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Research Article

ANTIBACTERIAL AND ANTIFUNGAL SCREENING OF *AMOORA ROHITUKA, MELIA AZEDARACH* AND *SOYMIDA FEBRIFUGA* STEM BARKS

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ABSTRACT

Human kind has been subject to infections by micro-organisms since before the dawn of recorded history. One presumed that mankind has been searching for suitable therapy for all infections. The *Amoora rohituka*, *Melia azedarach* and *Soymida* febrifuga belongs to same family i.e. Meliaceae. The Meliaceae plants are known to be rich sources of limonoids. A number of limonoids have been isolated from several genera of Meliaceae and some of this exhibit anticancer, antimalarial, cytotoxic, antiprotozoal, antifeedant and many various activities. The stem barks of *Amoora rohituka, Melia azedarach* and *Soymida febrifuga* exist these common therapeutic activities. The antibacterial and antifungal screening of alcoholic, hydroalcohalic (40:60) and aqueous extracts of *Amoora rohituka, Melia azedarach* and *Soymida febrifuga* stem barks were studied against gram's positive bacteria, gram's negative bacteria and fungal strains using Agar cup method. Alcoholic, hydroalcohalic (40:60) and aqueous extracts of *Amoora rohituka, Melia azedarach* and *Soymida febrifuga* stem barks extracts were used at dose of 500 mcg/cup, where the standard antibacterial drug was Kanamycin at dose of 500 mcg/cup and 30 mcg/cup and standard antibacterial drug was Ketoconazole at dose of 100 mcg/cup and 30 mcg/cup. The results indicated that the hydroalcoholic extracts of *Amoora rohituka, Melia azedarach* and *Soymida febrifuga* stem barks have significant antibacterial and antifungal activities when compared to standards.

Keywords: *Amoora rohituka, Melia azedarach, Soymida febrifuga, Antibacterial, Antifungal*

1. INTRODUCTION

Human kind has been subject to infections by micro-organisms since before the dawn of recorded history. One presumed that mankind has been searching for suitable therapy for all infections. This was a desperately difficult enterprise given the acute nature of most infections and the nearly total lack of understanding of tier origins, which was prevalent until the last century. The *Amoora rohituka, Melia azedarach* and *Soymida febrifuga* belongs to same family *i.e. Meliaceae* are known to be rich sources of limonoids. *Meliaceae* family has 40 genera and 600 species. A number of limonoids exhibit anticancer, antimalarial, cytotoxic, antiprotozoal, antifeedant and many more activities [1].

Amoora rohituka (Roxb.) wight & arnet Family *meliaceae* is a large handsome evergreen tree, with a dense spreading crown and a straight cylindrical bole up to 15m in height and 1.5-1.8m in girth. In India it is distributed in the sub-himalayan tract from Gonda Eastword to Bengal, Sikkim and Assam, Western Ghats, Chhota Nagpur, Andaman and adjoining hills from Poona to Southword to Tinnevelly.

Petroleum ether extract of the air dried bark gave a new tetranortriterpenoid; aphanamixinin ($C_{27}H_{34}O_7$). The bark appears to be an effective immunosuppressive drug similar to prednisolone. The bark is strongly astringent and used in disease of the liver and the spleen and for tumors and abdominal complaints [2].

Melia azedarach *Linn*. Family *Meliaceae,* (common name is Chinaberry tree, Pride of India) is deciduous tree up to 9-12 m tall with a spreading crown and sparsely branched limbs. Bark was smooth, greenish-brown when young, turning gray and fissured with age. It is distributed in Bangladesh, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, SriLanka, Thailand, and Vietnam [3].

Melia azedarach is often planted as an ornamental shade tree. Several compounds from Chinaberry have been isolated for medical purposes. Meliacine, a peptide isolated from leaves of *Melia azedarach*, exhibits potent activity against herpes simplex type 1 (HSV-1) and as an abortifacient, an antiseptic, a purgative, a diuretic, an insect repellent, etc [4].

Soymida febrifuga A. Juss. Family *Meliaceae,* (common name is Indian redwood) is a lofty deciduous trees with 22-25m height with rough bark exfoliating in large plates

or scales. It is distributed in Andhra Pradesh, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Tamil Nadu and Uttar Pradesh.

The stem bark contains mainly Alkaloids, Flavanoids, Saponin and Cyanogenic glycoside. The stem bark also gives positive result of Tannins. The chemical constituents of *Soymida febrifuga* stem bark are Deoxyandirobin, epoxyfebrinin B and its 14, 15-dihydro derivative, febrinolide, methylangolensate, Beta-sitosterol, lupeol, febrifugin, myricetin, ampelopsin, naringenin and quercetin phytochemicals. Bark is used in the treatment of diarrhoea, dysentery and fever and also as a general tonic; decoction used in gargles, vaginal infections, rheumatism swellings and as enemata [5].

Fig. no. 1: Plant of *Amoora rohituka*

Fig no. 2: Plant of *Melia azedarach*

Fig no. 3: Plant of *Soymida febrifuga*

2. MATERIAL AND METHODS

2.1. Plant material

The stem bark of *Amoora rohituka* (Roxb.) wight & arnet, *Melia azedarach* Linn. and *Soymida febrifuga* A. Juss. family *Meliacea*e was collected from Jawahar Lal Nehru Krishi Vishwavidyalaya, Jabalpur M.P. India. The authentication was done by Dr. A.B. Tiwari, Jawahar Lal Nehru Krishi Vishwavidyalaya, Jabalpur M.P. India.

2.2. Preparation of extract

Dried and coarsely powdered stem barks (250 gm) were extracted with solvents alcohol (99%), aqueous and hydro alcohol (40:60) using hot soxhlet extraction method, for 24 hrs. Filtered the extract then vacuum evaporated the filtrate and got the crude extracts [6].

2.3. Microorganisms

The microorganisms were collected from institute *i.e.* IMTECH, Chandigarh, India. The organisms used in study were listed in table no. 1

2.4. Standard drugs

Kanamycin is indicated for short term treatment of bacterial infections caused by one or more of the following pathogens *E. coli*, *Proteus* species (both indole-positive and indole-negative), *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Acinetobacter* species.

Table No. 1: Microorganisms and their characteristics

Microorganisms	Microorganisms no.	Character	Incubation Temp.^{0}C	Incubation Time
Escherichia coli	MTCC2690	Gram (-)ve bacteria	37	24 hrs
Pseudomonas auruginosa	MTCC4676	Gram (-)ve bacteria	37	24 hrs
Staphylococcus aureus	MTCC3160	Gram (+)ve bacteria	30	48 hrs
Bacillus cereus	MTCC1790	Gram (+)ve bacteria	26	24 hrs
Candida albicans	MTCC227	Fungal strain	25	48 hrs
Aspergilus nigar	MTCC282	Fungal strain	25	72 hrs
Aspergilus claratus	MTCC1323	Fungal strain	25	48 hrs

Ketoconazole is an antifungal medication which is used primarily to treat fungal infections. Ketoconazole is sold commercially as a tablet for oral administration (although this use has been discontinued in a number of countries), and in a variety of formulations for topical administration, such as creams (used to treat tinea; cutaneous candidiasis, including candidal paronychia; and pityriasis versicolor) and shampoos (used primarily to treat dandruff-seborrhoeic dermatitis of the scalp).

2.5. Culture media

For the study, nutrient broth (NB), nutrient agar (NA), and saboguraud broth and agar media were used [7].

Table No. 2: Different culture media and their composition used for bacterial and fungal strains

Media	Composition	g/L of Distilled Water
Nutrient broth(NB)	Peptone	5.00
	Sodium chloride	5.00
	Beef extract	1.50
	Yeast extract	1.50
	pH(at 25˚C)	7.4 -7.6
Nutrient agar (NA),	Peptone	5.00
	Sodium chloride	5.00
	Beef extract	1.50
	Yeast extract	1.50
	Agar	15.0
	Final pH(at 25˚C)	7.4-7.6
Sabouraud dextrose broth (SDB)	Peptone	10.0
	Dextrose	20.0
	Final pH(at 25˚C)	5.6-5.8
Sabouraud dextrose agar (SDA)	Mycological peptone	10.0
	Dextrose	40.0
	Agar	15.0
	Final pH(at 25˚C)	5.6-5.8

Direction for preparation of media

- Nutrient broth: Suspended 13 gm of NB in 1000 ml of distilled water. Boiled to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 min.
- Nutrient agar: Suspended 28 gm NA in 1000 ml of distilled water. Boiled to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 min.
- Sabouraud dextrose: Suspended 65 gm SDA in 1000 ml of distilled water. Boiled to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 min.

Anti-bacterial screening of samples

- Nutrient agar media was prepared, sterilized and poured into sterile petri dishes to obtain nutrient agar (NA) plates.
- Nutrient broth culture of the respective test organism was firmly seeded over the NA plate surfaces using a sterile cotton swab so as to make a lawn.
- Calculated the CFU/ml and maintained the CFU/ml is 10^5 by serial dilution of microbial colonies using suitable broth (liquid) culture media.
- Five holes of 5 mm diameter were punched on these inoculated plates using a sterile gel puncher.
- Using a sterile micropipette poured Kanamycin 30 mcg/cup and extracts sample 500 mcg/cup, total 50 µl of each samples of each concentration were poured into separate hole along with their respective negative control. The plates were incubated at desirable temperature for 24-36 hrs as per the bacterial strains.
- All the operations were carried out in aseptic environment.
- The plates were observed for a clear zone around the holes which indicate a positive antibacterial activity of the sample [8].

Anti-fungal screening of samples:

- The above procedure was also followed for screening for antifungal activity of sample using sabouraud dextrose Agar (SDA) plates.
- Agar (SDA) media was prepared and poured into sterile petridishes to prepare SDA plates.
- In case of yeast like fungi, e.g. *Candida*, lawn culture was prepared on SDA plates by firmly seeding the test organism from sabouraud dextrose broth using a sterile cotton swab.
- Five holes of 5 mm diameter were punched on these inoculated plates using a sterile gel puncher.
- Using a sterile micropipette poured Ketocanazole 100 mcg/cup and extracts sample 500 mcg/cup, total 50 µl of each samples of each concentration were poured into separate hole along with their respective negative control. The plates were incubated at desirable temperature for 24-72 hrs as per the fungal strains.
- All the operations were carried out in aseptic environment.
- Measureed the zone of inhibition of microbial growth for all extracts antibacterial and antifungal agents and also calculated the % inhibition of microbial growth in comparison to Kanamycin and Ketocanazole.

Table No. 3: Zone of inhibition of alcoholic extracts of *Amoora rohituka, Melia azedarach* and *Soymida febrifuga* stem bark in mm with SEM

Micro–organism	R1	R2	R3	K	KZ
Escherichia coli	9.51±1.15	12.00±1.52	13.51±2.30	18.50±1.10	-
Pseudomonas aeruginosa	11.31±0.58	15.53±1.23	12.21±2.03	20.12±1.23	-
Staphylococcus aureus	10.35±1.52	12.12±1.42	11.50±3.20	20.51±1.32	-
Bacillus cereus	9.63±0.59	10.34±0.89	9.52±3.20	18.00±2.12	-
Aspergilus nigar	12.50±0.69	17.5±0.96	16.00±1.58	-	21.50±1.10
Aspergilus claratus	11.51±1.23	16.50±1.56	15.36±2.13	-	21.50±1.63
Candida albicans	8.81±2.08	7.52±2.88	7.00±2.51	-	17.00±1.58

R1= Alcoholic extract of *Amoora rohituka* stem bark respectively at 500 mcg/cup; **R2**=Alcoholic extract of *Melia azedarich* stem bark respectively at 500 mcg/cup; **R3**=Alcoholic extract of *Soymida febrifug* stem bark respectively at 500 mcg/cup; **K** = Kanamycin 30 mcg/cup **KZ** = ketocanazole at 100 mcg/cup.

Table No. 4: The percentage inhibition (%) of microbial growth as compare to standard. For alcoholic extracts of *Amoora rohituka, Melia azedarach* and *Soymida febrifuga* stem bark

Microorganism	R1	R2	R3	K	KZ
E. coli	51.1	64.8	73.0	100	-
P. aeruginosa	56.2	77.1	60.6	100	-
S. aureus	50.4	59.0	56.0	100	-
B. cereus	53.5	57.4	52.8	100	-
A. nigar	58.1	81.3	74.4	-	100
A. claratus	53.5	76.7	71.4	-	100
C. albicans	51.8	44.2	41.1	-	100

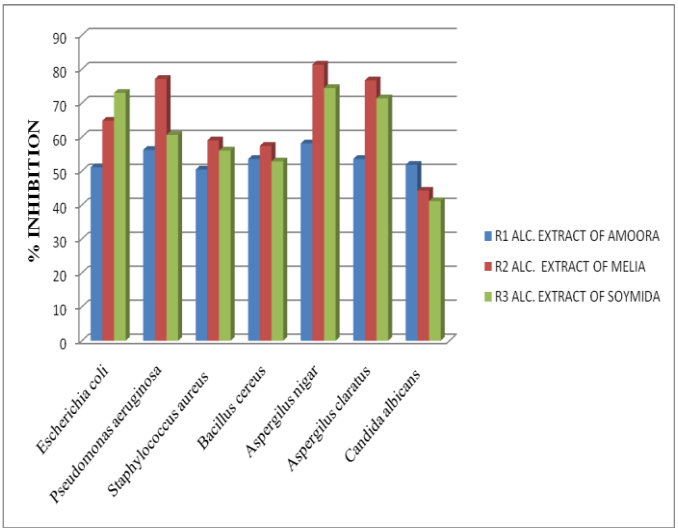


Fig. 1: The percentage inhibition of microbial growth as compared to standard

Table No. 5: Zone of inhibition of Aqueous extracts of *Amoora rohituka, Melia azedarach and Soymida febrifuga* stem bark in mm with SEM

Micro -organism	R4	R5	R6	K	KZ
Escherichia coli	9.31±1.14	11.60±1.58	11.51±2.03	18.00±0.70	-
Pseudomonas aeruginosa	10.61±1.58	15.41±1.02	11.01±2.53	17.00±0.59	-
Staphylococcus aureus	10.05±1.58	15.41±1.89	11.00±2.20	20.01±1.10	-
Bacillus cereus	8.01±1.57	9.84±0.80	8.30±1.21	18.50±1.12	-
Aspergilus nigar	11.60±0.96	13.50±0.58	12.00±1.13	-	21.70±0.58
Aspergilus claratus	8.41±1.59	7.31±1.45	8.20±2.01	-	18.00±1.26
Candida albicans	9.81±1.08	8.52±1.23	8.20±1.51	-	17.30±1.10

R4= Aqueous extract of *Amoora rohituka* stem bark respectively at 500 mcg/cup; **R5=** Aqueous extract of *Melia azedarich* stem bark respectively at 500 mcg/cup; **R6=** Aqueous extract of *Soymida febrifug* stem bark respectively at 500 mcg/cup; **K** = Kanamycin 30mcg/cup **KZ** = Ketocanazole at 100 mcg/cup.

Table No. 6: The percentage inhibition of microbial growth as compare to standard. For aquerous extracts of *Amoora rohituka*, *Melia azedarach* and *Soymida febrifuga* stem bark

Microorganism	R4	R5	R6	K	KZ
E. coli	51.6	64.4	63.8	100	-
P.aeruginosa	62.3	90.5	64.7	100	-
S.aureus	50.0	61.5	55.0	100	-
B. cereus	43.2	52.9	44.8	100	-
A. nigar	53.4	62.2	55.2	-	100
A.claratus	46.6	40.5	44.4	-	100
C.albicans	56.7	49.2	47.3	-	100

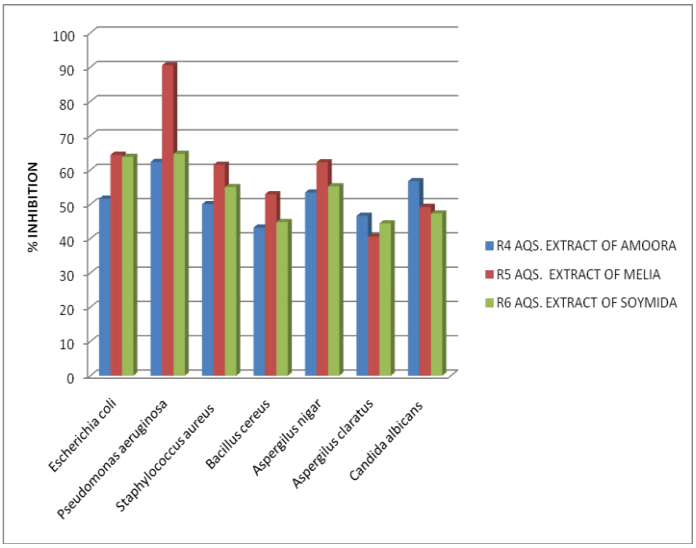


Fig. 2: The percentage inhibition of microbial growth as compared to standard

Table No. 7: Zone of inhibition of Hydroalcoholic extracts of *Amoora* rohituka, Melia azedarach and Soymida febrifuga stem bark in mm with SEM

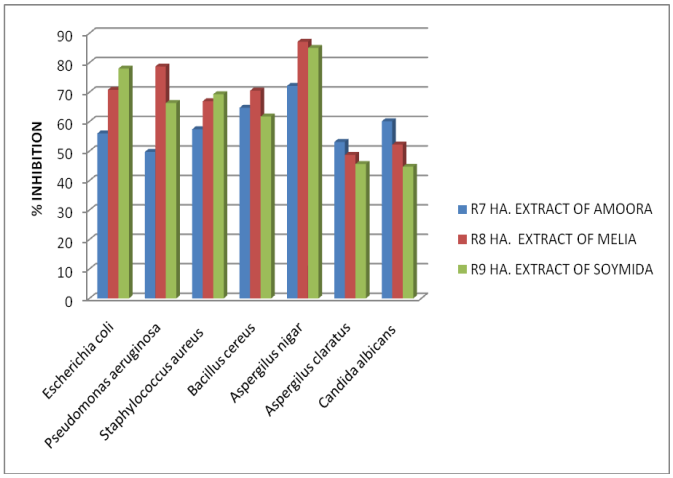
Micro -organism	R7	R8	R9	K	KZ
Escherichia coli	10.20±0.5	12.90±1.12	14.21±0.56	18.20±0.56	-
Pseudomonas aeruginosa	10.05±2.0	16.51±1.98	14.00±1.10	21.11±1.22	-
Staphylococcus aureus	12.01±1.0	14.00±1.58	14.01±3.01	20.90±0.74	-
Bacillus cereus	11.01±1.59	11.94±1.80	10.30±0.98	17.01±1.02	-
Aspergilus nigar	14.00±1.23	16.90±2.58	16.50±0.69	-	19.40±0.85
Aspergilus claratus	8.51±1.08	7.81±3.52	8.20±2.31	-	18.00±1.30
Candida albicans	9.81±1.2	8.52±1.35	7.30±1.47	-	16.30±1.66

R7= Hydroalcoholic extract of *Amoora rohituka* stem bark respectively at 500 mcg/cup; **R8=** Hydroalcoholic extract of *Melia azedarich* stem bark respectively at 500 mcg/cup; **R9=** Hydroalcoholic extract of *Soymida febrifug* stem bark respectively at 500 mcg/cup; **K** = Kanamycin 30mcg/cup **KZ** = Ketocanazole at 100 mcg/cup.

Table No. 8: The percentage inhibition (%) of microbial growth as compare to standard. For hydro alcoholic extracts of *Amoora rohituka, Melia azedarach* and *Soymida febrifuga* stem bark

Microorganism	R7	R8	R9	K	KZ
E.coli	56.0	70.8	78.0	100	-
P. aeruginosa	49.7	78.7	66.3	100	-
S. aureus	57.4	66.9	69.3	100	-
B.cereus	64.7	70.5	61.7	100	-
A. nigar	72.1	87.1	85.0	-	100
A. claratus	53.1	48.7	45.6	-	100
C.albicans	60.1	52.2	44.7	-	100

Fig. 3: The percentage inhibition of microbial growth as compared to standard



3. RESULTS AND DISCUSSION:

The alcoholic extract of *Amoora rohituka* stem bark showed good antibacterial activity against *Pseudomonas aeruginosa* and *Bacillus cereus* with 56% and 53% inhibition of bacterial growth, it is also having good antifungal activity against *Aspergilus nigar* with 58 % inhibition of fungal growth.

The alcoholic extract of *Melia azedarach* stem bark showed good antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus cereus* with 64 %, 77%, 59% and 57% inhibition of bacterial growth respectively, it also having good antifungal activity against *Aspergilus nigar* with 81 % inhibition of fungal growth.

The alcoholic extract of *Soymida febrifuga* stem bark showed good antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus* with 73%, 60 and 56 % inhibition of bacterial growth respectively; it is also having good antifungal activity against *Aspergilus nigar* with 74% inhibition of fungal growth.

The hydroalcoholic extract of *Amoora rohituka* stem bark showed good antibacterial activity against *Escherichia coli, Staphylococcus aureus and Bacillus cereus* with 56%, 57% and 64 % inhibition of bacterial growth respectively, it is also having good antifungal activity against *Aspergilus nigar* and *Candida albicans* with 72% and 60 % inhibition of fungal growth.

The hydroalcoholic extract of *Melia azedarach* stem bark showed good antibacterial activity against *Escherichia coli , Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus cereus* with 70%, 78%, 66% and 70 % inhibition of bacterial growth respectively, it is also having good antifungal activity against *Aspergilus nigar* with 87% inhibition of fungal growth.

The hydroalcoholic extract of *Soymida febrifuga* stem bark showed good antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus cereus* with 78%, 66%, 69% and 61 % inhibition of bacterial growth respectively, it is also having good antifungal activity against *Aspergilus nigar* with 85% inhibition of fungal growth.

The aqueous extract of *Amoora rohituka* stem bark showed good antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa* with 51% and 62% % inhibition of bacterial growth respectively, it is also having good antifungal activity against *Aspergilus nigar* and *Candida albicans* with 53% and 56% inhibition of fungal growth.

The aqueous extract of *Melia azedarach* stem bark showed good antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus with* 64%, 90% and 61 % inhibition of bacterial growth respectively, it also having good antifungal activity against *Aspergilus nigar* with 62% inhibition of fungal growth.

The aqueous extract of *Soymida febrifuga* stem bark showed good antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus with* 63%, 64% and 55 % inhibition of bacterial growth respectively, it also having good antifungal activity against *Aspergilus nigar* with 55% inhibition of fungal growth.

The alcoholic, hydroalcoholic and aqueous extracts of *Amoora rohituka* stem bark; *Melia azedarach* stem bark and *Soymida febrifuga* stem bark having good antimicrobial activity against various bacterial and fungal microorganism**.** The hydroalcoholic extracts of each stem barks having good antimicrobial activity in comparison to the other alcoholic and aqueous extracts of stem barks.

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