

Phytochemical Evaluation and Effect of Antipyretic Activity on *Murraya koenigii* spreng. Leaves Extracts

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ABSTRACT

The main aim of this study is to evaluate antipyretic activity on *Murraya koenigii* spreng. leaves extracts. *Murraya koenigii* spreng. leaves were collected from rural area and extracted with Petroleum ether, Chloroform, Ethanol and the extract was screened for antipyretic activity by Yeast induced hyper pyrexia method. Paracetamol (150mg/kg) was used as standard drug. It was observed that Petroleum ether and chloroform extracts did not show significant decrease in elevated temperature with respect to corresponding control. The Ethanolic extract gives significantly reduced elevated temperature. The present study indicates that *Murraya koenigii* spreng. leaves Ethanol extract possess good significant antipyretic activity as compared to control group, where as Petroleum ether extract, Chloroform extract.

Keywords: *Murraya koenigii* Spreng, Antipyretic, Phytoconstituents, Paracetamol, Evaluation.

INTRODUCTION

Plant derived medicines are useful therapeutic options and often provide a safe form of therapy, and in many instances specific phytomedicines have been shown to be clinically effective. There are many reasons for increased use of herbal medicines. These may range from appeal of product from 'nature' and the perception that such product is safe [1]. Based on the Traditional System of medicine, the use of a crude plant for a particular therapeutic activity, its extraction and fractionation may be carried out. The fractions of extract may prove to be effective, and less toxic for a particular ailment, when compared to an already existing alternative drug [2]. *Murraya koenigii* is genus of tree, native to tropical Asia from Himalaya foothill's of India to Shrilanka eastward through Myanmar, Indonesia, Southern China and Hainan. Leaves are bipinnately compound, 15-30 cm long each bearing 11-25 leaflets alternate on rachis, 2.5 - 3.5 cm long ovate lanceolate with an oblique base. Margins irregularly crenate.

Petioles 2 - 3 mm long [3-4]. Leaves are aromatic and contain proteins, carbohydrates, fiber, minerals, carotene, nicotinic acid and vitamin C. It is rich in vitamin A. and calcium The leaves contain high amount of oxalic acid, leaves also contains crystalline glycosides, carbazole alkaloids, koenigin, resin, fresh leaves contain yellow color 2.5 % volatile oil [5]. It also contains girinimbin, iso-mahanimbin, koenine, koenigine, koenidine and koenimbine [6]. Mahanimbicine and bicyclomahanimbicine, phebalosin, coumarine as Murrayone imperatoxin etc isolated from leaves [7]. Triterpenoids alkaloids cyclomahanimbine, tetrahydromahanmbine also presents in the leaves [8-9]. Murrayastine, murrayaline and many other chemical compounds have been reported in the leaves of *Murraya koenigii* [10]. The leaves and roots are bitter, acrid, cooling, anthelmintic, analgesic, it cures piles, allays heat of the body, thirst, inflammation and itching. It also useful in leucoderma and blood disorders. An

infusion of the toasted leaves in used to stop vomiting [3].

MATERIALS AND METHODS

Apparatus

Soxhlet, Test tubes, Desiccators, Specific gravity bottle, silica dish, Grinder, TLC plate, TLC camber, UV chamber,

Materials

Animal, Petroleum Ether extract, Chloroform extract, Ethanol extract, Paracetamol, Brewer's Yeast.

Methodology

Yeast induced hyperpyrexia method

Procurement and Authentication

The Leaves of *Murraya koenigii* was procured from the rural area of Chopda, Dist- Jalgaon, and authenticated from the Department of Botany, Agharkar Research Institute, pune, The Voucher No. is L-053.

Drying and Size Reduction

The leaves were dried in shade and reduced to coarse power using mechanical grinder and passed through a sieve No. 40 to obtain about powder of desired particle size.

Extraction

The powdered material was subjected to successive hot extraction (soxhlet) with various solvents in increasing order of polarity from Petroleum ether, Chloroform and Ethanol. The extraction was continued till the solvent in the thimble becomes clear indicating the completion of extraction. After the complete extraction, the solvent was distilled off and concentrated on a water bath. Some part of the total extracts was reserved for phytochemical investigation and assessment of antipyretic activity [11].

Preliminary Phytochemical Screening

The extracts were subjected to preliminary phytochemical screening for the detection of

various plant constituents [12], and there results are in Table 2.

Analytical Parameters

Ash values

Weigh accurately 2 gm of air dried seeds in tarred silica dish and incinerate at a temperature not exceeding 450°C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way exhaust the charred mass with hot water, collect the residue and on an ash less filter paper, incinerate the residue and filter paper until the ash is white or nearly so, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450°C. Calculate the percentage of ash with reference to the air dried drug.

$$\text{Ash \%} = \text{Loss in weight} / w \times 100$$

Where w= weight of the seeds powder in grams.

Loss on drying

Accurately weighed 5mg air dried seeds of *Murraya koenigii* Spreng. was taken in a tarred glass bottle and initial weight of sample with bottle was taken. The sample was heated at 105°C in an oven and weighed. This procedure was repeated until a constant weight was obtained. The moisture content of the sample was calculated with reference to air dried drug and the results were taken in Table 3.

$$\text{Loss on drying (\%)} = \text{loss in weight} \times 100 / w$$

Where w= weight of the seeds powder in grams.

Identification of active Principle by Thin Layer Chromatography

Preparation of Plate

The adsorbent used for preparation of thin layer plate (TLC) as a stationary phase was silica gel G. Silica gel G (25gm) in distilled water (35mL) was allowed to swell for about 15-20 minutes in a glass mortar, until it become homogeneous. Then distilled water (15mL) was added with stirring. The silica

gel G suspension was spread with a spreader on thin layer chromatographic plates. The prepared plates were air-dried and activated in an oven at 110° for 30 minutes. The activated plates were kept in desiccators till required.

Application of Sample

For applying test sample on plates, glass capillaries were used. The distance between two spots was kept at minimum of 1 cm. The spots were marked on the top of the plate to know their identity.

Chromatographic Chamber and Condition of Saturation

Chromatographic rectangular glass chamber was used in the experiment. To avoid insufficient chamber saturation and the undesirable edge effect a smooth filter paper approximately 15x40cm was placed in the chromatographic chamber (in a 'U' shape) and soaked in the solvent. The moistened filter paper was pressed to the chamber walls so that it adhered to the walls. The chamber was allowed to saturate for sufficient amount of time before use. The experiments were carried out at room temperature in diffused daylight.

Solvent System

Numbers of solvent systems were tried; the solvent system with satisfactory resolution was obtained by using following solvent systems.

Detection of Spot

Observe the plates under UV chamber, marking of colored spots and calculate the R_f values [13], and there results are in Table 4.

Acute Toxicity Study

Acute toxicity study was carried out according to OECD guidelines (Organization for economic co-operation and development), revised draft guidelines 423, revised from Committee for the Purpose of Control and Supervision of

Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The extracts were suspended in saline. The extracts were administered at a dose level of 500, 1000 and 2500 mg/kg body weight, to groups of 4 animals. After administration of extracts the rats were observed for gross behavioral, neurological, autonomic and toxic effects. The toxicological effects were observed in terms of mortality. No death occurred within 24 h of dose of 500, 1500 mg/kg but at a dose of 2500 mg/kg 50% mortality was observed. As dose was increased further up to 5000 mg/kg, at that dose all the animals were died. Hence 2500 mg/kg dose was considered as LD_{50} . $1/10^{th}$ of the LD_{50} was considered as an effective dose i.e. 250 mg/kg [14].

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°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°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, 90 and 120 min. post dosing [15] Standard drug selected was Paracetamol 150 mg/kg body weight. The various extracts were dissolved in 10% propylene glycol prior to administration [16], and there results are in Table 5.

Group I: Served as control and received 1mL, water p.o.

Group II : Treated with Carrageenan only.

Group III : Standard group Ibuprofen 50mg/kg p.o.

Group IV : Petroleum ether extract 250 mg/kg.

Group V : Chloroform extract 250 mg/kg.

Group VI : Ethanol extract 250 mg/kg.

Statistical Analysis

Data generated during the above investigations were subjected to appropriate statistical tests to decide the significance of the differences between the groups. $P < 0.05$ was considered significant in all cases.

RESULTS AND DISCUSSION

- Pet. ether extract : Gums, mucilage, fats, oil, caumarin glycosides, flavonoids, Phenolic compounds.
- Chloroform extract : Carbohydrates, gums, mucilage, Anthraquinone glycosides, Saponins, flavonoids, tannins and phenolic compounds.
- Ethanol extract : Carbohydrates, gums, mucilage, proteins, sterols and Triterpenoids, cardiac glycosides, alkaloids, flavonoids, Phenolic compounds.

Ethanol was subjected to thin layer chromatography for detecting the presence of alkaloids. The ethanol extract showed good significant reduction in elevated temperature as compared to control group, where as Petroleum ether extract, Chloroform extract, has showed comparatively less significant reduction in elevated temperature. In the view of the observations made in the present work also claims made in traditional systems of medicine, it can be said that *Murraya koenigii* Spreng. is a safe antipyretic agent.

Table 1: Showing the Percentage yield of petroleum ether, chloroform and ethanol extracts

Solvent	Nature of Extract	%Yield
Pet. Ether	Semisolid	3.9
Chloroform	Semisolid	3.1
Ethanol	Semisolid	6.3

Table 2: Showing the qualitative chemical investigation

of *Murraya koenigii* Spreng. Leaves extracts

Extracts	Constituents
Petroleum ether	Gums, mucilage, fats, oil, coumarine, glycosides, flavonoids, Phenolic compounds.
Chloroform	Carbohydrates, gums, mucilage, anthraquinone glycosides, Saponins, flavonoids, tannins and phenolic compounds.
Ethanol	Carbohydrates, gums, mucilage, proteins, sterols and Triterpenoids, cardiac glycosides, alkaloids, flavonoids, phenolic compounds.

Table 3: Showing the ash Values

Analytical parameters	% w/w
Total ash	0.92 %
Acid insoluble ash	1.775 %
Water soluble ash	6.205 %

Table 4: Showing R_f values in different Solvent Systems

Solvent System	Spot No.	R _f Values
Benzene: Chloroform (1:1)	1	0.06
	2	0.1
	3	0.46
Benzene:Chloroform:Ethanol (10:15:1)	1	0.594
	2	0.625
Benzene:Chloroform:Ethanol (2.8:5.7:1.5)	1	0.52
	2	0.67
	3	0.81
	4	0.86
	5	0.89
Toluene:Chloroform:Ethanol (5.1:12.4:2)	1	0.59
	2	0.62
Toluene:Chloroform:Ethanol (3:12:2)	1	0.60
	2	0.65

This system gives the several spots but out of which one of the spot gives the specific R_f value nearby 0.60 it may be indizoline alkaloid. So this plant may contain the carbazole alkaloids which are already reported in literature.

Table 5: Showing antipyretic activity of extracts

Time in Min.	Control Treated	Standard	Petroleum ether extract	Chloroform extract	Ethanolic extract
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
0.00	33.10 ± 0.21	35.92 ± 0.24	36.30 ± 0.24	35.50 ± 0.25	35.20 ± 0.21
30	33.80 ± 0.23	35.10 ± 0.23	35.48 ± 0.25	35.22 ± 0.24	34.70 ± 0.24
60	34.60 ± 0.28	34.52 ± 0.23	35.45 ± 0.25	35.20 ± 0.22	34.32 ± 0.21
90	35.10 ± 0.29	33.86 ± 0.27	35.43 ± 0.26	35.05 ± 0.24	34.70 ± 0.19
120	35.70 ± 0.21	33.58 ± 0.35	35.40 ± 0.25	34.80 ± 0.23	33.72 ± 0.25

It was observed that Petroleum ether and chloroform extract did not show significant decrease in elevated temperature with respect to corresponding control. The Ethanolic extract gives significantly reduced elevated temperature.

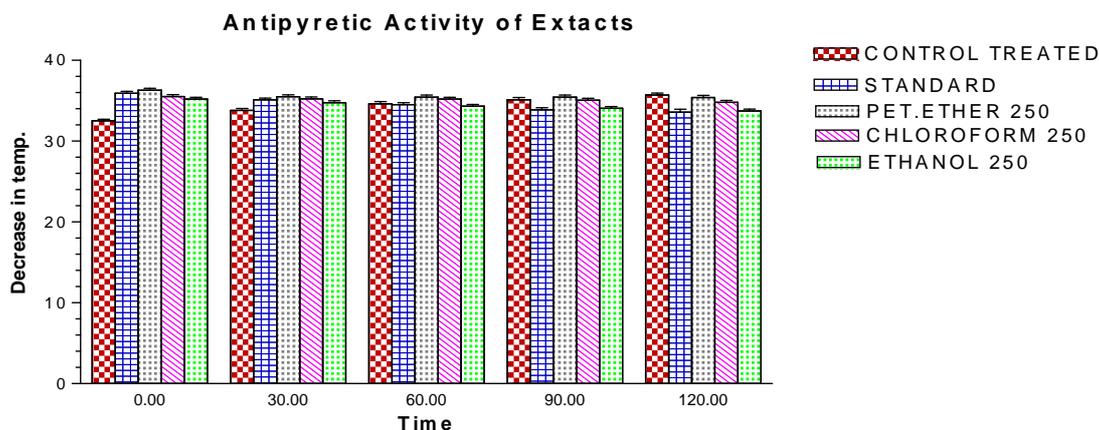


Fig. 1: Showing effect of *Murrya koenigii spreng.* leaves extracts

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