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Research Article

ANTHELMINTIC AND ANTIMICROBIAL ACTIVITIES IN SOME SPECIES OF MULBERRY

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ABSTRACT

The present study explores the anthelmintic and antimicrobial activity of sequential leaf extracts of three *Morus* species *viz*. *Morus alba, Morus serrata* and *Morus laevigata.* Different concentrations of the extracts were tested for anthelmintic capacity by the determination of time of paralysis and death of Indian earthworms, *Pheretima posthuma*. Albendazole was used as the standard. The antimicrobial activity of the extracts was screened against five bacterial strains *viz*. *Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Salmonella typhi, Shigella flexneri* and two fungal strains *viz*. *Candida albicans* and *Aspergillus niger*. The zone of inhibition was determined against the microorganisms. The effects of these extracts were compared to standard drugs, streptomycin and fluconazole. The order of anthelmintic and antimicrobial efficacy in the three species of mulberry were *Morus alba* > *Morus serrata* > *Morus laevigata* and the results are discussed.

Keywords: Anthelmintic activity; Antimicrobial activity; Medicinal plants; *Pheretima posthuma;* Albendazole; Streptomycin;

INTRODUCTION

Mulberry is a medicinally important plant belonging to genus *Morus*. It is widely distributed in India, China, Japan, North Africa, Arabia, South Europe *etc*. There are about a dozen of species found in genus *Morus*. The plants can be grown both in tropics and in the temperate regions. The white mulberry *Morus alba* is renowned as the primary food source for silkworms and is widely cultivated in its native China and India. The root bark of this species is used as an anthelmintic, purgative and vermifuge[1]. Red mulberry *Morus rubra* is native to the United State of America and grows in forests; it is renowned as a rich source of flavones, namely rubraflavones[2]. The black mulberry is indigenous to Iran but was exported to Britain more than 500 years ago. Black mulberry is mostly used for making processed foods such as pekmez, marmalades, juices, liquors, natural dyes, and frozen fruits for ice cream[3]. It has also been reported that leaf extract of *Morus alba* inhibited biofilm formation by *Streptococcus* mutants and *Streptococcus sanguinis*[4]. The present study was aimed at evaluation of *in vitro* anthelmintic and antimicrobial activities using leaf extracts of three different species of mulberry.

MATERIALS AND METHOD

Extract Preparation

The plant materials were collected form Mulberry Germplasm Center, Central Silk Board, Hosur, Tamil Nadu and leaves were shade dried. The dried leaf materials were powdered and subjected for hot soxhlet extraction utilizing petroleum ether, chloroform and methanol sequentially.

Organisms used

All the experiments were carried out on Indian adult earthworms (*Pheretima posthuma*) collected from Earthworm Rearing Center, Dummalli, Shimoga (Karnataka). Pure culture of bacteria used for this study were *Pseudomonas aeruginosa* (*NCIM 2945), Proteus vulgaris* (*NCIM 2027), Bacillus subtilis* (*NCIM 2920), Salmonella typhi* (*NCIM 2501), Shigella flexneri* (*NCIM 4924),* and two fungal species *Candida albicans* (*NCIM 3103) and Aspergillus niger* **(***NCIM 789).* These pathogens were obtained from National College of Industrial Micro organisms, Pune, India.

Anthelmintic activity

The anthelmintic assay was carried out as per the method described by Ghosh *et al* [5]. The animals were divided into forty seven groups containing six earthworms each. Different concentration of extracts was poured in to different Petri dishes. Group I received standard drug (Albendazole, 20mg/ml), group II received 20% Tween-80 which served as control , group III – VII, VIII - XII and XIII - XVII received petroleum ether, chloroform and methanol extract of *Morus alba* leaves; groups XVIII – XXII, XXIII - XXVII and XXVIII - XXXII received petroleum ether , chloroform and methanol extract of *Morus serrata* leaves, while group XXXIII - XXXVII , XXXIII - XXXII and XXXXIII - XXXXVII received petroleum ether , chloroform and methanol extract of *Morus laevigata* leaves. Observations were made for the time taken for paralysis (Paralysis was said to occur when worm did not revive in normal saline) and death (Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^{o}C))[6](Table 2).

Statistical analysis

The observations of the anthelmintic activity are reported as mean ± SEM. Differences between group's means were assessed by one-way analysis of variance (ANOVA). The results obtained were compared with the control group. $P < 0.05$ was considered statistically significant.

Antimicrobial activity

The *in-vitro* antimicrobial activity of all the extracts at different concentrations (20,40,60 and 80 mg/ml) were studied by agar well diffusion method[7,8], against five bacterial species *viz. Pseudomonas aeruginosa* (gm-ve), *Proteus vulgaris* (gm-ve), *Bacillus subtilis* (gm+ve), *Salmonella typhi* (gm-ve), *Shigella flexneri* (gm-ve), and two fungal species *Candida albicans and Aspergillus niger.* Organisms were obtained from stock cultures which were maintained on nutrient agar medium and Sabouraud's dextrose agar medium at 40^{o} C, then subcultures in nutrient broth at 37^{o} C, prior to each antimicrobial test. The antibacterial activity of all the extracts was compared with standard drug Streptomycin and antifungal activity with Fluconazole. The zone of inhibitions was determined by measuring scale as per standard procedure[9]. The experiment was repeated thrice, the mean values were tabulated.

RESULTS

Results of qualitative phytochemical analysis of different extracts of different species of mulberry revealed the presence of steroids, glycosides, terpenoids, saponins, alkaloids, flavonoids and tannins (Table 1).

In regard to anthelmintic activity, all the extracts showed dose dependent effects and comparable to standard drug Albendazole (Table 2). For paralysis among the three plants leaf extracts, methanol and chloroform has exhibited highest effect than petroleum ether extract. The methanol extract of *Morus alba* at 40-100 mg/ml (26.25±1.8, 24.18±1.6, 22.18±1.11 and 16.25±2.3) showed significant reduction in mean values whereas the chloroform extract at 60-100 mg/ml (30.62±1.29, 23.56±1.5, 18.18±1.25) showed significant (P<0.05) reduction in mean values

when compared to that of standard. While among *Morus serrata* extracts, methanol extract at 80-100 mg/ml (29.42±1.95, 23.26±0.98) showed significant reduction in mean values whereas the chloroform extract at 60-100 mg/ml (33.44±2.6, 27.46±1.5, 22.06±1.75) showed significant reduction in mean values when compared to standard. Among *Morus laevigata* leaf extracts, methanol extract at 40-100mg/ml (26.25±1.8, 24.18±1.6, 22.18±1.11 and 16.25±2.3) showed significant (P<0.05) reduction in mean values while the chloroform extract at 80-100 mg/ml (31.75±3.23 and 21.06±1.25) showed significant (P<0.05) reduction in mean values when compared to that of standard.

Similar trend is also observed in regard to the time taken for death among the three species. Among *Morus alba* leaf extracts, methanol extract showed significant reduction in mean values of 29.56±2.5, 27.23±1.2, 26.43±2.2 and 18.43±3.5 min at 40, 60, 80 and 100mg/ml concentrations respectively, whereas the chloroform extract registered significant (P<0.05) reduction in mean values of 46±1.5, 36.06±1.2 and 23.87±1.5 at 60, 80, and 100mg/ml when compared to that of standard. Among *Morus serrata* leaf extracts, methanol extract at 40-100mg/ml (42.22±1.88, 38.82±2.33, 34.62±2.38, 28.16±1.45) and Chloroform extract at 60-100mg/ml (49.62±2.05, 41.72±1.05, 26.52±1.5) showed significant effects. Among *Morus laevigata* leaf extracts, methanol extract at 40-100mg/ml (46.56±1.25, 40.43±1.25, 33.25±1.75 and 23.5±0.5) showed significant (P<0.05) reduction in mean values while the chloroform extract at 80-100 mg/ml (43.06±2.04 and 28.81±1.25) showed significant (P<0.05) reduction in mean values when compared to that of standard.

The results of the antimicrobial activity revealed that all the extracts showed noticeable anti microbial activity in dose dependant manner against the organisms studied (Table 3). In both petroleum ether and methanol extract of all the three plants, *Salmonella typhi* was found to be more susceptible whereas *Shigella flexneri* was found to be more resistant when compared to rest of the organisms. While among the chloroform extracts, all the extracts showed significant effect against *Pseudomonas aeruginosa* when compared to other tested organisms whereas *Bacillus subtilis* showed maximum resistance. With respect to antifungal activity, *Candida albicans* was more susceptible to all the extracts than *Aspergillus niger*.

The comparison of results in all three species revealed the superior anthelmintic and antimicrobial activity in the order of *Morus alba >Morus serrata > Morus laevigata.*

Table 2: Anthelmintic activity in different species of Mulberry

Extract	Group	Conc.	paralysis (Min)	Death(Min)
Standard	I	20mg	34±1.08	50.5±1.56
Control	II	-	-	-
MAPE	III	20mg	71.625±1.5	87.5±2.02
	IV	40mg	66.43±2.25	83.31±1.75
	V	60mg	61.5±4.58	72.85±3.5
	VI	80mg	55.37±4.5	65.43±3.25
	VII	100mg	46.37±3.04	58.75±3.5
MACH	VIII	20mg	56.06±2.25	64.75±2.5
	IX	40mg	42.31±2.25	56.62±2.29
	X	60mg	30.62±1.29*	46±1.5*
	XI	80mg	23.56±1.5*	36.06±1.2*
	XII	100mg	18.18±1.25*	23.87±1.5*
MAME	XIII	20mg	36.75±2.4	51.31±1.65
	XIV	40mg	26.25±1.8*	29.56±2.5*
	XV	60mg	24.18±1.6*	27.23±1.2*
	XVI	80mg	22.18±1.11*	26.43±2.2*
	XVII	100mg	16.25±2.3*	18.43±3.5*
MSPE	XVIII	20mg	82.52±2.33	95.52±2.55
	XIX	40mg	73.55±1.05	86.66±1.5
	XX	60mg	68.41±1.75	75.32±2.05
	XXI	80mg	54.35±1.5	67.06±1.75
	XXII	100mg	48.26±2.05	61.12±1.75
	XXIII	20mg	62.32±2.4	68.16±2.5
MSCH	XXIV	40mg	46.36±2.3	62.04±1.5
	XXV	60mg	33.44±2.6*	49.62±2.05*
	XXVI	80mg	27.46±1.5*	41.72±1.05*
	XXVII	100mg	22.06±1.75*	26.52±1.5*
	XXVIII	20mg	45.75±1.05	55.26±2.05
MSME	XXIX	40mg	38.63±2.03	42.22±1.88*
	XXX	60mg	34.62±2.88	38.82±2.33*
	XXXI	80mg	29.42±1.95*	34.62±2.38*
	XXXII	100mg	23.26±0.98*	28.16±1.45*
MLPE	XXXIII	20mg	87.5±4.25	98.5±4.5
	XXXIV	40mg	78.12±3.44	86.56±3.25
	XXXV	60mg	66.5±2.5	77.5±2.5
	XXXVI	80mg	57.68±3.25	69.18±2.25
	XXXVII	100mg	49.5±2.51	64.25±1.75
MLCH	XXXVIII	20mg	66.81±2.25	72.56±2.25
	XXXIX	40mg	48.06±2.25	60.12±2.44
	XXXX	60mg	41.62±1.5	51.06±1.95
	XXXXI	80mg	31.75±3.23*	43.06±2.04*
	XXXXII	100mg	25.06±1.25*	28.81±1.25*
MLME	XXXXIII	20mg	43.62±1.29	54.25±1.41
	XXXXIV	40mg	40.31±1.25	46.56±1.25*
	XXXXV	60mg	30.56±1.25*	40.43±1.25*
	XXXXVI	80mg	25.54±1.5*	33.25±1.75*
	XXXXVII	100mg	17.37±1.94*	23.5±0.5*

Values are Mean ±SEM; n=6; Std Vs. test* P<0.05

Table 1: Qualitative phytochemical analysis in different species of Mulberry

Tests	MAPE	MACH	MAME	MSPE	MSCH	MSME	MLPE	MLCH	MLME
Steroids	-	+	+	-	+	+	-	+	+
Glycosides	+	+	+	+	+	+	+	+	+
Terpenoids	-	+	+	-	+	+	-	+	+
Saponins	-	+	+	-	-	+	-	+	+
Alkaloids	-	+	+	-	+	+	-	+	+
Flavonoids	+	-	+	-	-	+	+	-	+
tannins	+	+	+	-	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+
Proteins	-	-	+	-	-	+	-	-	+
aminoacids	-	-	+	-	-	+	-	-	+

Where,

MAPE: *Morus alba* petroleum ether extract, MACH: *Morus alba* chloroform extract,

MAME: *Morus alba* methanol extract, MSPE: *Morus serrata* petroleum ether extract,

MSCH: *Morus serrata* chloroform extract, MSME: *Morus serrata* methanol extract,

MLPE: *Morus laevigata* petroleum ether extract, MLCH: *Morus laevigata* chloroform extract,

MLME: *Morus laevigata* methanol extract

Table 3: Antimicrobial activity in different species of Mulberry

		Zones of Inhibition of bacteria in mm					Zones of Inhibition of fungi in mm	
Extract	**Conc.in Mg/ml**	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Proteus vulgaris***	***Salmonella typhi***	***Shigella flexneri***	***Aspergillus niger***	***Candida albicans***
Standard	20	34	22	33	29	29	36	42
Control	-	-	-	-	-	-	-	-
MAPE	20	9	6	9	6	6	12	18
	40	8	8	10	10	7	14	18
	60	11	12	10	13	9	16	20
	80	13	15	12	19	14	20	20
MACH	20	10	9	7	9	7	16	16
	40	12	13	14	14	11	18	18
	60	15	19	15	19	16	18	20
	80	17	25	22	23	23	20	24
MAME	20	14	16	10	18	13	18	18
	40	21	18	13	21	14	20	20
	60	23	22	18	25	17	22	24
	80	28	24	26	28	22	24	28
MSPE	20	6	7	8	7	3	10	10
	40	7	7	8	9	6	12	14
	60	9	10	9	12	7	14	16
	80	12	13	12	14	9	14	22
MSCH	20	9	9	6	9	9	9	12
	40	10	11	10	11	10	11	14
	60	13	15	12	11	11	16	18
	80	15	22	17	14	13	18	22
MSME	20	9	10	7	10	7	10	14
	40	10	14	9	12	9	14	18
	60	13	17	12	13	12	18	20
	80	16	20	17	23	15	20	24
MLPE	20	6	9	6	9	3	10	9
	40	6	10	6	11	5	10	11
	60	7	12	9	12	6	12	13
	80	12	15	12	15	6	13	18
MLCH	20	7	9	6	9	6	11	12
	40	7	9	7	9	7	13	14
	60	9	14	8	10	9	13	16
	80	10	18	13	14	13	15	17
MLME	20	8	8	6	8	7	10	11
	40	11	10	7	11	7	16	14
	60	12	11	8	13	7	20	20
	80	16	15	13	22	16	20	22

DISCUSSION

The present study revealed that the sequential extracts of *Morus alba, Morus serrata* and *Morus laevigata* possess potent anthelmintic property in a dose dependent manner for the parameters studied *viz*. paralysis and death which is quite comparable with standard anthelmintic drug in the organism tested. It is due to the presence of active principles in the plant extracts. It acts as potent anthelmintic, because the extracts of the plant contains flavonoids, triterpenoids, alkaloids, steroids and tannins. Specifically, tannins and flavonoids present in the extracts may be attributed to the pronounced anthelmintic activity[10, 11]. Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cuticle of parasite[12], and cause death. The demonstration of antibacterial activity against bacteria and antifungal activity may be indicative of the presence of broad spectrum antibiotic compounds[13]. The activities could be attributed to the presence of flavonoids, triterpenoids, alkaloids, steroids, phenolic compounds and tannins which have multiple biological effects, including antioxidant, wound healing etc. which are toxic to the microorganisms. Flavonoids, phenolic compounds in particular are important for the plant growth and defense against infection and injury. These compounds while exhibiting antioxidant property are usually also act as good antimicrobial agents [14-19]. The underlying mechanisms could be enzyme inhibition by oxidation[20]. Further, the variation in antimicrobial sensitivity may be due to the differences in the chemical nature of the cell wall and cell membrane of each micro organism[21].

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