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ANTIMICROBIAL ACTIVITY OF FRUITS OF *Sapindus emarginatus*

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Abstract- Worldwide, infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Resistant to conventional antibiotics is an important health concern and reason for the problem of multidrug resistance (MDR) reside on the fact that the current arsenal of antibiotics has been largely designed on limited chemical scaffold with only few innovation leaving an opportunity for pathogens to develop and spread antibiotic resistance mechanisms worldwide. The complex molecules in nature have evolved over millennia to provide robust, disease-fighting mechanisms, intense biological activity as a result of natural selection. In present study *Sapindus emarginatus* fruits commonly known as soap berry, ritta whose aqueous extract of pericarp is used as detergent that is evaluated for its antimicrobial potential and found to be have significant antimicrobial potential with lowest of 7.8 mg/ml against yeast *Candida albicans*, at 15.6 mg/ml against dermatophyte *Trichophyton rubrum,* at 62.5 mg/ml against *Epidermophyton floccosum* and found not active against bacteria selected.
Keywords- *Sapindus emarginatus, antimicrobial, yeast, dermatophyte, bacteria*

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Introduction

Discovery and development of new therapeutic agent is a continuing process inspite of the fact that at present we have at our command a formidable array of modern drugs [1]. Classically, higher plants have played a dominant role in introduction of new therapeutic agent [2]. The herbal medication is more popular as it tackles health problems, is economical, less toxic and has reduced adverse effect and long duration effectiveness. The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main resources of drugs [3]. World health organization (WHO) has estimated that, atleast 80 % of the world population rely on traditional systems of medicine for their primary health needs. These systems are largely plant based and are the local heritage with global importance, world is endowed with a rich wealth of medicinal plants [4].

Species from Sapindaceae family are known for their traditional medicinal uses as a diuretic, stimulant, expectorant, natural surfactant, sedative, vermifuge and against stomachache and dermatitis in many parts of the world. Chemical investigations of this family have led to the isolation of saponins, diterpenes and flavonoids, among other secondary metabolites. Several saponins and acyclic sesquiterpene and diterpene oligoglycosides have been isolated as main secondary metabolites of several Sapindaceae species used in traditional oriental medicine [5].

Sapindus emarginatus (*SE*) Linn. family Sapindaceae is medium-sized deciduous tree found in south India. It is called soapnut tree. High content of saponins and sugars have been reported in the pericarp [6,7]. The saponin moiety is characterized as hederagenin group of glycosides [8]. The pericarp is reported for various medicinal properties. It is reported to possess emetic, tonic, astringent and used in the treatment of asthma, colic due to indigestion, diarrhea and paralysis of limbs, a thick watery solution of the pulpy mesocarp is introduced into the nose of the patients for the

relief of hemicrania and for restoring consciousness during epileptic and hysteric fits in the folklore literature, it is also reported that there was more or less relief in almost every case of hemicrania in which the solution was tried, but the cases of hysteria and epilepsy benefited by it were very less [9]. *SE* has been reported for its antispermatogenic, antiandrogenic activities [6, 10, 11], antirheumatic activities [12], antinociceptive [13, 14]. The pericarp of this fruit is known to contain 10- 11.5% of saponins [15]. The structures of different saponins present in the pericarp of this fruit, have been recently established [16]. These saponins have a common structural skeleton containing a pentacyclic triterpenoid part substituted with different carbohydrate side chains. The aqueous extract of the pericarp of *SE* fruits shows an acidic pH (3.8), which is due to the presence of a COOH group in the triterpenoid part. This acidic nature of the fruits extract prompted the present study, which describes the *in vitro* antimicrobial activity of extracts from dried pericarp and seeds of the fruits of *SE.* Although, *S. emarginatus* is used as regularly used as soap but there has been no published antimicrobial data on organisms in present study.

Materials and methods

Drugs and chemicals

DMSO (Fischer chemicals), Sabouraud Dextrose Agar, SDA (Oxoid chemicals) media for fungi, Nutrient agar, NA media for bacteria, Griseofulvin (97%, Acros organics), Ketaconazole (Sigma), Miconazole (Sigma), Amphotericin B (Sigma). The chemical structures of the test drugs are shown in Fig. 1.

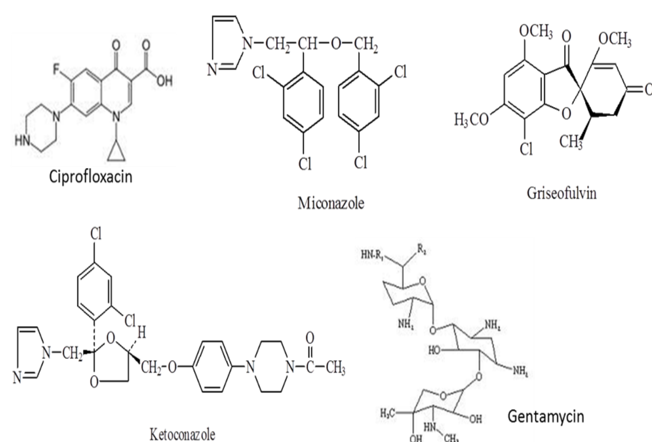

Fig. 1- The chemical structures of the test drugs

Organisms

Dermatophytes:Trichophyton rubrum, Epidermophyton floccosum; *Bacteria:Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Escheriachia coli; Yeast:Candida albicans.*

Preparation and fractionation of extract from fruits

The fruits of *SE* were collected in and around Gulbarga University campus, Gulbarga and authenticated by taxonomist from botany department. The fruits were separated as pericarp, seeds and coarsely powdered which were extracted with petroleum ether and methanol successively in a soxhlet apparatus. The resultant extracts were petroleum ether extract of pericarp (SEPP), oil from petroleum ether extraction of seeds (SESO), methanol extract of pericarp (SEPM) and methanol extract of seeds (SESM). To the SEPM and SESM on addition of distilled water slowly dropwise, solid precipitated out which on filtration found to be syrupy, thus this was extracted with n-butanol and it was found to be rich in saponins, labeled as *Sapidus emarginatus* pericarp butanol fraction (SEPB) and *SE* seeds butanol fraction.

In vitro susceptibility testing as determined by MICs

The minimum inhibitory concentrations (MICs) were determined by agar dilution technique. [17]

Preparation of extract dilution series

Extract stock solution

Dissolve 200 mg of plant extract in 10 ml DMSO with glass beads, vortex to homogenize. Dilution series: Pipette 5 ml stock solution into 5 ml purified water. Vortex for 30 seconds. Repeat doubling dilutions with successive 5 ml aliquots of purified water. Table 1

Table 1- Plant extract dilution in agar

Solution	Volume of solution (ml)	Diluent (ml)	Concentration in dilution (mg/ml)	Conc. of extract in agar (mg/ml)
Stock	**200 mg of plant extract**	**10 ml of DMSO**	**20000**	**--**
A	5 of stock	5 ml PW	10000	1000
B	5 of A	5 ml PW	5000	500
C	5 of B	5 ml PW	2500	250
D	5 of C	5 ml PW	1250	125
E	5 of D	5 ml PW	625	62.5
F	5 of E	5 ml PW	312	31.2
G	5 of F	5 ml PW	156	15.6
H	5 of G	5 ml PW	78	7.8
I	5 of H	5 ml PW	39	3.9
J	5 of I	5 ml PW	19.5	1.9

PW - Purified water, A-I are plant extract / dilution in serial order.

Preparation of microorganism inoculums

a) Dermatophyte stock suspension: Using a 7-10 day culture of dermatophyte, grown on SDA at 30°C, add enough saline solution, onto the surface of the growth and then gently agitated with the tip of a sterile cotton swab to create a suspension
b) Bacterial stock suspension: Using a 24 hour culture of bacteria, grown in nutrient broth at 37°C, add saline solution, then gently agitated with the tip of a sterile tip to create a suspension.
c) Yeast stock suspension: Using a 2-3 day culture of dermatophyte, grown on SDA at 30°C, add enough saline solution, onto the surface of the growth and then gently agitated with the tip of a sterile cotton swab to create a suspension.

Preparation of test plates

a) A suitable number of sterile 9 ml aliquots of suitable agar media were prepared in 30 ml glass universals (temperature held at 50-55°C). To each suitable agar media aliquot, 1 ml of extract dilution was added, then gently inverted to mix (without introducing air bubble).
b) For each series of extract/agar suspensions a number of double-compartment plastic/glass non-compartmental petri dishes were suitably labelled. Into petri dish the extract/agar suspension was added. The plates were left to set for 30 minutes at

room temperature.

c) Each plate was dried under laminar flow for 5-10 minutes with lid open if media is not inoculated. Each plate was stored in fridge until needed if not inoculated.

Inoculation of test plates

- Dermatophyte inoculation: From each dermatophyte, working suspension of 20 ml aliquot is pipetted onto the agar surface of each petri-dish compartment. Each compartment will have 3 aliquots representing the 3 different dematophyte. Plates were left to adsorb the dermatophyte spore.
- Yeast inoculation (*Candida albicans*): From yeast working suspension of 20 ml aliquot is pipetted onto the agar surface of each petri dish. Plates were left to adsorb the dermatophyte spore.
- Bacterial inoculation: From bacterial working suspension a 20 ml aliquot is pipetted into agar then it is mixed gently, inverted and poured the agar with microorganism into petri dish.

Incubation of test plates

- Once dry, plates are inverted and incubated at 30°C for minimum of 4 days or until growth is seen on control plates of (TVAC) in case of dermatophyte.
- Once dry, plates are inverted and incubated at 30°C for minimum 48 hrs or until growth is seen on control plates (TVAC) in case of yeast.
- Once dry, plates are inverted and incubated at 37°C to 40°C for minimum 24 hr or until growth is seen on control plates (TVAC) in case of bacteria.
- Plates can be incubated in loosely bound plastic bags to prevent excessive moisture loss in case of longer incubation.

Controls

- TVAC control: Use a serial pour plate method, with suitable agar, to determine total viable aerobic control (TVAC) of microorganism stock suspension. The plates are incubated along with test samples (to demonstrate sufficient organism concentration).
- Media control: Media agar plates (without extract dilution or organism) are incubated along with the test samples (to demonstrate media sterility). Agar plates (without extract dilution) inoculated with microorganism, as in test procedure, are incubated along with the test samples (to demonstrate media suitability and microorganism viability within the test system).
- Solvent control: Series of plates using dilutions of DMSO (without extract) prepared according to test procedure. Plates incubated along with test samples, (to demonstrate lack of solvent interference).
- Antibiotic/process control: MIC of antibiotic taken under study was determined during every experiment. A stock solution of antibiotic in DMSO was prepared (1000 mg/ml). This was diluted in purified water to produce a doubling dilution series and plates prepared according to test procedure (concentration 0.2 to 100 mg/ml). Incubated along with test samples, (to demonstrate process repeatability between day-to-day testing and comparability to published MIC values).

Table 2- Antimicrobial activity of Sapindus emarginatus different extracts compared with standards by agar dilution method and inhibition concentration expressed in mg/ml.

Sr.no.	Test sample	Ca	Ec	Sa	Sp	Tr	Kp	Ef
1	SEPP	250	1000	2000	2000	1000	1000	500
2	SEPM	31.2	1000	2000	1000	125	1000	250
3	SEPB	7.8	500	2000	1000	15.6	1000	62.5
4	SESO	500	2000	1000	2000	500	2000	1000
5	SESM	125	1000	1000	2000	250	2000	500
6	SESB	62.5	1000	500	2000	250	2000	500
7	Ketaconazole	<3.91	-	-	-	0.04	-	10
8	Miconazole	-	-	-	-	0.08	-	1.25
9	Griseofulvin	-	-	-	-	1.25	-	20
10	Ciprofloxacin	-	5	<3.91	<3.91	-	5	-
11	Gentamycin	-	5	<3.91	<3.91	-	5	-

Ca- Candida albicans; Ec- Escherichia coli; Sa- Staphylococcus aureus; Sp-Streptococcus pyogenes; Tr- Trichophyton rubrum; Kp- Klebsiella pneumoniae; Ef- Epidermatophyton floccosum

Recording results

The test plates are removed from the incubator and laid out in such a manner that the dilution series is apparent. The MIC value is the concentration of first plate in the dilution series to exhibit no growth (negative).

No growth is interpreted as no visible growth or a very faint haze, when compared to control plates.

The MIC value is concentration of first plate in dilution series to exhibit no growth (negative). The presence of colonial growth of organism on the area where dilution was applied (in case of fungi) and on whole plate (in case of bacteria) is recorded as growth (positive).

Results and discussion

Against Yeast- Among all the test extracts in present study SEPB found to be very active against *Candida albicans* at MIC of 7.81 mg/ml and standard ketaconozole at <3.91 mg/ml. SEPM and SESB were also active at MIC of 31.2 and 62.5 mg/ml., SESO, SEPP, SESM extracts could inhibit the growth of *Candida albicans* but at higher concentrations of 500, 250 and 125 mg/ml .

Against *dermatophytes:* Among all the test extracts in present study SEPB found to be active against *T. rubrum* at MIC of 15.6 mg/ml and standard ketaconozole at 0.04 mg/ml, Miconazole at 0.08 mg/ml and Greisufulvin at 1.25 mg/ml. All other extracts could inhibit the growth of *T. rubrum* but at higher concentrations above 125 mg/ml. Among all the test extracts SEPB found to be active against *Epidermophyton floccosum* at MIC of 62.5 mg/ml and standard Ketaconozole at 10 mg/ml, Miconazole at 1.25 mg/ml and Grisufulvin at 20 mg/ml. All other extracts could inhibit the growth of *T. rubrum* but at higher concentrations above 250 mg/ml.

Against bacteria- In present study bacteria selected are *Staphylococcus aureus, Streptococcus pyogens, Escherichia coli, Klebsheilla pneumonia.* The standard drugs undertaken for comparison are Ciprofloxacin and Gentamycin. The MIC inhibition found at 5 mg/ml for Ciprofloxacin and Gentamycin against *Escherichia coli, Klebsheilla pneumonia.* The MIC inhibition found < 3.91 mg/ml for Ciprofloxacin and Gentamycin against *Staphylococcus aureus, Streptococcus pyogens.* The pericarp and seeds extracts of *S. emarginatus* could inhibit the bacteria growth between 500 to

2000 mg/ml MIC range.
S. emarginatus, fruit pericarp and seeds were extracted with petroleum ether was defatted and later was extracted with methanol successively by hot soxhlet extraction. The extracts were tested against *Candida albicans*, *Staphylococcus aureus, Streptococcus pyogens, Escherichia coli, Klebsheilla pneumonia, T. rubrum and Epidermophyton floccosum* microorganisms. The pericarp of fruit found to be more active against test organisms over seeds of *S. emarginatus* and the polar extracts with saponin rich fraction was found to be more active over the non polar ones .

Conclusion

The saponin rich fraction of pericarp of *S. emarginatus* fruit found to be significantly active against *Candida albicans, Trichophyton rubrum* and *Epidermophyton floccosum,* antibacterial action found to be moderate over seeds and also corresponding petroleum ether fractions. This gives valuable information of scientific reasoning for the use of pericarp of fruit of this plant as detergent with antimicrobial property for greener and healthier use.

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