Short Communication

Development and Comparative Antibacterial Evaluation of Tincture of *Jatropha gossypifolia* L. Leaves

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Abstract

Objective: To compare antibacterial activity of tincture of *Jatropha gossypifolia* L., leaves against marketed tincture.

Methods: The tincture was formulated using methanolic extract of *J. gossypifolia* leaves. It was evaluated for its density, dry matter, pH, refractive index and content of methanol using appropriate formulae. Further, the tincture of *J. gossypifolia* leaves was compared with marketed tincture, Povidone Iodine for antibacterial activity using agar well diffusion method against strains such as *E.coli*, *E.faecalis*, *B.subtilis* and *S.aureus*.

Results: The results observed from the study complies prerequisites of a tincture and showed comparative antibacterial activity with that of marketed tincture, Povidone Iodine.

Conclusion: *J. gossypifolia* leaves have different pharmacological activities. The comparative evaluation of formulated tincture against marketed tincture showed significant antibacterial activity. Further, this study suggests isolating, identifying and evaluating the phytochemical substances and related pharmacological activity.

Keywords: *Jatropha gossypifolia*, Antibacterial efficacy, Tincture

Introduction

*Jatropha gossypifolia* L. (Euphorbiaceae), the common name is pignut or fignut, and in Yoruba land it is commonly known as Lapalapa [Odebiyi and Sofowora, 1998]. It was reported to possess various medicinal and pesticidal properties and is commonly known as Bellyache bush in Queensland [Csurhes, 1999]. The leaf decoction is used for bathing wounds [Morton, 1968]. The stem sap stops bleeding and itching of cuts and scratches [Morton, 1981; Horsten et al., 1996]. The roots are employed against leprosy, as an antidote for snakebite and in urinary complaints. It possesses significant anticancer, hepatoprotective and pesticidal activity [Hartwell, 1969; Chatterjee et al., 1980; Panda et al., 2009]. A decoction of the bark is used as an emmenagogue and leaves for stomach ache, venereal disease and as blood purifier [Kirtikar and Basu, 1996; Banerjee and Das, 1993]. In Southern Nigeria, the extract from fresh leaf applied with crushed leaf is routinely used by herbalists and local people to
stop bleeding from the skin and nose. The leaf extracts were also reported to possess anticoagulant activity [Oduola et al., 2005] and local anaesthetic activity [Rasheed et al., 2012]. This study aimed to explore the comparative antibacterial effect of tincture of *Jatropha gossypifolia* leaves against the marketed tincture.

**Materials and Methods**

**Collection and Authentication of Plant Material**
Fresh leaves of *Jatropha gossypifolia* L. were collected from Potharlanka village near Repalle, Guntur District, Andhra Pradesh, India and they were identified and authenticated by Dr. S M Khasim, Professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur and the specimen was deposited in the department for future reference. They were cleaned, dried under shade and powdered by mechanical grinder. Based on the increasing polarity of solvents, the powdered leaves were subjected to soxhlet extraction.

**Determination of Physico-chemical Constants**
The physico-chemical constants such as extractive values and ash values were performed as per the standard protocol followed in the Ayurvedic pharmacopoeia.

**Phytochemical Studies**
Freshly prepared methanolic extract of *J. gossypifolia* leaves was carried out for identification of phytoconstituents using qualitative tests as per standard protocol.

**Formulation of Tincture of Gossypifolia**
Dissolve herbal extract in methanol in 1:10 proportion and filter the solution and the obtained tincture was sterilized by moist heat sterilization. The tincture was packed in an amber coloured bottle and it was used for further evaluation.

**Evaluation Methods of Tincture of Gossypifolia**

**Determination of Density**
By using differential weighing method, the mass of given volumes of the tincture (m) at the temperature 20±0.1°C was determined. The density was calculated using the formula,

\[ \rho_{20} = \frac{m}{M} \times 0.99703 \pm 0.0012 \text{ g/cc} \]

Where, \( \rho_{20} \) Density of tincture expressed in g/cc; 
\( m \) Mass of tincture expressed in grams; 
\( M \) Mass of water expressed in grams.

**Determination of Dry Matter**
The dry matter [%] was determined using the formula,

\[ \%DM = \frac{w}{W} \times 100 \]

Where, DM Dry Matter expressed in percentage; 
\( w \) Dry Weight of Tincture expressed in grams; 
\( W \) Total Weight of Tincture expressed in grams.

**Determination of Refractive Index**
The refractive index was measured using Abbe’s refractometer.

**Determination of pH**
The pH value was determined by using Digital pH meter made by Systronics, model 361.
Determination of the Content of Methanol
The content of methanol was measured by using the formula,
\[ X = \frac{50 \times \rho_{20} \times Y}{q} \]
Where, 
- \( X \) Content of Methanol in Tincture expressed in the mass percentage;
- \( Y \) Content of Ethanol in mass percentage corresponding to the density;
- \( \rho_{20} \) Relative Density of the obtained distillate (50 mL) at 20°C;
- \( q \) Amount of Sample.

Comparative Antibacterial Activity of Tincture of Gossypifolia against Marketed Tincture
The comparative antibacterial activity was performed using Agar well diffusion method against strains such as \( E. \ coli, \ E. \ faecalis, \ B. \ subtilis \) and \( S. \ aureus \) in the evaluation of formulated tincture of gossypifolia and marketed tincture. Zone of inhibition of formulated tincture of gossypifolia was compared with that of marketed tincture.

Results and Discussion
The processed conceived powdered material carried out for physicochemical evaluation such as extractive values and ash values. The calculated extractive values were tabulated (Table 1). The different ash values were determined with standard protocol and values were tabulated (Table 2).

<table>
<thead>
<tr>
<th>Name of Extraction</th>
<th>Values (in gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol soluble extraction</td>
<td>0.16 gm</td>
</tr>
<tr>
<td>Water soluble extraction</td>
<td>0.05gm</td>
</tr>
<tr>
<td>Ether soluble extraction</td>
<td>0.04gm</td>
</tr>
</tbody>
</table>

Table 2. Different Ash Values of \( Jatropha \ gossypifolia \) Leaves

<table>
<thead>
<tr>
<th>Name of the Ash</th>
<th>Values (in gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash value</td>
<td>1.76</td>
</tr>
<tr>
<td>Acid insoluble ash value (Dil.Hcl)</td>
<td>1.67</td>
</tr>
<tr>
<td>Sulphated ash value (( H_2SO_4 ))</td>
<td>1.65</td>
</tr>
<tr>
<td>Water soluble ash value (( H_2O ))</td>
<td>1.70</td>
</tr>
</tbody>
</table>

Table 3. Evaluation Values of Formulated Tincture of gossypifolia

<table>
<thead>
<tr>
<th>Extract</th>
<th>Density</th>
<th>Dry matter</th>
<th>Refraction</th>
<th>pH</th>
<th>Content of Methanol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>1.646</td>
<td>0.77</td>
<td>1.33</td>
<td>6.7</td>
<td>47.34</td>
</tr>
<tr>
<td>PIT</td>
<td>1.65</td>
<td>0.8</td>
<td>1.421</td>
<td>6.9</td>
<td>48</td>
</tr>
</tbody>
</table>

TG Tincture of Gossypifolia; PIT Povidone Iodine Tincture (Marketed Tincture)

The preliminary phytochemical screening was performed to know the phytochemicals present in various solvent extracts. Here, the preliminary phytochemical screening shows majorly steroids, flavanoids, vitamin C and saponins and moderately glycosides, alkaloids, resins and carbohydrates. Further, the methanolic extract was taken for the formulation of tincture of gossypifolia and it was evaluated to know the standards for a tincture. The evaluation tests were carried out with various parameters such as density, dry matter,
refraction, pH and content of methanol (%) by using standard protocol followed in the earlier reported work. The results of evaluation parameters were tabulated (Table 3). Further, the formulated tincture of gossypifolia was compared with marketed antibacterial tincture to know the antibacterial efficacy against the strains such as E. coli, E. faecalis, B. subtilis and S. aureus using agar well diffusion method. The results observed from the study showed comparatively having significant antibacterial activity against used microorganisms and the results were tabulated (Table 4).

### Table 4 Comparison of Marketed Tincture and Formulated Tincture of Gossypifolia

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Concentrations</th>
<th>E. coli</th>
<th>E. faecalis</th>
<th>B. subtilis</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (1g/100ml)</td>
<td>100ug/ml</td>
<td>2.3mm</td>
<td>2.5mm</td>
<td>1.9mm</td>
<td>2.0mm</td>
</tr>
<tr>
<td>PIT (1g/100ml)</td>
<td>100ug/ml</td>
<td>2.6mm</td>
<td>2.8mm</td>
<td>2.2mm</td>
<td>2.2mm</td>
</tr>
</tbody>
</table>

TG Tincture of Gossypifolia; PIT Povidone Iodine Tincture (Marketed Tincture)

### Conclusion
Though, *Jatropha gossypifolia* leaves have different pharmacological activity. The preliminary phytochemical, physico-chemical and antibacterial studies were not reported earlier. Hence, this attempt gives preliminary idea about antibacterial and phytochemicals which are responsible for the activity. The comparative evaluation of formulated tincture against marketed antibacterial tincture showed significant antibacterial activity. Further, this study suggests evaluating, isolating and identifying the resulted phytochemical substances and related pharmacological activities.

### References
Banerjee J and Das B. 1993. MAPA, Dept. of Chemistry, University College of Science, Calcutta, India. 15:1002-1017.