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HPTLC FINGERPRINTING, CYTOKINE ESTIMATION, EVALUATION OF ANALGESIC, ANTIINFLAMMATORY AND MEMBRANE STABILIZING ACTIVITIES OF *ANTHOCEPHALUS CADAMBA* MIQ. ROOT BARK

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Abstract
Anthocephalus cadamba (family- Rubiaceae), popularly known as Kodom or Kodamba in Assam has been widely used for the treatment of various diseases including fever, uterine complaints, blood diseases, skin diseases, eye inflammation, diarrhoea, anaemia etc. The plant is a storehouse of several phytoconstituents which are believed to be responsible for its analgesic and anti inflammatory activities. The phytochemical screening of methanolic, chloroform and hydroalcoholic extracts of root bark of A.cadamba showed the positive result for alkaloids, glycosides, phenolic compounds, flavonoids, sterols and terpenoids. The HPTLC fingerprinting of hydroalcoholic and chloroform extracts of A. cadamba was performed and it generated 9 and 4 numbers of peaks respectively. The test drug HRBE at the dose of 200 and 400 mg/kg showed an anti-inflammatory activity that became significant ($p<0.05$) 3 h after the injection of carrageenan. Test drug 2 (400mg/kg) and Test drug1 (200mg/kg) showed maximum effect of 52% and 32% respectively at 24 h. The test drug at 200 and 400mg/kg body weight has shown a significant alteration in IL-10 and IL-4 level at 24 h of inflammation as compared to the standard drug Indomethacin. In Hot Plate Test the response of both the doses were found to exhibit a significant ($p < 0.05$) and dose dependent increase in latency period as compared to standard drug diclofenac sodium. Moreover, the test drug has very potent human erythrocytes membrane stabilizing activity. In conclusion, it can be stated that root of A. cadamba possess potent analgesic, anti-inflammatory and membrane stabilizing effect.

Key Words: *Anthocephalus cadamba*, HPTLC, Cytokine, Analgesic, Antiinflamatory activities.

Introduction:

Anthocephalus cadamba (family- Rubiaceae) is a large tree with a broad crown and straight cylindrical bole. In Assam, it is popularly known as *Kodom* or *Kodamba*. It is found all over India and abundantly grows in Assam, North and Eastern Bengal and Western Ghats. *Kadamba* is also native to Bangladesh, Nepal, Sri Lanka, Southern China,

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and South East Asia including Cambodia, Laos, Myanmar, Thailand, Vietnam, Indonesia and Malaysia (Kirtikar *et al* 1933).

In folk medicine, it is used in the treatment of fever, uterine complaints, blood diseases (Kirtikar *et al* 1933; Majumdar 2002), skin diseases (Bhandary *et al* 1995), eye inflammation, diarrhoea (Pal *et al* 2000), anaemia, leprosy, dysentery and stomatitis (Slkar *et. al.* 1992). A decoction of the leaves is good for ulcer and wounds and recommended as gargle. The other reported traditional uses of this plant are hypoglycemic (Kapil *et al* 1995), antimalarial (Sianne *et al* 2002), antimicrobial, wound healing, antioxidant (Sanjay *et al* 2007), anthelmintic and analgesic-anti inflammatory (Gunasekharan *et al* 2006).

Anthocephalus cadamba primarily consist of indole alkaloids, terpenoids, sapogenins, saponins, terpenes, steroids, fats and reducing sugars. A new pentacyclic triterpenic acid has been isolated from the stem bark *Anthocephalus cadamba* and named cadambagenic acid. Along with this acid quinovic acid and β sitosterol have also been isolated. Glycosidic indole alkaloids; cadambine, 3α-dihydrocadambine, isodihydrocadambine and two related non-glycosidic alkaloids; cadamine and isocadamine have been isolated from the leaves of *Anthocephalus cadamba* (Dubey *et al* 2011).

Traditionally in Assam, a paste prepared from the root bark is applied externally to the swollen tonsil and believed to reduce the inflammation and pain very promptly (information collected from local traditional healer). This stimulated us to investigate the analgesic anti-inflammatory activity of the root bark of this plant.

MATERIALS AND METHODS

Plant Material

The plant material (root) was collected from Nakhanda region of Barpeta District during the month of January 2015. A herbarium of the plant was prepared by conventional herbarium technique for identification and it was identified by Dr. L.R. Saikia, Professor, Department of Life Science, Dibrugarh University. A voucher specimen of the herbarium (DU/PHSc/HRB-03/2015) is deposited in the Department of Pharmaceutical Sciences. After authentication, fresh roots were collected in bulk, washed thoroughly and bark was separated. The bark was dried in the shade and pulverized in a mechanical grinder to obtain a coarse powder.

Preparation of Extract

The powdered root bark was successively extracted with hexane, chloroform and methanol in a Soxhlet apparatus. Hydroalcoholic extract was obtained by cold maceration with 70% alcohol. Following extraction, liquid extracts were evaporated to dryness on a

water bath. The methanolic, chloroform and hydroalcoholic extracts of root bark of *Anthocephalus cadamba* were used for further studies.

Phytochemical Screening

Standard methods were used for the preliminary phytochemical screening of the different extracts (Kokate *et al* 2004) to know the nature of phytoconstituents present in them.

HPTLC Fingerprinting

HPTLC fingerprinting of hydroalcoholic and chloroform extracts was carried out at Directorate of Forensic Science, Kahilipara, Guwahati by using CAMAG 4 equipment. The solvent system for hydroalcoholic and chloroform extract was butanol: acetic acid: water (4:1:1) and benzene: chloroform: ethyl acetate (2:5:2) respectively.

Acute Oral Toxicity Studies

Acute oral toxicity study was performed as per OECD-425 guidelines (OECD 2002) on female rats. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level 5mg/kg body weight by oral route of administration and observed for 14 days for skin changes, morbidity, aggressiveness, increase oral secretion, sensitivity to the sound and pain as well as respiratory movements and mortality.

Evaluation of Antiinflammatory Activity

Requirements

Chemicals

Indomethacin, carrageenan (S.D Fine chemicals, Bombay), Diclofenac Sodium, Sterile Normal Saline, Hydroalcoholic root bark extract of *A.cadamba* Miq(HRBE) prepared in normal saline.

Animals

Healthy albino rats (Wistar strain) of either sex, weighing from 100-150 gm were obtained from M/S Chakraborty Enterprise, Kolkata, India. The animals were housed individually in a room maintained under environmentally controlled conditions of 24±1° C and 12 hr light-12 hr dark cycle with free access to food and water *ad libitum* during the course of experiments. Animals were fed with standard laboratory diet and water *ad libitum* (filter water). The places where the experiments conducted were kept very hygienic by cleaning with antiseptic solution. The husk for the purpose of keeping as a bed to the animals was cleaned daily before the experiment.

Carrageenan induced Rat Paw Oedema (Winter *et al* 1962)

Animals of either sex were divided into 5 groups of 6 animals each. The first group received the normal saline and served as Vehicle treated group. Second group was treated with 0.1 ml of 1% carrageenan in normal saline by injection on the sub-plantar region of

the hind paw and treated as only carrageenan treated group. Third group was given indomethacin at a dose of 10mg/kg body weight and served as standard drug treated group. Fourth and fifth groups were treated as test group 1 and test group 2 and received test drug (HRBE) at the dose of 200mg/kg and 400mg/kg body weight respectively. After 1 hour of administration of the drugs, 0.1ml of 1% carrageenan was injected to the sub-plantar region of the hind paw of the rats of all the groups except the vehicle treated group. Hind paw volume was measured using a plethysmometer before injection and at 0, 3, 6, and 24 h after carrageenan injection. The difference between the initial and subsequent paw volume reading gave the actual oedema volume. The percent inhibition of inflammation was calculated using the formula

$$\% \text{ Inhibition} = V_c - V_t / V_C \times 100$$

Where V_c represents oedema of only carrageenen treated group, V_t is the oedema of drug treated group tested with hydroalcoholic or indomethacin. The results are shown in Table 5,6 and figure 5,6.

Estimation of cytokines

During Carrageenan Induced Paw Edema experiment, after talking the paw volume measurement, rats were anaesthetized with diethyl ether and blood samples were collected from orbital sinus. Two ml of blood from each rat was collected in sterile vials. The serum was separated by allowing blood to clot followed by centrifugation and was stored at -80^o C until use. TNFα, IL4, IL10, IL2, IFNϒ were estimated by Cytometric Bead Array using Fluorescence Activated Cell Sorter.

Analgesic Activity

Hot Plate Method

The evaluation of Analgesic activity of the hydroalcoholic extract of root bark of *A. cadamba* was carried out by Hot Plate method (Eddy *et al* 1953). The Control Group of rats (n=6) received 0.5 ml of normal saline orally. Rats in the test groups 1 and 2 were given the test drug HRBE (200mg/kg and 400mg/kg body weight respectively) orally. Diclofenac was given as standard at a dose of 20mg/kg body weight. Animals were then placed on the hot plate maintained at 55^o±1^o C. The reaction time (in seconds) or latency period was determined as the time taken for the rats to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before (0 min) and at 30, 60 and 120 minutes after the drug administration. The maximum reaction time or cut-off time was fixed at 35 sec to prevent any injury to the tissues of the paws. If the reading exceeds 35 sec, it would be considered as maximum analgesia (Gaur *et al* 2009). The maximum possible analgesia (MPA) was calculated as follows:

Maximum Possible Analgesia= (Test Latency-Control Latency)/Cut-off time-control latency) x100

Statistical Analysis: Results were expressed as Mean ± SD, statistical significance was calculated by applying one way ANOVA. $P<0.05$ was considered as statistically significant.

Membrane Stabilizing Activity:

Membrane stabilizing activity was assessed by using hypotonicity induced haemolysis of human erythrocytes.

Preparation of Erythrocyte Suspension

Fresh whole blood (3 ml) collected from one of the author vein puncture into a EDTA coated vials and was centrifuged at 3000 rpm for 10 min. A volume of normal saline equivalent to that of the supernatant was used to dissolve the red blood pellets. The volume of the dissolved red blood pellets obtained was measured and reconstituted as a 40% v/v suspension with isotonic buffer solution (10 mM sodium phosphate buffer, pH 7.4). The buffer solution contained 0.2 g of $NaH_2\ PO_4$, 1.15 g of $Na_2\ HPO_4$ and 9 g of NaCl in 1 litre of distilled water. The reconstituted red blood cells (resuspended supernatant) were used as such.

Hypotonicity Induced Haemolysis (Anishoke A *et al* 2012)

The effect of the hydroalcoholic extract of root bark of *Anthocephalus cadamba* tasted on human erythrocytes were subjected to hypotonic and heat stress. Aspirin was used as positive controls while isotonic saline was used as negative control. Samples of the extract used in this test were dissolved in distilled water (hypotonic solution). The hypotonic solution (5 ml) containing graded doses of the test drug HRBE (0.1g/ml, 0.2g/ml) were put into the centrifuge tubes. Isotonic solution (5 ml) containing graded doses of the extracts (01g/ml, 0.2gm/ml) were also put into the centrifuge tubes. Control tubes contained 5 ml of the vehicle (distilled water) and 5 ml of 350 µg/ml aspirin respectively. Erythrocyte suspension (0.5 ml) was added to each of the tubes and mixed gently. The mixtures were incubated for 10 min at room temperature (37°C), and afterwards, centrifuged for 10 min at 3000rpm. Absorbance (OD) of the haemoglobin content of the supernatant was estimated at 540 nm using colorimeter. The percentage heamolysis was calculated by assuming the heamolysis produced in the presence of distilled water as 100%. The percent inhibition of haemolysis by the extract was calculated thus:

$$\% \text{ Inhibition of haemolysis} = 1 - (OD_2 - OD_1 / OD_3 - OD_1) \times 100$$

Where,

OD_1 = absorbance of test sample in isotonic solution

OD_2 = absorbance of test sample in hypotonic solution

OD_3 = absorbance of control sample in hypotonic solution

RESULTS

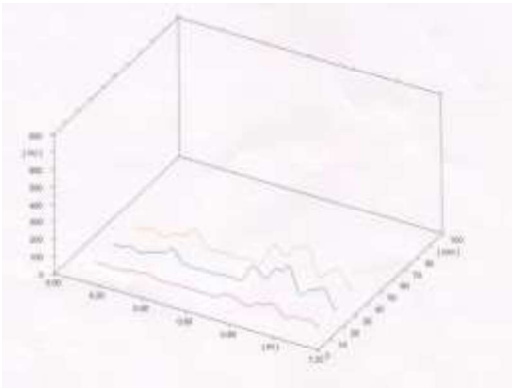
Phytochemical Screening: The phytochemical Screening of methanolic, chloroform and hydroalcoholic extracts of root bark of *A.cadamba* showed the positive result for alkaloids, glycosides, phenolic compounds, flavonoids and sterols and terpenoids (Table-1). The Maximum number of phytoconstituents were found to be in the hydroalcoholic and methanolic extracts.

Table-1. Preliminary phytochemical screening of different extracts of *A. cadamba* root bark.

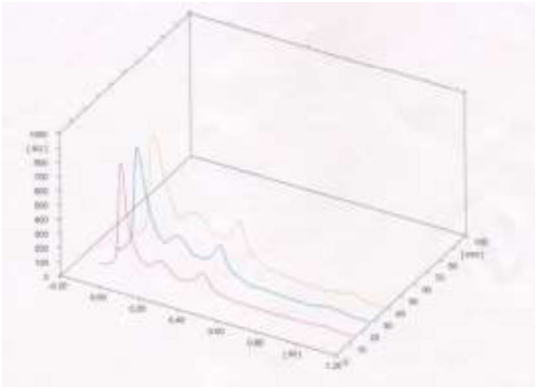
Plant constituent	Chloroform	Methanol	Hydroalcoholic
Alkaloid	+	+	-
Glycoside	-	-	+
Phenolic compounds	-	+	+
Flavonoids	-	+	+
Carbohydrates	-	+	+
Sterols	+	-	-
Fats and Lipids	-	-	-
Saponin	-	+	+

HPTLC Chromatogram:

The HPTLC fingerprinting of Hydroalcoholic and Chloroform extracts of *A.cadamba* generated 9 and 4 numbers of peaks respectively. The $^{\lambda}$Max and R_f values of the peaks are shown in the below tables (Table-2 & Table-3) and figure (Fig-1).



(A)



(B)

Fig-1: HPTLC peak of (A) hydroalcoholic extract and (B) chloroform extract.

Acute Oral Toxicity

The Hydroalcoholic extract of root bark of *A.cadamba* Miq. was found safe at all test doses (50mg, 300mg and 2000mg/kg body weight). During 24 hour assessment time, test animals were found normal.

Table-2: HPTLC peaks of hydroalcoholic Extracts of *Anthocephalus cadamba* Miq. root bark with R_f values.

Peak	**R_f value**	**λ Max**
1	0.90	241nm
2	0.74	200nm
3	0.84	200nm
4	1.00	200nm
5	0.36	200nm
6	0.60	200nm
7	0.18	326nm
8	1.00	200nm
9	0.27	200nm

Table-3: HPTLC peaks of chloroform extract of *Anthocephalus cadamba* root bark.

Peak	**R_f values**	**λ Max**
1	0.01	290nm
2	0.44	293nm
3	0.22	243nm
4	1.00	314nm

Anti-inflammatory Study

Carrageenan Induced Paw Edema

The anti-inflammatory activity of test doses (200 and 400mg/kg body weight.) of *A.cadamba* root bark is presented in Table-4 and Figure-2 with the average volume of paw edema. The percentage inhibition is presented in Table-5 and Figure-3. The injection of carrageenan in paw created an inflammatory edema which gradually increased. The test drug HRBE at the dose of 200 and 400 mg/kg showed an anti-inflammatory activity that became significant ($p<0.05$) 3 h after the injection of carrageenan and was maintained all along the experiment. Test drug 2 (400mg/kg) showed maximum effect of 52% at 24 h whereas Test drug1 (200mg/kg) exhibited 32 % inhibition at 24h. Standard drug Indomethacine exhibited maximum inhibition of 34% at 6h which reduced to 30% at 24h. The two test doses showed greater inhibition as compared to the standard drug.

Table-4: Mean Paw Volume of the experimental rats in various groups

Groups	Mean Paw volumes* after-			
	0 hr	3 hr	6 hr	24 hr
Control	0.44±0.13	0.42±0.4	0.40±.01	0.35±0.03
Std. Treated	0.46±0.03	0.41±0.06	0.40±0.04	0.34±0.03
Carr. Treated	0.46±0.06	0.64±0.03	0.67±0.16	0.74±o.14
Test 1	0.51±0.08	0.50±0.06	0.43±0.04	0.41±0.03
Test 2	0.38±0.05	0.39±0.13	0.34±0.1	0.31±0.06

*Data are reported as Mean± SD for group of six animals.

Cytokine Estimation

Our study has revealed a significant dose dependant decrease in the concentration of pro-inflammatory cytokines IL10 and IL4 (Table-6 and Fig-4) whereas no detectable amount of TNF-α, IFN-γ and IL2 expression has counted in the sera samples from both the untreated control and drug treated groups. In this study, SDL6 and SDH6 has represented the experimental rats from which sera was collected at 6 h of inflammation after treated with 200 and 400mg/kg of test drug (HRBE) respectively whereas SDL24 and SDH24 groups has represented the experimental rats from which sera was collected at 24 h of inflammation after the administration of the HRBE drug in same doses as mentioned earlier. The test drug at two different doses (i.e. 200 and 400mg/kg body weight) has shown a significant alteration in IL-10 and IL-4 cytokines at 24 h of inflammation as compared to the standard drug Indomethacin treated group.

Table-5: Percentage inhibition of inflammation by HRBE of *A. cadamba*

Groups	% Inhibition of Inflammation*		
	3 hr	6 hr	24 hr
Control	-	-	-
Std. Treated	40	34	30
Carr. Treated	-	-	-
Test 1	23	27	32
Test 2	34	38	52

*Data are reported as Mean± SD for group of six animals.

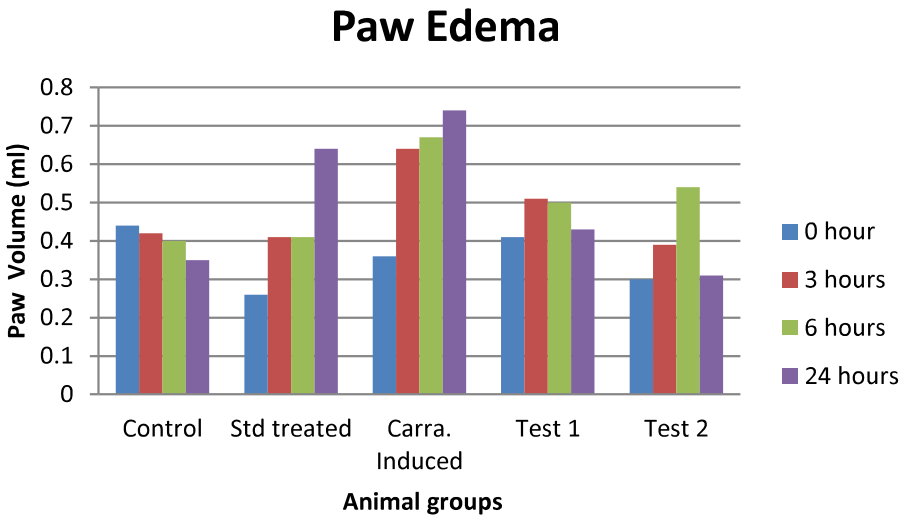


Figure-2: Figure showing Paw oedema of different groups on carrageenan induction.

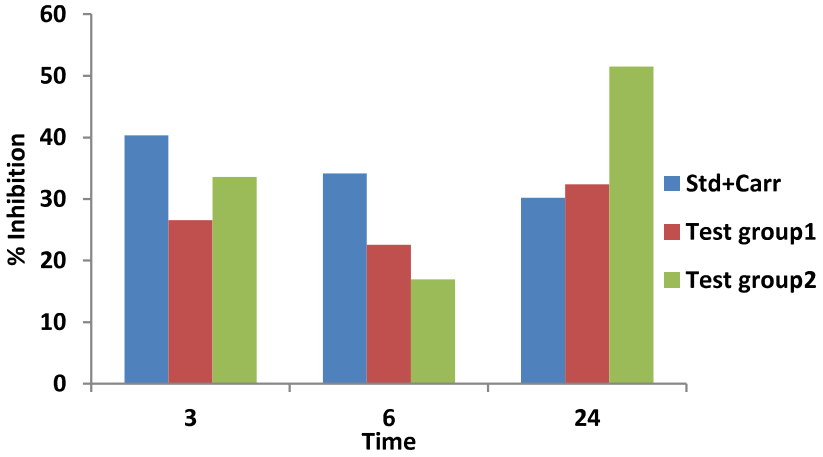


Figure-3: Figure showing % Inhibition of paw oedema of Test drugs compared to Standard.

Table-6: Different cytokine levels in test serum samples.

Experimental groups	Concentration (pg/ml)				
	IFN-γ	IL-10	IL-4	TNF	IL-2
CONTROL	0	136.26	12.67	0	0
C6	0	0	1.08	0	0
C24	0	0	2.27	0	0
C24+INDOM	0	395.1	7.66	0	0
C6+INDOM	0	306.8	17.35	0	0
SDH 24	0	0	0	0	0
SDL 24	0	96.93	0	0	0
SDH 6	0	340.43	30.2	0	0
SDL 6	0	20.58	0	0	0

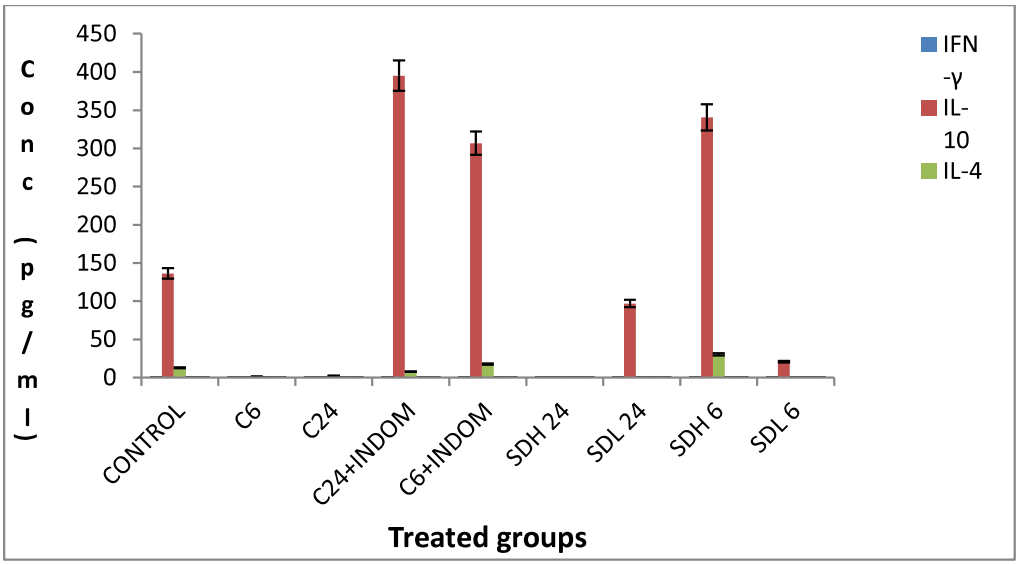


Figure-4: Different levels of cytokines in test serum samples.

Analgesic Activity

Hot Plate Method

In Hot Plate Test the response of both the doses was found to exhibit a significant ($p< 0.05$) and dose dependent increase in latency period. The result of the hot plate test revealed that the latency time was significantly ($p<0.05$) increased from 5% to 87% at the dose of 200mg/kg to 400mg/kg body weight. The effect was dose dependant and the maximum effect was observed after 60 minutes. Test drug at the dose 200mg/kg maximum analgesia (57.7%) at 60 mins whereas, the dose 400mg/kg showed maximum

analgesia at 120 mins which was 87%. Both the doses exhibited significant result as compared to standard drug Diclofenac Sodium (20mg/kg). The result of Hot Plate test is given in Table-7 and Figure-5.

Table-7: Results of Hot Plate Test showing Latency Period and percentage Analgesia

Group	Dose (mg/kg)	Mean Latency before and after drug administration*			% Analgesia		
		30 mins	60 mins	120 mins	30 mins	60 mins	120 mins
Gr. A	Normal Saline	4.49±0.85	3.57±1.08	3.62±0.26	-	-	-
Gr. B	STD 20mg/kg	6.27±0.74	3.57±1.08	11.19±0.6	5.8	0	24
Gr. C	TEST1 200 mg/kg	7.59±0.75	21.71±1.51	21.38±1.30	11.30	57.7	56.5
Gr. D	TEST2 400 mg/kg	11.52±0.65	30.99±1.49	21.11±1.10	23	87	55.7

*The values are given as Mean± SD for group of six animals. The data are analyzed by ANOVA followed by Dunnet's test.

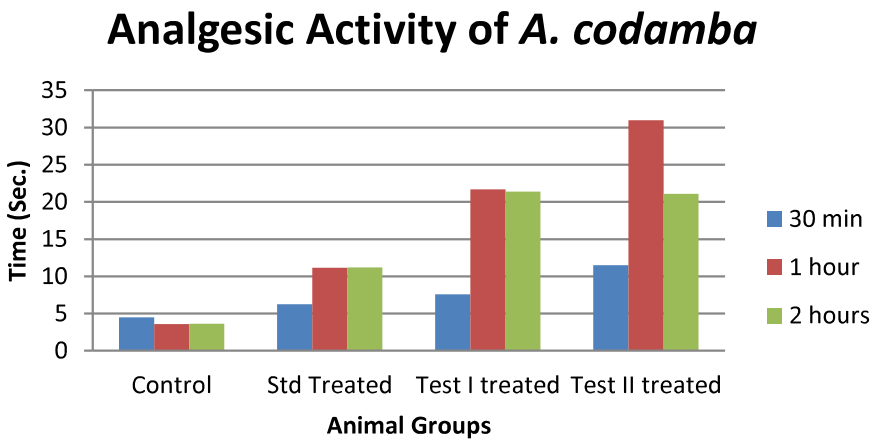


Figure-5: Result of Hot Plate Test showing Latency Period and percentage analgesia. The values are given as Mean± SD for group of six animals. The data are analyzed by ANOVA followed by Dunnet's test.

Membrane Stabilizing Activity (Anishoke *et al* 2012)

The result of Membrane Stability Test showed that the hydroalcoholic extract of root bark of *Anthocephalus cadamba* Miq. has very potent human erythrocytes membrane

stabilizing activity as compared to standard drug Aspirin. The result is given in Table-8. Both the test drugs exhibited greater inhibition when compared to test drug aspirin. Test drug at the dose of 0.1g/ml showed maximum inhibition of 93.27%.

Table-8: Result of Membrane Stabilizing Activity showing percentage inhibition of hemolysis.

GROUP	OD_1	OD_2	% INHIBITION
Standard	0.015	1.580	17
Test 1 (.01g/ml)	0.74	0.499	93.27
Test 2 (0.2/ml)	0.136	0.253	76.40

DISCUSSION

HPTLC fingerprinting is an essential and useful parameter for crude drug evaluation. In the present study of fingerprinting reveals 9 and 4 distinct peaks respectively in hydroalcoholic and chloroform extract of *A. cadamba* root bark. The $^{\lambda}$max of all the peaks fall between 200 -326 nm and R_f values between 0.01 – 1.00. This could be used as reference standard for further study.

Preliminary phytochemical studies showed the presence of alkaloids, flavonoids, glycosides, phenolics, sterols and saponins in different extracts. The hydroalcoholic extract of root bark of *Anthocephalus cadamba* Miq. has marked analgesic-antiinflammatory and Membrane stabilizing effect with a reasonable safety profile.Thermal nociception model was used to evaluate the analgesic activity. To assess the central mechanism of the compound in producing analgesia, Hot Plate method was employed. Hot Plate test involves higher brain function and is considered to be a supraspinally organized response. μ, k3 and d2 are the opioid receptor sub-type primarily responsible for supraspinally mediated analgesic action which can be seen in Hot plate method whereas spinal analgesia appears to be mediated through μ2, k1 and d2 receptor (Wani *et al* 2012). The hot-plate method is useful in elucidating centrally mediated anti-nociceptive responses, which focuses mainly on changes above the spinal cord level. The significant increase in pain threshold produced by ethanolic extract of root bark of *A.cadamba* suggests involvement of central pain pathways. Number of complex processes including opiate, dopaminergic, descending nor-adrenergic and serotonergic systems are involved in centrally modulated pain. Receptor systems involved in central or peripheral mechanisms involving inhibition of prostaglandins, leukotrienes, and other endogenous substances that play a major role in inflammation and pain were responsible for the analgesic and anti-inflammatory effect of the extract (Pasero *et al* 1999). In this study the

hydroalcoholic extract of root bark of *A.cadamba* (200mg/kg, 400mg/kg) exhibited significant result in the thermal nocieption model.

The molecular mechanism of the carrageenan-induced inflammation is well-characterized, and these models of inflammation are standard models of screening for anti-inflammatory activity of various experimental compounds. The early phase of carrageenan edema is related to the production of histamine, leukotrienes, and possibly cyclooxygenase products, while the delayed phase of the carrageenan-induced inflammatory response has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals, such as hydrogen peroxide, superoxide, and OH radicals, as well as to the release of other neutrophil-derived mediators (Vinegar *et al* 1969). A reduction in paw swelling volume is a good index in determining the protective action of anti-inflammatory agents (Liao *et al* 2011). Studies have shown that TNF-α is a candidate marker in inflammation which is capable to induce immune responses by the influx of neutrophil populations at the site of inflammation followed by the stimulation of antigen presenting cells (APCs) and T-cell subsets and alter the expressional profiling of other inflammatory cytokine genes, such as IL-6 and IL-1β in a paracrine mode of regulation. TNF-α is also a mediator in carrageenan-induced inflammatory response and induces further release of kinins and leukotrienes, which are suggested to play an important role in the maintenance of inflammatory response (Dawson *et al* 1991). In this study experimental rat treated with different doses of novel drug HRBE has shown a significant reduction in edema in both the doses (200mg/kg and 400mg/kg body weight.) but the maximum reduction has observed at 24^{th} h of inflammation (52%). Moreover, our study has shown that administration of HRBE is significantly associated with the alternation in the expressional profiling of IL-4 and IL-10 cytokines in experimental rats treated with carrageenan.

Test drugs at concentrations of 0.1 g/ml – 0.2 g/ ml protected the human erythrocyte membrane against lysis induced by hypotonic solution. During inflammation, there are lyses of lysosomes which release their component enzymes that produce a variety of disorders. Non-steroidal anti-inflammatory drugs (NSAIDs) exert their beneficial effects by either inhibiting the release of lysosomal enzymes or by stabilizing the lysosomal membranes (Mounnissamy *et al* 2008). Exposure of red blood cells (RBCs) to injurious substances such as hypotonic medium, heat, methyl salicylate or phenylhydrazine results in the lysis of the membranes, accompanied by haemolysis and oxidation of haemoglobin (Feirrali *et al* 1992). The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Injury to red cell membrane will render the cell more susceptible to secondary damage through free

radical induced lipid peroxidation (Halliwell *et al* 2004). Membrane stabilization leads to the prevention of leakage of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory mediators (Chaitanya *et al* 2011). In this study, root extract of *A.cadamba* at concentrations of 0.1g/ml and 0.2g/ml protected the human erythrocyte membrane against lysis induced by hypotonic solution.

The preliminary phytochemical screening showed the presence of therapeutically important phytoconstituents like alkaloids, glycosides, ohenolic compounds, flavonoids, sterols, terpenoids and saponins. In conclusion, our study suggests that root of *A.cadamba* possess analgesic,anti-inflammatory and membrane stabilizing effect.

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