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Research Article

EXTRACTION AND ISOLATION OF GLYCOSIDE MADLONGISIDE-A FROM BARK OF *MADHUCA LONGIFOLIA* AND ITS PHARMACOLOGICAL SCREENING

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ABSTRACT
The study was carried out to identified the presence of isolate compound madlongiside-A from bark parts of *Madhuca longifolia* by TLC, IR,NMR and chemical test. Further single compound can be screened in anti inflammatory and analgesic activity by rat model. As such no pharmacological activity can be done previously and the result shown that the identified isolated compound have dose dependent activity.

KEY WORDS: *M.longifolia* bark, madlongiside-A, anti inflammatory, analgesic.

INTRODUCTION

Buttercup tree or mahua, *M.longifolia* Family, Sapotaceae , is a large shady tree both wild and cultivated in all over INDIA. The tree is valued for its seeds, flower and bark. The tree is approx 10-15 m tall, bark is rough, brown in color, inner bark red. The astringent bark extract is used for dental-related problems, rheumatism, and diabetes.

Previous phytochemical studies reported that *M.longifolia* seeds included fatty acids, lipid soluble bioactives, sapogenins, carbohydrates, triterpenoids, saponin, flavonoids and glycoside.

MATERIAL AND METHODS

Animals

Wistar rats of both sexes (150-250 g) were maintained under standard animal house condition (Reg no-1254/ac/09/CPCSEA), fed standard pellet diet (ZRC, Ahmadabad) and allowed water ad libitium. Fasted animals were deprived of food for at least 16 h, but were allowed free access to water. The study was approved by the CPCSEA and & Institutional Animal Ethics Committee (IAEC) of B.Pharmacy College, Rampura, kakanpur,Godhra.

Collection and authentication of plant:

The fresh bark and seeds of plant *Madhuca longifolia* (Koenig) were collected from Trichy, Tamilnadu, in the month of January. The plant bark were authenticated by Prof. P.Jayaraman, Director, Plant Anatomy Research Centre (PARC), Medicinal Plant Research Unit, west Tambaram, Chennai. This plant is continuing for further pharmacological screening.

After authentication the bark sample were dried at room temperature until they were free from moisture. The bark was subjected to size reduction to get coarse powder of desired particle size. The coarse powder was then stored in a clean dry air tight container.

Phytochemical studies [4,5,6]

The air-dried bark(4.5 kg) of *M. longifolia* were defatted with petroleum ether(boiling point range 60-80°C). The powdered material was subjected to maceration using different solvent (methanol/acetone/hexane etc.) for seven days. The extract was filtered reddish brown syrupy mass was obtained and it was finally dried at low temperature under reduced pressure in a rotary evaporator (microwave oven). The residue (370 g/430 g) was suspended in water and extracted successively with methanol and acetone (3× 1 L, each). Solutions were evaporated to dryness in vacuo to provide (only bark extract) alcoholic soluble (55 g), acetone soluble(90 g) and water-soluble portion(180 g).

Qualitative chemical identification can be done by for flavonoids (shinoda test, flurescence test, *FeCl3 test, Lead acetate solution test)* Glycosides (*Modified Borntrager's Test, Legal's Test, Balget Test, Killer killani Test)* Saponins(*Froth's Test).* Chromatographic examination can done by TLC and preparative TLC plate.

The yellow coloured crystaline compounds isolated by preparative TLC method at R_f – 0.86 and 0.78 from the methanol extract of *M.longifolia* were subjected to general chemical tests for flavonoids/saponin and TLC studies. The melting point of isolated compounds were taken and then they were characterized with the help of instrumental techniques like UV spectroscopy, IR spectroscopy, and NMR spectroscopy

Anti inflammatory study [7,8]

Male wistar rats (195-250g) were used, and the rats were to be be fasted with free access to water at least 16 hr prior to experiments. The test compound suspended in 1 mL of 0.5% methyl cellulose and 0.025% Tween 20 (Sigma), dosed orally via an 18-G gavage needle at 2 hr prior to induction of inflammation. Edema will then induced by injection of 0.1 mL of 1% carrageenan suspension in sterile saline via a 27-G needle into the plantar tissue of the right hind footpad. Paw volume will measured by a displacement plethysmometer at 3 hour after injection of carrageenan, and the mean inhibition values will be determined on the basis of an average of rats (experimental design shown in table).

Analgesic study [8,9,10]

Hot-plate method

The reaction time is measured to the nearest fifth of a second,10 and 5 minutes before s.c inj or oral administration of a drug and at 10,20,30,45,60,90,120,180 and 240 min thereafter. The volume of solution used is 0.1 ml per 10 gm of body weight. The normal time is the average of the reaction times 10 min and 5 min before giving the drug. The

response is considered positive when the reaction time after injection is longer than 30 sec at least once or when three or more readings exceeds the normal reaction time by a factor of at least three.

RESULTS & DISCUSSION

The methanol extract bark of *M.longifolia k.* prepared by maceration solvent extraction technique was used to isolate saponin.10µl of the methanol extract was spotted on TLC plate. It was developed in Toluene: Ethyl acetate: Acetone: Formic acid (5: 2.5: 7.5: 0.5) mobile phase. The plate was observed spraying with anisaldehyde-sulphuric acid reagent and heating at 100˚C till the colored bands appear. The R_f values and color of the resolved bands are shown.

Graded doses of the C1 of *M.longifolia* bark varying from 4.0 mg to 64.0 mg/kg increased significantly in dose dependent manner the time taken for withdrawal of tail in rats shown as area under the curve.

Carrageenen induced rat paw oedema is used widely as a working model of inflammation in the search of new anti inflammatory drugs. The present study indicates the development of oedema in the paw of rat after the injection of carrageenan is due to release of histamine and prostaglandins like chemical mediators. Significantly high anti inflammatory activity of compound 1 may be due to presence of inhibitory substance in plant seeds or inhibitory potential activity of the C1, which inhibit the release of chemical mediators.

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Table:-1. Anti-inflammatory activity of isolated compound-1 from *Madhuca longifolia* bark.

Group	Treatment	Increase in paw volume (ml)			
		1 h	2 h	3 h	4 h
I	Control vehicle	0.43±0.07	0.73±0.01	0.87±0.31	0.77±0.41
II	C-1(100µg/kg)	0.39±0.23	0.33±0.11	0.26±0.10	0.22±0.15
III	C-1(200 µg/kg)	0.30±0.16	0.26±0.09	0.23±0.14	0.19±0.09
IV	C-1(300 µg/kg)	0.25±0.07	0.22±0.04	0.18±0.07	0.14±0.08
V	Diclofenac sodium(100 µg/kg)	0.20±0.03	0.18±0.02	0.15±0.09	0.12±0.01

Each value is represented as mean± SEM, No of animals (n) = 6-8,

Table:-2. Analgesic activity of isolated compound-1 from *Madhuca longifolia* bark.

Dose (mg/kg, *i.m*)	Area under curve (in cm^2 ±S.E.)	Area under curve (in cm^2 ±S.E.)
	Normal	Diclofenac
Saline	48.22 ± 0.78	53.66 ± 0.26*
C-1 of *M.longifolia*		
4	49.36 ± 0.05	—
8	55.88 ± 0.72*	—
16	56.18 ± 0.68**	73.66 ± 0.31** xx
32	87.44 ± 0.70**	91.20 ± 0.18** xx
64	83.05 ± 0.73**	—

Each value is represented as mean± SEM, No of animals (n) = 6-8,

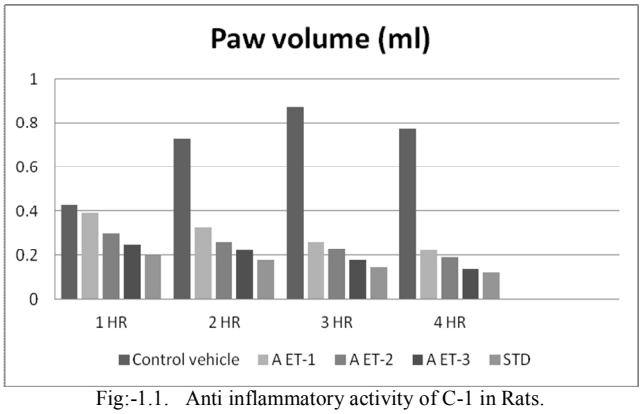

Fig:-1.1. Anti inflammatory activity of C-1 in Rats.

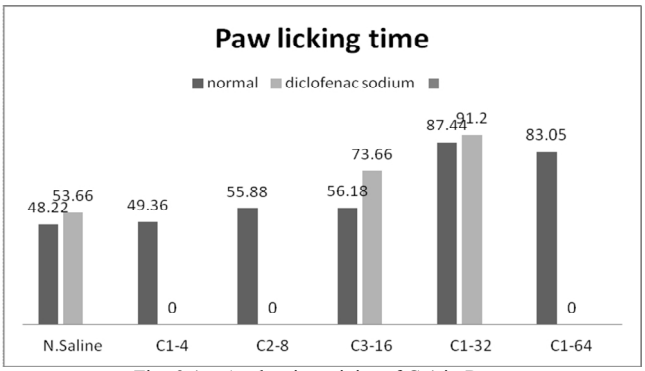

Fig:-2.1. Analgesic activity of C-1 in Rats.

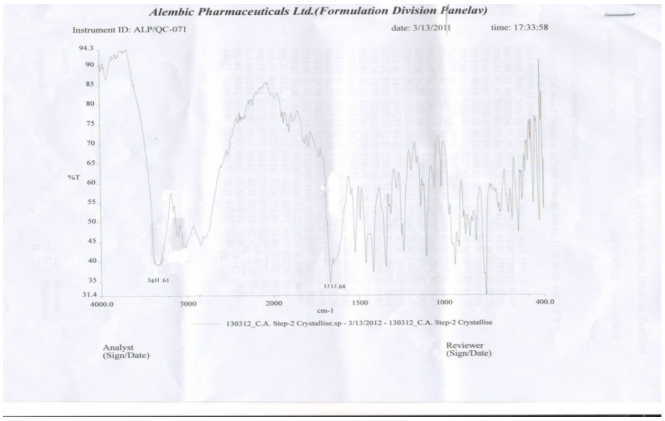

Fig:- IR graph of isolated C -1 compound.

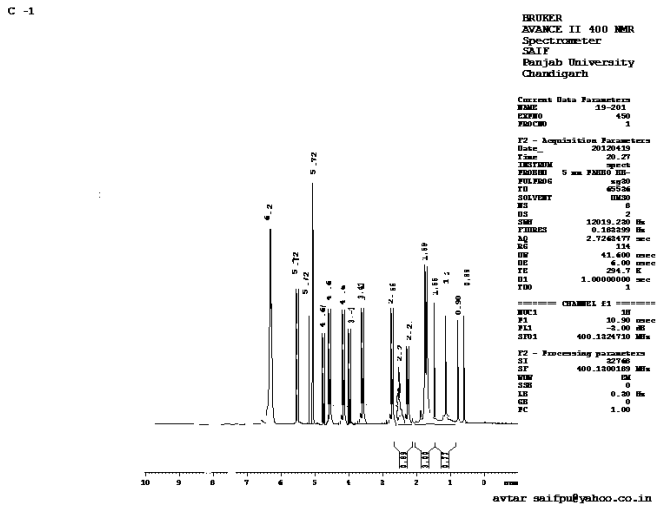

Fig:- NMR spectra of compound C-1

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