Antimicrobial and Antihelminthic Activities of Various Extracts of Leaves and Stems of *Abutilon indicum* (Linn.)

Pusapati Madan Ranjit¹*, Y. Ankamma Chowdary¹, Harika Krapa¹, Sindhu Nanduri¹, Himabindhu Badapati¹, Kola Phani Kumar², Priyanka Bommadevara¹, Madhuri Kasala¹

¹NRI College of Pharmacy, Pothavarappadu (V), Agiripalli Mandal, Krishna (Dist.), A.P, India
²ANU College of Pharmaceutical Sciences, Nagarjuna Nagar, Nagarjuna University, Guntur (Dist.), A.P, India

Received 28 Oct 2012; Revised 07 Feb 2013; Accepted 18 Feb 2013

ABSTRACT

Our present study carried out the antimicrobial and antihelminthic activity of leaves and stems of the plant *Abutilon indicum* (Linn.) Sweet (Malvaceae). Different parts of the plant are used for treatment of various ailments in ethnomedicine. We are carried out the antimicrobial activity chloroform extract prepared form leaves using agar-well diffusion method against both gram positive and gram negative microorganisms and antihelminthic activity was performed by using alcoholic extract of stems, ethyl acetate and carbon tetra chloride fraction of aqueous extracts from the leaves of plant against *Pheretima posthuma*.

Key words: Antimicrobial activity, Antihelminthic activity, ethnomedicine, *Abutilon indicum, Pheretima posthuma*.

INTRODUCTION

*Abutilon indicum* (Linn.) Sweet (Malvaceae) is a shrub distributed throughout India and other topical regions of the world. The various parts of the plant (leaves, roots, seeds and seed and seed oil) are widely used in variety of ailments in traditional system of medicine such as Ayurveda and Siddha. The roots are useful in treating uterine hemorrhagic discharges. Leaves are useful in treatment toothache, lumbago, piles and all kinds of inflammation. Bark is used as anthelmentic and diuretic [1]. In addition it also showed antispasmodic, cardiac depressant, estrogenic, antifungal and hepatoprotective properties [2-5].

Dried whole plant was used as laxative and demulcent [6], febrifuge, anthelmentic, diuretic, anti-inflammatory, especially in uterine discharges, piles, lumbago [7] anti inflammatory, immuno stimulant, piles and gonorrhea treatment. Roots and bark are used as aphrodisiac, anti diabetic, nerve tonic, and diuretic. Seeds also used as aphrodisiac, in treatment of urinary disorders [8]. The whole plant is reported to have analgesic [9], hypoglycemic [10], hepatoprotective [11], hypolipidemic activity [12]. The various leaves extract was reported to contain alkaloids, flavonoids, steroids, triterpenoids, and glycosides [13-15]. The other phytochemical constituents present in this plant are include fatty acids, abutilin A, flavonoids, quercetin, glycosides, alkaloids, steroids, terpenoids, saponins, sesquiterpenes, lactones, gallic acid, β-sitosterol, geranoin, caryophylline and phenolic compounds [16-18]. The aim of the present research is, to determine the preliminary phytochemical constituents, antimicrobial and antihelminthic activity of various extracts of the leaves and stems of *Abutilon indicum*.

MATERIALS AND METHODS

Collection of plant material:

*Abutilon indicum* (linn.) leaves and stem are collected in the month of July and August from the area Pothavarappadu (V), Agiripalli (M), Krishna District, Vijayawada. The collected parts of the plant materials were authenticated by Dr. K. Madhava Chetty, Asst. Professor, Dept. of Botany, Sri Venkateswara University, Tirupati (DRM/NRI/15/08/2011/AC2). Herbarium specimen was deposited in the Department of Pharmacognosy, NRI College of Pharmacy. (Specimen no.NRI/COL/P.COG/AILS).

*Corresponding Author: Pusapati Madan Ranjit, Email: madanranjit@gmail.com*
Preparation of extracts:
The various parts of the plant dried under the shed and subjected to grinding. The powered material was subjected to extraction in soxhlet extractor by using solvents. The leaves are extracted with chloroform (Yield of extract 7.15g) and water. The aqueous extract was further fractioned by using ethyl acetate (Yield of extract 4.23g) and carbon tetrachloride (Yield of extract 1.4g). Likewise, they were stems powered and extracted with alcohol (Yield of extract 2.7g).

Preliminary phytochemical investigation:
The qualitative chemical tests of various extracts of plant were carried out using standard procedures [19]. Preliminary phytochemical investigation shows the presence of alkaloids, saponins, carbohydrates flavonoids, phenolic compounds, steroids and tannins.

ANTIBACTERIAL ACTIVITY:
Test organisms:
Antibacterial activity was performed by using chloroform extract of the leaves against selected NCIM (National Collection of Industrial Microorganisms) type bacterial stains. These are four Gram positive micro-organisms (Staphylococcus aureus NCIM 2079, Bacillus subtilis NCIM 2063, Bacillus pumilis NCIM 2327, and Micrococcus lutes NCIM 2871) and three Gram Negative microorganism (Escherichia coli NCIM 2067, Pseudomonas aeruginosa NCIM 2037, and Proteus vulgaris NCIM, 2027).

Preparation of inoculums:
The in vitro screening of antibacterial activity was carried out using Cylinder-plate assay method. For antibacterial activity, the inoculums or microbial suspension is prepared according to the procedure given in the I.P (Indian pharmacopoeia-2010). The test organism (one loop full) were seeded to the nutrient agar (HIMEDIA) at temperature between 40° and 50° and immediately pour the inoculated medium into the Petri plate (8 Inch) to give a depth of 3 to 4 mm and allowed to solidify and punched with a sterile cork borer (6.0 mm diameter) to make open cavities. Each plate should have maximum seven cavities with appropriate distances.

Preparation of test and standard solutions:
The stock solution of test was prepared by dissolving the dried chloroform extracts of leaves of Abutilon indicum (Linn.) at concentration of 50,100,200,300 and 500μg/ml in dimethyl sulphoxide (DMSO). The stock solution of reference standards (Cefexime) was prepared at a concentration of 20μg/ml in sterile water. Antibacterial activity was screened by adding 0.05 ml stock solution to each cup by micropipette. The 20μg/ml Cefexime was used as positive control and 0.05 ml of DMSO was used as negative control. Antibacterial activity was screened by adding 0.05 ml of both test and standard solution to each cavity of the plate using micropipette. Each microbial culture was inoculated into three petri plates. All the plates were kept for 1 to 4 hours at room temperature and then incubate them for about 24 hours at the incubator (33-34°C). After incubation, the bacterial inhibition zone diameters were measured and take the average of inhibition zone diameters of three plates of each organism was noted.

ANTHELMINTIC ACTIVITY:
Alcoholic extract of the stems, ethyl acetate and carbon tetra chloride fraction of aqueous extracts from the leaves plant of Abutilon indicum were investigated for their anthelmintic activity against Pheretima posthuma. Various concentrations (10, 20, 40 and 80 mg/ml) of each extracts were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Albendazole was included as a standard reference and saline water as control. The anthelmintic assay was carried as per the method of [20] with minor modifications. The assay was performed on adult Indian earthworm, Pheretima posthuma due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [21-22]. The experimental design of the investigation was carried out in seventeen groups with six worms in each group and carried out in the following regimes.

Group 1: Normal saline.
Group 2: Albendazole 10 mg/ml.
Group 3: Albendazole 20 mg/ml
Group 4: Albendazole 40 mg/ml
Group 5: Albendazole 80 mg/ml
Group 6: Alcoholic extract of the stems 10 mg/ml
Group 7: Alcoholic extract of the stems 20 mg/ml
Group 8: Alcoholic extract of the stems 40 mg/ml.
Group 9: Alcoholic extract of the stems 80 mg/ml.
Group 10: Ethyl acetate fraction of aqueous extract of leaves 10 mg/ml
Group 11: Ethyl acetate fraction of aqueous extract of leaves 20 mg/ml
Group 12: Ethyl acetate fraction of aqueous extract of leaves 40 mg/ml
Group 13: Ethyl acetate fraction of aqueous extract of leaves 80 mg/ml
RESULTS AND DISCUSSION

Antimicrobial activity:

Chloroform extract of Abutilon indicum leaves showed antimicrobial activity against only on Gram +Ve microorganism. When compared within the microbial strains, chloroform extract showed inhibition zone diameter 19.3±0.5mm in diameter on Staphylococcus aureus NCIM 2079 at 500μg/ml. The extract showed growth inhibition zones against other strains in a dose dependent manner. But this extract did not show growth inhibition against Gram –Ve microorganisms, when compared with standard drug cefexime (10μg/ml). Here we are using a crude extract of plant. It is only preliminary activity for identification of antibacterial activities of the Abutilon indicum.

Table 1: Zone of inhibition of various concentrations of chloroform extract of Abutilon indicum leaves

<table>
<thead>
<tr>
<th>Type of strain</th>
<th>Zone diameters (mm) with respect to Conc. of the Chloroform extract</th>
<th>Control</th>
<th>Conc. of Cefexime 10μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50μg/m</td>
<td>100μg/ml</td>
<td>200μg/ml</td>
</tr>
<tr>
<td>B.S</td>
<td>9±0.0</td>
<td>11±0.0</td>
<td>12±0.0</td>
</tr>
<tr>
<td>B.P</td>
<td>10.6±0.5</td>
<td>12±0.0</td>
<td>14±1.0</td>
</tr>
<tr>
<td>M.L</td>
<td>10.6±0.5</td>
<td>13.6±0.5</td>
<td>16±0.5</td>
</tr>
<tr>
<td>S.A</td>
<td>11±0.0</td>
<td>13.6±0.5</td>
<td>15.6±0.5</td>
</tr>
<tr>
<td>P.A</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>E.C</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>P.V</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

(Fig 1) shows antibacterial activity of Chloroform extract of leaves of Abutilon indicum at different concentrations against selected microbial strains. B.S:Bacillus subtilis NCIM 2063; B.P:Bacillus pumilis NCIM 2327; M.L:Micrococcus lutes NCIM 2871; S.A: Staphylococcus aureus NCIM 2079; P.A: Pseudomonas aeruginosa NCIM 2037; E.C: Escherichia coli NCIM 2067; P.V: Proteus vulgaris NCIM 2027.

Antihelminthic activity:

The antihelminthic activity of alcoholic extract of Abutilon indicum showed good response on earth worms. The time taken for standard drug albendazole for paralysis of worms was 13±0.5 min and time taken for death was 19±0.5min at 80mg/ml concentration. When compared with standard drug, alcoholic extract of stems showed time taken for paralysis was 11±0.5min and time taken for death was 15±0.5min at same dosage, like ethyl acetate fraction of aqueous extract of leaves, carbon tetra chloride fraction of aqueous extract of leaves also showed the time taken for paralysis 20±0.5, 38±0.5min and time taken for death was 27±0.5, 47±0.5 respectively at 80mg/ml concentration. antihelminth activities of various extracts of leaves and stem are compared with Albenbdazole as standard. The valores of each
concentration of mean ± standard deviation Dunnett’s test (Paralysis at 80 mg/kg). *(SD). P<0.05, one way ANOVA followed by

Table 2: Antihelminthic activity of various extracts of leaves and stem compared with Albenbdazole as standard

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration mg/ml of extract for each group</th>
<th>Time taken for paralysis (min)</th>
<th>Time taken for death (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water)</td>
<td>Nil</td>
<td>Nil</td>
<td>nil</td>
</tr>
<tr>
<td>Albenbdazole</td>
<td>10 27±0.5</td>
<td>37±0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 20±1.0</td>
<td>27±0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 17±0.5</td>
<td>22±0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 13±0.5</td>
<td>19±0.5</td>
<td></td>
</tr>
<tr>
<td>Alcoholic extract of the stems</td>
<td>10 25±1.0</td>
<td>30±1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 20±0.5</td>
<td>24±0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 15±0.0</td>
<td>21±0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 11±0.5e</td>
<td>15±0.5</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate fraction of leaves</td>
<td>10 40±0.0</td>
<td>58±1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 35±1.0</td>
<td>37±0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 25±0.5</td>
<td>30±0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 20±0.5*</td>
<td>27±0.5*</td>
<td></td>
</tr>
<tr>
<td>Carbon tetra chloride fraction of leaves</td>
<td>10 65±0.5</td>
<td>70±1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 54±0.5</td>
<td>62±1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 47±1.0</td>
<td>59±0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 38±0.5*</td>
<td>47±0.5*</td>
<td></td>
</tr>
</tbody>
</table>

The valves of each concentration of mean ± standard deviation (SD). *P<0.05, one way ANOVA followed by Dunnett’s test (Paralysis at 80 mg/kg).

CONCLUSION
Chloroform extract of *Abutilon indicum* leaves showed antimicrobial activity against only on Gram + Ve microorganism. Like this, various extracts of this plant showed antihelminthic properties, when compared with standard albendazole. Further studies are needed for the confirmation of antibacterial action and antihelminthic activity of plant by isolating pure chemical constituents and also for the identification of the compound, that responsible for the properties of crude extract of the *Abutilon indicum*.

ACKNOWLEDGEMENT
The authors are thankful to the Management and Principal of NRI College of Pharmacy, Pothavarappadu, Agiripalli mandal, Krishna District, Andhra Pradesh, India, for the financial support (Ref no. NRIP/2012/Proj-22) and providing the research facilities.

REFERENCES

Pusapati Madan Ranjit et al / Antimicrobial and Antihelminthic Activities of Various Extracts of Leaves and Stems of *Abutilon indicum* (Linn.)

17. Pengelly, A. Triterpinoids and saponins, in the constituent of medicinal plants, CABI publishing, USA, 2004; 74 PP.