COMPARATIVE EVALUATION OF IN-VITRO ANTHELMINTIC POTENCY OF *DELONIX REGIA* (RAFIN) AND *CAESALPINIA PULCHERRIMA* (LINN) FLOWER EXTRACTS BY AQUEOUS AND METHANOL AS A SOLVENT

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ABSTRACT
The aim of present study was to evaluate the in-vitro anthelmintic potency of aqueous and methanolic extracts of the flower Delonix regia (Rafin) and Caesalpinia Pulcherrima (Linn) against Pheretima posthuma. Three concentrations (25, 50 and 100 mg/ml) of each extracts were used for this study which involved the determination of time of paralysis (vermifuge) and time of death (vermicidal activity) of the worms. Extracts obtained from both flowers not only paralyzed but also killed the earthworms. Both extracts exhibited anthelmintic activity in a dose-dependent manner. The most significant activity was observed at the highest concentration of 100 mg/mL against the worms. Piperazine Citrate (10 mg/ml) and distilled water were included as standard reference and control respectively. The results conclude that aqueous extracts of Delonix regia (Rafin) took less time to cause paralysis of the earthworm than that of methanolic extract but Caesalpinia Pulcherrima (Linn) methanolic extract took less time to cause paralysis of the earthworm than that of aqueous extract. Thus the present study demonstrate that the traditional claim of Delonix regia (Rafin) and Caesalpinia pulcherrima (Linn.) as an anthelmintic has been confirmed as the aqueous and methanolic extracts displayed activity against the earthworm used in study.

Key words: Delonix regia (Rafin), Caesalpinia pulcherrima (Linn) flower, anthelmintics, Tannin, Paralysis, Death of earthworm.

INTRODUCTION
Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on the anthelmintic drug is sparse. According to the WHO, only a few drugs are used in treatment of helminthes in humans. Anthelmintics from natural sources could play a key role in the treatment of these parasite infections. Helminthiasis is a
worldwide and one of the common diseases of all ages especially in third world countries. The parasitic diseases cause severe morbidity by affecting population in endemic areas with major economic and social consequences [3]. Helminthes infections are among the most common infections in man, affecting a large proportion of the world’s population. In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travelers who have visited those areas and some of them can develop in temperate climates [2].

The disease is highly prevalent particularly in third world countries [3] due to poor management practices. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, increasing problems of development of resistance in helminthes [4, 5] against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. The plants are known to provide a rich source of botanical anthelmintics [6, 7]. A number of medicinal plants have been used to treat parasitic infections in man and animals [8, 9, 10].

Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm or tapeworm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs, infected people excrete helminthes eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation [11]. Other people can then be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Parasitic diseases cause severe morbidity, including filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis [12]. As per WHO only synthetic drugs are frequently used in the treatment of helminthes infestations in human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effect.

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The plant Delonix regia (family: leguminosae, sub family: fabaceae) also known as royal Poinciana. May flower plant or Flamboyant, is many branches, broad, spreading, flat crowned deciduous tree and well known for its brilliant display of red-orange bloom, literally covering the tree from May to June [13]. It is commonly known as ‘Gulmohar’ in Hindi and Marathi. The Delonix regia will provide fullest flowering and best growth when planted in full sun location [14]. The literature survey reveals that Delonix regia bark contain β-sitosterol, saponins, alkaloids, carotene, hydrocarbons phytotoxins and flavonoids. Flowers of Delonix regia also contain carotenoides, tannins, saponins, flavonoids, steroids, alkaloids and β-sitosterol, seed consists of saponins galactomannon and plant used as an catharatic, antirheumatic and flatulalence (Singh).

Caesalpinia pulcherrima L. Swartz (Leguminosae) is an ornamental plant due to its variety of flowers [18] and it is a common medicinal plant in India, Taiwan and South- East Asian countries. In alternative medicine, the different parts of this plant have been used in the treatment of anti-inflammatory, abortifacient, emmenagogue, bronchitis and malarial infection while fruits are employed to cure diarrhea and dysentery. Phytochemical investigations on Caesalpinia pulcherrima have revealed the presence of various phytoactive constituents such as glycosides, retinoids, isoflavones, flavanones, chalcones, flavanols, flavones and sterols [19, 20]. Seeds have shown Antiviral activity by Chiang [20], stem shown cytotoxic activity by Pherson [21], Leaves shown Antitumor by Chiang [20], Antimicrobial activity by [22], Antiviral activity by Chiang [20].

Flowers shown Antimicrobial Antifungal activity [23], fruits shown Antiviral activity by Chiang [20]. Bark showed Antimicrobial, Cytotoxic activity [24].

Among the most widespread of all chronic infection are those caused by various species of parasitic helminthes (worms). Inhabitants of tropical or subtropical, low income countries are most at risk; children often become infected with one or more species almost as soon as they are born and may remain infected throughout their lives. In some
cases these infection results mainly in discomforts and does not causes substantial ill health, but others such as schistosomiasis and hookworm disease, can produce very serious morbidity. Worm infestations are also a major cause for concern in veterinary medicine, affecting domestic pets form animals [25]. Parasitic diseases cause severe morbidity by affecting population in endemic areas with major economic and social consequences [26]. A number of medicinal plants have been used to treat parasitic infections in man and animals [8, 27]. Antihelminthics are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them. In view of this, an attempt has been made to study the anthelmintic activity property in Delonix regia (Rafin) and Caesalpinia Pulcherrima (Linn) flower extract by water and methanol as solvents.

**MATERIALS AND METHODS**

**Collection of Plant Material**
The whole plant of Delonix regia (Rafin) and Caesalpinia Pulcherrima (Linn) flower was collected from local areas of Bangalore and Mysore, Karnataka.

**Preparation of aqueous extract**
The shade dried flower powder was extracted with water and methanol for 72hr, filter and the same supernatant was removed in vacuum at 40°C by using rotary evaporator and dried sample used for further pharmacological studies.

**Animals**
Indian adult earthworms (Pheretima posthuma) were used to study anthelmintic activity according to [28]. The earthworms were collected from moist soil in Mysore and washed with normal saline to remove all fecal matter. Earthworms 3-5 cm in length and 0.1-0.2 cm in width were used for all experimental protocol.

**Chemicals**
Piperazine Citrate Methanol

**Phytochemical screening**
The phytochemical screening tests were done according to Deepika Kannan [29].

**Experimental control treatment**
Distilled water was pour in sterile Petri dish containing Indian adult healthy earth worm to carry out experiment.

**Experimental standard treatment**
A 10mg/ml Piperazine Citrate in distilled water was used as experimental standard.

**Anthelmintic activity**
Methanol and aqueous extracts from the flower of Delonix regia (Rafin) and Caesalpinia Pulcherrima (Linn) were investigated for anthelmintic activity against Pheretima posthuma. Various concentrations (25, 50 and 100 mg/ml) of each extract were tested by bioassay, which involved determination of time of paralysis and time of death of the worms. The anthelmintic activity was performed according to the method [30]. On adult Indian earth worm Pheretima posthuma as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Pheretima posthuma was placed in separate petridishes containing three different concentrations (25, 50, 100mg) of Delonix regia and Caesalpinia Pulcherrima (methanol, and water extract) solutions. Each petridish was placed with 6 worms and observed for paralysis (or) death. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. In the same manner Piperazine Citrate was included as reference compound. The test results were compared with reference compound Piperazine Citrate (10mg/ml) treated sample.

**RESULTS AND DISCUSSION**
Preliminary phytochemical analysis showed the presence of alkaloids, phenols, flavonoids, steroids, tannins like phytoconstituents in the extract of Delonix regia (Rafin) and Caesalpinia Pulcherrima (Linn) flower. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity. From the above study it was seen that the aqueous and methanolic extract showed dose dependent antihelmentic activity as compared to a
standard drug piperazine citrate. Piperazine citrate exhibits anthelmintic activity by blocking glucose uptake and depletion of glycogen stores in test parasite. Comparison was carried by selecting earthworm for the antihelmintic activity and indicating it is most sensitive to the methanol extract of *Delonix regia* (Rafin) and aqueous extract of *Caesalpinia Pulcherrima* (Linn) as can be seen in Table-2&3.

**Table 1: Qualitative phytochemical tests for flower *Delonix regia* (Rafin) and *Caesalpinia Pulcherrima* (Linn).**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytochemicals</th>
<th><em>Delonix regia</em> (Rafin)</th>
<th><em>Caesalpinia Pulcherrima</em> (Linn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reducing sugar</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>Proteins</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Amino acids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Cardiac glycosides</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Alkaloids</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

(+) indicates presence at good concentration, (++) indicates presence at high concentration, (+++) indicates presence at very high concentration.

**Table 2: Anthelmintic potency of *Delonix regia* (Rafin) flower.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mg/ml)</th>
<th><em>Delonix regia</em> (Rafin) flower</th>
<th>Paralyzing time (min)</th>
<th>Death time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Piperazine Citrate</td>
<td>10</td>
<td>27.00±0.05</td>
<td>68.95±0.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>87.95±0.08</td>
<td>161.83±0.12</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>50</td>
<td>48.88±0.05</td>
<td>65.03±0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>14.06±0.06</td>
<td>23.07±0.05</td>
<td></td>
</tr>
<tr>
<td>Methanoilc extract</td>
<td>25</td>
<td>72.95±0.16</td>
<td>147.89±0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>42.03±0.03</td>
<td>58.98±0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>13.39±0.15</td>
<td>20.17±0.14</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Anthelmintic potency of *Caesalpinia Pulcherrima* (Linn) flower.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mg/ml)</th>
<th><em>Caesalpinia Pulcherrima</em> (Linn) flower</th>
<th>Paralyzing time (min)</th>
<th>Death time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Piperazine Citrate</td>
<td>10</td>
<td>27.00±0.05</td>
<td>68.95±0.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>40.25±0.25</td>
<td>67.36±0.28</td>
<td></td>
</tr>
</tbody>
</table>

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The crude extracts samples, which were used to evaluate anthelmintic activity, showed variable times at different concentrations and the mean time values were calculated for each parameter. The crude extracts of aqueous and methanol showed the significant anthelmintic effect causing death of the worm at all the concentrations but the time of death was different in each case. However, when observed the response of worms in case of paralysis, there was significant variation among the results produced by the extracts at different concentrations like 25, 50, 100mg/ml.

<table>
<thead>
<tr>
<th></th>
<th>Aqueous extract</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Time (min)</td>
<td>20.87±0.14</td>
<td>51.49±0.22</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>28.01±0.07</td>
</tr>
<tr>
<td>Concentration</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Time (min)</td>
<td>13.00±0.04</td>
<td>45.96±0.06</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>25.05±0.09</td>
</tr>
<tr>
<td>Concentration</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Time (min)</td>
<td>18.95±0.12</td>
<td>32.65±0.34</td>
</tr>
</tbody>
</table>

Fig-1: IN-VITRO ANTHELMINTIC ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACT (mg/ml) OF DELONIX REGIA (RAFIN) FLOWER.

Fig-2: IN-VITRO ANTHELMINTIC ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACT (mg/ml) OF CAESALPINIA PULCHERRIMA (LINN) FLOWER.
The effect of extracts on the paralysis (or) helminthiasis of the worm, according to the results table-1 and table-2 may be indicated as methanol>water and water>methanol extracts respectively. In the meantime piperazine citrate at a dose of 10 mg/ml cause paralysis and death in the above helminthes in 27 and 68.95 minutes respectively. The anthelmintic activity of the aqueous and methanolic extracts of Delonix regia (Rafin) and Caesalpinia Pulcherrima (Linn) may be due to the presence of polyphenolic compounds [31].

Tannins chemically polyphenolic compounds [31], were shown to produce anthelmintic activities [32]. Reported anthelmintic effect of tannins, can bind to free proteins in the gastrointestinal tract of host animal [33] or glycoprotein on the cuticle of the parasite and may cause death. Further studies are under process to identify the possible phytocstituents responsible for anthelmintic activity.

Tannins, the secondary metabolite, occur in several plants have been reported to show anthelmintic property by several investigators [34, 35]. Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminthes parasites by uncoupling oxidative phosphorylation [36] or, binds to the glycoprotein on the cuticle of parasite [37] and cause death.

Coming to the chemistry of nematode surface, it is a collagen rich extracellular matrix (ECM) providing protective cuticle that forms exoskeleton, and is critical for viability, the collagen is a class of proteins that are modified by a range co-and post – translational modification prior to assembly into higher order complexes (or) ECMS [38]. The mammalian skin also consists largely of collagen in the form of fibrous bundles. In leather making industry, vegetable tannins are commonly used in the tanning operation of leather processing that imparts stability to collagen of skin matrix through its reactivity and hence make the collagen molecule aggregate into fibers. This results in the loss of flexibility in the collagen matrix and gain of mechanical property with improved resistance to the thermal (or) microbial/enzymatic attack.

Similar kind of reaction is expected to take place between the nematode cuticle (the earth worm) and the tannin of Delonix regia (Rafin) and Caesalpinia Pulcherrima (Linn), possibly by linking through hydrogen bonding, as proposed in this study. This form of reactivity brings toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death. Most worm expellers like piperazine citrate cause paralysis of the worms so that they are expelled in the feces. The aqueous and methanolic extracts of the plants not only demonstrated this property but also killed the worms. Hence further investigation and proper isolation of the active principles might help in the findings of new lead compounds, which will be effective against various parasitic infections.

CONCLUSION

In this investigation the preliminary phytochemical tests revealed the presence of alkaloids, sterol, phenol, flavonoids and tannins in aqueous and methanolic extract of Delonix regia (Rafin) and Caesalpinia Pulcherrima (Linn) were used to evaluate anthelmintic activity by using the above models and prove its significant anthelmintic activity. The wormicidal activity of Delonix regia (Rafin) and Caesalpinia Pulcherrima (Linn) flower extracts suggests that, maybe it is effective against parasitic infections of humans. With this background we conclude that, the Current study gives the evidence that it may be a fruitful medicine of tomorrow. Further research needed to isolate the possible active phytocstituent, which are responsible for the anthelmintic activity and its mechanism and its pharmacological actions which is still transparent to research.

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