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ANALGESIC, ANTIPYRETIC AND ANTI INFLAMMATORY ACTIVITIES OF COLUMN FRACTION OF *BABYLONIA ZEYLANICA* (BRUGUIERE, 1789) IN ALBINO RATS

Santhi V.[1], V Sivakumar[2*], R D Thilaga[3*], A Thangathirupathi[4*]*

[1] *P.G. Department of Zoology, J.A. College for Women (Autonomous), Periyakulam-625601, India*
[2] *P.G. and Research Department of Zoology, V.O. Chidambaram College, Thoothukudi-628008, India*
[3] *P.G. and Research Department of Zoology, St.Mary's College,Thoothukudi, India.*
[4] *Dept of Pharmacology, S.B.College of Pharmacy, Sivakasi - 626130*
***Corresponding Author Email:**

ABSTRACT

The analgesic, antipyretic and anti-inflammatory effect of the benzene: methanol extracts of gastropod Babylonia zeylanica was experimented to albino rats shown promising results. In acute toxicity test oral administration of doses up to 800mg/kg did not show any toxic symptoms and could not provoke any significant change in their behavior. The extract of Babylonia zeylanica at the concentration of 100 and 200mg/kg, p.o decreased the pain in analgesic activity which was compared with Pentazocine for narcotic type by tail-immersion method. With same dose it reduced the Yeast induced Pyrexia raised body temperature in albino rats in antipyretic activity which was compared with paracetamol. The same dose in the anti-inflammatory activity against carragennan induced paw edema was compared with diclofenac sodium reduced the paw edema in albino rats. These promising results reveals, the presence of alkaloid, ester, nitrogen, terpene and steroid compounds by GC-MS analysis from benzene: methanol column purified fraction of Babylonia zeylanica might be responsible for analgesic, antipyretic and anti- inflammatory effect in Albino rats. In all the methods, both the doses showed good results with statistical significant.

KEYWORDS

Analgesic· Anti-pyretic· Anti-inflammatory· Mollusc·

INTRODUCTION

Marine organisms encompass roughly a half of the total biodiversity, thus offering a vast source to discover useful therapeutics. Academic researchers began to collaborate with pharmacologists in 1989 and the potential of the oceans became clear with many unique bioactive substances being extracted from marine plants and invertebrates (Fenical, 1997). Due to unsystematic use of anti microbial drugs; microorganisms are resistance to many antibiotics. They reduce the susceptibility to antibiotics and made it difficult to give treatment against the infectious diseases (Sieradzki *et al*., 1999). The critical events are the emergence of *Staphylococcus aureus* with decreased sensitivity to methicilin (Roder *et al.,* 1999), worldwide resistance to penicillin in *Staphylococcus pneumonia* and several resistances to *Mycobacterium tuberculosis*. The cost of manufacture of synthetic drugs is also high and they may also produce adverse side effect, compared to bioactive naturally derived drugs. Therefore, naturally originated agents with very little side effects are required to substitute chemical therapeutics. The knowledge acquired in the past two decades and the discovery of new groups of drugs makes natural antibiotics the basic element of a novel generation of drugs for the treatment of human diseases (De Lucca, 2000; Hancock 2000; Welling *et al.,* 2000). Inflammatory responses

are mostly associated with pathological disorders (Akah & Nwamble, 1994). The complex events and intermediaries involved in the inflammatory reaction can be induced, sustain or intensify many diseases (Sosa *et al.,* 2002). Therefore, development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary. Hence much attention has been paid recently to the biologically active compounds derived from plants and animals used in the medicine (Essawi, 2000).

The most interesting living things with respect to pharmacologically active marine compounds include bacteria, fungi, algae, sponges, soft corals, tunicates, molluscs and bryozaons (Faulkner, 2000). Among the marine invertebrates, the molluscs are potential sources and the bioactive compounds isolated from the gastropods are considered to have a role in a chemical defense of the animals against their predators. Many promising lead compounds have been isolated from marine organisms such as manoalide, (Potts and Faulkner, 1992) pseudopterosins, topsentins and scytonemin (Mayer and Lehmann, 2001), diterpene (Loukaci, et *al*., 2000). Inadequate literatures concerning the analgesic, antipyretic and anti-inflammatory properties of molluscs initiated the present study to find out the analgesic, antipyretic and anti-inflammatory potential of column fractionated extract of *B. zeylanica (*gastropod marine mollusc) of Tuticorin coast, Gulf of Mannar, Tamil Nadu in an animal model with adult Wister Albino rats.

MATERIAL AND METHODS

The crude extract of *B. zeylanica* was isolated and subjected to silica gel column chromatography using hexane: chloroform (F1), chloroform (F2), benzene (F3), benzene: methanol (F4), and methanol (F5) in order of polarity. These fractions were tested against bacterial pathogens. The most potent column chromatography extract of the test animal was subjected to FT-IR, GC-MS and pharmacological studies such as analgesic, antipyretic, and anti-inflammatory activities.

FT-IR

The solid sample obtained from column chromatography fraction (F4) of *B. zeylanica* was subjected to IR spectrographs (FT-FR, Shimadzu, Japan) which helped to identify the presence of different functional groups. GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system.

Identification of Compounds

Interpretation on mass spectrum GC-MS was conducted using the data base of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The unknown components with electrons impact fragmentation patterns of the mass spectra of column fractioned test mollusc were matched with the spectrum of the known components stored in NIST ver.21 National Institute of Standard Technology, the mass spectra library and predicted from Dr.Duke's phytochemical and Ethnobotanical databases.

Selection of Experimental Animals

Adult Wistar albino rats of either sex weighing between 150 and 180 gm maintained at Sankaralingam Bhuvaneswari College of Pharmacy animal house, Sivakasi were used for the study with prior approval of Institutional Animal Ethics Committee (IAEC). The selected animals were housed under standard environmental conditions (temperature of 22±1^{o}C) maintained by giving uniform pelette diet, water ad libitum with an alternating 12 hrs light dark cycle and relative humidity of 60± 5%.

Acute toxicity of column fractionated extracts of test animals

Acute toxicity study was performed in Albino rats divided into different groups of 6 each. After an overnight starvation, the column

fractionated extract in 0.5% (W/v) suspension of Sodium Hydroxide was administered orally in graded doses (100 mg to 800 mg/kg body weight) to Albino rats. They were observed continuously for the first 2 hour for toxic symptoms and up to 24 hours for mortality.

Analgesic Testing (Narcotic Type of Analgesic Activity)

Wister albino rats were screened for its sensitivity by placing the tip of the tail (last 1 – 2 cm) gently in warm water maintained at 55°C (±2°C). Any albino rats flicking the tail within 5 sec were selected for the study. The selected rats were divided into four groups of six animals each. Group I and Group II received distilled water (Control) 1ml/kg and Pentazocine (Standard) 4 mg/kg i.p respectively. Group III and Group IV received column fractionated test drug of *B. zeylanica* in 0.5% Sodium Hydroxide suspended extracts at 100 and 200 mg/kg p.o. respectively. After drug treatment, the basal reaction time of all groups of animals was noted at different time intervals such as 1 hr, 2 hrs, 3 hrs and 4 hrs.

Antipyretic Testing

Either sex of Albino rats having the temperature between 36.5°C and 38.5°C were selected. The animals were divided into four groups of six animals each. All six albino rats in each group were injected in the nape subcutaneously with 20% aqueous suspension of Brewer's yeast (20 ml/kg). The animal developing 0.5°C and more rise in rectal temperature, 18 hours yeast injection was selected for further studies. Group I received distilled water (1ml/kg) alone and Group II received paracetamol 45 mg/kg p.o. The other 2 groups were treated with column fractionated test drug of *B. zeylanica* in 0.5% Sodium Hydroxide suspended extracts of 100 mg and 200 mg/kg p.o. respectively. The rectal temperature was recorded at 0, 1, 2, 3, & 4 hours after the administration of test drugs.

Anti – Inflammatory Testing

Anti - inflammatory activity was assessed by the method suggested by Winters *et al*., (1962) using Carrageenin as phlogestic agent. The adult Wistar albino rats of either sex weighing between 150 & 180 gm were housed in groups of six animals each. They were starved overnight during the experiment but had free access to water. The volume of paw of each animal was determined before giving any drug. Group I and II were given orally the distilled water (Control) 1mg/gm and suspension of Diclofenac Sodium (Standard) (10 mg/kg b.w) respectively. III and IV group of animals received the chloroform fractionated test drugs of *B. zeylanica* extract of suspended in 0.5% Sodium Hydroxide at 100 and 200 mg/kg respectively. The extract was suspended in 0.5% W/v Sodium Hydroxide and administered orally 30 minutes before injection of Carrageenin (0.1 ml of 1% W/v solution) in normal saline into sub planter region of left hind paw of each rat (Ocete *et al*., 1989).

The degree of edema formation at the hind paw volume was measured by plethysnographically at each hour, for 4 hour after Carrageenin was injected. The percentage inhibition of edema has been calculated by the following formula.

$$A - B = \frac{C}{A} \times 100$$

Where A represents the average increase in paw volume of control and B represents the average increase in paw volume after the administration of drug.

Statistical Analysis

All the data were expressed as mean ± S.E. Statistical significance of the difference between control and treated groups were accessed by the method of analysis of one way ANOVA followed by Dunnet's t-test. $P<0.001$ was considered as statistically significant.

RESULTS

The FT-IR, and GC-MS result of the column fractionated extracts of *B.zeylanica* (F4), showed the presence of alcohol, alkaloid, alkene, plasticizer, oleic acid ester, terpene, steroid, aldehyde and amide compounds. In acute toxicity study, the fractionated extracts were found to be safe and no mortality was observed at a dose as high as 800 mg/kg.

Figure (1) shows the FT-IR spectrum of F4 fraction of *B. zeylanica*. A peak at 1112.85cm^{-1} indicates C-O stretching .A strong absorption of plane bending bands appear from 1300-1000cm^{-1} region is the most prominent plane bend (1222.79cm^{-1}) of aromatic compound. A peak at 1643.24cm^{-1} indicates C=O stretching and another peak at 1710 .74cm^{-1} indicates the C=O stretching and five member ring or cyclic. A peak at 2923.88cm^{-1} shows aliphatic CH-CH_2 symmetric stretching lipids or protein. Any heteroaromatic N-H stretch absorption between 3500 and 3220cm^{-1} results from (3394cm^{-1}) pyrines, pyrazine, furans, thiophenols and pyrroles.

F4 fraction (**Table 1**) from *B. zeylanica* revealed ten compounds based on the mass spectra library matched to the terpene compound a-D-Manno furonoside farnesyl, an alkaloid 2-Piperidene, a Nitrogen compound 3-Hexadecycloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion an amide compound 9-octadecenamide,(Z)- and a plasticizer compound 1, 2- benzene dicarboxilic acid diisoocyl ester, Plasticizer compound, diethyl phthalate, oleic acid ester hexadeconoic acid methyl ester,aldehyde-undecanal 2-methyl,and oleic acid in trace amounts were also identified (Table 1). A terpene a-D manno furanoside farnesyl was the most abundant (18.12%) compound found in this fraction.

Analgesic Testing

The analgesic effect of the column purified extracts of *B.zeylanica* (F4) on albino rats were presented in **Table 1**. The extract of the test mollusc, at the concentration of 100 & 200 mg/kg p.o. showed significant decrease in pain when compared to that of control, at a four hour of experiment. The results were comparable with that of control and the drug was a good analgesic activity, with larger reaction time values of the test extract (12±0.41, 7±0.41) than control group (4.5± 0.5).The difference between the extract and control group was significant at P<0.005 and P<0.001 (**Table 1**).

Anti-pyreting Testing

The results of effect of *B.zeylanica* (F4 fraction) on yeast induced pyrexia in albino rats was depicted in **Table 2**. F4 fraction of *B.zeylanica* produced significant (P<0.05) antipyretic effect in both the concentrations. The sub-cutaneous injection of yeast suspension markedly elevated the rectal temperature after 18 hour of administration. Treatment with *B.zeylanica* (F4) extract at a dose of 200 mg/kg caused significant (P<0.005) lowering of body temperature at 4 hour following its administration (36.93 ± 0.017) than 100 mg/kg (37.04± 0.001).

Anti-inflammatory Testing

The anti-inflammatory effect of the *B.zeylanica* (F4) was experimented on albino rats and the result was depicted in Table 3. The extract at the concentration of 100 and 200 mg/kg p.o. of the test animals showed significant decrease in the paw thickness when compared to that of control, at 4th hour of experiment. The results were comparable with that of control and the percentage inhibition of paw thicknesses was found to be 57.67% and 59.79% in *B. zeylanica*, at concentration of 100 and 200 mg/kg p.o. respectively. In the present investigation 200mg of *B. zeylanica* extract controlled the inflammation maximum than that of 100mg/kg. The extract of the test animal exhibited a significant (P<0.001) reduction in paw thickness

at 4th hour in Carrageenan induced paw edema, when compared to control (**Table 3**).

DISCUSSION

Pharmaceutical industry now accepts the world oceans as a major frontier for medical research. Pharma MAR (Spain and USA) has taken leading position in the development of drugs from the sea. It has four novel compounds, Yondelis, Aplidin, kahalaide F and ES-285 in clinical trails and a rich pipeline of preclinical candidates. The results indicated that the extracts of *B.zeylanica* mollusc was active against all the experimentally induced laboratory models of analgesic, anti pyretic and anti inflammation and the extract at the two doses 100 and 200mg/kg used in the study significantly increases the duration response in albino rats.

In the present study for analgesic, anti inflammatory and anti pyretic *B.zeylanica's* (F4) extract showed significant activity when compared to the standard. The extract contains alkaloids, steroids, terpenes, amino compounds with sulphur linoleic acid ester and oleic acid ester. The analgesic, antipyretic and anti inflammatory activity exhibited by the extracts of the test molluscs in the present study may be due to the presence of above mentioned compounds because they are known to cause significant anti pyretic, analgesic and anti- inflammatory effects (Xiano and Peng, 2004; Raja and Mustafa, 2005). The analgesic, anti pyretic and anti inflammatory activities may be attributed to the presence of alkaloids, carbohydrates, glycosides and steroids in the tested animals (Kakate, 1996; Plaisted and Philip, 1996).

Further *B.zeylanica* extract produced significant analgesic activity at 200mg/kg than that of control. In the earlier reports alkaloid extract was found to possess analgesic activity (Vohera *et al*., 1984). It is therefore, possible that the extract from *B.* zeylanica exert analgesic effect by inhibition synthesis or action of prostaglandins. The difference in the rectal temperature of rats between the control and drug treated groups at 4 hour interval were observed as statistically significant in the test molluscs (P< 0.001). This suggests short acting anti pyretic effect of the drug but it denotes the potential therapeutic application in pain related diseases. Clin Rheumatol (2006) reported that freeze- dried green lipped mussel *Perna canaliculus* powder controls rheumatoid and osteoarthritis patients.

The anti pyretic activity of *Lapartea crenulata* is due to the presence of steroids (Chattopadhyay *et al*., 2005). In the present study also presence of steroids might be responsible for the anti-pyretic action. Oral administration of extracts of the test animal showed a significant (P< 0.01, P< 0.05 and P<L 0.01) inhibition of Carrageenan induced in the paw inflammation at 100 mg and 200 mg. At the dose of 100 mg/kg orally the F4 fraction of *B.zeylanica* extract produced 57.67% inhibition in case of the Carrageenan induced edema (P<0.001).The results showed that the extract of the test animal contains substances capable of inhibiting carragenin induced inflammation. Halpern (2000) reported that a lipid rich extracts prepared by supercritical fluid (CO2) extraction of freeze-dried stabilized mussel powder (Lyprinol) has shown anti-inflammatory activity when given to animals and humans. Kumar (2003) has reported that the methanolic extracts of *Cypraea errones* and *Cypraea arabica* exerted moderate anti-inflammatory effect against Carrageenan-induced inflammation. Similar result was reported by Chellaram and Edward (2009) from acetone column purified fraction of *Drupa margariticola*.

Based on the results obtained from this present investigation it can be concluded that the extract of *B.zeylanica* (200mg/kg) possessing significant (P<0.001) analgesic, anti pyretic and

anti inflammatory activity when compared to the control. Further purification of Benzene: methanol fraction of this gastropod extract may be more potent than the standard drug and to evaluate the real usefulness of these extracts in the therapy of pain release. It is also understood that, the rich diversity of marine biota with its unique physiological adaptations to the harsh marine environment provides a fruitful source for the discovery of life saving drugs

Table 1: Analgesic action of Benzene: Methanol extract of *B.zeylanica* by tail immersion method

Treatment	Dose mg / kg	Basal Reaction Time (in Second)				
		0 hr	1 hr	2 hr	3 hr	4 hr
Control	1 ml / kg P.O.	2.75 ± 0.25	3.75 ± 0.25	4 ± 0.8	4.25 ± 0.62	4.5 ± 0.5
Pentazocine i.p.	4 i.p.	2.5 ± 05	5.5 ± 0.28	8.25± 0.47	10 ± 0.4	12 ± 0.4 *
***B.Zeylanica P.O.* S1**	100	2.25 ± 0.25	4.5 ± 0.28	5 ± 0.41	6 ± 0.41	7 ± 0.41
***B.Zeylanica P.O.* S2**	200	4 ± 0.7 *	4.75± 0.5	7 ± 2.7	7.5 ± 2.7	9 ± 2.45 *
One way Anova	F	2.7619	1.162	1.6557	2.913	5.9629 ••
	DF	12, 3	12, 3	12, 3	12, 3	12, 3
	P	NS	< 0.05	< 0.05	< 0.05	< 0.05

All values are expressed as Mean ± S.E,
P < 0.05 • * P < 0.001
One way Anova followed by Dunnet's t-test and NS – Non significant Vs Control group.
6 animals were used in each group.

Table 2: Anti-pyretic action of Benzene: Methanol of *B.zeylanica* against Brewer's yeast induced pyrexia in albino rats

Treatment	Dose Mg / kg PO	Reduction in Rectal Body Temperature (°C)			
		1 hr	2 hr	3 hr	4 hr
Control	1 ml	37.53±0.012	37.41±0.012	37.29±0.012	37.17±0.012
Paracetamol (33mg / kg) PO	10	37.39±0.012 *	37.12±0.017 *	36.87±0.03 *	36.67±0.012 *
***B.Zeylanica* S1**	100	37.25±0.012 *	37.17±0.017 *	37.09±0.017 *	36.98±0.02 *
***B.Zeylanica* S2**	200	37.25±0.012 *	37.13±0.012 *	36.95±0.912 *	36.81±0.01 *
One way Anova	F	80.8•	83.45•	86.76•	93.20•
	DF	12, 3	12, 3	12, 3	12, 3
	P	< 0.05	< 0.05	< 0.05	< 0.05

All values are expressed as Mean ± S.E,
P < 0.05 • * P < **0.001**
One way Anova followed by Dunnet's t-test and NS – Non significant Vs Control group.
6 animals were used in each group.

Table 3: Anti-Inflammatory action of Benzene: Methanol of *B.zeylanica* against Carrageenan induced paw oedema in albino rats

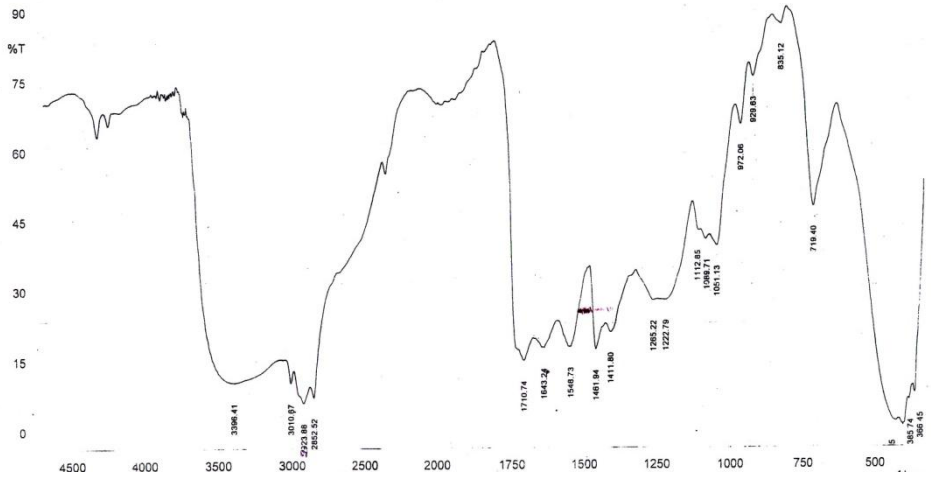
Treatment	Dose Mg / kg PO	Reduction in Paw Thickness and Percentage of oedema			
		1 hr	2 hr	3 hr	4 hr
Control	1 ml	0.21 ± 0.013	0.39 ± 0.013	0.73 ± 0.013	0.945 ± 0.009
Diclofenac Sodium PO	10	0.08 ± 0.016 ^{NS} 61.9 %	0.185 ± 0.033 * 52.56 %	0.28 ± 0.033 * 62.32 %	0.4 ± 0.022 * 57.67 %
P.persica S3	100	0.175 ± 0.01 ^{NS} 16.66 %	0.34 ± 0.086 ^{NS} 12.82 %	0.32± 0.08 * 56.84 %	0.595 ± 0.08 * 37.03 %
P.persica S4	200	0.165 ± 0.04 ^{NS} 21.42 %	0.385 ± 0.043 ^{NS} 35.89 %	0.32 ± 0.04 * 54.79 %	0.49 ± 0.09 * 48.15 %
One way Anova	F	5.03 $^{\bullet}$	13.08 $^{\bullet}$	20.16 $^{\bullet}$	12.65 $^{\bullet}$
	DF	12, 3	12, 3	12, 3	12, 3
	P	< 0.05	< 0.05	< 0.05	< 0.05

All values are expressed as Mean ± S.E,
$P < 0.05$ $^{\bullet}$ $^{*}P <$ **0.001**
One way Anova followed by Dunnet's t-test and NS – Non significant Vs Control group.
6 animals were used in each group.

Table 4: Components identified in the Benzene: Methanol column fraction of *B.zeylanica* by GC-MS

S.No	RT	Name of the compound	Peak Area %	Compound Nature
1	2.32	Glycerin	20.48	Alcohol
2	4.30	2-Piperidinone	6.55	Alkaloid
3	9.19	Diethyl Phthalate	0.73	Plasticizer compound
4	12.67	9-Hexadecenoic acid, methyl ester, (Z)-	1.83	Oleic acid ester
5	12.91	Hexadecanoic acid, methyl ester	4.66	Palmitic acid ester
6	16.34	9-Octadecenamide, (Z)-	0.94	Amide compound
7	17.70	Undecanal, 2-methyl-	0.94	Aldehyde compound
8	18.04	Oleic Acid	0.73	Oleic acid
9	21.27	1,2-Benzenedicarboxylic acid, diisooctyl ester	2.46	Plasticizer compound
10	22.34	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	3.88	Nitrogen compound
11	26.49	á-D-Mannofuranoside, farnesyl-	18.12	Terpene compound
12	29.40	Cholesterol	16.82	Steroid

Fig. 1: FT-IR Analysis of Column Extract (Benzene: Methanol) of *B.zeylanica*



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***Corresponding Author:**
V.Santhi,
Associate Professor,
P.G Dept of Zoology,
Jayaraj Annapackiam College for Women. (Autonomous)
Periyakulam – 625 605.
Theni District,
TamilNadu.