ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Anti-Pyretic Activity of Anthocephalus cadamba Roxb. Leaves Extracts

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ABSTRACT

Anthocephalus cadamba (Family: Rubiaceae) is commonly known as Kadamba-vriksha. Antipyretics compounds in the market still present a wide range of undesired effects leaving an open door for new and better compounds. Natural products are believed to be an important source of new chemical substance with potential therapeutic applicability. Several plant species traditionally used as Antipyretics. The purpose of the present study was to evaluation estimation and anti-pyretic activity of anthocephalus cadamba Roxb. Leaves extracts using Yeast induces hyperpyrexia method. Paracetamol was kept as standard. The study was carried out in Wister strain weighing 150-200gm. The results obtained from the Yeast induced pyrexia method indicated that Chloroform, ethanol, distilled water extracts have significant onset of action as reduction of temperature by these extracts was found within 30 minutes. Whereas the reduction of temperatures with Petroleum ether and Solvent ether extracts was late. In all extracts the temperatures were reduced to normal till 180 minutes. Even Paracetamol also has significantly reduced rectal temperature to the extent of 37.7^0c from 30 min. to 180 minutes. All the results were compared with Control group.

Keywords: Anthocephalus cadamba, Investigation, TLC, Paracetamol, Phytoconstituents.

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ISSN: 0975-8585

INTRODUCTION

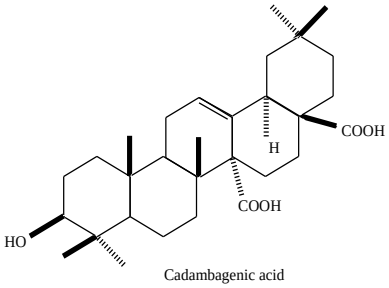
Throughout human history, people have relied on natural products and plants in particular, to promote and maintain good health and to fight sickness, pain and disease. The past 200 years have witnessed not only acceleration in the rate of extinction of plant and animal species, but also the erosion of traditional knowledge related to the medicinal properties and uses of plants and other natural products [1]. Today, there are thousands year old traditions and records of popular healing methods that have maintained their importance despite new developments and progress in the field of chemistry, pharmaceutics and medicine. The role of natural products in the development of drugs used in modern medicine is unsurpassed even when synthetic chemistry has been developed beyond expectations. Indeed, interest in herbal drugs is increasing [2].

Table 1: Showing list of plants of antipyretic activity

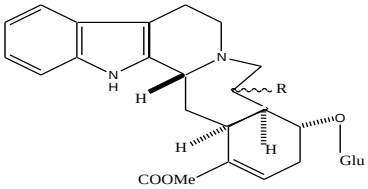
Name of plant	Part used	Reference
Acanthus illicifolius	Whole plant	MAPA 2004-01-0444
Capraria biflora	Leaves	MAPA 2004-02-0644
Peristrophe bicalyculata	Whole plant	MAPA 2004-02-0765
Aconitum carmichaeli	Flower, buds	MAPA 2004-02-0787
Couroupita guianensis	Flower, bark	MAPA 2004-03-1140
Acalypha indica	Leaves	MAPA 2004-03-1141
Vitex negundo	Leaves	MAPA 2004-05-2079
Auxemma oncocalyx	Heartwood	MAPA 2004-05-2083
Aeolanthus suaveolens.	Whole plant	MAPA 2004-05-2212
Celastrus peniculatus	Flower	MAPA 9501-0161
Tecomella undulata	Whole plant	MAPA 9501-0161
Astragalus siculus Biv.	Whole plant	MAPA 9501-0171
Adansonia digitata	Fruit pulp	MAPA 9502-0916
Reseda phyteums	Whole plant	MAPA 9503-1599
Ecballium elaterium	Whole plant	MAPA 9605-2740
Psychotria colorata	Flower, Fruit, Root	MAPA 9602-0774
Pistacia integerimagalls	Whole plant	MAPA 9605-2743
Cuscuta chilensis,	Whole plant	MAPA 9605-2747
Cestrum parqui	Aerial part	MAPA 9605-2747
Psoralea glandulosa	Aerial part	MAPA 9605-2747
Afrormosia laxiflora	Leaves	MAPA 9605-2770
Cyathula prostrata	Whole plant	MAPA 9605-2770
Ficus glomerata	Leaves	MAPA 9605-2770
Lantana Camara	Leaves	MAPA 9605-2770
Lippia geminata	Leaves	MAPA 9605-2770
Spilanthes acmella	Stems	MAPA 2005-01-0086
Curcuma amada	Rhizomes	MAPA 2005-03-1260
Eupatorium adenophorum	Leaves	IJNP-21 (1) 6
Acharas sapota	Bark	IJNP-21 (3) 6
Rhinacanthus nasutus	Whole plant	IJNP-21 (3) 6
Basella rubra linn	Whole plant	ID-41 (9), 04

ISSN: 0975-8585

Anthocephalus cadamba (Family: Rubiaceae) is commonly known as Kadamba-vriksha and is a genus of trees, distributed throughout the Indo-Malaysian region. A medium sized tree attaining 2 m girth and 18 m height, branches spreading horizontally and slightly enlarged at their junction with the main stem [3]. It is cultivated all over India. Leaves are about 7.5-18 X 4.5-16 cm in size, flower head globes, yellow, solitary, terminal, 3.7 cm in diameter consisting of small, yellow or orange colored, scented flowers; fruits are fleshy, orange, globes pseudo carp of compressed angular capsules with persistent calyx; seeds small, muriculate [4]. The leaves are used in stomatitis [5], in hydrocoele [6] and in Pyorrhoea [7]. The decoction of leaves is good for ulcers, wounds and metrorrhea [8]. The leaves and bark contained 0.2 and 0.18% of alkaloids respectively [9] and hentriacontanol, β-sitosterol [10] and two non-glycoside alkaloids Cadamine and Isocadamine [11].

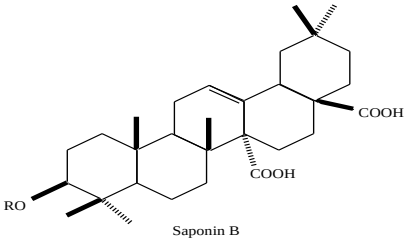


Cadambagenic acid



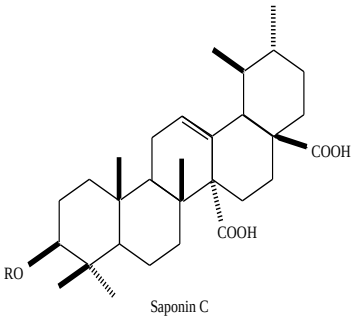
3ß-Dihydrocadambine

R=ß-OH



Saponin B

R=Fuc(3➔1) Rha(2➔1)Rha

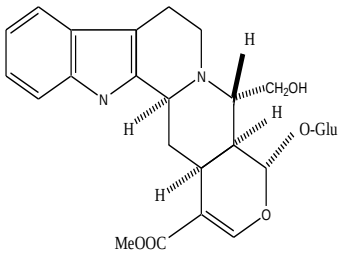


Saponin C

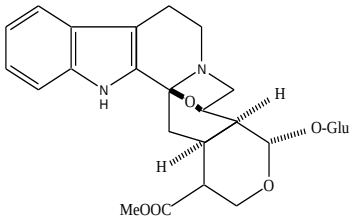
R=Fuc(3➔1) Glu(4➔1)Glu

Saponin D

R=Fuc(4➔1) Glu(2➔1)Glu(4➔1)Rha



Isodihydrocadambine



Cadambine

MATERIALS AND METHODS

Procurement and authentication of drug

The leaves of Anthocephalus cadamba Roxb. were collected from local area in Jalgaon and were authenticated from botanical Department, Pune.

Drying and size reduction

In the present study, 1 kg dried leaves of Anthocephalus cadamba Roxb. were reduced to coarse powder using mechanical grinder and passed through a sieve no.40 to obtain powder of desired particle size.

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Extraction

The powdered material was subjected to successive hot extraction (soxhlet) with various solvents in increasing order of polarity from Petroleum ether, Solvent ether, Chloroform, Ethyl alcohol and Dist. Water to 30 cycles per batch. The extraction was continued till the solvent in the thimble becomes clear indicating the completion of extraction and Shown in Table 2. After the complete extraction, the solvent was distilled off and concentrated on a water bath to a dry residue. Some part of the total extracts was reserved for phytochemical investigation and assessment of antipyretic activity.

Table 2: Showing the percentage yield of various extracts of Anthocephalus cadamba Roxb. Leaves

Extracts	Weight	% yield	Colour
Petroleum ether	27gm	2.7%	Yellowish brown
Solvent Ether	29gm	2.9%	Green
Chloroform	20gm	2.0%	Blackish green
Ethanol	42gm	4.2%	Dark green
Distilled water	45gm	4.5%	Brown

Phytochemical investigation of Anthocephalus cadamba Roxb. Leaves

The various extracts were subjected to qualitative chemical investigation for the identification of the active principles [12], shown in Table 3.

Table 3: Showing the qualitative chemical investigation of Anthocephalus cadamba Roxb. Leaves extracts

Tests / Extracts	Pet. Ether Extract	Ether Extract	Chloroform Extract	Ethanol Extract	Distilled water Extract
Test for sterols					
1. Salkowski's test	-	-	+	+	+
2. Sulphur test	-	-	+	+	+
3. Liebermann Burchards's	-	-	+	+	+
Test for Triterpenoids	-	-	-	-	-
1. Salkowski's test	-	-	-	-	-
2. Libermann-Burchard test					

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Test for Glycosides					
a) Cardiac glycosides					
1. Baljet's test	+	+	+	+	+
2. Keller- Killiani test	+	+	+	+	+
3. Raymond's test	+	+	+	+	+
4. Bromine water test	+	+	+	+	+
5. Legal's test	+	+	+	+	+
b) Anthraquinone glycosides					
1. Borntrager Test	-	-	-	+	+
2. Modified Borntrager test	+	-	-	+	+
c) Saponins glycosides					
1. Foam Test	-	+	+	+	+
2. Haemolysis test	-	+	+	+	+
d)Cynogenetic glycosides					
1. Guignard reaction or sodium picrate test	-	-	-	-	-
2. Test soln. + 3% Aq. Mercurous nitrate soln.	-	-	-	-	-
e)coumarine glycosides					
1)Alcoholic extract + Alkaline	-	-	-	-	+
Test for carbohydrates					
1. Molish's Test	-	-	-	-	-
Test for Alkaloids					
1. Mayer's test	-	-	-	-	-
2. Wagner's test	-	-	-	-	-
3. Dragendorff's test	-	+	+	+	+
4. Hager's test	+	+	+	+	+
5. Murexide test for Purine alkaloids	-	-	-	+	-
Test for Flavonoids					
1. Lead acetate test	-	-	-	+	+
2. Shinoda test	-	-	-	-	-
3. Test soln.+ Excess NaOH	-	-	-	-	-
Test for Tannins and Phenolic compounds					
1. 5% Ferric Chloride test	-	-	-	-	-
2. Gelatin test	-	+	+	+	+
3. Lead acetate	-	+	+	+	+
4. Bromine water	-	+	+	+	+
5. Acetic acid soln.	-	-	-	+	-
6. Potassium dichromate	-	+	+	+	+
7. Dilute Iodine sol	+	+	+	+	+
8. Dilute Nitric acid	-	+	+	+	+
9. Dilute Ammonium hydroxide + Potassium ferrocyanide soln.	-	+	+	+	+
10. Ammonium hydroxide + Excess 10% Silver nitrate, Heat	-	-	-	-	+
Test for Proteins					
1. Biuret test	-	-	-	-	-

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Tests for Gums and Mucilage's					
1. Drug + Ruthenium red					
2. Drug + Water	-	-	-	-	-
3. Drug + Aqueous Potassium	-	-	-	-	-
hydroxide	-	-	-	-	-
Test for Amino acids					
1. Ninhydrine test.	-	-	-	-	-
Test for Fats and Fixed oils					
1. Spot test.	-	-	-	-	-
2. Saponofication test	-	-	+	+	+

Identification of active Principle by Thin Layer Chromatography [13]

Adsorbent – Silica gel G (activated)
Plate size – 20 cm x 8 cm
Plate thickness – 0.2 mm
Solvent system – Chloroform
Spraying agent – Sulphuric acid: Water (1:1)
(For detecting the presence of Sterols)
– Methanol: Conc. Ammonium hydroxide (200: 3)
(For detecting the presence of Alkaloids)
Developing time – At 110^0c for 10 min

The R_f values of Anthocephalus cadamba Roxb. Leaves extracts for Sterols and Alkaloids shown in Table 4.

Table 4: Showing the R_f values of Anthocephalus cadamba Roxb. Leaves extracts for Sterols and Alkaloids

Extracts	Sterols		Alkaloids	
	R_f Value	Colour of Spot	R_f Value	Colour of Spot
Petroleum ether extract	0.46	0.0	Pinkish blue	------
Ether extract	0.56	0.723	Pinkish blue	Brown
Chloroform extract	0.45	0.892	Pinkish blue	Yellowish brown
Ethanol extract	0.42	0.769	Pinkish blue	Yellowish brown
Distilled water extract	0.46	0.0	Pinkish blue	------

Assessment of anti-pyretic activity

Animal selection

Wister strain weighing 150-200gm were used for anti-pyretic models. Rats were kept in polypropylene cages and led on standard laboratory diet and ad libitum. The animals were exposed to 12 hours of darkness and light each. The bedding materials of cages were changed every day. Rats were divided into groups of six.

ISSN: 0975-8585

Antipyretic activity

Yeast induced hyperpyrexia method

A 15% suspension of Brewer's yeast in 0.9% saline was prepared. Seven groups of 6 rats of either sex with body weight of 150-200 gm were used. By insertion of a thermocouple to a depth of 2cm into the rectum the initial rectal temperature were recorded. The animals were fevered by injection of 10 mg/kg of brewer's yeast suspension subcutaneously in the back below the nape of the neck. The sight of injection was massaged in order to spread the suspension beneath the skin. The room temperature was kept at 22-24^{o}c. Immediately after yeast administration, food was withdrawn 18 h. post challenge, the rise in rectal temperature the measurement was repeated after 30 min. Only animal with a body temperature of at least 38^{o}c are taken into the test. The animals receive the test compound or standard drug by oral administration. Rectal temperature was recorded again 30, 60, 120, and 180 min. post dosing [14].

Standard drug selected was Paracetamol 150 mg/kg body weight. The various extracts were dissolved in 10% propylene glycol prior to administration [15].

Seven groups were made each containing six animals

Group I	:	Control
Group II	:	Standard (Paracetamol)
Group III	:	Petroleum ether extract
Group IV	:	Solvent ether extract
Group V	:	Chloroform extract
Group VI	:	Ethanol extract
Group VII	:	Distilled water extract

RESULT AND DISCUSSION

Phytochemical investigation

The results of qualitative chemical investigation of leaves of Anthocephalus cadamba Roxb. were as follows

Petroleum ether extract	: Glycosides, Alkaloids, Tannins, Phenolic compounds.
Solvent ether extract	: Glycosides, Alkaloids, Tannins, Phenolic compounds.
Chloroform extract	: Steroids, Glycosides, Alkaloids, Tannins, Phenolic compounds.
Ethanol extracts	: Steroids, Glycosides, Alkaloids, Tannins, Phenolic compounds, Flavonoids.
Distilled water extract	: Steroids, Glycosides, Alkaloids, Tannins, Phenolic compounds, Flavonoids.

ISSN: 0975-8585

Pharmacological screening

Antipyretic activity

Yeast induced pyrexia method

The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 18 hours of administration. Treatment with various extracts was carried out at dose of 200 mg/kg body weight. The result obtained from both, the standard drug and various extracts were compared with the control group and a significant reduction in yeast induced elevated rectal temperature was observed.

The results obtained from the Yeast induced pyrexia method indicated that Chloroform, ethanol, distilled water extracts have significant onset of action as reduction of temperature by these extracts was found within 30 minutes. Whereas the reduction of temperatures with Petroleum ether and Solvent ether extracts was late. In all extracts the temperatures were reduced to normal till 180 minutes. Even Paracetamol also has significantly reduced rectal temperature to the extent of 37.7^0c from 30 min. to 180 minutes. All the results were compared with Control group.

The details of the results are indicated in the Table 5 and Figure 1.

Table 5: Showing the effect of Anthocephalus cadamba Roxb. Leaves extracts on yeast induced hyperpyrexia method

Group	Rectal Temp ^{0}C		Time after administration				
	Initial	**18 hr after Yeast injection**	30 Min	60 Min	90 Min	120 Min	180 min
Control	37.30 ±0.02	39.90 ±0.03	39.90 ±0.02	39.80 ±0.05	39.80 ±0.04	39.78 ±0.03	39.80 ±0.04
Paracetamol	37.27 ±0.02	39.90 ±0.03	38.93 ±0.02	38.36 ±0.02	37.80 ±0.03	37.72 ±0.02	37.70 ±0.06
Pet. Ether	37.28 ±0.04	39.80 ±0.04	39.62 ±0.02	39.20 ±0.03	38.42 ±0.02	38.22 ±0.06	37.85 ±0.04
Solvent Ether	37.26 ±0.02	39.74 ±0.03	39.12 ±0.02	38.50 ±0.04	38.18 ±0.05	37.50 ±0.04	37.50 ±0.02
Chloroform	37.25 ±0.02	39.71 ±0.06	38.81 ±0.02	37.68 ±0.02	37.36 ±0.06	37.30 ±0.03	37.30 ±0.04
Ethyl alcohol	37.28 ±0.07	39.90 ±0.03	38.80 ±0.05	38.18 ±0.03	37.81 ±0.02	37.60 ±0.03	37.42 ±0.03
Distilled Water	37.30 ±0.02	39.90 ±0.04	38.82 ±0.04	37.90 ±0.04	37.65 ±0.04	37.61 ± 0.02	37.30 ±0.04

The results given are mean±S.E.M.; number of animals used (n=6) P<0.01 Experimental groups were compared with control.

ISSN: 0975-8585

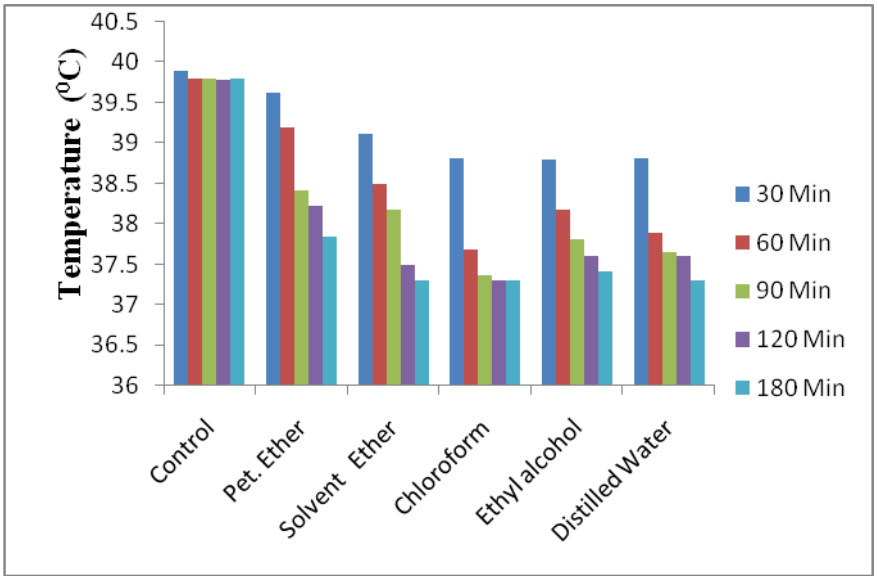

Figure 1: Showing the effects of Anthocephalus cadamba Roxb. Leaves extracts on yeast induced hyperpyrexia method

SUMMARY AND CONCLUSION

In the present study, leaves of Anthocephalus cadamba Roxb. were collected and shade dried. It was reduced to required particle size and then subjected to the successive extraction with the various solvents like Petroleum ether, Ether, Chloroform, Ethanol, Distilled water according to the polarity in soxhlet extractor.

Some part of the various extracts were subjected to preliminary phytochemical investigation, for the identification of various Phytoconstituents and rest of extracts were utilized for pharmacological screening for assessment of antipyretic activity, using following method.

The various extracts after the preliminary phytochemical investigation have shown the presence of following active principles.

Petroleum ether extract	: Glycosides, Alkaloids, Tannins, Phenolic compounds.
Solvent ether extract	: Glycosides, Alkaloids, Tannins, Phenolic compounds.
Chloroform extract	: Steroids, Glycosides, Alkaloids, Tannins, Phenolic compounds.
Ethanol extracts	: Steroids, Glycosides, Alkaloids, Tannins, Phenolic compounds, Flavonoids.
Distilled water extract	: Steroids, Glycosides, Alkaloids, Tannins, Phenolic compounds, Flavonoids.

The antipyretic action of all the extracts was profound; Chloroform, Ethanol and Distilled water extracts gave especial early onset of action as compared to Petroleum ether and Solvent ether extract. All the extracts were compared with the Control group.

Hence, to put into nutshell, the active principle of leaves of Anthocephalus cadamba Roxb. like glycoside, sterols, carbohydrates, saponins may be responsible for the antipyretic activity. However, it needs isolation, structural elucidation and screening of any of the above mention active principle to pin point the activity of drug.

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