Anti-Inflammatory Formulation and Characterization of an Herbal Formulation

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Authors’ contributions

This work was carried out in collaboration between both authors. Author SP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors LM and SP managed the analyses of the study. Author LM managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Anti-inflammatory refers to group of medication that is utilized for curing pain and inflammation. Many synthetic products are used as anti-inflammatory agents but the effects caused by them is not satisfactory. Cordis obliqua is an anti-inflammatory herb that is enormously involved in pain reduction. The present paper aims to develop a topical gel formulation comprising herbs i.e. Cordis obliqua and curcumin with potent anti-inflammatory activity. The gel formulation was then subjected to characterization studies which involve pH, viscosity, excrudability, spreadability and in vitro analysis. pH of gel formulation was found to be 7.2±1.01 which is almost equivalent to the skin pH. In viscosity test, it was observed that viscosity of gel formulation was found to be effective. Moreover, spreadability co-efficient of the formulation satisfactory which indicates that sanitizer gel easily adheres on the skin and covers the bacteria residing on the skin, if any.

Keywords: Anti-inflammatory; cordis oblique; curcumin; gel formulation.

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1. INTRODUCTION

Cordia (Basic Name-Clammy) obliqua plant belonging to a family of Boraginaceae Strawberry and Lasora are a well distributed tree with high growth and medium height. It has a variety of diagnostic practises in line with the conventional method. Phytochemical and antipsychotic, antipyretic trials were tested on Cordia obliqua and anti-inflammatory activity of leaf is determined by using methanol [1].

There are approximately 2,700 varieties in the Boraginaceae group, distributed across the earth in tropical, subtropical and colder areas. It consists of roughly 130 genera as well as six sub-families with one Cordioideae. The Cordia family is made up of evergreen plants and trees. Worldwide, approximately 300 species of the Cordia genus were described. There are 13 organisms in India of this genus [2,3].

This is a moderate fecestic tree, which has been found in the middle of Himalya at about 1,470 metres in height. In Himachal Pradesh there are two main species of Cordia obliqua Volda, and the existence of different forms is really the fruit width, one having smaller fruit. The fruit of the tree is sweet and has diuretic, anthelmintic, purging, expectorant activity and are effective for dry cough, heart and urethra disorders, gallbleness and persistent fever and joints. It's fine in spleen disorders, according to the Yunani system [4]. It's useful for all respiratory ailments and are used to replace Cordia walelichii. According to Ayurvedic medicine Phenolics, flavonosides, pyrrolyzidines, tannins, triterpenoids, and phenylpropanoid products are found and in phytochemical investigation. A Tamilnadu research identified Cordia obliqua Willd's berries, var. Obliqua and spec [5,6]. The Boraginaceae family's tomentosa (known as specific names Virusu and Kal disease) are being used as paillyar digestive.

Turmeric Plant is an herbaceous perennial of the ginger piperaceae Family and is widely grown in southeastern and southeastern tropical Asia. The rhizome is perhaps the most valuable component which has been used in the culinary spice for millennia and is often referred to it as the roots of the herb. It was used internally and as a topical salt for a host of diseases. Modern Indian nutraceutical medicine is generally used for the treatment of hepatic infections, anorexia, cough, respiratory infections, psoriatic arthritis and conjunctivitis. Slaked lime, turmeric powder is a common home remedies for inflammatory and wound healing [7]. Old Indian medicines texts explain the use of turmeric in inflammatory disorders, wound cure and stomach problems.

Curcumin, the antioxidants ingredient for turmeric, has been used for generations in India as a culinary spice and a topical formulation treatment ointment for inflammatory. Turmeric (diferuloylmethinic), however, disintegrate into acetone, alcohol, and is practically insoluble inside water. Turmeric contains 10–20% turmeric, communication failures and methoxycurcumin in industrial grades, and is as powerful as pure curcumin. A normal oral dosage of 3.6 g turmeric was successful for colorectal cancer and promotes its development into Clinical trials on the basis of a variety of clinical study in tumorigenesis [8,9]. In addition to the influence of tumor, turmeric has been beneficial in both in vitro activity and preclinical trials for a range of disease problems. Many formulations are available in the market for treating the inflammation. Marie Guhmann and co-workers developed diclofenac taste-masked orodispersible tablets (ODTs) possessing rapid drug release features. Various taste-masking techniques and formulation conceptions were screened in vitro for suitable drug selection. Though it was effective in treating the inflammation but people suffered from adversities like gastric ulcers, stomach pain. Thus there is a need to develop a formulation that cures inflammations effectively and overcoming the problems caused due to synthetic medicines.

However, most of them are synthetic based products that significantly affect the individual's skin or does not effectively treat the inflammation. Thus the present paper aims to formulate a topical gel of Cordia oblique leaf extract in combination with curcumin and study their pysiochemial parameters.

2. METHODOLOGY

2.1 Design

The samples of Cordia obliqua and curcumin along with the excipients were collected from the market and then the extract of the Cordia obliqua was prepared after the preparation the formulation was prepared by a dispersion method. Then the prepared formulation was subjected to characterization studies in order to determine the efficacy of the drug formulation.
2.2 Samples

The leaves of *Cordia oblique* were collected from raw material Herbarium and museum. The curcumin was procured from a research laboratory, Carbomer, ethyl parabene, propylene glycol 500, triethanolamine (TEA) along with the solvents were procured from a chemical industry in Delhi. Instruments involved were Brookfield viscometer, pH METER, and diffusion cell apparatus of orchid Scientificus.

2.3 Extracting Method

About 1 kilo of raw leaf medication was soaked and coarsely powdered. The leaf material extraction process was performed with soxhlet. Extraction devices and solvents have been used to improve Polarity to remove both phytoconstituents (hexane, chloroform, methanol and water). All four substances were then focused separately to rotary evaporator and desiccator holding vacuum evaporator. Methanolic the yield for the extract was 1.2% w/w and not sticky and of tone of dark green. Methanol extract is obtained from prior research. It was observed to have a greater antioxidant effect in phyto-constituents. Some leaf extracts are compared to. It also has a beneficial affect Pain, edoema and fever care[2]. So extract of leaf methanol. The topical gel formulation has been chosen.

Dispersion approach was adopted for topical gel formulation since the polymers of carbomer can be spread quickly by blending them in the atmospheric temperature [2]. Leaf extract was mixed with carbomer in the 50 ml of water with a steady stirring followed by addition of methyl Paraben respectively to obtain a solution. The obtained solution was heated on a boiling water to obtain an extract. The extract was then blended in and volume was made with distilled water. Lastly, the persistent inclusion of Triethanolamine (TEA) in the solution was done and stirred to obtain an anti-inflammatory formulation [10].

2.4 Biochemical Analysis

pH of the formulation was taken by digital pH meter (Labman, India), in which 2 gm of the formulation was stored in a container and was then measured via pH electrode [10]. Viscosity of gel formulation was determined by utilizing viscometer at 27±1.5°C [11]. Spreadability of the formulation was procured by the following the process which comprises having two glass slides in which the area of 2 cm on the primary slide was marked followed and the gel formulation was spreaded. Then secondary slide was placed over the primary slide and pressed to spread the gel between the two slides. Finally, the area of spread was noted [12]. Extrudability was evaluated via collapsible frame. Extrudability is defined as amount of fluid extruded from the foil. Weight was measured that is responsible for extruding at least 0.5 cm gel within 20 sec [12].

2.5 Anti-microbial Assay (herbal extract)

Specific microbes were included for procuring antimicrobial activity of *Cordia oblique* extracts. Dip well process was espoused for executing anti-microbial activity of *Cordia oblique*. Antimicrobial activity of *Cordia oblique* extract was taken on three separate sterile plates. Micro-organisms included were *E. coli*, *S. aureus*, and *Salmonella*. This is necessary to cover. MacConkey agar and muller hington agar media were prepared and solidified. Further, sub cultured micro-organism were inoculated on nutrient agar followed by inoculating three discs with *Cordia oblique* extracts. Then plates were incubated for one complete night at 37°C. Zone of inhibition was perceived and MIC was calculated after 24 hrs of incubation [11].

2.6 Anti-bacterial Efficacy (Gel Formulation)

MIC is defined as lowermost concentration of prepared sample required to retard micro-organism expansion during a 24-hour incubation cycle. The control of minimum inhibitory extracts amount was intended by preparation within nutrient broth of specific extract concentrations of 200 μg, 400 μg and 800 μg, respectively. In Muller Hinton agar reservoirs were seeded with uniform inoculum of sample bacteria, 1 ml amount of each dilution was applied aseptically. Triplicate tests were conducted. At 37°C, 24 hours agar plates have been incubated. The lowest extent of MIC were considered in samples presenting a strong inhibition area. Spread plating was performed to determine anti-bacterial efficacy of the sample. 100 ml of gel sample was applied to hands of subject. After sanitization, collected samples were allowed to grow on nutrient agar medium overnight at 37 degree celsius and colony forming units were counted [11].
2.7 Cyto-toxicity Assay on Human Cells

For the evaluation of cytotoxicity on human cells, Human BJ cell lines of fibroblast origin which was derived from Human foreskin was procured from the American Type Culture Collection (ATCC) and was maintained in Eagle’s Minimum Essential Medium (EMEM) in addition with 10% Fetal Bovine Serum (FBS) in normal mammalian cell culture conditions. Cells were then treated with varying concentrations of poly-herbal extracts (0,100,350,500,750 and 1000 µg/ml) after 48 h. MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was conducted to determine the percentage of viable cells.

2.8 Anti-oxidant Assay

2.8.1 DPPH assay (1,1-diphenyl-2-picrylhydrazyl)

DPPH assay was performed for the formulation. 400 µl of gel formulation sample and 600 µl of Tris-HCl buffer (pH 7.4) was taken in a test tube followed by addition of DPPH solution and stirred for at least 15 seconds to obtain a solution. The solution was then stored in dark environment at room temperature for 30 min. After that, absorbance of the solution was measured at 520 nm. Blank used herein is mixture of 1.3 ml of ethanol and 799 µl of Tris-HCl buffer. Experiments were conducted in triplicate. Inhibition ratio was calculated through formula:

\[
\text{Inhibition ratio (\%)} = \frac{(Ac-As)}{Ac} \times 100
\]

2.9 Stability

To check the stability of the sample, the gel formulation samples were stored at differing temperatures ranging from 50 °C, 25°C and 37°C for duration of 6 weeks.

3. RESULTS AND DISCUSSION

3.1 Biochemical Analysis Results

After conducting the pH test, it was seen that the pH of the gel formulation was optimum which is exactly 7.2±1.01. Viscosity of anti-inflammatory gel sample was conducted in which it was observed that viscosity of gel formulation sample is exactly 65c Pascal. The spreadability of gel formulation was determined by spreading the sample over the plate followed by pressing via second plate over first plate to analyse the area covered by gel formulation by spreading. Spreading co-efficient analyzed herein is 16.55. This clearly indicates that gel formulation easily spreads on the hands and thus penetrates within skin through pores thereby completely curing inflammation. Anti-inflammatory gel sample was subjected to extrudability test in which amount of gel extruded from the blister was measured. It was perceived that, extrudability of the sample was 22.27 gm/cm². The reason of increased viscosity i.e. higher the viscosity highest is the extrudability.

3.2 Anti-microbial Assay

According to the inhibition zone produced from gel formulation sample against various bacterial species, an enhanced functionality was showcased by herbal gel formulation i.e. Cordia oblique leaf extract in combination with curcumin thus stating that the herbal formulation possess great anti-microbial property against pathogens. The herbal formulation produced three zones of inhibition against E. coli, S. aureus, P. aeruginosa. It was observed that Cordia oblique leaf extract broadest inhibition zone against E.coli with diameter of 3.7 cm. Then the inhibition zone of 3.1 cm and 2.9 cm was produced by Cordia oblique leaf extract for S. aureus, P. aeruginosa and C. albicans respectively. After that the mean value was evaluated for all the three inhibition zones. It was observed that: mean value of zone of inhibition is 3.23 cm which is acceptable for inhibition of bacterial species. Table 1 shows inhibited zone area of different bacterial species by Cordia oblique leaf extract.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Bacterial species</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>E.coli</td>
<td>3.7 cm</td>
</tr>
<tr>
<td>2.</td>
<td>S. aureus</td>
<td>3.1 cm</td>
</tr>
<tr>
<td>3.</td>
<td>P. aeruginosa</td>
<td>2.9 cm</td>
</tr>
</tbody>
</table>

Also prepared gel formulation i.e. Cordia oblique leaf extract in combination with curcumin exhibited maximum zone of inhibition against S. aureus which is 4.6cm followed by 4.2, 4.0 for E. coli, P. aeruginosa, respectively. Average zone of inhibition of the anti-inflammatory gel sample was 4.26 cm. This clearly indicates the anti-bacterial efficacy of the sample. Thus this proves that the gel possess enhanced anti-
bacterial activity against aforementioned mentioned pathogens. Table 2 represents zone inhibition surface of bacterial species by herbal gel formulation.

**Table 2. Zone inhibition area of microbial species by herbal sanitizer, inhibition zone of* E. coli, S. aureus, P. aeruginosa *is 4.6 cm, 4.2 cm, 4.0 cm**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Bacterial species</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>E. coli</em></td>
<td>4.6 cm</td>
</tr>
<tr>
<td>2.</td>
<td><em>S. aureus</em></td>
<td>4.2 cm</td>
</tr>
<tr>
<td>3.</td>
<td><em>P. aeruginosa</em></td>
<td>4.0 cm</td>
</tr>
</tbody>
</table>

Anti-oxidant activity of the herbal formulation was found to be 15.76% and that of *Cordia oblique* leaf extract was found to be 13.68%. Curcumin also have anti-oxidant activity that was found to be 9.99%. (Table 3). Application of the gel formulation on certain group of people for 7 regular days exhibited an effective response. Fig. 1 shows effect of herbal gel formulation on different people in different days.

The gel formulated possess both anti-inflammatory and anti-bacterial activity. It clearly indicated that prepared herb possess immense potential to kill the germs and cure inflammation. As many cosmeceuticals contain materials that are harmful for skin. The newly formulated herbal gel contains extracts of natural herbs that are beneficial for skin and do not damage skin. Natural remedial steps are very safe and useful for skin as well as inflammation.

**Table 3. Representation of anti-oxidant activity of* Cordia oblique* leaf extract in combination with curcumin, *Cordia oblique* leaf extract and curcumin wherein highest anti-oxidant activity is of herbal gel formulation i.e. *Cordia oblique* leaf extract in combination with curcumin (14.67%)**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Bacterial species</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Herbal gel formulation</td>
<td>15.76%</td>
</tr>
<tr>
<td>2.</td>
<td><em>Cordia oblique</em> leaf extract</td>
<td>13.68%</td>
</tr>
<tr>
<td>3.</td>
<td>Curcumin herb</td>
<td>9.99%</td>
</tr>
</tbody>
</table>

### 3.3 Cytotoxicity Assay

Human BJ cells treated with varying concentrations of poly-herbal extracts for 48 h yielded negligible cytotoxicity (Fig. 2). Thereby, it can be presumed that the poly-herbal extracts being used as an anti-inflammatory agent is safe to use.

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**Fig. 1. Representation of effect of herbal gel formulation on different subjects at different days. The graph represents increase in effect of gel on different pathogenic species, as the days of the gel increases its effect on pathogenic species also increases**
Fig. 2. Cytotoxicity assay on Human BJ cells. Human BJ cells of fibroblast origin were treated with an increasing concentrations of the poly-herbal extracts (0,100,350,500,750 and 1000 µg/ml) after 48 h. The poly-herbal extract showed negligible cytotoxicity even after 48 h.

The anti-inflammatory herbal gel formulated in the present work was found to be efficacious and also exhibited increased extrudability and spreadability results, wherein extrudable and spreadable co-efficient of 22.27 gm/cm² and 16.55 which is quite satisfactory on commercial scale. All these results thus indicated that, the herbal gel formulation was effective for the treatment of inflammation and also due to presence of curcumin the gel also possess anti-bacterial activity thereby treating the inflammation as well as eradicating the harmful bacterial species residing over skin and thus also protect the skin from any damage.

The combination of *Cordia oblique* leaf extract and curcumin used herein provides anti-bacterial and anti-inflammatory properties which will be very useful for people who utilizes it [13,14]. Also the gel will be cost-effective as it involves utilization of herbs that are widely available. This formulation as chemicals based will seriously serve as a potent formulation in killing the pathogenic species in contact with the skin and reduce the inflammation.

**4. CONCLUSION**

*Cordia obliqua* wild. is most known and generally found trees. Traditionally numerous medicinal activities and its fruits are utilised in various forms such as pickel, vegetables and alike. Despite all these it also possess various antibacterial well as anti-inflammatory activity. The present research discusses about a herbal formulation that is prepared by mixing *Cordia obliqua* wild. with curcumin to treat the inflammation. The gel formulation developed also possess anti-bacterial properties due to presence of curcumin. From the test that were conducted it was found that the pH of gel formulation was found to be skin friendly which is almost equivalent to the skin pH. In viscosity test, it was observed that viscosity of gel formulation was found to be 65c Pascal. Also spreadability co-efficient of the formulation was found to be satisfactory which indicates that sanitizer gel easily adheres on the skin and covers the bacteria residing on the skin, if any. Extrudability of the sample was also accurate that provides the user to easily extrude the formulation for further use. The herbal gel also showed immense potential in anti-inflammatory and anti-bacterial analysis i.e. it was able to inhibit growth of bacterial species which preferably includes *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans*.

Thus to conclude it is said that the prepared gel formulation possess anti-bacterial and anti-inflammatory activities and thus is suitable for long term use without causing any harm to human skin. Thus it aids in preventing people from harmful diseases.

**CONSENT**

It is not applicable.

**ETHICAL APPROVAL**

It is not applicable.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.
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