



# AMERICAN JOURNAL OF PHARMTECH RESEARCH

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## Evaluation of Antibacterial, Antifungal and Anthelmintic Activity of a Medico-religious plant, *Corchorus depressus* Linn. (Tiliaceae)

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### ABSTRACT

The present study describes screening for antimicrobial and anthelmintic activity of the petroleum ether and alcoholic extracts of *Corchorus depressus* Linn. (Tiliaceae). The plant has been used in the traditional Indian system of medicine as an antibacterial, antifungal and anthelmintic drug, as a tonic, cooling medicine in fevers; its mucilage is prescribed in gonorrhoea. Root is rubbed on stone and smeared over forehead to get relief in migraine; extract of plant is applied as a paste in healing of wounds. The HPLC chromatograms of the methanolic extract shows a single large peak with a retention time 6.451 min along with other components. The Anthelmintic activity was studied on adult Indian earthworms, '*Pheretima posthuma*'. The methanolic extract produced more significant Anthelmintic activity than petroleum ether extract and the activities are comparable with the reference drug Piperazine citrate. The methanolic whole plant extract exhibited significant anti bacterial, antifungal activity, comparable to the standard drug tetracycline. The present study confirms the folklore claim of the plant utilized for its antimicrobial activity

**Keywords:** Antimicrobial; Anthelmintic; HPLC; Tetracycline; Piperazine citrate.

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Received 29 December 2016, Accepted 06 January 2017

Please cite this article as: Khuntia T *et al.*, Evaluation of Antibacterial, Antifungal and Anthelmintic Activity of a Medico-religious plant, *Corchorus depressus* Linn. (Tiliaceae). American Journal of PharmTech Research 2017.

## INTRODUCTION

Parasitoses have been of concern to the medical field for centuries and the helminthes still cause considerable problems for human beings and animals. The most of the medicinal preparation now-a-days available in the market are either not effective up to the mark or has developed resistance resulting in reoccurrence again.<sup>1</sup>

Several plants with phenolic compounds such as Flavonoids, phenolic acids, diterpenes and tannins have been used in folklore medicine as anti-bacterial and anti-fungal agents and they play an important role in health services around the globe. Therefore attempts are being taken to study promising plants, which may lead to develop newer or safer drugs.<sup>2,3</sup>

*Corchorus depressus* Linn. (Tiliaceae), commonly known in Sanskrit as Bedani; Odiya as Bojoromuli, is a perennial herb, 15-23 cm, prostrate, much branched from the base; twisted, imbricate, woody, found in drier parts of India like Odisha, Punjab, Gujarat.

The plant is treated as sacred and worshipped by the married women of Odisha, India, in the rituals called as “**Jama Jutia**”; the traditional ancient method of worshiping the Lord “**Yamaraj**” (The Lord of Death) following which they softly beat their family members with the worshiped plant and it is believed that by doing so the family members will be free from attack of any disease and have a long life. (Slide 1-3). With the aim of authenticating the medicinal use of the medico-religious plant and to evaluate the possible mechanism of its action, a study on different literatures revealed that in Indian system of medicine “**Ayurveda**”, the selected plant is described as sweetish, hot sharp, acrid; removes tumours and pain; cures piles. It is given as a cooling medicine in fever.<sup>4</sup> (**Kirtikar and Basu Indian Medicinal Plants**)

The plant has been used in the Indigenous system of medicine as a tonic, cooling medicine in fevers; its mucilage is prescribed in gonorrhoea. Root is rubbed on stone and smeared over forehead to get relief in migraine.<sup>5</sup> It is also used to increase the viscosity of seminal fluid, to set-up menstrual disorder.<sup>6</sup> An extract of plant is applied as a paste in healing of wounds.<sup>7</sup>

It has been used as antibacterial, antifungal, anthelmintic drug in Folklore medicine,<sup>8</sup> as antimalarial,<sup>9</sup> has cardiotoxic activity,<sup>10</sup> as tonic,<sup>11</sup> in treatment of gonorrhoea,<sup>12</sup> as veterinary medicine<sup>13</sup> and possesses diuretic activity.<sup>14</sup>

Lack of sufficient scientific evaluation data with respect to the anti bacterial, anti fungal and anthelmintic activity of various extracts of *Corchorus depressus* Linn., encouraged us for the study of its activity against different microorganisms.

## MATERIALS AND METHOD

### Plant Material

*Corchorus depressus* Linn. (Tiliaceae) were collected from the coconut gardens of Salipur, Odisha in the month of August 2009. The plant was identified, authenticated by Botanical Survey of India, Central National Herbarium, Howrah (No- CNH/I-I/28/2009/Tech.II/93) and a voucher specimen was kept in the herbarium of Sri Jayadev College of Pharmaceutical Sciences, Naharkanta, Bhubaneswar, Odisha. (Slide 4-7)

Leaves were 6-20 by 6-12mm, roundish, usually wrinkled, glabrous, irregularly crenate-serrate, the serratures not appendaged, base rounded or cuneate; petioles 1.2-2.5 cm long, very slender; stipules subulate. Flowers numerous, on leaf-opposed cymes; buds obovoid, apiculate; bracts lanceolate-subulate; peduncles and pedicels very short. Capsules 1-1.5 cm long, cylindrical, beaked, glabrous, often curved upwards, 4-valved; seeds trigonous black.<sup>3</sup> The whole Plant material was washed with distilled water to remove epiphytes and dirt particles.

### Extract Preparation

The whole plants were collected and washed thoroughly in water, chopped, air dried for a week at 35-40<sup>0</sup>C and pulverized in electric grinder. 150 gm. of the powder subjected to Soxhlet apparatus using methanol and petroleum ether as solvents. The solvents were then removed under reduced pressure, which obtained a greenish- black colored residue. The yield was 5.4% and 4.7% respectively. The prepared extracts were used for the antimicrobial and anthelmintic activity.

## EXPERIMENTAL DESIGN

### Anthelmintic Activity Study

The Anthelmintic activity was done on adult Indian earth worm ' *Pheretima posithuma* ' due to its anatomical and physiological resemblance with the intestine round worm parasites of human beings.<sup>15, 16</sup>

### Study Protocol

Four groups of approximately equal size earthworms consisting of six earthworms in each group were used for the present study.

**Group-1** Control (normal saline)

**Group-2** Standard (Piperazine citrate- 10mg/ml)

**Group-3** Pet. ether extract of different concentration (10mg/ml, 50mg/ml, 100mg/ml)

**Group-4** Methanolic extract of different concentration (10mg/ml, 50mg/ml, 100mg/ml)

Observations were made for the time taken to paralysis and death in individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body color.<sup>17, 18</sup>

### **Anti-microbial Study**

#### **Micro Organisms**

Three strains of E.Coli, Bacillus Subtilis, Staphylococcus aureus were used for assessing the anti microbial activity standard tetracycline (10µg/ml). Two fungal strains *Asperigillus niger* and *Candida albicans* were used for anti-fungal activity. The microorganisms were obtained from the Department of Microbiology, Sri Jayadev College of Pharmaceutical Sciences, Naharakanta, Bhubaneswar, Orissa.

#### **Study Protocol**

Antimicrobial activity was determined by Disc Diffusion method. Muller Hinton and Saboured Dextrose Broth were used as medium for bacterial and fungal strains respectively.<sup>19, 20</sup> Positive control experiment was carried out under the similar condition by using tetracycline (10µg/ml).

The petridishes with the bacteria and fungal cultures were incubated at  $37\pm 2^{\circ}$  C for 24 hrs and  $27\pm 2^{\circ}$  C for 48 hrs respectively. The assessment of anti microbial activity was based on the measurement of diameter of inhibition zone formed. The experiment was repeated thrice and the results were taken as mean of three readings.<sup>21, 22</sup>

### **QUALITATIVE PHYTOCHEMICAL ANALYSIS**

The preliminary phyto chemical studies were performed for testing different chemical groups present in methanolic extract.<sup>23, 24</sup>

### **CHROMATOGRAPHIC STUDY**

#### **Preparation of sample solution**

Preparation of sample solution were optimized to achieve good fingerprinting and also to extract the marker compounds efficiently. Since the marker compound was soluble in methanol, methanolic extract was prepared by accurately weighing 5 g of the powdered drug and extracted with 50 ml of methanol, for 3 hour under reflux on a water bath. The methanolic extract was filtered through what man filter paper, filtrates were combined, concentrated under vacuum and the volume was made up to 25 ml in a volumetric flask.

#### **HPLC method**

HPLC system, (Waters 515) equipped with Empower 2 software consist of a quaternary solvent delivery pump, and a 2998 photo-diode array (PDA) detector, were used for the chromatographic

analysis. All separations were carried out on Waters Spherisorb C<sub>18</sub> column (4.6mm x 250mm, 5µm).

Mobile phase was acetonitrile-water (60:40, v/v). It was filtered and ultrasonically degassed before use. The flow rate was 0.5 ml/min, column temperature was maintained at 25°C, and injection volume was 10 µl. Identification of the peaks was based on the retention time and the PDA spectrum presented in the chromatogram.

## RESULTS AND DISCUSSION

From the anthelmintic activity study, the alcoholic extract at a dose of 100mg/ml has significant anthelmintic activity where as petroleum ether showed moderate activity. (Table-1)

**Table 1: Anthelmintic Activity of *Corchorus depressus* extract.**

Group	Treatment	Con. (mg/ml)	Paralysis time (min.)	Death time (min.)
1 <sup>st</sup>	Control (Normal saline)	-	-	-
2 <sup>nd</sup>	Piperazine citrate	10	20.36±0.20	26.46±0.13
3 <sup>rd</sup>	Methanolic Extract	10	86.41±0.21	90.35±0.30
		50	67.73±0.36	78.9±0.29
		100	39.86±0.22	51.0±0.23
4 <sup>th</sup>	Petroleum ether Extract	10	91.21±0.33	100.63±0.17
		50	70.13±0.32	90.5±0.24
		100	57.26±0.13	70.96±0.43

The results were expressed as mean± standard deviation.

The results of antimicrobial activity of petroleum ether and alcoholic extracts of *Corchorus depressus* were studied and it was found that methanolic extract of 10mg/ml produced potent antimicrobial activity as it shows more inhibitory zone as compared to other individual concentrations of petroleum ether. The activities are comparable with the reference drug Piperazine citrate. (Table-2) The preliminary Phytochemical studies show the presence of alkaloids, Flavonoids, carbohydrates, glycosides, phenols and Tannins. (Table-3)

**Table 2: Anti microbial activity of *Corchorus depressus* extract report.**

Treatment	Concentration	Diameter of Zone of Inhibition (cm.)					
		Bacteria			Fungi		
		B. Subtilis	E. Coli	S. Aureus	C. Albicans	A. Niger	
Test	Pet.ether	5mg/ml	1.73	2	1.93	1.86	2.73
Corchorus depressus	extract	10mg/ml	1.9	2.15	2.1	2.0	2.83
	Methanolic extract	5mg/ml	1.96	2.06	1.93	1.90	2.80
		10mg/ml	2.03	2.33	2.13	2.16	3.20
Standard Tetracycline		10µg/ml	2.23	2.5	2.23	2.8	3.46

The results were expressed as mean.

The chromatograms obtained for HPLC analyses of the methanolic extract of *Corchorus depressus* shows a single large peak with a retention time 6.451 min along with other components. (Figure 1)

**Table 3: The preliminary Phytochemical studies for testing different chemical groups present in methanolic extract.**

Plant constituents	Results	Inference
<b>Tests/Reagents used</b>		
<b>1 GLYCOSIDES</b>		
<b>Borntrager's Test:</b> To 3ml of extract dil H <sub>2</sub> SO <sub>4</sub> was added and filtered + equal volume of chloroform added. Organic solvent separated and ammonia added	Ammonical-layer turned to pink	+
<b>2 PHENOLIC GLYCOSIDES</b>		
<b>Ferric sulphate Test:</b> 10 ml of methanolic extract is evaporated to dryness and the residue is dissolved in 10 ml of water. To 1ml of aqueous solution, a crystal of ferric sulphate is added	At first violet colour appears and then a precipitate is formed	<b>Presence of phenolic glycosides</b>
<b>3 ALKALOIDS</b>		
<b>Dragendorffs Test:</b> Alcoholic extract was evaporated and dil. HCl was added shaken and filtered. Few drops of Dragendorffs reagent added to it	Orange brown ppt. found	+
<b>4 FLAVONOIDS</b>		
<b>(i)Shinoda Test:</b> To dry powder of extract 5ml of 95% ethanol was added and with few drops of HCl & 0.5 g. of magnesium turning were added	Pink colour was observed	+
<b>(ii) Lead acetate :</b> Lead acetate was added to small quantities of extract	Yellow colour observed	+
<b>(iii) Sodium hydroxide Test :</b> To the residue sodium hydroxide was added	Yellow colour observed	+
<b>5 CARBOHYDRATE</b>		
<b>Molish's Test:</b> To the 2-3 ml of aqueous extract few drops of $\alpha$ -naphthol was added, shaken then con. H <sub>2</sub> SO <sub>4</sub> was added from sides of the test tube	Violet ring formed at the junction of two liquid	+
<b>6 TANNINS</b>		
<b>Ferric Chloride Test:</b> 2 ml of alcoholic extract is diluted with 3 ml of water and 3 drops of dilute ferric chloride is added	Green black colour	Presence of catechol (condensed tannins)
<b>Nacl Test:</b> 2 ml of alcoholic extract is diluted with 3 ml of water and treated with 5 ml of 2%	Precipitate disappears after addition of excess gelatin	

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Nacl solution. Precipitate is formed and filtered through filter paper and 5 ml of 1% gelatin added which gives precipitate.		+
<b>7 Test for Phenol</b>		
<b>Methanolic Ferric chloride Test:</b>		
To the 3ml of alcoholic extract 5% methanolic ferric chloride was added	Deep blue colour formed	+

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+ Mark represents the presence of the Phytochemicals.

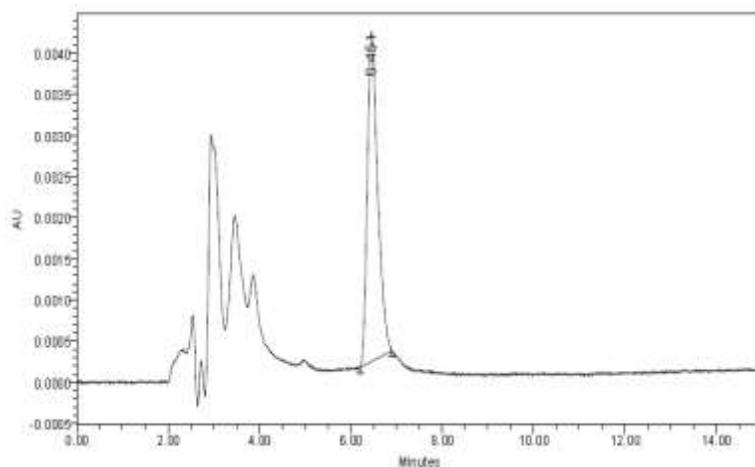


Figure 1: HPLC chromatogram of methanolic extract of *Corchorus depressus*.

**PHOTO SLIDES:**



**Slide: 1**



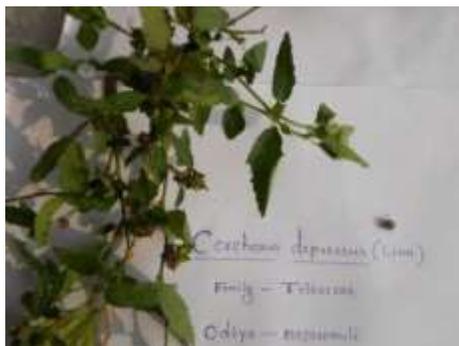
**Slide: 2**



**Slide: 3**

**Slide (1, 2 &3):** The photographs of *C. depressus* Linn. (Tiliaceae), worshipped by the people of Odisha, India, in the festival 'JAMAJUTIA'.

**PLANT PHOTO SLIDES:**

**Slide 4****Slide 5****Slide 6****Slide 7**

**Slide(4-7): Photographs of the whole Plant *Corchorus depressus* Linn. (Tiliaceae).**

Plant derived drug serve as prototype to develop more effective and less toxic medicines. The antibacterial activity of the methanolic and petroleum ether extracts of *C. depressus* was compared with standard broad spectrum antibiotic Tetracycline and the anthelmintic activity was compared with the standard Piperazine citrate. From both the extracts, methanolic one had shown marked antibacterial activity against *B. Subtilis*, *S. aureus* and *E.coli* at the dose level of 10 mg/ml.

As the methanolic extract was producing significant activity, hence it was subjected to HPLC analysis. The chromatogram showed a single large peak with a retention time 6.451 min along with other components.

Flavonoids and phenol constituents of the plant extracts have shown potent antimicrobial properties<sup>25</sup>. The observed antimicrobial activity could be attributed to the phenol and flavonoid constituent of the extracts.<sup>26, 27</sup> The antimicrobial activity observed in the present study justified the traditional uses of *C. depressus* Linn. for wound healing activity.

## CONCLUSION

Though there are a number of antibacterial, anti-fungal and Anthelmintic drugs available in the market, they produce many side effects; hence to improve the status of therapy, various ailments of plant extracts like *Corchorus depressus* will be much useful. The present study confirms the

folklore claim of the plant utilized for its antimicrobial activity. Further studies in this regard will develop some useful drugs for the treatment of bacterial, fungal and helminthes infections.

## ACKNOWLEDGEMENT

The authors are thankful to the Department of Microbiology, Sri Jayadev College of Pharmaceutical Sciences, Naharakanta, Bhubaneswar, Odisha; Institute of Pharmacy and Technology, Salipur and Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj, Odisha, India for providing the necessary facilities and support to carry out this work.

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