PHARMACOGNOSTIC STANDARDIZATION, PHYTOCHEMICAL INVESTIGATION AND ANTHELMINTIC EVALUATION OF THE EXTRACT OF *MADHUCA INDICA J. F. (GMEL)* FLOWERS

Swati Katiyar,* Manisha Tandon , Amol Chandekar and Neeraj Upmanyu

R.K.D.F. College of Pharmacy, Hoshangabad Road, Bhopal (M.P.)-462047 India

***Corresponding Author**

Swati Katiyar, R.K.D.F. College of Pharmacy,

NH-12, Hoshangabad Road, Bhopal (M.P.)-462047

Email: drneerajupmanyu@gmail.com

Summary

Pharmacognostic evaluation and preliminary phytochemical investigation on flower of *Madhuca indica* J. F. Gmel was carried out using macroscopy, fluorescence analysis, extractive value, successive solvent extraction and preliminary phytochemical screening by chemical tests. The ethanol and methanol extract of the flowers of *Madhuca indica* J. F. Gmel (Sapotaceae) was investigated for its possible anthelmintic activity in *Pheretima posthuma (*Indian Earth Worm). Three concentrations (20, 40 and 60 mg/ml) of each extract were studied in a bioassay, which involved the determination of time of paralysis and time of death of the worms. Both extracts showed significant anthelmintic activity but methanolic extract demonstrated the best anthelmintic activity in both the parameters. Mebendazole was included in the assay as standard reference drug.

Keywords: *Madhuca indica (Gmel)*, Mebendazole, Ethanol, Methanol.

Introduction

Helminthiasis or worm infestation, is one of the most prevalent disease and one of the most serious public health problems in the world. Hundreds of millions if not billions of human infections by helminthes exist worldwide and increased due to world travel and immigration from the developing countries [1]. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, increasing problems of development of resistance in helminths [2-3] against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity.

The Plant *Madhuca indica (Gmel)* flower belongs to the family Sapotaceae commonly knowm as Mahua. It is a large deciduous tree reaching 20 meters in height with a spreading crown. This plant is economically important because of the role it plays in yielding country liquor, edible succulent corollas and oil from the seeds [4-5]. Kernels of green- color egg size fruits of Mahua (*Madhuca indica Gmel.*) contained 55-65 % of soft yellow oil that is widely used locally for cooking and tallow [6]. Mahua flowers are regarded as cooling tonic and demulcent and are used in coughs , colds and bronchitis [7] *Madhuca indica* flower is traditionally considered as tonic, both nutritional and cooling and also in treatment of helminthes, acute and chronic tonsilitis, pharyngitis [8] as well as bronchitis [9]. The medicinal properties attributed to this plant are stimulant, demulcent, laxative and astringent. The bark is good remedy for itch, swelling, fracture and snake bite poisoning, internally employed in diabetes mellitus. Its bark is used to cure leprosy and wounds. Its flowers are prepared to relieve coughs and heart-trouble while its fruits are given in cases of blood diseases [10-11].

The aim of the present study is to investigate the Pharmacognostic, phytochemical study and anthelmintic evaluation of *Madhuca indica (Gmel)* flower.

Material and Methods

Plant Collection and Authentication

The flowers of *Madhuca indica* Flower were collected in the month of January 2011 from the garden of Bharatpur, Jalaun District, Uttar Pradesh and were authenticated by Dr. Anil Prakash, Dept. of Biotechnology, Barkatullah University, Bhopal (M.P.).

Preparation of Plant Extract

The flowers of plant were washed with tap water to remove soil and other adhering dirt under shade. Flowers were converted into moderately coarse powder by mechanical grinder. The coarsely powdered plant material (about 50gm) was defatted with Petroleum Ether (40-60°C). Complete defatting of drug was ensured by placing a drop from the thimble on a filter paper to give any oily spot. The deffated plant material was again subjected to continuous extraction with ethanol and 95% methanol. The solvent was removed in vaccum at 40°C by using rotatory evaporator (Rota Evaporator Buchi Type) which gave brown color of both ethanol and methanol extracts. The percentage yield was 45% w/w in ethanol and 0.965% w/w in methanol.

Table 1 :- Extractive values of different extracts of *Madhuca indica (Gmel)* flower

S.N.	Solvent	Color of the extract	Yield of the extract (in gm)	Percentage yield(%w/w)
1.	Ethanol	Dark brown	22.5	45%
2.	Methanol	Brown	00.42	0.965%

Worms Collection and Authentication

Indian earthworm *Pheritima posthuma* (Annelida) were collected from the water logged areas of soils. Indian earthworms were identified at Department of Zoology, Barkatullah University, Bhopal (M.P.).

Pharmacognostic Studies

Macroscopy

The following macroscopic characters for the flower were noted: size, shape, color, surface, corolla, texture, odor, taste [12].

Physico-chemical Parameters

Physico-chemical values such as the percentage of total ash, water soluble ash and loss on drying, water soluble extractive and alcohol soluble extractive values were calculated as per the Indian Pharmacopoeia [13].

Fluorescence Analysis

Fluorescence characteristics of the powered drug with different chemicals were observed in day light and ultraviolet light. Various solvent extracts were also subjected to day light and ultraviolet light for its fluorescence characteristic. The powdered flower was treated with various solvents like concentrated sulphuric acid, concentrated hydrochloric acid, solution of nitric acid and ammonia solution, concentrated sodium hydroxide, iodine solution, 50% nitric acid v/v, ammonia solution and observed under day light and also in UV at 254 nm and 366 nm. The fluorescence analysis of the ethanol and methanol extracts of *Madhuca indica (Gmel)* flower were observed under day light and also in UV at 254 nm and 366 nm. These fluorescence analyses were observed and recorded [14-16]. (Table 3)

Preliminary Phytochemical Screening

The extracts thus obtained were subjected to preliminary phytochemical study following the methodology of Khandelwal (2008) and Kokate (2005). The results obtained in the present investigation (Table 4) ethanol and methanol extract of the flower of *Madhuca indica (Gmel)* showed the presence of alkaloid, tannin and phenolic compound, protein and carbohydrate.

Anthelmintic Activity

The anthelmintic activity was carried out on adult Indian earthworms, *Pheretima posthuma* in view of its physiological resemblance with the intestinal round worm parasites in human beings [18]. The anthelmintic assay was carried out as per the method of Patil *et al*. 2010 with minor modifications. Group of test organisms each containing six earthworms of approximately equal size were released into 10 ml of desired preparation. The dose suspensions were prepared using Carboxy Methyl Cellulose Sodium (1% CMC), which is nontoxic and nonirritant used in oral and other formulations. Each group was treated with the following vehicles (1% CMC in normal saline) and solution of ethanol and methanol (20, 40, 60 mg/ml each) extracts of *Madhuca indica Gmel* flowers. Mebendazole (20, 40, 60 mg/ml in 1% CMC) was used as standard reference. Observations were made regarding the time taken for paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C) [19].

Results and Discussion

Macroscopic Study

The color, odour, taste, surface and corollas of flower of *Maduca indica* were reported in Table 2.

Table 2 : Organoleptic study of *Madhuca indica* flowers

S.N.	Morphological characters of flower	*Madhuca indica*
1.	Color	Brown
2.	Odour	Sweet
3.	Taste	Sweet
4.	Surface	Longitudinal
5.	Corollas	Fleshy

Physical Evaluation

The loss on drying, total ash ,water soluble ash, crude fibre, alcohol soluble extractive, water soluble extractive of flower powder of *Madhuca indica* were reported in Table 3.

Table 3: Physicochemical parameters of flowers of *Madhuca indica*

S. No.	Parameters	Values obtained(%w/w)
1.	Loss on drying	18%
2.	Total ash value	0.184%
3.	Water soluble ash	0.080%
4.	Crude fibre content	15.5%
5.	Alcohol soluble extractive	0.680%
6.	Water soluble extractive	0.664%

Fluorescence Analysis

Many drugs exhibit fluorescence when their powder is exposed to ultraviolet radiation. It is important to observe that all materials on reaction with different chemical reagents under U.V. light show fluorescence. The fluorescence characteristics of powdered drug and different extracts were studied under day light and U.V. light after treating with different chemical reagents and reported in Table 4 and 5.

Table No 4: Fluorescence analysis of powdered *Madhuca indica (Gmel)* flower

S.No.	Flower powder	Visible light	Under UV light	
			254 nm	366 nm
1	Conc. Sulphuric acid	Green	Light green	Dark Brown
2	Conc. Hydrochloric acid	Green	Whitish	Light Brown
3	Solution of Nitric acid and Ammonia	Green	Whitish	Light Brown
4	Conc. Sodium hydroxide	Greenish	Whitish	Light Brown
5	Iodine solution	Greenish	Brown	Brown
6	50% Nitric acid v/v	Greenish	Whitish	Whitish
7	Ammonia solution	Greenish	Whitish	Light Brown
8	Ethanolic Sod. Hydroxide	Dark Brown	Yellowish	Brown
9	Ethanol	Dark Brown	Yellowish	Brown
10	Pet. Ether	Dark Brown	Yellowish	Brown
11	Methanol	Dark Brown	Whitish	Brown

Table No 5: Fluorescence analysis of the different extracts of *Madhuca indica (Gmel)* flower

S. No.	Extract	Visible light	Under UV light	
1	Ethanol	Dark brown	Parrot green	Green
2	Methanol	Light brown	Sea green	Green

Preliminary Phytochemical Tests

Preliminary phytochemical tests of *Madhuca indica* flowers showed the presence of phytochemicals in Table 6. In this table, ethanolic extract of *Madhuca indica* showed the presence of alkaloids, tannins, proteins, carbohydrates. Methanolic extract showed the presence of alkaloids, tannins, carbohydrates. Some of these phytoconstituents may be responsible for a potent anthelmintic activity.

Table No 6: Qualitative Phytochemical analysis of the extracts of *Madhuca indica (Gmel)* flowers

S.No.	Phytoconstituents	Tests	Ethanolic Extract	Methanolic Extract
1.	Alkaloids	Tannic acid test	+ve	+ve
2.	Tannins	Lead acetate solution test	+ve	+ve
		Acetic acid solution	+ve	+ve
3.	Proteins	Xanthoprotein test	+ve	-ve
		Biuret test	+ve	-ve
4.	Flavonoids	Alkaline reagent test	-ve	-ve
5.	Carbohydrates	Molisch's test	+ve	+ve
		Test for pentose's	+ve	+ve
6.	Amino acids	Ninhydrin reagent test	-ve	-ve
7.	Volatile oil	Sudan red III test	-ve	-ve

(+ve): Present, (-ve): Absent

Thin Layer Chromatography

In the present experiment, different solvent systems were tried to resolve the components of ethanolic extract of *Madhuca indica* . TLC of ethanolic extract was performed by using different solvent systems and visualized by UV Chamber. TLC of ethanolic extract of *Madhuca indica* was shown in Table 7.

Table 7: TLC solvent systems for Ethanolic extract of *Madhuca indica* .

S.No.	Phytoconstituents	Solvent System	No of spots Detected	R_f Value
A	**Alkaloids**			
1	Colchicine Alkaloids	Benzene : Ethylacetate : Diethyl amine (70 : 20 : 10)	2	0.239, 0.891
2	Pyrrolidine, Pyridine and Piperidine alkaloids	Chloroform : Ethanol (95 : 5)	2	0.595, 0.957
		Chloroform : Methanol : Acetic acid (60 : 10 : 1)	2	0.439, 0.512
		Benzene : Chloroform : Methanol (70 : 30 : 20)	2	0.2, 0.866
		Benzene : Methanol : Diethyl amine (93 : 1 : 5)	1	0.161
3	Tropane Alkaloids	Chloroform : Diethylamine (90 : 10)	2	0.3, 0.6
4	Pyrrolizidine Alkaloids	Benzene : Ethylacetate: Diethyl amine (70 : 20 : 10)	1	0.466
5	Quinolizidine Alkaloids	Chloroform : Methanol (80 : 20)	2	0.595, 0.380
6	Alkaloids of the Papaveraceae	Benzene : Methanol (90 : 10)	1	0.209
		Chloroform : Ethanol (75 : 25)	4	0.119, 0.023, 0.333, 0.904
		Benzene : Methanol (80 : 20)	2	0.195, 0.239
		Chloroform : Ethanol (90 : 10)	2	0.195, 0.512
7	Opium Alkaloids	Chloroform : Diethylamine (90 : 10)	2	0.3, 0.6
8	Bis	Chloroform : EthylAcetate :	2	0.531,

	(Benzylisoquinoline) Alkaloids	Methanol (40 : 40 : 20)		0.936
9	Ipecacuanaha Alkaloids	Chloroform : Methanol (85 : 15)	1	0.275
10	Amaryllidaceae Alkaloids	Chloroform : EthylAcetate : Methanol (40 : 40 : 20)	2	0.531, 0.936
		Chloroform : Methanol : Diethylamine (92 : 3 : 5)	1	0.225
		Ethanol : Methanol : Diethylamine (65 : 10 : 5)	1	0.619
11	Indole Alkaloids	Chloroform : Methanol (95 : 5)	3	0.155, 0.533, 0.777
		Benzene : Ethylacetate : Diethylamine (70 : 20 : 10)	2	0.239, 0.891
12	Rauwolfia Alkaloids	Chloroform : Diethylamine (90 : 10)	2	0.3, 0.6
13	Vinca Alkaloids	Chloroform : Ethylacetate (50 : 50)	1	0.812
		Ethyl acetate : Absolute Ethanol (50 : 50)	1	0.434
14	Ergot Alkaloids	Chloroform : Methanol (70 : 30)	2	0.3 , 0.85
15	Oxindole Alkaloids	Ethyl acetate : Chloroform (90 : 10)	1	0.85
		Ethyl acetate : Chloroform (95 : 5)	1	0.923
16	Cinchona Alkaloids	Benzene : Methanol (80 : 20)	2	0.195, 0.239

		Chloroform : Ethanol (90 : 10)	3	0.222, 0.266, 0.444
		Chloroform : Methanol : Diethylamine (80 : 20 : 10)	1	0.84
17	Furanoquinoline Alkaloids	Chloroform : Methanol (98 : 2)	1	0.815
		Benzene : Methanol (90 : 10)	1	0.209
18	Sterol Alkaloids	Chloroform : Methanol (95 : 5)	3	0.155, 0.533, 0.777
B	**Carbohydrates**			
1	Oligosaccharides	n-Butanol : Formic acid : Water (33 : 50 : 17)	1	0.520
		Ethyl acetate : Methanol : Water (68 : 23 : 9)	2	0.480, 0.961
2	Amino sugars	Ethanol : Water : Conc Ammonium Hydroxide (80 : 20 : 1)	1	0.230
3	Acetates and Benzoates	Methanol : Benzene (3 : 97)	2	0.119, 0.190
4	Miscellaneous Derivatives	Benzene : Methanol (90 :10)	1	0.209
C	**Phenolics**			
1	Phenolics	Chloroform : Ethylacetate : Diethyl amine (93 : 5: 2)	1	0.465
		Benzene : Methanol (95: 5)	3	0.229, 0.369, 0.916
		Benzene	3	0.222, 0.377, 0.844

Anthelmintic Activity Evaluation

Ethanolic and Methanolic extracts in dose dependent manner showed anthelmintic activity and the results were comparable with the standard drug Mebendazole. Phytochemical analysis of the crude extract revealed the presence of tannins among other chemical constituents contained within them which were shown to produce anthelmintic

activities in the in vitro anthelmintic activities. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of the host animal or glycol protein on the cuticle of the parasite and may cause death.

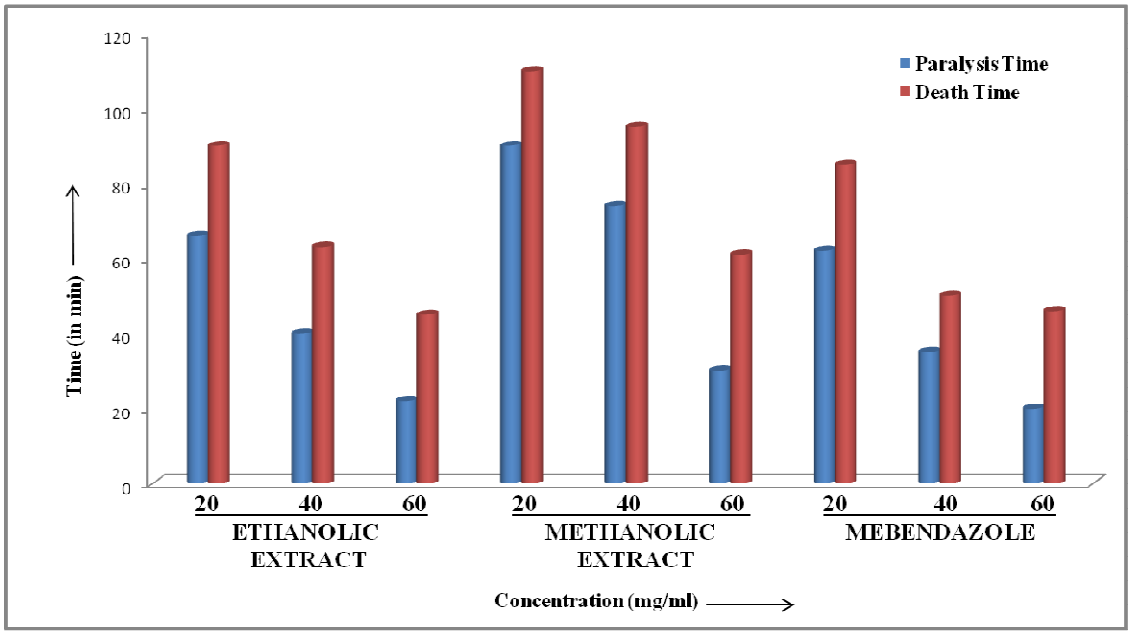
Statistical Analysis

The data presented as Mean ± SEM. The activities of all extracts were compared with the control. All the extracts showed significant anthelmintic activity,longer duration of paralysis and death.

Table 8: Anthelmintic activity of Ethanolic and Methanolic extracts of *Madhuca indica Gmel* flowers .

Treatment	Concentration in mg/ml	Time taken for Paralysis (P) and Death (D) of *Pheritima posthuma* worms in min (Mean ± SEM)	
		P	D
Ethanolic Extract	20 mg/ml	66 ± 0.61	90 ± 0.28
	40 mg/ml	40 ± 0.40*	63 ± 0.40*
	60 mg/ml	22 ± 0.81**	45 ± 0.20**
Methanolic Extract	20 mg/ml	90 ± 0.61	110 ± 0.76
	40 mg/ml	74 ± 0.81**	95 ± 0.40**
	60 mg/ml	30 ± 0.76**	61 ± 0.61**
Mebendazole	20 mg/ml	62 ± 0.61	85 ± 0.28
	40 mg/ml	35 ± 0.36	50 ± 0. 24
	60 mg/ml	20 ± 0.20	46 ± 0.26
Control(Normal Saline)	_	_	_

All values are Mean ± SEM; n=6 in each group. Values are significantly different from reference standard (Piperazine citrate) *p<0.05; **p<0.01.



Graph-1 Anthelmintic activity of Ethanolic and Methanolic extracts of *Madhuca indica Gmel* flowers

Preliminary phytochemical screening indicates the presence of alkaloids, tannins, flavonoids, triterpenes and reducing sugars. The crude extract samples, which were used to evaluate anthelmintic activity, showed variable times at different concentrations. The crude extract of ethanol showed the significant anthelmintic effect, causing death of the worm at all the concentrations but the time of death was different in each case. However, when observed the response of worms in case of paralysis, there was significant variation among the results produced by the different extracts at different concentrations like 20, 40 and 60 mg/ml. The ethanol extract showed more significant effect on paralyzing the worms, in terms of paralysis time. The effect of extracts on the paralysis (or) helminthiasis of the worm, according to the results Table 7 may be indicated as ethanol > methanol extracts . In particular, the ethanol extract exhibited similar paralytic as well as helminthiatic effect over Mebendazole at the given concentrations (20, 40, 60 mg/ml). The data presented in the Table 8 and observations made thereof, lead to the conclusion that the different degree of helminthiasis of the different extracts are due to the level of tannins present in the compounds. Tannins, the secondary metabolite, occur in several plants have been reported to show anthelmintic property by several investigators. Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation or binding to the glycoprotein on the cuticle of parasite and cause death. Coming to the chemistry of nematode surface, it is a collagen rich extracellular matrix (ECM) providing protective cuticle that forms exoskeleton, the collagen is a class of proteins that are modified by a range of post-translational modification prior to assembly into higher order complexes (or) ECMS . The mammalian skin also consists largely of collagen in the form of fibrous

bundles. Similar kind of reaction is expected to take place between the nematode cuticle (the earth worm) and the tannins of *Madhuca indica*, possibly by linking through hydrogen bonding. This form of reactivity brings toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death. Hence, further investigation and proper isolation of the active principles might help in finding of new lead compounds, which would be effective against various parasitic infections. In conclusion, the traditional use of the flowers of *Madhuca indica* Gmel as an anthelmintic has been confirmed.

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