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J. Sci. Trans. Environ. Technov. 2010, 3(4): 194-202

Scientific Transactions in Environment and Technovation

Pharmacological screening of acrid latex from the mangrove species, *Excoecaria agallocha* L.

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Abstract

In vivo and *in vitro* evaluation of pharmacological properties of acrid latex from the mangrove plant *E. agallocha* were carried out in the green frog (*Rana hexadactyla*) and albino rat. Acrid latex of *E. agallocha* produced local anesthetic activity in frog at 1:500, 1:100, and 1:10 concentrations and cardiac depressant activity when tested in the frog's heart. It was found to have a good effect on the ciliary muscle for the cholinergic action in the buccal cavity of frog *(viz*., lower the concentration the higher the potency). The acrid latex of *E. agallocha* produced partial purgative activity at concentrations 1:500 and 1:100. It was found to have central nervous system depressent action at 1:500 and 1:100 concentrations and at 1:10 concentration it produced total lethargy. It was also found to have relaxant property in the skeletal muscle and smooth muscle of frog and rat, respectively.

Keywords: acrid latex, cardiac depressant, cholinergic, CNS depressant, *Excoecaria agallocha*, local anesthetic, purgative

INTRODUCTION

The mangrove species, *Excoecaria agallocha* L. is specifically worshipped in Lord Nataraja's temple (Lord Shiva) as a 'sacred tree' at Chidambaram, Tamil Nadu State, South India in the form of rock carvings. There was a belief that a dip in the temple's pond water that was under the influence of the mangrove species cures many 'incurable' human diseases (Kathiresan and Bingham, 2001; Kathiresan, 2002). However, the milky latex exuded from the bark of *E. agallocha* is poisonous which may cause blindness or blistering and irritation of the skin where the sap is contacted and promote tumour (Jayaweera, 1980; Kathiresan and Thangam, 1987; 1990; Erickson *et al*., 1995). According to Guillet *et al.* (1985) the sap of *E. agallocha* causes a reaction characteristic of a burn on contact with eye and produces severe conjunctivitis, which, if complicated by secondary infection, could result in loss of sight.

E. agallocha possesses various traditional uses such as an uterinetonic, purgative, for treatment of epilepsy, conjunctivitis, dermatitis, haematuria, leprosy, toothache, nervous disorders, skin diseases, blood pressure, diabetes, malaria and ulcers, anti-diarrhoea, anti-helminthic, anti-dotes for venomous bites, as a piscicide, dart poison, and skin irritant (Reddy *et al*., 1991; Bandaranayake, 1998). In Sri Lanka, the smoke of its burning wood has been used in the treatment of leprosy, while the root pounded with ginger has been used as an embrocation for swelling hands and feet (Jayaweera, 1980). The bark and wood of *E. agallocha* have been used in the traditional medicine in Thailand for the treatment of flatulence (Erickson *et al*., 1995; Karalai *et al*., 1994), while its leaves and latex have been used as a fish poison in Malaysia (Kawashima *et al*., 1971), India (Prakash *et al*., 1983) and New Caledonia (Ohigashi *et al*., 1974). *E. agallocha* was found to have significant analgesic activity but was less effective when compared to morphine (Padmakumar *et al*., 1993). The extract of *Excoecaria* plant is also used for treating rheumatism, paralysis and purgative and as an abortificant (Padmakumar and Ayyakkannu, 1985).

Acrid latex of *E. agallocha* was reported to have many medicinal properties such as antiviral, antioxidant, analgesic, antimicrobial activity activity against tumour growth, human immono deficiency and biotoxicity towards fish and field crabs (Thangam and Kathiresan, 1992; Premanathan *et al*., 1993; Konishi *et al*., 2003). This paper details the *in vivo* and *in vitro* evaluation of pharmacological activities of acrid latex from the mangrove plant *E. agallocha viz.*, local anesthetic activity, skeletal muscle activity, buccal ciliary muscle activity, cardiac muscle activity, effects on central nervous system and purgative activity in the intestine of the green frog (*Rana hexadactyla*) and smooth muscle activity in the albino rat.

MATERIALS AND METHODS

Collection of acrid latex

The acrid latex was collected from the young stems of *E. agallocha* obtained from Cuddalore district at Poondiyakuppam Village (Lat. 11°37' to 30.42"N; Long. 79°43' to 39.32"E). Sterilized screw capped glass bottles, kept in hot air oven at 60°C for 12 h were used for collecting the acrid latex. The young tender stem was cut opened and the drops of acrid latex was collected

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carefully into the bottle. Approximately 5 ml of the acrid latex was collected and kept in the refrigerator at -40°C until the experiment was started.

Dilution of acrid latex

The acrid latex on injecting directly into the living system is toxic because of its high concentration and poisonous nature. Hence, it was diluted with the distilled water to reduce the concentration to the ratio of 1:500 (T1), 1:100 (T2), and 1: 10 (T3) for the pharmacological evaluation on the green frog (*Rana hexadactyla*) and albino rat.

Animals used for pharmacological studies

The green frog (*Rana hexadactyla*) and albino rats (Male Wistar strains) were used to examine local anesthetic, cardiac depressant, cholinergic, purgative, CNS depressant and antagonistic activities of the acrid latex. Male Wistar strains of rat, weighing about 150-200 g obtained from the small animal's breeding centre of Institutional Animal Ethics Committee, Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India were used for smooth muscle relexant study. Rats were kept in the animal house at room temperature of 25 - 30°C and at 45 - 55% relative humidity for 12 h each of dark and light cycle in the Department of Pharmacy, Annamalai University. The rat and frog were fed with rat pellet feeds (Hindustan Lever Limited, Bangalore, India) and filtered water. The rats were kept as per the stipulations of the Animal Ethical Committee (IAEC) constituted under the guidelines of Committee for the Purpose of Control and Supervision on Experimental Animal (CPCSEA), Ministry of Environment, Govt. of India, New Delhi. IAEC No: 409 / 160 / 1999 / CPCSEA / dated; 16.11.2006.

Effects of crude acrid latex from *E.agallocha* in the sciatic nerve of frog

Local anesthetic activity was studied by Lumbar plexus anesthesia or Nerve block anesthesia method in frog (Bulbring and Wajda, 1945). The frog's brain and upper one eighth of the spinal cord was pithed and both the sciatic nerves were exposed. The frog was pinned on the frog board vertically with two of its hind legs hanging free from the board. After 5 minutes the reflex time for each foot was recorded by dipping it in 0.1 N hydrochloric acid solution. The foot was rinsed with 0.7% saline after each exposure to the acid solution. A small cotton pledget soaked in the standard drug xylocaine of 1% w / v was laid on the sciatic nerve for 5 minutes and the time taken for blockade of reflex was noted. The same procedure was repeated with the test extracts T1, T2 and T3 each with separate healthy frogs and the results were compared with that of the standard drug xylocaine.

Effects of crude acrid latex from *E. agallocha* in the skeletal muscle of frog

Since the antimigraine drugs were reported to have skeletal muscle activity, this experiment was attempted to assess the effect of acrid latex from *E. agallocha* in frog *rectus abdominis* muscle preparation. The experiment was carried as per Kulkarni *et al*., (1998). The rectus abdominal muscle was exposed and was cut into a rectangular shape. A thread was tied to the top and the bottom of the muscle preparation before detaching the muscle from the body of the frog. The preparation was mounted in an up-right position in the organ bath containing frog's ringer solution (NaCl = 6.0 g; KCl = 0.14 g; $CaCl_2$ = 0.12 g; $NaHCO_3$ = 0.2 g and glucose = 2 g and under a tension of 1 g). There is no need to maintain the bath temperature, since it is an amphibian tissue preparation. The organ bath was bubbled with air by using the air pump. The tissue was relaxed for 45 minutes during which the tissue was periodically washed with fresh quantum of ringer for at least four times. The concentration of the acetylcholine was recorded by using simple side way lever. Ninety second contact time and five minutes time cycle was used for proper recording the responses. Four responses were recorded at increasing doses of acetylcholine. The maximum response was achieved if one gets the same (or) slightly less response with a higher concentration. The graphs were labeled and the tracings were fixed with the help of the fixing solution and kept for drying. The height of the response was measured (mm) and expressed in terms of percentage. Similar concentration response curves were prepared to observe the activity for the concentrations of the test extracts T1, T2 and T3 in the presence of acetylcholine because the test extracts, T1, T2 and T3 itself did not produce any response with the skeletal muscle. The obtained concentration response curves were labelled and fixed as explained before. The responses of acrid latex with the absence and presence of acetylcholine were compared.

Effect of crude acrid latex on the isolated frog heart

Effect of test extract on the isolated frog heart was tested as per Kulkarni *et al*., (1998). A midline incision was made on the abdomen. The pectoral girdle was removed and the heart was exposed. The pericardium was carefully removed and a few drops of frog ringer were poured over the heart. The inferior vena cava was traced and a thread was tied around it. Then a small incision was made in order to insert the venous cannula, which was in turn connected to a perfusion bottle containing frog ringer. The cannula was inserted in the vein and the thread was tied to assure the cannula in place. A small incision was made in one of the aortae for the perfusion to come out. A venous pressure of 2-4 cm was kept by altering the height of the perfusion bottle. The effective venous pressure was the height in cm from

level of the venous cannula and the ringer level in the perfusion bottle. The use of Marriott's bottle helps in attaining the constant pressure by opening the screw clamp attached to the tube to start the perfusion. A thin pin hook was passed through the tip of the ventricle and with the help of a fine thread the hook was tied to the free limb of the universal lever, which was fixed to a stand. Proper tension and magnification were adjusted by altering the height of the lever.

The normal contraction of the heart was recorded on the smoked drum first and for each dose of the standard drug 0.1 ml of the stock solutions of adrenaline, acetylcholine, calcium chloride and potassium chloride were injected in a sequential order and the change in the rate and amplitude of the contraction of the heart was noted. Five minutes gap was maintained between the administration of each dose of the standard drug. The standard drug was administered by injecting the drug into the perfusion tube, very close to the venous cannula. Precautions were taken to avoid any leakage of the standard drug from the tube. Accordingly the experiment was repeated with the test extract, acrid latex from *E. agallocha* T1, T2 and T3 concentrations in a sequential order as mentioned earlier and the change in the rate and amplitude of the contraction of the heart was recorded on the smoked drum. The tracings were labelled and fixed with the fixing solution.

Effect of crude acrid latex from *E. agallocha* on the ciliary muscle of frog

The experiment was conducted as per Kulkarni *et al*., (1998). The frog was decapitated and was pinned on the frog board on its back. The lower jaw was pinned in the abdomen. The buccal cavity was cut opened sufficiently and the oesophagus was opened and normal saline was spread throughout. The point from the lower jaw to the beginning of the oesophagus was fixed. The distance from the lower jaw to the beginning of the oesophagus was kept constant. To a marked spot in the jaw a poppy seed was placed. The stopwatch was switched on and the time taken by the poppy seed to reach from the initial point to the oesophagus was noted. The experiment was repeated several times. A few drops of neostigmine were sprayed on the buccal cavity and the same experiment was repeated after ten minutes. The difference in the time taken by the poppy seed to move between the marked distance in the buccal cavity in the presence of saline and atropine were observed. The same experiment was then repeated with the test extract of crude acrid latex from *E. agallocha* T1,T2 and T3 concentrations. The difference in the time taken by the poppy seed to move between the marked distances in the buccal cavity in the presence of the test extract at T1, T2 and T3 concentrations were noted. The time taken by the poppy seed for the ciliary movement in the buccal cavity for the test extract was compared with the standard drug neostigmine and atropine.

Effect of crude acrid latex from *E. agallocha* on the ion action of frog

Normal healthy frogs were selected for assessing the ion action and the experiment was carried as per Kanakambal (2001). One of the hind limb was tied to a fixed pole. The normal activities like jumping reflex was observed in the frog. One ml of 25 % magnesium sulphate solution was injected intra muscularly in one of the hind limb. A few minutes later, the frog becomes lethargic and unable to retain its posture. Later to the same animal one ml of 10 % calcium gluconate solution was injected intra muscularly in another hind limb. The animal regains its normal activities within few minutes. One ml of T1 concentration of the test extract was injected intra muscularly in one of the hind limb of another healthy frog. The same experiment was repeated with the test extracts T2 and T3 and the activities of the frog were observed. After observing, the same animal was tested for confirming the normal activities by injecting the standard drug, calcium gluconate and magnesium sulphate.

Effect of crude acrid latex from *E. agallocha* on the small intestine of frog

The experiments were carried out as per Kanakambal Sivasubramaniam (2001). The abdomen was opened to expose the intestine. Four ligatures were formed at equal distances to form three loops of intestine. In the first loop 0.25 ml of tap water was injected, the next loop was left free and in the last loop 0.25 ml of magnesium sulphate was injected. The intestine was placed in the abdomen and the abdomen was closed with sutures and cotton. The loops were re-examined after one hour and the changes produced were observed. The same experiment was repeated with another healthy frog by the test extracts T1, T2 and T3 in the last loop. The loops were re-examined after one hour and the changes produced were observed and compared with the standard drug magnesium sulphate.

Effect of crude acrid latex from *E. agallocha* on isolated rat colon

Effect of acrid latex on isolated rat colon was evaluated as per Kanakambal (2001). The rat was sacrificed by cervical dislocation. The abdomen was cut opened and the colon was identified. The right flexure *i.e.* the subhepatic region of the colon where the ascending colon turns to become the transverse colon was cut out and placed in a shallow dish containing the modified ringer solution (NaCl = 9 g; KCl = 0.42 g; $CaCl_2$ = 0.06 g; $NaHCO_3$ = 0.5 g; glucose = 0.5 g). The lumen was gently cleaned and about 3 cm long tissue was mounted in the organ bath containing modified ringer solution (pH 7.4) maintained at 25°C and bubbled with carbogenated air.

The preparation was allowed to equilibrate for 45 minutes to fewer than 500 mg tension. The concentration dependent dose response by acetylcholine using frontal writing lever was recorded. The contact time was 60 seconds and 5 minutes was kept for the tissue cycle for the proper recording of the graded responses. Later the test extracts T1, T2 and T3 were administered to the inner organ bath and the responses were recorded.

The kymograph was labeled and the tracing was fixed with the help of the fixing solution and kept for drying. The height of the response was measured (mm) and expressed in terms of percentage height. In the same way the activity for the concentration of the test extract was recorded in the presence of acetylcholine because the test extract produces responses only in the presence of acetylcholine. So, the test extract T1 and acetylcholine were administered in increasing doses and the responses were recorded. The responses were also recorded in the same way for the test extracts T2 and T3. The obtained concentration response curves were labeled, fixed and was compared with the two concentration response curves of acetylcholine *i.e.* in the presence and absence of acrid latex at concentrations T1, T2 and T3.

RESULTS

The test extract acrid latex from *E. agallocha* produced good local anesthetic effect similar to the standard drug xyclocaine (higher concentrations produced higher effects) (Table 1). T1 (1:500) takes three minutes and T2 (1:100) takes two minutes for the complete blockade for both left and right hind leg while, T3 (1:10) takes one minute for the complete depressing (or) blocking the sensory nerves of the frog. The standard drug xyclocaine takes three minutes for the left hind leg and four minutes for the right hind leg for the complete blockade of the sensory nerves in the frog. Results obtained showed that local anesthetic activity of the crude acrid latex of *E. agallocha* increased according to the following trend: T1<T2<T3 (1:500<1:100<1:10).

Administration of the test extract with the sub maximal dose of acetylcholine produced skeletal muscle relaxant (antagonist) activity. But the test extract combined with the standard drug acetylcholine produces partially antagonist activity. The data confirms that the test extracts T1 and T2 partially block the normal acetylcholine responses and partially produce skeletal muscle contraction (Table 2). The high concentration of the test extract T3, completely blocked the responses of the normal acetylcholine and produces skeletal muscle relaxation (Fig. 1), property.

The test extract T1 produces cardiac depressant activity. Accordingly the test extract T2, produces a decrease in the rhythmic contraction of the heart slowly. The test extract T2 when added along with the standard drug acetylcholine, also produces the same effect like T1. So, on further administration of the test extrac T3 (high dose concentration), it gradually decreases the rhythmic contraction of the heart (Fig. 2). On further contact with the test extract T3, it becomes toxic to the cardiac muscle and hence it arrest or block the function of the heart resulting in paralysis of the cardiac muscle.

The test extract, acrid latex from *E. agallocha* produces cholinergic activity in the ciliary muscle of the frog's buccal cavity. The test extract T1 and T2 produces excellent ciliary movements when compared to the standard drug neostigmine (choline esterase enzyme inhibitor). When the test extracts were administered *viz.*, T1, T2 and T3, the time taken by the particle (poppy seed) to travel 1cm in the ciliary muscle were 16.25 sec., 26.25 sec. and nil, respectively (Table 3). Thus cholinergic activity of acrid latex was more when compared to the neostigmine. T1 produced higher cholinergic activity (16.25 sec.) when compared to T2 (26.25 sec.) and neostigmine (93.75 sec.).

The test extract T1, proves that it posses the same effect of the standard drug magnesium sulphate, with regard to the central nervous depressant effect on systemic administration (Table 4). In another frog, when 0.2ml of the test extract T2 was injected, there was no change in the frog's normal motor activities (Table 4). After the administration of magnesium sulphate the frog becomes lethargic. Even at less concentrations, the test extracts T1and T2 produced lethargic effect in the frog because it partially suppresses the central nervous action. The test extract T3 (high concentration dose) even at 0.1ml produced a complete central nervous depressant effect.

The test extract T1, partially produced an osmotic purgative action, whereas the test extract T2 produced a week osmotic purgative action because it retains very minute water in the intestine by its salt action (Table 5). After the administration of the test extract T3, at 0.1ml there were no osmotic purgative actions because the test extract T3 becomes toxic due to high concentration. The test extract T3 did not show any osmotic purgative action when compared to T2 because it does not retain water in the intestine by its salt action. Thus the osmotic purgative action increases according to the trend T1>T2>T3 (1:500>1:100>1:10) and when compared with the standard drug magnesium sulphate the trend is magnesium sulphate > T1>T2>T3 (Table 5).

The test extracts T1 and T2, produced a tremendous decrease in antagonistic activity. One ml of acetylcholine produced 72.22 % response, while 0.1 ml of test extract T1 with acetylcholine produced 20.90 % response. The test extract T2 (0.8 ml) with acetylcholine at 0.8 ml on rat colon preparation showed 21.83 % response (Table 6). The test extract T3, produced lesser response and it completely blocked (or) caused paralysis in the colon because, the concentration was higher and toxic in nature. Thus the antagonist activity of the test extract

Table 1. Local anesthetic activity of different dilutions of crude drug acrid latex from *E. agallocha*

Drug	Hind leg responses in 0.1N Hydrochloric acid	Reflex responses at different times (minutes)						
		0	1	2	3	4	5	6
Before drug administration	Left leg	+	+	+	+	+	+	+
	Right leg	+	+	+	+	+	+	+
After test drug administration T1 (1:500)	Left leg	+	+	+	+	-	-	-
	Right leg	+	+	+	+	-	-	-
After test drug administration T2 (1:100)	Left leg	+	+	+	-	-	-	-
	Right leg	+	+	+	-	-	-	-
After test drug administration T3 (1:10)	Left leg	+	+	-	-	-	-	-
	Right leg	+	+	-	-	-	-	-
After xyclocaine administration	Left leg (1:10)	+	+	+	-	-	-	-
	Right leg (1:100)	+	+	+	+	-	-	-

+ indicates withdrawal of legal (Presence of reflex)
- indicates no withdrawal of leg (Absence of reflex)

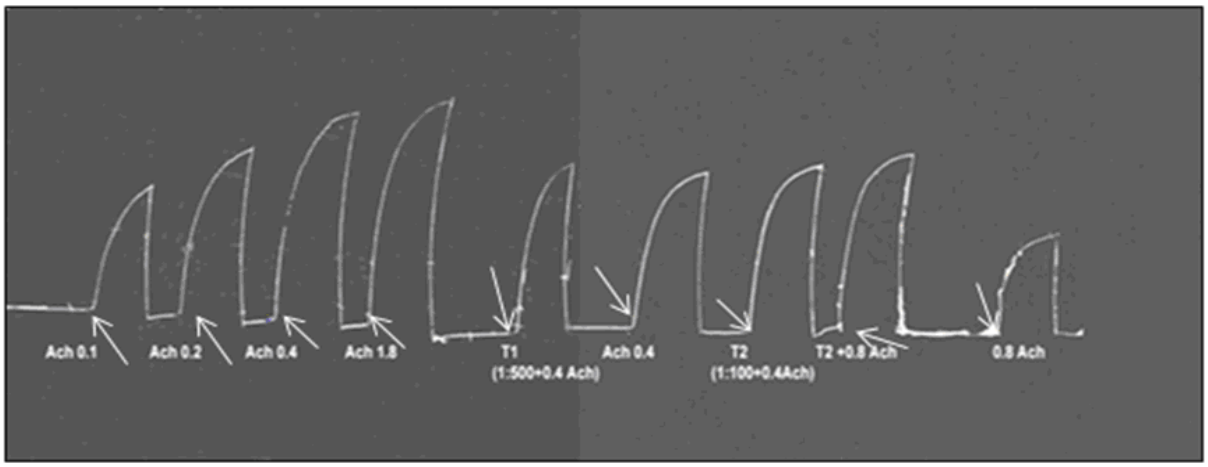

Figure 1. Effects of different dilutions of acrid latex from *Excoecaria agallocha* on the frog's isolated *rectus abdominis* muscle (skeletal muscle)
Ach – Acetylcholine; T1 - 1:500, T2 – 1:100, and T3 – 1:10 concentrations of acrid latex from *E. agallocha*

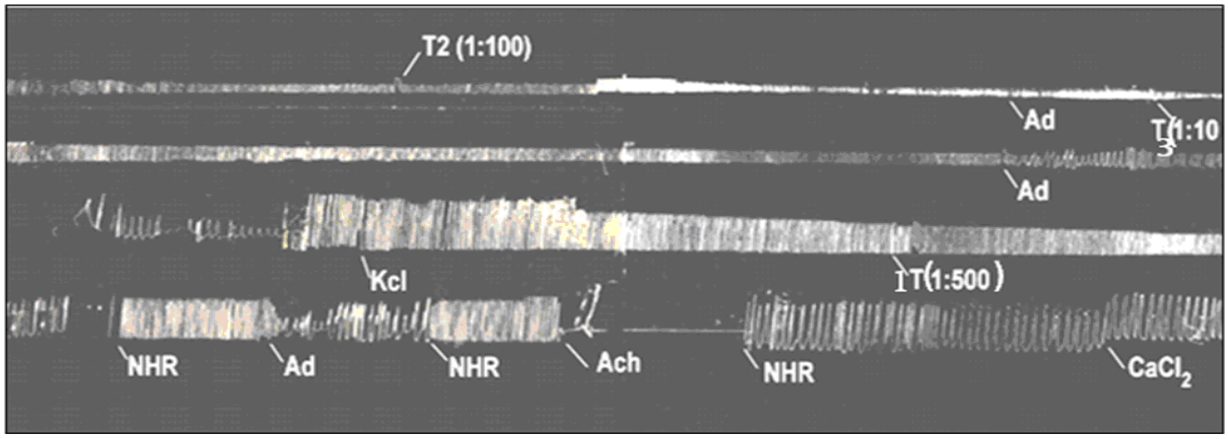

Figure 2. Effects of different dilutions of acrid latex from *E. agallocha* on the isolated frog heart
NHR- Normal Heart Rate; Ad- Adrenaline; Ach- Acetylcholine; $CaCl_2$- Calcium chloride; KCl- Potassium chloride; Ach – Acetylcholine; T1 - 1:500, T2 – 1:100, and T3 – 1:10 concentrations of acrid latex from *E. agallocha*

Table 2. Effects of different dilutions of acrid latex from *E. agallocha* on the activities of frog *rectus abdominis* muscle

Drug	Dose (ml)	Per cent Responses in terms of height of the peak
Acetylcholine	0.1	53.44±1.55[a]
Acetylcholine	0.2	73.59±0.69
Acetylcholine	0.4	93.88±1.30
Acetylcholine	0.8	99.52±0.58
Test drug (T1)	0.4	Nil
T1+Ach	0.4+0.4	73.55±1.08
Acetylcholine alone after administration of T1+ Ach	0.4	67.15±0.64
T2+Ach	0.4 +0.4	71.98±0.35
T2+Ach	0.8+0.8	74.35±0.99
Acetylcholine alone after administration of T2 + Ach	0.8	42.68±0.58
T3 +Ach	0.4 +0.4	69.58±0.45

[a]Means of triplicate samples ± standard deviation.
T1 -1:500, T2 -1:100, T3 -1:10 concentrations of acrid latex from *E. agallocha*; Ach - acetylcholine

Table 3. Effects of different dilutions of acrid latex from *E.agallocha* on the ciliary muscle in frog buccal cavity

Treatment	Ciliary muscle activity (movement of poppy in seconds)
Saline (Control)	64
Atropine sulphate (10 mg / ml)	83
Neostigmine (10mg / ml)	33.75
Test drug T1 (1:500)	16.25
Test drug T2 (1:100)	26.25
Test drug T3 (1:10)	Nil

Table 4. Effects of different dilutions of acrid latex from *E. agallocha* for the ion action in frog

Experiment	Drugs Administered	Observations in the frog
I	Step 1: Administration of 0.2 ml of Magnesium sulphate	CNS depressant and lethargy
	Step 2: Administration of 0.2 ml of Calcium gluconate	Active
II	Step 1: Administration of 0.2 ml of T1	CNS depressant and lethargy
	Step 2: Administration of 0.2 ml of Calcium gluconate	Active
III	Step 1: Administration of 0.2 ml of T2	Active
	Step 2: Administration of 0.2 ml of Magnesium sulphate	CNS depressant and lethargy
	Step 3: Administration of 0.2 ml of Calcium gluconate	Active
IV	Administration of 0.2 ml of T3	CNS depressant, lethargy and toxic

T1 = 1:500, T2 = 1:100, and T3 = 1:10 concentrations of acrid latex from *E. agallocha*

increases up and down as the concentration dose increases i.e. T1<T2<T3 (Fig. 3).

DISCUSSION

It was evident from the results that the present investigation shows the test extract acrid latex from *E. agallocha* produced local anesthetic activity in frog at T1 (1:500) and T2 (1:100) as per the nerve block anesthesia method suggested by Sollaman, (1918) and Bulbring and Wajda, (1945) for ~6 hrs. Earlier researchers have also found that Bulleyaconitine A (BLA), an active ingredient of *Aconitum bulleyanum* plant at 0.375 mM with 2 % lidocaine (~80 mM) or epinephrine (1:100,000) produces local anesthetic activity in the sciatic nerve for ~4 hrs (Wang *et al*., 2007). Thus, the test extract acrid latex of *E. agallocha* is more potent in terms of local anesthetic activity with higher the concentration of acrid latex from *E. agallocha* is the higher the potency.

In the present study the test extract acrid latex from *E. agallocha* has been found to have cardiac depressant activity when tested in the frog heart. Earlier researchers have also stated that the alcoholic extract from the coastal plants produced significant positive ionotropic and negative chronotropic actions on frog's heart (Muralidharan and Dhananjayan, 2004).

In the present study, the test extract, acrid latex from *E. agallocha* was found to have a good effect on the ciliary muscle for the ciliary movement action in the buccal cavity of frog with lower the concentration the higher the potency. Earlier studies have also reported that prostaglandins increases matrix metalloproteinase (MMP) activities in the ciliary smooth muscle cells (Lindsey *et al*., 1996; Weinreb *et al*., 1997).

Results of the present study showed that the test extract acrid latex from *E. agallocha* produced partial purgative activity at T1 (1:500) and T2 (1:100) concentrations. Earlier Ghosh *et al.* (2003) found the roots of *Rumex nepalensis* to possess a purgative activity and suggested anthraquinones present in the plant extract might be responsible for such purgative activity. Similarly, in the traditional Chinese medicine, the drug Ta-Cheng-Chi-Tang (TCCT) was reported to have a strong purgative activity (Tseng *et al*., 2006).

In the present study the test extract acrid latex from *E. agallocha* was found to have central nervous system depressent action at T1 (1:500) and T2 (1:100) and T3 (1:10) resulted in total lethargy. Similarly, earlier researchers have also proved the crude hydro alcoholic extract (EH) of *Qualea grandiflora, Salvia aegyptiaca* to cause central nervous system depression (CNS) effect in mice (Al-Yousuf *et al*., 2002; Gaspi *et al*., 2006).

Results of the present study showed that the test extract acrid latex from *E. agallocha*, T1 (1:500) and T2 (1:100) with acetylcholine produced skeletal muscle relaxant property similar to the standard drug d-tubocurarine (or) Pancronium. Earlier researchers have also found that chloroform extract of *Ervatamia crispa* and alcoholic extract of *Chonemorpha macrophylla* revealed skeletal muscle relaxant effect on isolated frog *rectus abdominis* muscle preparation (Das *et al*., 2005 a b).

Results of the present study showed that the test extract acrid latex from *E. agallocha* produced smooth muscle relaxant effect. But the test extract with acetylcholine produced an antagonistic action i.e., 80% smooth muscle relaxant activity at T1 (1:500) and T2 (1:100) in the rat colon preparation at 0.1ml. Arranz *et al.* (2003) also obtained highly significant results and dose dependent relaxant activity on the smooth muscle of Guinea pigs from the dichloromethane extracts of *Huperzia saururus* (87% of relaxation at the dose of 10 mg / ml), *Satureja parvifolia* (95% of relaxation at 2.5 mg / ml), *Senecio eriophyton* (94% of relaxation at 5 mg / ml) and similar effects with the methanol extracts of *Haplopappus rigidus* (88% of relaxation at 10 mg / ml).

So, it is concluded that the mangrove plant, *E. agallocha* is a potent medicinal plant which could be used for various therapeutical purposes and could be a promising source for developing novel drugs for many curious and incurable diseases.

ACKNOWLEDGEMENT

Authors thank Prof. Dr. T. Balasubramanian, Director, Centre of Advanced Study in Marine Biology and Prof. Dr. R. Manavalan, Head, Department of Pharmacy, Annamalai University, for providing all the necessary facilities and constant encouragement.

REFERENCES

Al-Yousuf, M.H., Bashir, A.K., Ali, B.H., Tanira, M.O.M. and Blunden, G. 2002. Some effects of *Salvia aegyptiaca* L. on the central nervous system in mice, *J Ethnopharmacol.*, 81: 121-127.

Arranz, C., Balaszczuk, A., Costa, M., Coussio. J.D. and Ferraro, G. 2003. Argentinian plant extracts with relaxant effect on the smooth muscle of the corpus cavernosum of Guinea pig. *Intl. J. Phytotherapy and Amp Phytopharmacol.*, 10: 17-18.

Bandaranayake, W.M. 1998. Traditional and medicinal uses of mangroves. *Mang. and Salt Marshes*, 2: 133-148.

Bulbring, E. and Wajda, I. 1945. Biological comparison of local anaesthetics. *J. Pharmacol. Exp. Therap.*, 85: 78–84.

Das, A., Ray, N. and Ranendra Roy, 2005b. *Ervatamia crispa* revealed skeletal muscle relaxant effect on isolated frog. *Indian J. Pharmacol.*, 17: 32-33.

Das, A., Ray, N., Ranendra Roy, 2005a. Skeletal muscle relaxant effect of Chonemorpha macrophylla in experimental animals. *Indian J. Pharmacol.*, 12: 24-25.

Erickson, K.L., Beutler, J.A., Cardellina, J.H., Mc Mahon, J.B. and Newman, D.J. 1995. A novel phorbol ester from *Exoecaria agalloch. J. Nat. Prod. (Lloydia)*, 58: 769-777.

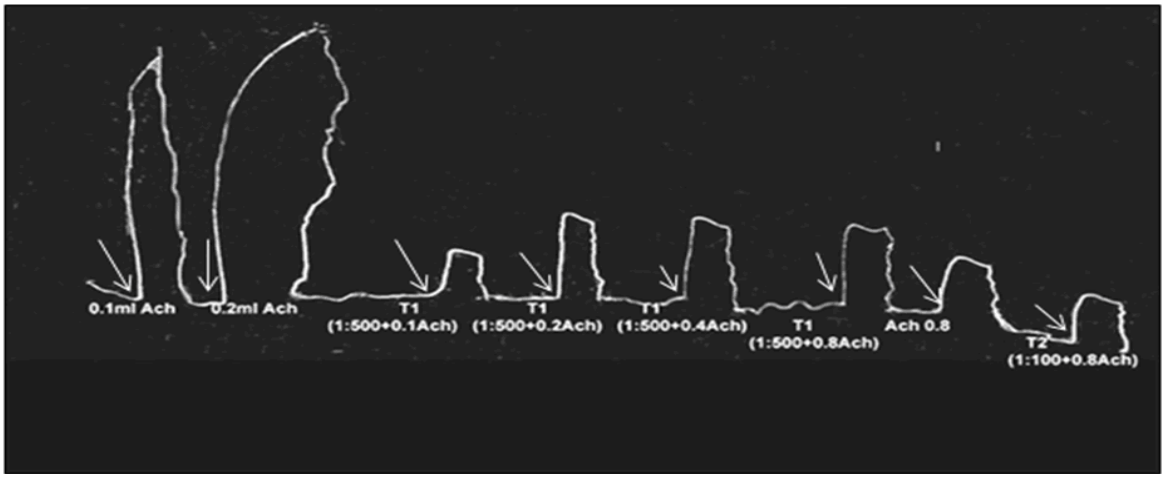

Figure 3. Effects of different dilutions of acrid latex from *E. agallocha* on the rat's isolated colon preparation (smooth muscle)
Ach - Acetylcholine; T1 - 1:500, T2 – 1:100 and T3 – 1:10 concentrations of acrid latex from *E. agallocha*

Table 5. Effects of different dilutions of acrid latex from *E. agallocha* for the salt action in frog's small intestine

Drug	Dose (ml)	Observations in frog's intestine
Magnesium sulphate	0.1	Osmotic purgation
T1	0.1	Partial osmotic purgation
T2	0.1	Week osmotic purgation
T3	0.1	No osmotic purgation and paralysis

T1 = 1:500, T2 = 1:100 and T3 = 1:10 concentrations of acrid latex from *E. agallocha*

Table 6. Effects of the crude drug acrid latex from *E. agallocha* on the rat colon preparation

Drugs	Dose (ml)	Percent Responses in terms of height of the peak	Responses in the height of the peak
Acetylcholine	0.1	72.07±0.17[1]	Increased
Acetylcholine	0.2	87.83±0.31	Increased
Acetylcholine	0.4	95.83±0.41	Increased
Acetylcholine	0.8	99.62±0.56	Increased
Test drug T1	0.1	Nil	Nil
T1+ach	0.1+0.1	20.90±0.30	Increased
T1+ach	0.2+0.2	36.12±0.24	Increased
T1+ach	0.4+0.4	35.10±0.46	Increased
T1+ach	0.8+0.8	34.98±0.26	Increased
Acetylcholine	0.8	23.45±0.57	Decreased
T2+ach	0.8+0.8	21.02±0.37	Increased
T3+ach	0.8+0.8	17.22±0.78	Increased

[1] Means of triplicate samples ± standard deviation.
T1 -1:500, T2 -1:100, and T3 -1:10 concentrations of acrid latex from *E. agallocha,* Ach - acetylcholine

Gaspi, F.O.G., Foglio, M.A., Carvalho, J.E. and Moreno, R.A. 2006. Pharmacological activities investigation of crude extracts and fractions from *Qualea grandiflora* Mart. *J. Ethnopharmacol.*, 107: 19-24.

Ghosh, L., Gayen, J.R., Murugesan, T., Sinha, S., Pal, M. and Saha, B.P. 2003. Evaluation of purgative activity of roots of *Rumex nepalensis. Fitoterapia,* 74: 372-374.

Guillet, G., Helenon, R. and Guillet, M.H. 1985. Dermatitis from *Hippomane mancinella. Ann de Dermatol et de Venerol,* 112: 51-56.

Harbone, J.B. 1998. Triterpenoids and Steroids. *Phytochemical methods*, 3rd edition, Chapman and Hall, Rajkamal Electric Press, Delhi.

Jayaweera, D.M.A. 1980. Medicinal plants used in Ceylon. *Natl. Sci. Council Sri Lanka*, 2: 214-215.

Kanakambal, S., 2001. *Experimental pharmacology*. Kanaga Publishers, Tirunelveli.

Karalai, C., Wiriyachitra, P., Operkuch, H.J. and Hecker, E. 1994. Cryptic and free skin irritants of the daphnane and tigliane in latex of *Excoecaria agallocha*. *Planta Med.*, 60: 351-355.

Kathiresan, K. 2002. Why are mangroves degrading?, *Curr. Sci.*, 83: 1246-1249.

Kathiresan, K. and Bingham, B.L. 2001. The Biology of mangroves and mangrove ecosystems. *In:* Southward, A.J., Tyler, P.A., Young, C.M. and Fuiman, L.A. (Eds.), *Advances in Marine Biology*, Academy Press, New York.

Kathiresan, K. and Thangam, T.S. 1987. Biotoxicity of *Excoecaria agallocha* L. latex on marine organisms. *Curr. Sci.*, 56: 314-315.

Kawashima, T., Takahashi, T., Inoue, Y., Kodama, M. and Ito, S. 1971. Euphorbiaceae, Constituents of *Excoecaria agallocha. Phytochem.,* 10: 3308-3309.

Kokate, C.K. 1994. *Practical Pharmacognosy*. Vallabh Prakash, Delhi.

Konishi, T., Yamazoe, K., Konoshima, T., Maoka, T., Fujiwara, Y. and Miyahara, K. 2003. New bis-secolabdane diterpenoids from *Excoecaria agallocha. J. Nat. Prod.*, 66: 108-111.

Kulkarni, S.K., Ninan, I. and Singh, A. 1998. The drug showed antianxiety activity with adaptogenic effect. *Indian J. Pharmacol.*, 30: 323-325.

Lindsey, J.D., Kashiwagi, K. and Boyle, D. 1996. Prostaglandins increase proMMP-1 and proMMP-3 secretion by human ciliary smooth muscle cells. *Curr. Eye Res.*, 15: 869-875.

Muralidharan, A. and Dhananjayan, R. 2004. Cardiac stimulant activity of *Ocimum basilicum* Linn. Extracts. *Indian J. Pharmacol.*, 36: 163-166.

Ohigashi, H., Katsumata, H., Kawazu, K., Koshimizu, K. and Mitsui, T. 1974. A piscicidal constituent of *Excoecaria agallocha. Agri. Biol. Chem.*, 38: 1093-1095.

Padmakumar, K. and Ayyakkannu, K. 1985. Bioactive compounds from marine plants. II. Antibiotic properties of *Excoecaria agallocha* L. from Pichavaram mangroves. In: Leela, J. and Bosale, P (Ed.), *Proc. Nat. Symp. Bio. Utiliz. Conserv. Mangroves*. Shivaji University, Kolhapur. pp. 319-324.

Padmakumar, K., Ramaswamy, S., Ayyakkannu, K. and Nair, P.G.V. 1993. Analgesic activity of marine plants, Nutrients and Bioactive Substances in Aquatic Organisms. In: Devadasan, K., Mukundan, M.K., Antony, P.D., Nair, P.G.V., Perigreen, P.A. and Joseph, J. (Eds.), *Paper Presented in the Symposium Held in Cochin*, India 16–17 September 1993, Society of Fisheries Technologists, Cochin, India. pp. 25-30.

Prakash, S., Khan, M.A., Khan, H. and Zaman, A. 1983. A piperidine alkaloid from *Excoecaria agallocha. Phytochem.*, 22: 1836-1837.

Premanathan, M., Kathiresan, K., Chandra, K. and Bajpai, S.K. 1993. Antiviral activity of marine plants against New Castle disease virus. *Tropical Biomedicine*, 10: 31-33.

Reddy, T.K.K., Rajasekhar, A., Jayasunderamma B. and Ramamurti, R. 1991. Studies on marine bioactive substances from the Bay of Bengal: Bioactive substances from the latex of the mangrove plant *Excoecaria agallocha* L, Antimicrobial Activity and Degradation. *In: Bioactive compounds from marine organisms with emphasis on the Indian Ocean*. Thompson, M.F., Sarojini, R. and Nagabhushanam, R. (Eds.), An Indo-United States Symposium, Goa India.

Sollaman, T. 1918. Text book of Pharmacological. *J. Pharmacol. Exp. Therap.*, 11: 1-4.

Thangam, T.S. and Kathiresan, K. 1992. Mosquito larvicidal activity of mangrove plant extract against *Aedes aegypti*. *International Pest Control*, 34: 116-119.

Tseng, Sung-Hui, Hsin-Hsueh Lee, Lih-Geeng Chen, Chih-Hsiung Wu, Ching-Chiung, 2006. Comparative investigations of hydroxylamine carane derivative and its r, s-diastereoisomers with strong local anesthetic activity. *Pol. J. Pharmacol.*, 53: 535-539.

Wang, Chi-Fei, Peter Gerner, Sho-Ya Wang, Ging Kuo Wang, Bulleyaconitine, 2007. A isolated from Aconitum plant displays long acting local anesthetic properties in vitro and in vivo. *J. Ethnopharmacol.*, 107: 82-90.

Weinreb, R.N., Kashiwagi, K. and Kashiwagi, F. 1997. Prostaglandins increase matrix metalloproteinase release from human ciliary smooth muscle cells. *Invest. Ophthalmol. Vis. Sci.*, 38: 2772-2780.