Evaluation of Anthelmintic Activity & Phytochemical Screening of the Peels of Citrus sinensis & Rhizomes of Curcuma longa

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ABSTRACT
Phytochemicals are secondary metabolites produced by all plants in which some have medicinal uses. The phytochemical analysis of peel & rhizome extracts in aqueous, ethanolic, acetone, hexane, and chloroform extracts of indigenous medicinally important plants of citrus sinensis (peels) & curcuma longa (dried rhizomes) were investigated. The phytochemical analysis revealed the presence of active constituents such as carbohydrates, flavonoids, alkaloids, terpenes, phytosterols, tannins, steroids, saponins, glycosides, phenols, and anthraquinones. This research supports the local use of the peel and rhizome extracts of orange and turmeric to show the potent nature of the sealants when using in combination to treat helminthiasis. These plants belong to family rutaceae & zingiberaceae respectively. The present study provides evidence that the solvent extract of citrus sinensis and curcumin along contains medicinally important bioactive compounds and this just if is the use of these plants in combination to treat helminthiasis & control mode growth in intestines.

Key words: Phytochemical screening, Indigenous,Citrus sinensis, Curcuma longa, peel & rhizome extract.

INTRODUCTION
The use of medicinal plants for the treatment of many diseases is associated to folk medicine from different parts of the world. Natural products from some plants, fungi, and other organisms, continue to be used in pharmaceutical preparations either as pure compounds or as extracts. An increasing interest in herbal remedies has been observed in several parts of the world and many of the herbal remedies have been incorporated into orthodox medicinal plant practice.

Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile convulsion, diarrhoea, dysentery, fungal and bacterial infections. Medicinal herbs considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils.

Helminths are the parasitic worms which are large in size and so called macroparasites. The adult worms can be seen with naked eye. Many of the mare transmitted via soil and infect the gastrointestinal tract, which makes the intestinal worms. Some parasitic worms including leeche sand monogeneans, areceta parasitesthes, they are not classified as helminthes, which are endoparasites. Any disease or infection caused due to ahelmint his known as helminthiasis, helminth infection. They often live in the gastrointestinal tract of their hosts, but they may also burrow in too their organs, where they induce physiological damage. Helminthiasis has been found to result in poor birth outcome, poor cognitive development, poor school and work performance, poor socio economic development, and poverty.

MATERIALS & METHODS
COLLECTION & PREPARATION
The fruit so citrus sinensis and rhizomes of curcuma along are purchased from the local market of visakhapatnam south India. The plant and the plant material were identified and authenticated by the department of botany. Citrus fruits were washed thoroughly by using tap water and were peeled off manually. All the peels were segregated in to whole halves where one half wares had edriedat room temperature for 10 to 12 days. The dried peels were further made into small size and stored in air tight bag for the later extraction process. The rhizomes of curcuma longa are washed thoroughly in water, cut into small pieces and air dried for 2 week sat 35 to 40'C and were stored in 4'C in air tight containers for further studies.

EXTRACTION
Extraction is the first step to separate the desired natural products from the raw materials. Solvent extraction is the most widely used method. The extraction of natural products progresses through the following stages: (1) the solvent penetrates into the solid matrix; (2) the solute dissolves in the solvents; (3) the solute is diffused out of the solid matrix; (4) the extracted solutes are collected. Soxhlet extraction is commonly used for the extraction process. Extractions use two immiscible phases to separate the substance from one phase into the other.

PHYTOCHEMICAL ANALYSIS
Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were
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carried out in extracts as well as powder specimens using the standard procedures.

**PHYTO CHEMICAL SCREENING**

**TEST FOR CARBOHYDRATES**

**MOLISCH’STEST**
To the test solution add few drops of alcoholic alphanapthol then add few drops of concentrate dsulphuric acid through the sides of test tube wall purple to violet colouring appears at the junction.

**BARFOED’S TEST:**
1ml of test solutionis heated with 1ml of barfoed’s reagent on water bath, if red cuprico xideis formed, mono saccharides present.Disaccharide on prolonged heating (about10min)may also cause reduction, owing to partial hydrolysesto mono saccharides.

**FEHLING’STEST**
Add 1 mleach offehling’s soluti of test solution A & B to1ml of test solution and heate dina water bath, if red precipitate of cupricoxide is formed, it indicates the presence of carbohydrates.

**TEST FOR ALKALOIDS**

**MAYER’STEST**
Alkaloids give cream colour precipitate with mayer’s reagent(potassium mercuric iodide solution)

**WAGNER’STEST**
Alkaloids give red dish brown precipitate with wagner’s reagent(iodine-potassium iodide solution)

**3)DRAGONDORFF’STEST**
Alkaloids give red dish brown precipitate with dragondorff’s reagent(potassium bi smuthiodide solution)

**TESTFORTANNINS**

**TEST WITH FERRIC CHLORIDE**
Tannins give bluish black or brownish green colour with ferric chloride.

**TEST WITH LEADACETATE**
Tannins are precipitated by salts of blood.

**TEST FOR FLAVONOIDS**
The extract(1ml) was diluted in 1 ml of diluted sodium hydroxide, formation of yellow precipitate indicated the presence of flavonoids.

**TEST FOR STEROIDS**
The extract (1ml) was dissolved in 2ml of chloroform in a test tube ,and then 1 ml of concentrated sulphuric acid was added ,formation of reddish brown colour at the inter-phase indicated the presence of steroids.

**TESTFORPHENOLS**

**TEST WITH FERRIC CHLORIDE**
The extract (1ml) was added with 1 ml of 10 % ferric chloride. The formation of a greenish brown precipitate indicated the presence of phenols.

**TEST FOR SAPONINS**

**FROTHING TEST**
2g of extract was mixed and boiled with 20 ml of water and then filtered.5ml of distilled water is added in 10 ml of this filtrate and was shaken vigorously for stable persistent froth.The formation off roth shows the presence of the saponin in extract.

**TEST FORANTHRAQUINONES**
0.5g of the extract was boiled with 10ml of sulfuric acid and filtered while hot 5ml of chloroform used to shake the filtrate.1ml of dilute ammonia was added in the chloroform layer the resulting solution was observed for colour changes.

**TEST FOR TERPENOIDS**
To 0.5 g each of the extract was added 2ml of chloroform. To form a layer, concentrated sulfuric acid (3ml) was carefully added. A reddish brown appearance of the interface indicates the presence of terpenoids.

**TEST FORGLYCOSIDES**

**TEST FOR CARDIAC GLYCOSIDES**

**LEGALS TEST**
Treat the test solution with pyridine and alkaline sodium nitroprusside solution, blood colour appears.

**TEST FOR SAPONINGLYCOSIDES**

**FROTH FORMATION TEST**
Place 2ml solution of drug in water in a test tube, shake well, stable for this formed.

**TEST FOR FLAVANOID GLYCOSIDES**

**TEST FOR PHYTOSTEROLS**

**SALKOWSKI TEST**
Dissolve cholesterol in 2 ml of chloroform in dry test tube. Add equal amount of concentrated sulphuric acid (H₂SO₄). Shake gently, the upper layer turns red and the sulphuric acid layer shows a yellow colour with agreen fluorescence.

**LIEBERMAN–BURCHARD TEST**
Dissolve 1 or 2 crystals of cholesterol in dry chloroform in a dry test tube. Add several drops of acetic anhydride and then 2 drops of concentrated H₂SO₄ and mix carefully which givesa deep green colour.
RESULTS & DISCUSSION

The data revealed that the various sex tracts obtained from the peels of citrusinensis & curcumalonga as a combinations how ed anthelminthic activity at 50 mg/ml, while the ethanolic extract showed significant results, which makes it as a standard solvent. The concentrations of 10mg/ml, 20 mg/ml, 50mg/ml paralyzed at the same time but the time taken for death differed, out of these three concentrations, the plant drugs how optimum anthelminthic activityat 50mg/ml concentration. Potency of the extract was inversely proportional to time take For paralysis and death of earth worms. The results were compared to standard drug ivermectin of various concentrations. There forethe activity shown by a combinational drug is more potent when compared to individu alone.

Tab 1: Anthelminthic activity of variou sextracts obtained from citrusinensis & curcumalonga with different solvents:

<table>
<thead>
<tr>
<th>S.N o</th>
<th>Plantsextracts</th>
<th>Con(c µ/ml)</th>
<th>Time taken for paralysis(min)</th>
<th>Time taken for death(min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle(control saline)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform extracts</td>
<td>10</td>
<td>42.16±0.61</td>
<td>75.51±0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>35.29±0.28</td>
<td>68.28±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>25.48±0.35</td>
<td>34.14±0.50</td>
</tr>
<tr>
<td>3.</td>
<td>Ethanol extracts</td>
<td>10</td>
<td>37.75±0.52</td>
<td>68.66±0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>27.25±0.21</td>
<td>50.18±0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>20.11±0.72</td>
<td>26.09±0.76</td>
</tr>
<tr>
<td>4.</td>
<td>Hexane extracts</td>
<td>10</td>
<td>30.14±0.16</td>
<td>51.61±0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>18.52±0.15</td>
<td>34.49±0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>9.41±0.13</td>
<td>30.12±0.62</td>
</tr>
<tr>
<td>5.</td>
<td>Acetone extracts</td>
<td>10</td>
<td>40.10±0.57</td>
<td>65.48±0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>32.51±0.27</td>
<td>52.20±0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>23.81±0.32</td>
<td>32.10±0.45</td>
</tr>
<tr>
<td>6.</td>
<td>Ivermectin</td>
<td>10</td>
<td>16.24±0.84</td>
<td>42.14±0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>14.19±0.21</td>
<td>24.13±0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>7.14±0.22</td>
<td>19.32±0.27</td>
</tr>
<tr>
<td>7.</td>
<td>Ethanolic extract of volatile orange oil</td>
<td>10</td>
<td>15.22±0.60</td>
<td>18.30±0.24</td>
</tr>
<tr>
<td>8.</td>
<td>Ethanolic extract of volatile curcuma oil</td>
<td>10</td>
<td>14.21±0.21</td>
<td>14.13±0.21</td>
</tr>
</tbody>
</table>

CONCLUSION

Ethanolic extract of citrusinensis at the conc of 10mg/ml showed the time of paralysis & death at 15 min and 18 min respectively. Ethanolic extract of curcumalonga at conc10 mg/ml showed the time of paralysis & death at 14.2 min and 14.3 min respectively. Finally it can be concluded that the combination of both citrusinensis & curcumalonga gives aptent extract which shows significant anthelminthic activity against earth worms. The current study leads to a conclusion that ethanolic extract of the plants possess a unique property when compared with the prevalented drug. Further investigations needed inorder to isolate the phyto chemical constituents responsible for anthelminthic activity.

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10. ZouX,DaiZ,DingC,ZhangL,ZhouY and Y etal. Put anthelminthic activity at 14.2 min and 14.3 min respectively. Finally it can be concluded that the combination of both citrusinensis & curcumalonga gives aptent extract which shows significant anthelminthic activity against earth worms. The current study leads to a conclusion that ethanolic extract of the plants possess a unique property when compared with the prevalented drug. Further investigations needed inorder to isolate the phyto chemical constituents responsible for anthelminthic activity.

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