethanol was added and extracted according to its boiling point i.e 56.53°C, for 6 hours. The sample was extracted with hexane to remove volatile oil for 2 hours. Hexane extract was discarded and the powder was re-extracted with acetone for 6 hours. After completion of extraction the dark brown extract was then cooled, filtered, concentrated using rotary evaporator, to get a crude dried 18g extract which was black orange in color.
Purification of curcuminoids: The extracted Curcuminoids were dissolved in methanol and heated. After complete dissolution, chloroform was added to get the methanol: chloroform ratio of 5:2 and kept at 5°C overnight. The crystals obtained were separated by filtration. The crystals were precipitated with petroleum ether. §

Thin Layer Chromatography

Test Sample: 10 mg of Curcuma longa extract obtained above was dissolved in 10ml of methanol, filtered and used.

Solvent System: Choroform: ethanol: Glacial acidic acid (Rankem) (94:5:1)

Procedure: Spot of both test and reference solution was applied on the silica gel plate after air-drying, kept for development in solvent system. After development, spot of test sample was identified.

Formulation of Gel

Formulation of gel based on synthetic gelling agents:

The gel based on synthetic gelling agents was prepared. Weighed amount of gelling agents were placed in known amount of distilled water. After complete dispersion, the polymer solution was kept in dark for 24 hours for complete swelling. Accurately weighed amount of drugs were dissolved in a specified quantity of suitable solvent. The drug solution was added slowly to the aqueous dispersion of polymer with the help of high speed stirrer (500 rpm) taking precaution that air did not entrap. Finally, the remaining ingredients were added to obtain a homogeneous dispersion of gel.

Evaluation of Gels:

Gels were evaluated for their clarity, pH, viscosity, spreadability, extrudability, drug content, in vitro diffusion studies by the standard procedure. All studies were carried out and average values were reported.

Clarity: The clarity of various formulations was determined by visual inspection and it was graded as follows; turbid: +, clear: ++, very clear (glassy): +++.

pH: 2.5 grams of gel was accurately weighed and dispersed in 25 ml of distilled water. The pH of dispersion was measured by using digital pH meter. (Systronics-362)

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

Spreadability: It was determined by wooden block and glass slide apparatus. § For the determination of spreadability, excess of sample was applied in between two glass slides and they were compressed to form a uniformly thick layer of gel. 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 to separate 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). Spreadability was calculated by using the formula:

\[ S = \frac{ML}{T} \]

Where, \( S \) = Spreadability

\( M \) = Weight tied to upper slide

\( L \) = Length moved on the glass slide

\( T \) = Time taken to separate the slide completely from each other

**Viscosity measurement:** Viscosity of the gels was determined using a Brookfield viscometer, by using small sample adapter having spindle.

**Drug content:** The gel (5 ml) was dissolved in 50 ml of phosphate buffer 7.4. The volumetric flask containing gel solution was shaken for 2 hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometerically.

**Extrudability:** The extrudability test was carried out by using Pfizer hardness tester. 10gms of gel was filled in aluminum tube. The plunger was adjusted to hold the tube properly. The pressure of 1kg/cm² was applied for 30 sec. The quantity of gel extruded was weighed. The procedure was repeated at three equidistant places of the tube. Test was carried out in triplicates.

**Drug diffusion study:** Phosphate buffer of pH of 6.8 was prepared according to the standard procedure given in IP. The solution was filled in the receptor compartment of Franz diffusion assembly. Cellulose nitrate membrane (Germany) was tied between the donor and receptor part and weighed amount (3gm) gel was filled in the donor compartment. Using a magnetic stirrer the medium was agitated in the receptor compartment and temperature was maintained at 37°C±1. Samples (5ml) from the receptor compartment were taken at various intervals over a 4hr period of time and assayed for drug released.

**Stability studies:** Stability is defined as the extent to which a product retains its efficacy within specified limits throughout the period i.e. shelf life. All the selected formulations were subjected to a stability testing for 6 weeks at room temperature. All selected formulations were analyzed for the change in pH, Spreadability, homogeneity or drug content by procedure stated earlier.

**Table 1: Determination of Quantitative data**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Physico-chemical parameter</th>
<th><em>Boswellia Serrata</em></th>
<th><em>Curcuma longa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Foreign matter</td>
<td>2%</td>
<td>Nil</td>
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</table>
Table 2: Thin Layer Chromatography of *Boswellia serrata* extract.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Distance travelled by solute (cm)</th>
<th>Distance travelled by solvent (cm)</th>
<th>Rf values obtained</th>
<th>Rf values reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3</td>
<td>12</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>2.</td>
<td>6.9</td>
<td>12</td>
<td>0.58</td>
<td>0.55</td>
</tr>
<tr>
<td>3.</td>
<td>9.0</td>
<td>12</td>
<td>0.75</td>
<td>0.78</td>
</tr>
<tr>
<td>4.</td>
<td>10.5</td>
<td>12</td>
<td>0.88</td>
<td>0.91</td>
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Table 3: TLC of *Curcuma longa* extract.

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Distance travelled by solute (cm)</th>
<th>Distance travelled by solvent (cm)</th>
<th>Rf values obtained</th>
<th>Rf values reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5</td>
<td>12</td>
<td>0.41</td>
<td>0.43</td>
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<tr>
<td>2.</td>
<td>6.6</td>
<td>12</td>
<td>0.55</td>
<td>0.60</td>
</tr>
<tr>
<td>3.</td>
<td>9.2</td>
<td>12</td>
<td>0.77</td>
<td>0.79</td>
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Table 4: Evaluation of gels.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Clarity</th>
<th>pH</th>
<th>Homogeneity</th>
<th>Spreadability</th>
<th>Extrudability</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>+++</td>
<td>6.9</td>
<td>Good</td>
<td>23.08</td>
<td>++</td>
</tr>
<tr>
<td>F2</td>
<td>++</td>
<td>6.3</td>
<td>Good</td>
<td>22.06</td>
<td>+++</td>
</tr>
<tr>
<td>F3</td>
<td>++</td>
<td>6.4</td>
<td>Good</td>
<td>27.27</td>
<td>++</td>
</tr>
<tr>
<td>F4</td>
<td>+++</td>
<td>6.5</td>
<td>Good</td>
<td>28.86</td>
<td>++</td>
</tr>
<tr>
<td>F5</td>
<td>+</td>
<td>6.6</td>
<td>Good</td>
<td>20.55</td>
<td>++</td>
</tr>
<tr>
<td>F6</td>
<td>+</td>
<td>6.8</td>
<td>Good</td>
<td>18.05</td>
<td>+++</td>
</tr>
<tr>
<td>F7</td>
<td>++</td>
<td>6.7</td>
<td>Good</td>
<td>18.07</td>
<td>+</td>
</tr>
<tr>
<td>F8</td>
<td>++</td>
<td>6.9</td>
<td>Good</td>
<td>25.45</td>
<td>+</td>
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Table 5: Drug release study of formulation.

<table>
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<tr>
<th>Time (min.)</th>
<th>% Drug release rate <em>Boswellia</em> extract</th>
<th>% Drug release rate wintergreen</th>
<th>% Drug release rate <em>Curcuma</em> extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>23.0</td>
<td>28</td>
<td>20.4</td>
</tr>
<tr>
<td>90</td>
<td>53.2</td>
<td>41.4</td>
<td>27.7</td>
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<td>180</td>
<td>60.5</td>
<td>50.1</td>
<td>34.1</td>
</tr>
<tr>
<td>240</td>
<td>64.2</td>
<td>60.5</td>
<td>53.6</td>
</tr>
<tr>
<td>300</td>
<td>68.2</td>
<td>69.6</td>
<td>67.5</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSIONS

The herbal gel was yellowish in color and translucent in appearance and gave smooth feel on application which was maintained after tested stability study. pH was maintained throughout the study which was found 6.2 – 6.9. Spreadibility was also measured and found to be less variation. The initial viscosities of developed gels were measured using Brookfield viscometer with spindle. Further stability test for two months has been carried out and results revealed that carbopol 934 gel showed better stability than other formulations. Initial viscosity for gel containing carbopol 934 and carrageenan were 27390 cps and 29640 cps respectively and after stability study there were not much variation. In vitro drug release of different formulations it was found that carbopol gels among synthetic gelling agents show highest drug release, better pH, clarity, spreadability and homogeneity. Also the carrageenan based gels among natural gelling agents show better results and thus is an optimized formulation. In formulation optimization process the studies on their release properties have revealed that the carbopol gel shows highest release rate in comparison to other synthetic and natural gelling agents.

CONCLUSION

Rheumatoid arthritis is a chronic disease therefore it becomes preferable to use medicines with long term safety. Although there are many plants which are effective in treatment of RA they have limitations such as lack of efficacy, excessive side effects and high cost. However, much of the current research is focused on the identification, isolation and characterization of active principle(s) from crude extracts of known medicinal plants or herbs, often overlooking the fact that strong synergism of several constituents in the crude drug may prove more potent and effective than any single purified compound and this may help to nullify the toxic effects of individual constituents.

Studies conducted so far have revealed promising results; however, there exists a scope for further anti-inflammatory studies and pharmacokinetic studies. The long-term safety and efficacy
of most of the herbal preparations commonly promoted as anti arthritic have not been established by placebo-controlled randomized trials in patients and indeed some of these may even interfere with the ongoing treatments. Therefore, it is imperative that scientific evidence regarding the safety and efficacy of herbal preparations commonly used by arthritis patients be studied and presented.

REFERENCES


