Preliminary Phytochemical Screening, *In Vitro* Antioxidant Activity, Topical and Oral Formulation of Extract of *Woodfordia fruticosa* and *Gardenia gummifera*

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Abstract. *Woodfordia fruticosa* and *Gardenia gummifera* are traditionally claimed to be useful in treatment of number of diseases. The main aim of this study was to evaluate preliminary phytochemical tests and antioxidant activity of ethanolic extract of *Woodfordia fruticosa* flowers and *Gardenia gummifera* leaves and finally extracts were formulated in Gels and Suspensions. *In vitro* antioxidant activity was evaluated by DPPH method. The different concentration (100, 200, 300, 400 and 500 μg/ml) of standard and test samples were prepared. The anti-oxidant activity is exhibited in percentage inhibition. The DPPH radical scavenging activity of both the extracts increases with increasing concentration. The prepared gels were evaluated physical appearance, homogeneity, Grittiness, Spreadability and Viscosity. The prepared suspensions were evaluated organoleptic properties, pH and Sedimentation Volume.

1. Introduction

*Woodfordia fruticosa* (Kurz.) family Lythraceae is a straggling leafy shrub, frequently used English names are Fire Flame Bush while locally known as Dhai is about 3.5 m in high and distributed abundantly throughout the North India, as well as in the majority of the East Asian countries. According to the Traditional system of medicine, the flowers of *Woodfordia fruticosa* are pungent, acrid, cooling, toxic, alexiteric, use as astringent in disorders of mucous membranes,, uterine sedative and anthelmintic and is useful in thirst, dysentery, leprosy, erysipelas, blood disease, leucorrhoea, menorrhagia and toothache [1,2]. In addition, *Woodfordia fruticosa* flowers are extensively used by tribal people for its wound, ulcer healing, analgesic and anti-rheumatic properties [3-5]. Pharmacological claims of *Woodfordia fruticosa* dried flowers can be ascribed for its important bioactive phytoconstituents such as flavonoids, sterols, anthraquinones, saponins and tannins [6-8]. Preclinical data from various studies indicates, that dried *Woodfordia fruticosa* flower extract possess anti-pyretic, Anti-inflammatory [9], Anti-tumor [10, 11], Antiviral [12], Immunomodulatory [13], Anti-fertility [14], Antibacterial [15], Hepatoprotective [16], Anti-hyperlipemic [17], Antidiabetic [18], Bronchoprotective [19], Wound healing [20] and Anti-asthmatic activity [21].

*Gardenia gummifera* (family- Rubiaceae) is geographically distributed in India, Burma, Bangladesh, Konkan region, North Kanara, and Malabar Coast. *G. gummifera* is claimed to have a number of medicinal properties which include anthelmintic, antispasmodic, carminative, diaphoretic, expectorant, potentiation of pentobarbitone induced sleep, antiepileptic, peripheral and central analgesic, cardiotonic, antioxidant and anti-hyperlipidemic. It is also claimed to be useful in dyspepsia, flatulence for cleaning foul ulcers and wounds and to keep off flies from wounds in veterinary practice [22, 23]. Dikamali is the gum resin obtained from the leaf buds of *Gardenia gummifera* [24]. A number of flavonoids such as gardenin A, B, C, D and E were isolated from the plant [25, 26].
2. Materials and Methods

2.1. Preliminary Phytochemical Screening

Collection and Authentication of Plant: The flowers of *Woodfordia fruticosa* was collected in the month of February 2014 from the Swarn Jayanti Park, near Sarvadharm colony, Bhopal (M.P.). Herbarium file of plant part was prepared and authenticated by Dr. Zia Ul Hasan (Professor, Department of Botany), Safia College Bhopal and the specimen voucher no. assigned was 460/Bot/Saif/14. The leaves of *Gardenia gummifera* Linn. was collected in the month of September from the Park of Bellaire Apartment, near Abbas nagar, Bhopal (M.P.). Herbarium file of plant part was prepared and authenticated by Dr. Zia Ul Hasan (Professor, Department of Botany), Safia College Bhopal and the specimen voucher no. assigned was 503/Bot/Saif/14.

Morphological Screening of Plants parts: Morphological screening of flower of *Woodfordia fruticosa* and leaf of *Gardenia gummifera* was completed such as colour, odor, taste, size and texture.

Figure 1. *Woodfordia fruticosa* (Left) and *Gardenia gummifera* (Right).

Drying and Size Reduction of Plant Material: The flowers of *Woodfordia fruticosa* and leaves of *Gardenia gummifera* were dried under shade in college laboratory. It was pulverized to coarse powder. The coarse powder of flowers and leaves was passed through sieve No.20 to maintain uniformity and packed into airtight container and stored in cool and dry place. These powders of flowers of *Woodfordia fruticosa* and powder of leaves of *Gardenia gummifera* were used for extraction.

Screening of Powders: Screening of powder of *Woodfordia fruticosa* flowers and *Gardenia gummifera* leaves was completed such as Loss of drying, Total Ash Value (Acid Insoluble & Water Soluble) and Foaming Index.

Preparation of Crude Extract: Soxhlet apparatus was used for the solvent extraction and ethanol was selected as a solvent for extraction while Petroleum ether was used for defatting of the waxy materials.

Figure 2. Extract of *W. fruticosa* flower (left) and *G. gummifera* leaf (right).
Screening of Crude Extracts (Qualitative Phytochemical Analysis): The crude extracts obtained by solvent extraction were subjected to various qualitative tests to detect the presence of common chemical constituents such as Alkaloids, Glycosides, Carbohydrates, steroids & sterols, Saponins, Tannins, Flavonoids, Proteins & amino acids etc [27, 28].

2.2. Anti-oxidant activity by DPPH method

Preparation of standard solution: Required quantity of Ascorbic acid was dissolved in methanol to give the concentration of 100, 200, 300, 400 and 500μg/ml.

Preparation of test sample: Stock solutions of samples were prepared by dissolving Ethanolic extract (10 mg) in methanol (10 ml) to give concentration of 1mg/ml. Separately all the samples were diluted in 10 ml volumetric flask to give (100, 200, 300, 400 and 500 μg/ml concentration.

Preparation of DPPH solution: 3.94 mg of 2, 2- diphenyl-1-picrylhydrazyl radical (DPPH), a stable radical was dissolved in methanol (100ml) to give a 100 µm solution: it was protected from light by covering the test tubes with aluminium foil.

Protocol for estimation of DPPH scavenging activity: 150μl DPPH solution was added to 3 ml methanol and absorbance was taken immediately at 517 nm for control reading. Diluted test sample with methanol up to 3 ml. 150μl DPPH solution was added to each test tube. Absorbance was taken at 517 nm in UV-visible spectrophotometer (Systronics 2203) after 15 min using methanol as a blank.

The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

\[
% \text{ (FRSA)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100
\]

Each experiment was carried out in triplicate and results are expressed as mean and percent antiradical activity [29-31].

2.3. Formulation development

2.3.1. Topical Formulation development: Preparation of 0.1% Gel of Ethanolic extract of *Woodfordia fruticosa* & *Gardenia gummifera* [32].

Chemicals:
- Carbopol 934
- Ethanol
- Methyl paraben
- Propylene glycol 400
- EDTA
- Triethanolamine

Procedure: 1 g of Carbopol 934 was dispersed in 50 ml of distilled water kept the beaker aside to swell the carbopol for half an hour and then consent steering (300 RPM for 30 min) was done to mix the carbopol. Required quantity of methyl paraben and EDTA was dissolved in 5 ml of distilled water by heating on water bath. Solution was cooled and Propylene glycol 400 was added. Further required quantity of extract (100mg for 0.1%) was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. Finally full mixed ingredients were mixed properly to the Carbopol gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH and to obtain the gel at required consistency.
Evaluation of gel formulations

Physical Evaluation: All developed gels were tested for physical appearance (Appearance, Colour, Odour) and homogeneity by visual observation.

Homogeneity: Prepared gel was tested for homogeneity by visual inspection before transfer into container. They were tested for their appearance and presence of any aggregates.

Pharmaceutical Evaluation

Grittiness: A pinch of product is rubbed on skin and then observed with magnifying glass; if it is free from rashes or eruption then it is considered as free from grittiness.

Spreadability: Spreadability is a term expressed to denote the extent of area to which the ointments readily spreads on application to skin or affected part. The spreadability was expressed in terms of times in seconds taken by two slides to slip off from ointment. The spreadability of ointment was determined by measuring the time in seconds taken by two slides to slip off from ointment on placing the 1 gm of ointment between two horizontal glass plates. Lesser the time taken for separation of two slides, result the better spread ability. Spread ability was calculated by using the formula.

\[
\text{Spreadability} (S) = \frac{M \times L}{T}
\]

where \( S \) = Spreadability, \( M \) = Weight tied to upper slide, \( L \) = Length of glass slides and \( T \) = Time taken to separate the slides.

Viscosity: The viscosity was determined by CAP- 2000 Brookfield viscometer. Test sample was taken in a clean and dry 250 ml beaker and the viscosity of the test sample was determined by standard operating procedure of Viscometer by using spindle nos. 1 to 4. Each spindle was used for finding the viscosity of the sample at speeds of 10 round/minute and sample was about to settle over 30 minutes at the temperature before the measurement was taken.

Viscosity was calculated by using the following formula:

\[
\text{Viscosity} (\eta) = \frac{F}{S}
\]

where \( \eta \) = viscosity, \( F \) = Shear stress, \( S \) = Shear rate.

2.3.2. Oral Formulation development: Preparation of suspension of Ethanolic extract of *Woodfordia fructicosa & Gardenia gummifera* [33].

Procedure: Suspensions of extracts were prepared using 0.5% carboxyl methyl cellulose (CMC) as suspending agent. Calculated amount of extract was premixed in distilled water followed by addition of required amount of CMC, Sorbitol Solution (0.5 %) and Methyl Paraben; the mixture was agitated with the help of glass rod and was finally sonicated with help of ultrasonicator. Suspensions of both extract were prepared to get the test doses (50mg/kg per ml).
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>Required Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extract</td>
<td>5gm</td>
</tr>
<tr>
<td>2</td>
<td>Carboxyl methyl cellulose</td>
<td>500mg</td>
</tr>
<tr>
<td>3</td>
<td>Sorbitol Solution (0.5 %)</td>
<td>0.5ml</td>
</tr>
<tr>
<td>4</td>
<td>Methyl Paraben</td>
<td>100mg</td>
</tr>
<tr>
<td>5</td>
<td>Purified Water</td>
<td>Up to 100ml</td>
</tr>
</tbody>
</table>

Evaluation of suspension

**Organoleptic properties:** All the developed suspensions were evaluated for organoleptic properties such as colour, odour and taste.

**Pharmaceutical Evaluation**

**pH:** pH of the prepared formulation was measured using digital pH meter.

**Sedimentation Volume:** Fifty ml each of suspension was taken in 50 ml stopper graduated measuring cylinder. The suspension was dispersed thoroughly by moving upside down for three times. Later, the suspension was allowed to settle for three minutes and the volume of sediment was noted.

3. Results

**Table 1.** Morphological characteristics of *W. fruticosa* flower and *G. gummifera* leaf.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Character</th>
<th>Observations</th>
<th><em>W. fruticosa</em> flower</th>
<th><em>G. gummifera</em> leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Color</td>
<td>Bright Orange</td>
<td>Dark Green</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Characteristic</td>
<td>Offensive</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Acid</td>
<td>Pungent</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Size</td>
<td>1-1.5 cm. length</td>
<td>6-20 cm. long</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Texture</td>
<td>Soft</td>
<td>Smooth</td>
<td></td>
</tr>
</tbody>
</table>

3.1. Physiochemical analysis of powder

Physiochemical analysis of powder of *W. fruticosa* flower and *G. gummifera* leaf was done including determination of moisture content, Ash value in which percentage of Water soluble ash, Acid insoluble ash, Total ash and Foaming index. Results are shown in Table 2.

**Table 2.** Physiochemical analysis of powder of *W. fruticosa* flower and *G. gummifera* leaf.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Observation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>W. fruticosa</em></td>
<td><em>G. gummifera</em></td>
</tr>
<tr>
<td>1</td>
<td>Loss on drying</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>Total ash value</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash value</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash value</td>
<td>1.23</td>
</tr>
<tr>
<td>5</td>
<td>Foaming index</td>
<td>23 (ml)</td>
</tr>
</tbody>
</table>

3.2. Colour, Consistency and % yield of extracts

Yield of ethanolic extract of *W. fruticosa* (EEWF) was 4.7% and ethanolic extract of *G. gummifera* (EEGG) was 5.64%. Results are shown in Table 3.

**Table 3.** Colour, Consistency and % Yield of extract of *W. fruticosa* and *G. Gummifera*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Color</th>
<th>Consistency</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>W. fruticosa</em></td>
<td>Brown</td>
<td>Solid</td>
<td>4.7</td>
</tr>
<tr>
<td><em>G. gummifera</em></td>
<td>Brown</td>
<td>Solid</td>
<td>5.64</td>
</tr>
</tbody>
</table>
3.3. Phytochemical Screening

Both the extracts were subjected to various chemical tests for preliminary identification of various phytoconstituents. Both the extracts contain carbohydrates, Tannins, alkaloids, glycosides, flavonoids. Steroids and sterols were found absent in EEWF and EEGG. Proteins and amino acids were found absent in EEWF. Results are shown in Table 4.

Table 4. Phytochemical Screening of ethanolic extract of *W. fructicosa* and *G. Gummifera*.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Tests</th>
<th>EEWF</th>
<th>EEGG</th>
</tr>
</thead>
</table>
| 1      | Carbohydrates  
  i) Molisch’s Test  
  ii) Fehling’s Test  
  iii) Benedict’s test | (+) | (+) |
| 2      | Tannins  
  i) with 5%ferric chloride solution  
  ii) with 10% aqueous Potassium dichromate solution  
  iii) with 10% lead acetate solution | (+) | (+) |
| 3      | Alkaloids  
  i) Dragendorff’s Test  
  ii) Mayer’s Test  
  iii) Hager’s Test | (+) | (+) |
| 4      | Glycosides  
  i) Borntrager’s Test  
  ii) Legal Test  
  iii) Baljet Test | (+) | (+) |
| 5      | Flavonoids  
  i) Shinoda’s Test  
  ii) Alkaline reagent test  
  iii) Lead test | (+) | (+) |
| 6      | Steroids and Sterols  
  i) Libermann-Burchard Test:  
  ii) Salkowski Test: | (–) | (–) |
| 7      | Proteins and Amino Acids  
  i) Biuret Test:  
  ii) Ninhydrin Test:  
  iv) Millon’s Test | (–) | (–) |

(+)= Present, (–)= Absent

3.4. Evaluation of Topical Formulations

The herbal gel was prepared and subjected to evaluation of the various parameters. The Gel of EEWF was light yellowish & gel of EEGG was Light brownish in colour. Gel of EEWF & EEGG was Semi solid in appearance, odourless and had a cool and smooth feeling on application. Spreadibility was measured and found to be 4.8 g.cm/s of Gel of EEWF and 5.2 g.cm/s of Gel of EEGG. Viscosity was measured and found to be 4500 cp Gel of EEWF and 4700 cp of Gel of EEGG. Both the gel was non-irritant upon application on the skin of rats. Results are shown in Table 5.
Table 5. Evaluation of Topical (Gel) Formulations of ethanolic extract of *W. fructicosa* and *G. Gummifera*.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Evaluatory parameter’s</th>
<th>Gel (<em>W. fructicosa</em>)</th>
<th>Gel (<em>G. gummifera</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Appearance</td>
<td>Semi solid</td>
<td>Semi solid</td>
</tr>
<tr>
<td>2</td>
<td>Colour</td>
<td>Light Yellowish</td>
<td>Light brownish</td>
</tr>
<tr>
<td>3</td>
<td>Odour</td>
<td>Odourless</td>
<td>Odourless</td>
</tr>
<tr>
<td>4</td>
<td>Homogeneity</td>
<td>Aggregate free</td>
<td>Aggregate free</td>
</tr>
<tr>
<td>5</td>
<td>Grittiness</td>
<td>No rashes</td>
<td>No rashes</td>
</tr>
<tr>
<td>6</td>
<td>Spreadibility (g.cm/s)</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>7</td>
<td>Viscosity (cp)</td>
<td>4500</td>
<td>4700</td>
</tr>
</tbody>
</table>

3.5. Evaluation of Oral Formulations

The suspension dosage form was prepared and evaluated various parameters. Suspension of EEFW and EEGG was Dark brown in colour, Slight pungent in odour and Light Bitter in Taste. Suspension of EEFW was measured pH (6.4) and evaluated Sedimentation Volume (12 ml). Suspension of EEGG was measured pH (6.8) and evaluated Sedimentation Volume (15 ml). Results are shown in Table 6.

Table 6. Evaluation of the Oral (Suspension) Formulation of ethanolic extract of *W. fructicosa* and *G. Gummifera*.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Evaluatory parameter’s</th>
<th>Suspension (<em>W. fructicosa</em>)</th>
<th>Suspension (<em>G. gummifera</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Dark brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Slight pungent</td>
<td>Slight pungent</td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Light Bitter</td>
<td>Light Bitter</td>
</tr>
<tr>
<td>4</td>
<td>pH</td>
<td>6.4</td>
<td>6.8</td>
</tr>
<tr>
<td>5</td>
<td>Sedimentation Volume (ml)</td>
<td>12</td>
<td>15</td>
</tr>
</tbody>
</table>

3.6. DPPH scavenging activity

The results of the free radical scavenging potentials of extract of EEFW and EEGG were tested by DPPH method. Scavenging activity of EEFW and EEGG was evaluated and compared with ascorbic acid in term of % inhibition at concentration 100, 200, 300, 400 and 500 µg/ml. Results are shown in Table 7 and Fig. 4.

Table 7. DPPH scavenging activity of ethanolic extract of *W. fructicosa* and *G. Gummifera*.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>Antioxidant Results</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Standard (Ascorbic acid)</td>
<td>100</td>
<td>0.437</td>
<td>0.243</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.437</td>
<td>0.197</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.437</td>
<td>0.172</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.437</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.469</td>
<td>0.111</td>
</tr>
<tr>
<td>EEFW</td>
<td>100</td>
<td>0.437</td>
<td>0.345</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.437</td>
<td>0.312</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.437</td>
<td>0.271</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.437</td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.437</td>
<td>0.165</td>
</tr>
<tr>
<td>EEGG</td>
<td>100</td>
<td>0.437</td>
<td>0.348</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.437</td>
<td>0.276</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.437</td>
<td>0.241</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.437</td>
<td>0.189</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.437</td>
<td>0.162</td>
</tr>
</tbody>
</table>
4. Discussion

Many herbal remedies individually or in combination have been recommended in various medical expositions for the cure of different diseases. Plants are among the most important and common sources of potentially valuable new drugs. Therefore, there is a need to investigate the biological properties of medicinal plants in order to develop new drugs. Much work has been carried out on herbal treatment of various diseases, but still there is need to explore more [34].

Topical application of gel at pathological sites offer great advantage in a faster release of drug directly to site of action, independent of water solubility of the drug as compared to creams and ointments. Gels are highly biocompatible with a lower risk of inflammation or adverse reactions, easily applied and do not need to be removed [35, 36]. Gels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removed, emollient, non-staining and compatible with several excipients and water soluble or miscible [37, 38]. The oral route of drug administration is the most important method of administrating drugs for systemic effects. Except in few cases, parenteral route is not routinely used for self administration of medications [39].

It has been recognized that alkaloids and flavonoids shows antioxidant property and their effects on human nutrition and health care are considerable. Mechanism of action of alkaloids is through inhibition of peroxidation. Compounds such as flavonoids are responsible for inhibition of lipid peroxidation. Scavenging activity of free radicals of 1, 1 diphenyl-1,2-picryl hydrazyl (DPPH) has been widely used to evaluate the antioxidant activity.

The coarse powder of the shed dried parts of the plant was subjected to extraction by using soxhlet apparatus. The coarse powder of the shed dried parts of the plant was subjected to extraction by using soxhlet apparatus. The plant materials were treated with solvents for 24 hours. The plant material was extracted with ethanol.

5. Conclusion

In conclusion from this study, it has been found that the ethanolic extract of *Woodfordia fruticosa* flowers and *Gardenia gummifera* leaves have a considerable amount of antioxidant activity that is comparable to ascorbic acid. Result of the in-vitro antioxidant activities indicate that both the plant extract are significant source of natural antioxidant, which might be supportive in the prevention of several diseases. Further research is warranted to elucidate the specific phytoconstituent/s involved.
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References


