In helminthiasis disease a part of the body is infested with worms such as pinworm, roundworm or tape worm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs (Jaya and Yesuf, 2010). In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia (Bundy, 1994). The parasitic worms are divided into three groups: cestodes or tapeworms; nematodes or roundworms; and trematodes or flukes (Sharma, 2007). Parasitic diseases may cause severe morbidity, including lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis (Lukhoba et al., 2006). Most diseases caused by helminths are of a chronic nature; they probably cause more morbidity and even economic and social deprivation among humans and animals than any single group of parasites (Partap et al., 2012). Anthelmintics are drugs that act either locally to expel worms from the gastrointestinal tract or systemically to eradicate adult helminthes or developmental forms that invade organs and tissues (Brunton, 2001). Most of the existing anthelmintics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea (Bundy, 1994). Albendazole is a well tolerated drug, however gastrointestinal side-effects, dizziness have been noted in few patients. Also prolonged use in hydatid or in cysticerosis, causes headache, fever, alopecia, jaundice and neutropenia (Tripathi, 2008). In order to exterminate the harmful side-effects of these synthetic anthelmintic drugs, it is important for us to promote the studies of traditionally used anthelmintic plants which will lead to the development of new anthelmintic substances with ease of availability and lesser side-effects (Khan et al., 2011).
The anthelmintic activity was evaluated on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings and easy availability (Vidyasarathi, 1977).

*Calamus leptospadix* [Synonym: *Palmijuncus leptospadix* (Griff. ) Kuntze ] belonging to the family Arecaceae is commonly known as Lejaibet (Ass.), Jeyying (Adi.), Kukhre bet (Bhu.) and is an important ethnomedicinal plant of Assam. The plant is a slender, cluster forming climber with stems climbing to 25 m long and 2 cm diameter. Leaf sheaths are green with grayish brown hairs, with scattered to densely arranged, brownish, flattened to 2.5 (to 5 at sheath apices) cm long spines, those at sheath apices needlelike yellowish; ocreas present to 1.5 cm long densely bristly, with long, needlelike spines; flagella present; leaf rachis to 2 m long with 50–55 linear leaflets per side, these are regularly arranged, cirriform absent. Inflorescence to 4 m long, flagellate: bracts tubular, briefly open and spreading at the apices; flowering branches very short; fruits globose to 1.5 cm diameter, white or yellowish. Flowering and Fruiting season is in the month of March-October.

It is distributed in Bangladesh, Bhutan, Northeastern India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim, Tripura, West Bengal), Myanmar (Kachin) and Nepal; mostly on lowland or mountain forest along river margins to 1300 m elevation (Henderson, 2009; Haridasan, 2002). In Ayurvedic system Calamus species are used in fever, piles, dyspepsia, biliousness; Flowers are used as antiseptic, antibacterial, externally for cuts, burns, bruises, scalds (Khare, 2007). Young shoots of the plant are used as vegetable (Sarmah, 2010). In Assam, it is traditionally believed by the folk people that the tender shoots, leaves and seeds of this plant are used as vermicide. This plant is widely used by various communities in Assam, however no detailed study or reports alongwith scientific evidence are available with this important plant.

An exhaustive study was carried out in order to substantiate the therapeutic potential of the plant in terms of its anthelmintic activity against *Pheretima posthuma* using Albendazole as a reference standard.

**MATERIALS AND METHODS**

**Plant material**

The shoots of *Calamus leptospadix* were collected from Jokai, Dibrugarh, Assam, India during the month of July, 2012. The plant was identified and authenticated by Dr. N. Odyuo, Botanical Survey of India, Eastern Regional Centre, Shillong. A voucher specimen (Specimen no. Du/SB/2012/06, Reference no. BS1/ERC/2013/Tech/Plant identification/636) is kept in Department of Pharmaceutical Sciences, Dibrugarh University, Assam for future references. Young and tender shoots were cut into pieces, washed thoroughly with water and then dried partially under sunlight and partially under the shade for a week. The dried shoot pieces were then pulverized in mechanical grinder and stored in airtight containers free from moisture.

**Preparation of extract**

250 g of powdered crude drug of *Calamus leptospadix* Griff. (Arecaceae) shoots were extracted by soxhletation (Continuous hot extraction) with 1000 ml of methanol, 1000 ml of ethanol and 1000 ml of water for 18 hours after pretreatment with petroleum ether. After the extracts were concentrated, preliminary phytochemical tests were carried out with all the extracts in order to evaluate for the presence of different phytochemical constituents. The MECL (Methanolic extract of *Calamus leptospadix*) contains the major phytoconstituents like alkaloids, carbohydrates, fats & oils, glycosides, lignin, steroids, saponins, triterpenoids, tannins and phenolic compounds. The EECL (Ethanolic extract of *Calamus leptospadix*) contains the major phytoconstituents like alkaloids, fats & oils, carbohydrates, glycosides, steroids, saponins and tannins. The AECL (Aqueous extract of *Calamus leptospadix*) contains the major phytoconstituents like alkaloids, flavonoids, carbohydrates, glycosides, steroids, saponins and tannins.

**Indian adult earthworm as model for the experiment**

The anthelmintic activity was carried out in Indian Adult Earthworms (*Pheretima posthuma*) due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings and easy availability. The worms were collected from moist soil and washed with 0.9% w/v of normal saline to remove all dirt and faecal matters. The worms measure 3-5 cm in length and 0.2-0.3 cm in width (Pillai and Nair, 2011).

**Drugs and chemicals**

0.9% w/v of normal saline was prepared. Albendazole (Lupin Pharmaceuticals Ltd.) was prepared at three different concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml in distilled water and this was used as standard drug. Similarly MECL, EECL and AECL were prepared at the concentrations of 25 mg/ml, 50 mg/ml, 100 mg/ml in distilled water and these were used as test drugs for the activity.

**Evaluation of anthelmintic activity**

The anthelmintic assay was carried out as per the method of (Panda *et al.*, 2011) with slight modifications in the process. Forty eight Indian Adult Earthworms were collected and divided into twelve groups containing four earthworms in each group. 10 ml of each different concentrations of standard drug Albendazole and test drugs MECL, EECL and AECL were taken with pipette in twelve different petridishes. Four earthworms were placed in each of the twelve petridishes after they have been washed with 0.9% w/v of normal saline solution. Motility of the worms were observed and time for paralysis and time for death of the worms were noted down. Time for paralysis was noted when the worms showed no movements when shaken vigorously. Time for death was recorded when the worms showed no movements even when dipped in warm water at 50-60 °C temperature also with fading away of their body colour.
**Statistical analysis**

The results are expressed as Mean ± SEM of four worms in each group. Comparisons have been made between standard against test treated groups, P< 0.05 was considered significant. The observation table is shown in Table no. 1.

<table>
<thead>
<tr>
<th>Drug Treatments</th>
<th>Doses (Mg/ml)</th>
<th>Time Taken for Paralysis (Min)</th>
<th>Time Taken for Death (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard drug (Albendazole)</td>
<td>25</td>
<td>25.25 ± 0.21</td>
<td>28.25 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>20.5 ± 0.43</td>
<td>24.75 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>18.25 ± 0.21</td>
<td>23.25 ± 0.54</td>
</tr>
<tr>
<td>Methanolic extract of Calamus leptospadix Griff.</td>
<td>25</td>
<td>27.5 ± 0.25</td>
<td>29.75 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>22.25 ± 0.41</td>
<td>24.45 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>18.75 ± 1.29</td>
<td>22.5 ± 1.25</td>
</tr>
<tr>
<td>Ethanol extract of Calamus leptospadix Griff.</td>
<td>25</td>
<td>43.5 ± 0.75</td>
<td>50.5 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>37.5 ± 0.43</td>
<td>41.25 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>31.5 ± 0.43</td>
<td>35.75 ± 0.21</td>
</tr>
<tr>
<td>Aqueous extract of Calamus leptospadix Griff.</td>
<td>25</td>
<td>45.5 ± 0.25</td>
<td>59 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>50.5 ± 0.43</td>
<td>56.5 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>47.25 ± 0.41</td>
<td>51.5 ± 0.25</td>
</tr>
</tbody>
</table>

The result was expressed as Mean ± SEM. Statistical analysis was carried out with comparisons between standard and treated groups. P<0.05 was considered statistically significant and n=4 was taken in each group.

**RESULT AND DISCUSSION**

From the observation table given in table no.1, it was found that higher the concentration of the extract faster was the paralytic effect and shorter was the death time for all the earthworms. Crude methanolic, ethanolic and aqueous extracts with concentrations of 25mg/ml, 50mg/ml, 100mg/ml produced dose-dependent paralysis. The data given in the observation table no.1 showed that methanolic extract of the shoots of Calamus leptospadix Griff. gave shorter paralysis and death time at 100mg/ml as compared to that of ethanolic and aqueous extracts. Even at the minimum dose of 25mg/ml anthelmintic effect of extracts was found as compared to that of standard drug Albendazole (Das et al., 2011). This study was performed on Indian Adult Earthworms as these are easily available and possess anatomical and physiological resemblance with that of intestinal round worm parasites of human beings (Vidyasarathi, 1977). These parasitic helminths affect mankind and animals causing relentless infections to them. Many synthetic compounds and their derivatives have been developed but the problems associated with the use of such drugs leads to serious side-effects. Also sometimes these parasites develop resistance to the drugs leading to more severe infections. Thus, steps have been taken towards developing herbal medicines as a safer remedy to cure helminths (Partap et al., 2012). Researches and studies are going on in different parts of the world on medicinal plants. The results obtained in this study have shown hopeful results on anthelmintic activity. This plant could be used by human beings in controlling gastrointestinal nematode infections. Albendazole is variably and erratically absorbed after oral administration; absorption is enhanced by the presence of fatty foods and possibly by bile salts as well. After a 400mg oral dose, albendazole cannot be detected in plasma, because the drug is rapidly metabolized in the liver and possibly in the intestine as well to albendazole sulfoxide which has potent anthelmintic activity (Marriner et al., 1986; Redondo et al., 1999). Mean ± SEM values were calculated for the extracts and standard. All the three extracts showed anthelmintic activity in a dose-dependent manner taking shortest time for paralysis (18.75) and death (22.5) for MECL. Time taken by EECL at 100mg/ml concentration is (31.5) for paralysis; (35.75) for death and time taken by AECL at 100mg/ml concentration is (47.25) for paralysis; (51.5) for death in a dose-dependent manner as compared with that of standard drug Albendazole for paralysis (18.25) and death (23.25) at 100mg/ml respectively. As phytochemical investigation of the methanolic,ethanolic and aqueous extracts of Calamus leptospadix Griff. showed the presence of alkaloids (Acharya et al., 2011) which might be the reason for the paralysis of the worms, however presence of triterpenoids and tannins in the methanolic extract could be the reason for the faster death of the worms (Balumurugan and Selvaragavan, 2009; Sravani, 2011) as compared to that of ethanolic and aqueous extracts. Therefore from the present study conducted it can be suggested that the methanolic extract revealed higher anthelmintic activity possibly due to alkaloids, tannins and triterpenoids. Further isolation and characterization of the methanolic extract needs to be carried out in order to establish the possible active compound responsible for the use of Calamus leptospadix as an anthelmintic drug and in-vivo studies could also be carried out in the future in order to establish the effectiveness of this plant as an anthelmintic drug.

**CONCLUSION**

The methanolic extract of the shoots of Calamus leptospadix exhibited shortest anthelmintic activity against Indian adult earthworm (Pheretima posthuma). From the biological assay performed and observations noted, it can be concluded that Calamus leptospadix Griff. (Arecaceae) used by the people of Assam traditionally to treat intestinal worm infections, possesses significant anthelmintic activity when compared with the normally used drug and hence provides a basis for the traditional use of this plant as an anthelmintic.

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