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Research Article

Investigation and study of anti-nflammatory activity of *murraya koenigii* spreng. Leaves

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ABSTRACT
This work has been done for the investigation and study of anti-inflammatory activity of solvent extract of dried leaves of *Murraya Koenigii* Spreng. The leaves, root and bark are tonic, stomachic, inflammation, itching and carminative. Internally in dysentery also checking vomiting. For investigation the activity it includes preliminary phytochemical investigation of extracts and further screening them for possible anti-inflammatory potential by using carrageenan induced paw edema model in albino Wister rats (150-200g) Ethanolic extract shown good significant in anti-inflammatory activity. Where as Ibuprofen is standard drug. Thus our investigation suggests a potential benefit of ethanol extract of leaves of *Murraya Koenigii* in treating condition associated with inflammation.

Key words: *Murraya Koenigii* Spreng., Anti-inflammatory, Phytoconstituents.

INTRODUCTION

Since the origin of man on earth, man has been using plants and herbs as a food and then as a medicine. In 19th century research has been developed and life saving drugs has been isolated from these plants and herbs.[1] It has been estimated that from 25000 to 75000 species of higher plant, about 10% has been used in traditional medicine.[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.[3-4] 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 creative. Petioles 2 - 3 mm long.[3,5] 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, glycosides, carbazole alkaloids, koenigin, resin, fresh leaves contain yellow color 2.5 % volatile oil.[4] girinimbin, iso-mahanimbin, koenine, koenigine, koenidine and koenimbine.[6-7]Triterpenoid alkaloids cyclomahanimbine, tetra hydromahanmbinealso.[8-9] 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. The leaves and roots are bitter, acrid, cooling, anthelmintic, analgesic, leucoderma and blood disorders. An infusion of the toasted leaves in used to stop vomiting. The plant is credited with tonic and stomachic property.[5] The branches of *Murraya koenigii* are very popular for cleaning the teeth as *datun.*[10]

MATERIAL AND METHODS

Plant Material: The leaves of *Murraya Koenigii* Spreng. were collected from local areas of Chopda Maharashtra and authenticated by Department of Botany, Agharkar Research Institute, pune, The Voucher no. is L-053. 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.

Preparation of Leaves Extract[11]: The powdered material had been planned to charge into soxhlet apparatus to carry out exhaustive extraction using Petroleum ether (40-60˚C), Chloroform and Ethanol. The drug had been planned to extract with solvent till complete extraction affected. Then extract had been planned to concentrate by distilling of the solvent to obtain the crude extract. The color, consistency and percentage extractive values had been calculated. They are showing in Table No. 1

Preliminary Phytochemical Screening of Extracts[12] : The extracts were subjected to preliminary qualitative chemical analysis shows in Table No.2.

Analytical Parameters[13]**:**The extracts were subjected to analytical parameters shows in Table No.3.

Identification of Active Principle by Thin Layer Chromatography[13]: The extracts were subjected to identification of TLC shows in Table No.4.

Pharmacological Screening[14]: Animal Used: Acute oral toxicity study was carried out suing Albino Wister rat of weighing between 150 and 200 g. Rat were kept in

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Figure No. 1: Whole Leaf of *Murraya Koenigii* Spreng

Table No. 1: The percentage yield of *Murraya Koenigii* Spreng. Leaves extracts.

Sr. No.	Solvent	Nature of Extract	Color	%Yield
1	Pet. Ether (40-60˚C)	Semisolid	Greenish black	3.9
2	Chloroform	Semisolid	Dark green	3.1
3	Ethanol	Semisolid	Green	6.3

polypropylene cages and fed on standard laboratory diet. The bedding material of cages was changed everyday.

Materials Used: Extracts {Petroleum ether (40-60^{0}C), Chloroform and Ethanol} 250 mg/kg, Ibuprofen was used as standard (50 mg/kg), Carrageenan as phlogistic agent (irritants) and 1 % tween 80 as suspending agent.

Acute Toxicity Studies[15] : 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}. 1/10th of the LD_{50} was considered as an effective dose i.e. 250 mg/kg.

Carrageenan Induced Paw Edema Model in Rat[16]: Thirty minutes after drug or test compound administration, 0.1 mL. of 1% carrageenan in distilled water was injected into the subplantar region of right hind paws of all groups. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema volume was measured with the help of plethysmographic by mercury displacement method, at zero hours. (Immediately after injecting carrageenan).

The same procedure was repeated at 30 mins. 1, 2, 3 hours. The difference between 1 hours and subsequent hours reading was taken as actual edema volume. The percentage inhibition of paw edema in the various treated groups was then calculated by using the formula;

Percentage inhibition = (1 – Vt/Vc) X 100

Where Vt = is the edema volume in the drug treated group.

V = is the edema volume in the control group.

Group I : Served as control
Group II : Treating with Carrageenan
Group III : Standard group Ibuprofen 50 mg/kg
Group IV : Petroleum ether extract 250 mg/kg
Group V : Chloroform extract 250 mg/kg
Group VI : Ethanol extract 250 mg/kg

STATISTICAL ANALYSIS

The mean values ± SEM of changes in paw volume on 1, 2, 3 hr. after induction were evaluated for statistical significance by one-way ANOVA test followed by

Table No. 2: The qualitative chemical investigation of *Murraya Koenigii* Spreng. Leaves extracts

Test	Pet. Ether Extract	Chloroform Extract	Ethanol Extract
Tests for Carbohydrates			
a) Molish's test	+	+	+
b) Fehling's test	+	+	+
c) Benedict's test	-	+	+
d) Barfoed's test	-	-	-
e) Bial's orcinol test	-	-	-
f) Aniline acetate test	-	-	-
g) Phloroglucinol test	-	-	-
h)Tollen's phloroglucinol test for galactose	-	-	+
Test for Gums			
a) Fehling's test	+	+	+
b) Benedict's test	+	+	+
Test for Mucilage			
a) Powdered drug + ruthenium red.	-	+	+
b) Powdered drug + H_2O/ aq.KOH	+	+	+
Tests for Proteins			
a) Biuret test (General Test)	-	+	+
b) Millon's test	-	-	+
c) Xanthoprotein test	-	-	+
d) Test for proteins containing $_{SO4}$	-	-	-
Tests for amino acids			
a) Ninhydrin test	+	+	+
b) Test for tyrosine	-	-	+
c) Test for tryptophan	-	-	-
d) Test for cysteine	-	-	-
Test for Fats and oils			
a) Solubility Test:	+	+	+
b) Saponification Test	+	-	-
Test for Sterols and Triterpenoids			
a) Salkowski's test	-	-	-
b) Liebermann-burchards's test	-	-	+
c) Liebermann test	-	-	+
Tests for Cardiac Glycosides			
a) Legal's test	-	-	+
b) Keller-killiani test	-	-	+
Tests for Anthraquinone Glycosides			
a) Borntrager's test	-	+	-
b) Modified borntrager's test for C-Glycosides	-	+	+
Tests for Coumarin Glycosides			
a) Aromatic odor.	+	-	+
b) Alkaline test	+	-	-
Tests for Alkaloids			
a) Mayer's test	-	-	-
b) Wagner's test	-	-	+
c) Hager's test	-	-	+
d) Dragendorff's test	-	-	+
Tests for Saponins			
a) Foam test	-	+	-

(+) Present (-) Absent

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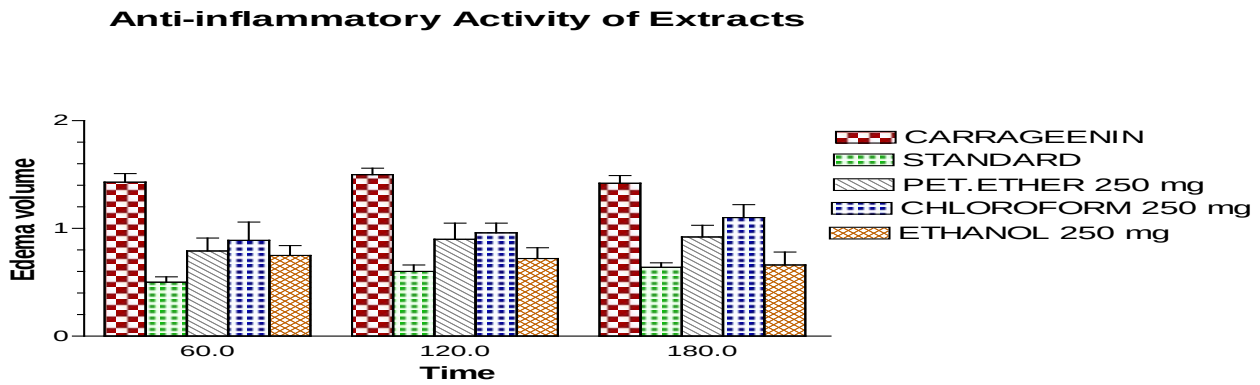

Figure: No. 2: Anti-inflammatory activity of *Murraya Koenigii* Spreng. Leaves extracts by using carrageenan induced rat paw edema method

Table No. 3: The Analytical Parameters of *Murraya Koenigii* Spreng

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

Table No. 4: R_f Values in Different Solvent Systems.

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

Table No. 5: The effect of *Murraya Koenigii* Spreng. Leaves extracts on carrageenan induced rat paw edema method.

Group No.	Treatment	Dose	1hr	2hr	3hr	Average reading	% Inhibition
			Mean± S.D.	Mean± S.D.	Mean± S.D.		
1	Carrageenan	0.1mL. 1% sol.	1.43±0.08	1.50±0.06	1.42±0.04	1.45	---
2	Ibuprofen	50mg/kg	0.50±0.05	0.60±0.06	0.64±0.04	0.58	60
3	Pet. Ether extract	250mg/kg	0.79±0.12	0.92±0.15	0.96±0.11	0.89	39
4	Chloroform extract	250mg/kg	0.89±0.17	0.90±0.09	1.10±0.12	0.93	36
5	Ethanol extract	250mg/kg	0.75±0.09	0.72±0.1	0.66±0.12	0.71	52

student-Newman Keul's post test. Differences of $P<0.05$ were taken as statistically significant.

RESULTS AND DISCUSSION

The Table No. 1 Shows the successive solvent extraction like different extracts petroleum ether (40-60^{0}C), chloroform, and ethanol of 3.9g, 3.1g and 6.3g respectively. Table No. 2 on qualitative chemical investigation of *Murraya Koenigii* Spreng. has indicated the presence of Carbohydrates, Alkaloids, Glycoside, Amino acid, flavonoids and Proteins. Sterols, fats, oil, Tannins, Gum, Mucilage, Saponins, Triterpenoids and Phenolic Compound. Table No. 3 Shows the analytical parameters. Table No. 4 shows the TLC values in different solvent system.

The anti-inflammatory activity of various extracts of *Murraya Koenigii* Spreng. leaves was assessed by carrageenan induced rat paw edema shows in Table No. 5 on the carrageenan- induced rat paw edema indicated that, ethanol extract shown good significant ($p<0.001$) reduction in paw edema to the extent of 52.7% at 250 mg/kg concentration, respectively from 3rd hour when compared to control group However, petroleum ether (60-80^{O}c) extract, chloroform extract has reduced the paw edema to the extent of 39% and 36% respectively at 250 mg/kg concentration when compared with control group. Where as Ibuprofen also significantly ($P<0.001$) reduced paw edema form 3rd hours when compared to control group.

CONCLUSION

It appears from the study that the *Murraya Koenigii* Spreng. Leaves ethanol extract (250 mg/kg) of treated group showed good significant anti-inflammatory activity as compared to standard group, where as petroleum ether extract and chloroform extract group showed anti-inflammatory activity.

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