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Isolation and Identification of Antidundruff Component from *Sapindus trifoliatus* [Ritha]

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Dandruff samples were collected from 40 subjects and were screened for *Malassezia furfur* infection. 10 isolates were obtained and extracts of *Sapindus trifoliatus*, were checked for anti-dandruff activity against these isolates. *Malessezia furfur* is considered to be the resident flora of the normal scalp, but the degree of infection is correlated with the degree of dandruff. Anti-fungal susceptibility testing was done using extracts prepared in various solvents like water, ethanol, acetone & hydro-alcohol. Their inhibition activity was checked using Paper disc method against the isolates as well as the standard culture *Malessezia furfur*ATCC14521. and with standard anti-fungal drugs. An attempt was made to separate the various components present in the plant extract and also to detect the active component using HPTLC. Preparative HPTLC was done to elute identified saponins from the extract of *Sapindus trifoliatus* and were found to be inhibiting *Malassezia furfur* invitro. The amount of saponins present in the plant extract was quantified using densitogram. Alcohol extract contained 7.7% saponin whereas water extract contained 0.83% saponin. This implicated that saponin is one of the components which was found to inhibit *Malassezia furfur*. *Sapindus trifoliatus* was found to be an effective remedy for *Piyriasis capitis* in- vitro.

Key words: *Sapindus trifoliatus,* solvent extract, *Malassezia furfur,* active component.

Dandruff is a common scalp condition that affects most people at sometime during and after puberty. On an average the scalp skin replaces itself about once every 28 days. If turnover speeds upto every 11 days, the net result is dandruff. If the metabolic rate is increased even further for e.g. in psoriasis, where the replacement is every four days, a patch of red thick adherent scale develops. Sometimes large flakes and itchy red areas scattered about the scalp appear and also on ears and eyebrows. This is referred to as seborrhoea or seborrhoeic dermatitis *Malassezia furfur* [*Pityrosporum ovale]* is the major cause of dandruff. An oily scalp supports the growth of *Pityrosporum ovale*. Since it is a normal flora of the scalp it cannot be eliminated completely, it can be only managed and controlled.

Previous studies have indicated that microbial flora of the scalp is a mixed one, comprising at least 25 species of bacteria, 15 species of yeast and 31 species of molds. Included among the yeast is *Pityrospoum ovale* which is found in 80% of human scalps.

Many chemicals like Zinc pyrithrone, selenium sulfide etc have been used commercially in shampoos to inhibit the yeast. Certain herbs are also good anti-dandruff agents. These include *Acacia concinna (*Shikakai), *Sapindus trifoliatus (*Aritha\ Soapnut), *Emblic myrobalan (*kachli*), Ecliptaerecta (*Maka*), Androprogon muricats*

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*(*Wada), rose petals*, Lawsonia inermis* (Henna*)* . *Malassezia furfur* (Pityrosporum ovale) - The culprit

Taxonomic Classification

Kingdom	:	Fungi
Phylum	:	Basidiomycota
Class	:	Hymenomycetes
Order	:	Tremellales
Family	:	Filobasidiaceae
Genus	:	*Malassezia*

Malassezia is a lipophilic yeast found on skin and body surfaces of humans and animals. It has been shown that colonization with *Malassezia* may occur as early as neonatal period . It is a member of the normal skin flora in as much as 90% of adults and may occasionally cause superficial and deep mycoses. *Malassezia* has no known teleomorphic phase. There are seven proposed species in the genus *Malassezia* based on molecular, morphological, and biochemical profiles. The most common and well-known species are *Malassezia furfur* and *Malassezia pachydermatis*. The genus *Pityrosporum* belongs to the family Cryptococcacea, was created by Sabouraud for the organism which malassez observed in case of pityriasis simplex. *Malassezia furfur* is a skin saprophyte, grows only if fatty acids of chain length greater than C^{10} are added to culture medium. The lipophilic nature of the organism was reported ,it was found that butterfat extracts facilitated growth on conventional medium. The fungus is composed of frequently budding, ovoid, minute spherical yeast-like elements, measuring 0.25-5.0mm in major diameter. Its size may range from 2m-3m x 4m-5m and it reproduces by single polar bidding. No mycelial forms are observed but chlamydospore formation is occasionally observed.

Malassezia infections are mostly endogenous and originate from the colonized skin. They may occur in otherwise healthy individuals as well as immunocompromised hosts, such as bone marrow transplant recipients, patients with cancer or AIDS

The most common clinical picture caused by *Malassezia furfur* is pityriasis versicolor . It may also cause seborrheic dermatitis , folliculitis, neonatal pustulosis, blepharitis and white piedra . Given the lipophilic nature of the fungus, fungemia, catheter-related infections and sepsis due to *Malassezia furfur* may occur particularly in patients who are on parenteral nutrition with lipids . Noteworthy, colonization of the catheters with *Malassezia* may occur in absence of lipid administration as well. *Malassezia globosa* and *Malassezia sympodialis* are also common causes of pityriasis versicolor in humans.

Malassezia pachydermatis is a distinctive species due to its well-known zoophilic nature. It causes canine otitis externa and is prevalent in carnivores. However, according to current knowledge, *Malassezia pachydermatis* is not the only *Malassezia* species associated with infections or colonization in animals. Some lipid-dependent species of *Malassezia* may also be isolated as occasional causes of canine otitis externa. *Malassezia pachydermatis* may cause disseminated infections in humans as well.

Sapindus trifoliatus

Latin name	*Sapindus trifoliatus* Linn
Family	Sapindaceae
Common name	Aritha, Ritha, Phenila
English name	Soap nut
Key applications	Natural detergent

Ritha *(Sapindus mukorossi)* with its large leaves, is a deciduous tree found in India. This tree belongs to the main plant order Sapindeae and family Sapindaceae. The species is widely grown in upper reaches of the Indo-Gangetic plains, Shivaliks and sub-Himalayan tracts at altitudes from 200m to 1500m. The trunk of Ritha is straight and cylindrical, nearly 4-5 m in height.

Bark

The bark of Ritha is shinning gray and fairly smooth when the plant is young. It is dark gray when the plant approaches maturity. Ritha leaves are long stalked odd pinnate. The rachis is nearly 30 to 50 cm long and bears 5 to 10 pairs of leaflets. Ritha flowers during summer. The flowers are small and greenish white, polygamous and mostly bisexual in terminal thyrses or compound cymose panicles. These are sub-sessile; numerous in number and at times occur in lose panicles at the end of branches. The fruit appears in July-August and ripens by November-December. These are solitary globose, round nuts 2 to 2.5 cm diameter, fleshy, saponaceous and yellowish brown in color. The seed is enclosed in a black, smooth and hard globose endocarp. The

fruit is collected during winter months for seed and or sale in the market as soap nut.

The dried fruit of Ritha is most valuable part of the plant. Its fleshy portion contains saponin, which is a good substitute for washing soap and is as such used in preparation of quality shampoos, detergents, washing woolen clothes. This is why some botanists have named the species as *Sapindus detergens*. Ritha foliage can be used as cattle fodder during drought. The fruit is of considerable importance for its medicinal value as well. Ayurvedic, Unani and Tibetan systems of medicine consider it to be useful for treating a number of diseases like common cold, pimples, epilepsy, constipation, nausea, etc. It is also used as expectorant and enthelmintic in small doses. The Central Drug Research Institute, Lucknow (India), has recently developed a contraceptive cream out of Ritha fruit. It is being marketed under the trade name 'Consap'.

MATERIAL AND METHODS

Preparation of plant extract

Pharmacognostically identified dried pericarp of fruits of *Sapindus trifoliatus* Linn, and pods of *Acacia concinna* were collected from the local market.

Plant extract was prepared by the method of Alade and Irobi with minor modification.100g of powdered plant material was soaked in 100ml of 70% alcohol, water, hydroalcohol for 72 hrs. The material was stirred every 24h using a sterile glass rod. At the end of extraction the extract was filtered using Whattmann filter paper no. 1. The alcoholic filtrate was concentrated in vacuo at 30ºC and stored at 4ºC until further use[24].

Selection of subjects

People with persistent problem of dandruff in the age group of 18-55yrs were selected for the studies with Pre- sampling period Patients did not do any scalp treatment.

Sampling

Dandruff samples were collected from Dr. Rajan's clinic at J.V.P.D. The number of dandruff samples collected were 40. After screening and identification only 10 isolates were obtained.

To check antifungal activity

Anti- fungal susceptibility testing was carried out using disc diffusion method.

Minimum inhibitory concentration of extracts

The minimum inhibitory concentration was found out using Broth macro dilution method.

Contact plate method

Expose the culture to plant extract, at concentration of MIC value. After the 0hrs, 2hr, 4hr, 6hr, 24hr and 48hr contact viable cell number was determined by plate technique. After incubation for 72hrs. Calculate percent reduction.

Separation of components of extracts

HPTLC was carried out in order to separate the components present in each extract. Readymade silica plates were used (20cmx 10cm plates were used to load the sample].

Sample preparation

500mg of extract dissolved in 5.0ml. of methanol

Standard preparation

10 mg. of saponin & Oleic acid dissolved in 5.0ml.of methanol.

Mobile phase preparation

Dry the solvent chamber completely.
In a conical flask add the following reagents in the ratio mentioned below.
Chloroform: acetic acid: methanol: water.
6.4: 3.2: 1.2: 0.8.

Shake it vigorously and dispense it in the solvent chamber. Allow the chamber to saturate for 5 mins.

Loading of sample

Sample was loaded on the silica plates using LINOMAT 3 HPTLC sample applicator. Rinse the syringe with methanol and load 10ml of each extract and the standards.

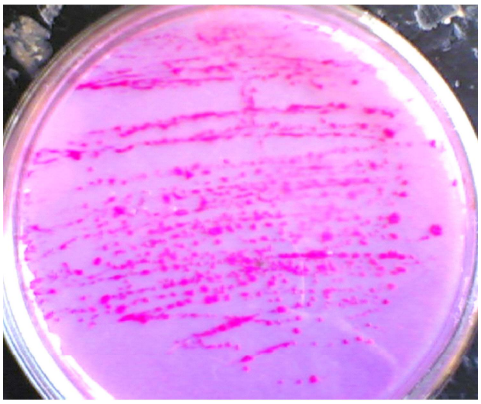
HPTLC

After loading the sample and allowing it to dry, dip the plate in the solvent system. Allow it to run till the solvent reaches 3/4th of the distance. After it reaches 3/4th distance dry the plate and observe the plate at 254nm and 366nm

Also scan the plates using CAMAG LINOMAT TLC Scanner. Derivitise the plate using anisaldehyde-sulphuric reagent by dipping the plate in it for 60 secs. Dry the plate. Observe the plate under UV and 366nm. Capture the image using LINOMAT image analyzer. Calculate the Rf of all the components and that of standards also.

Sapindus trifoliatus (Ritha) whole plant

Sapindus trifoliatus (Ritha) fruits



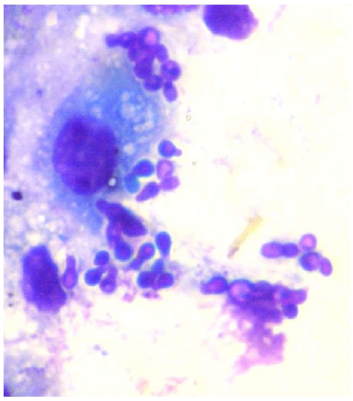


Fig. 1. Microscopic examination of *Malassezia furfur*

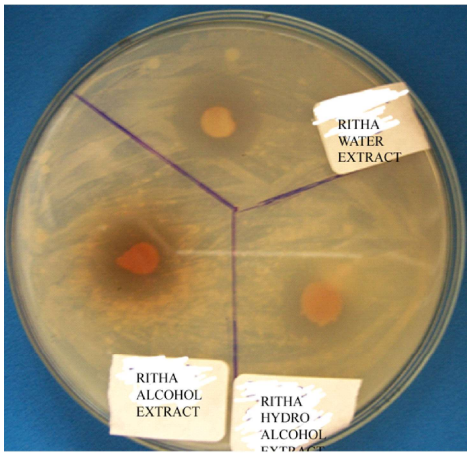
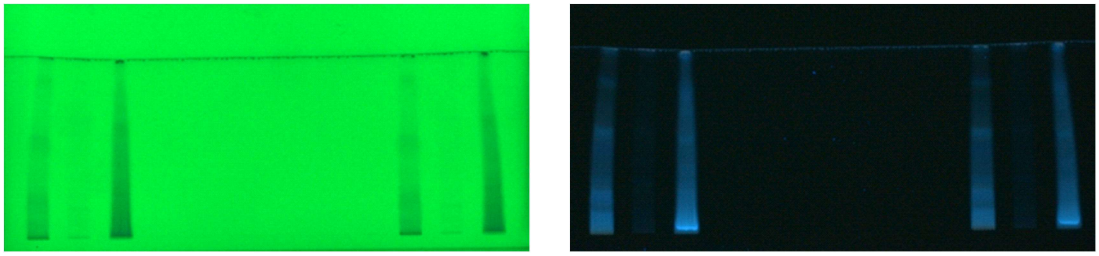
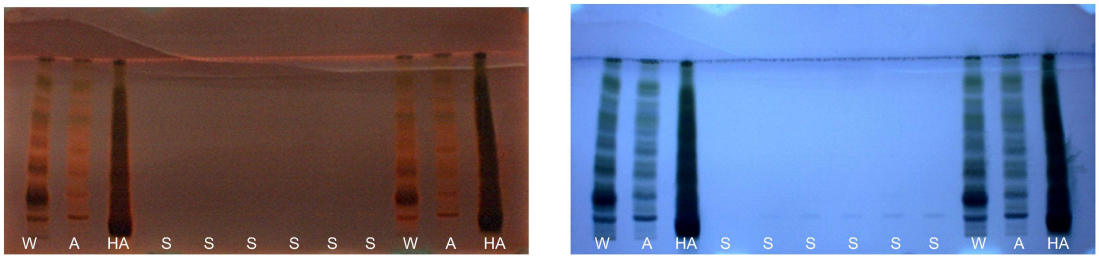


Fig. 2. Contact plate method, Viable count after different times of contact with extract



At 254 nm. At 366 nm.

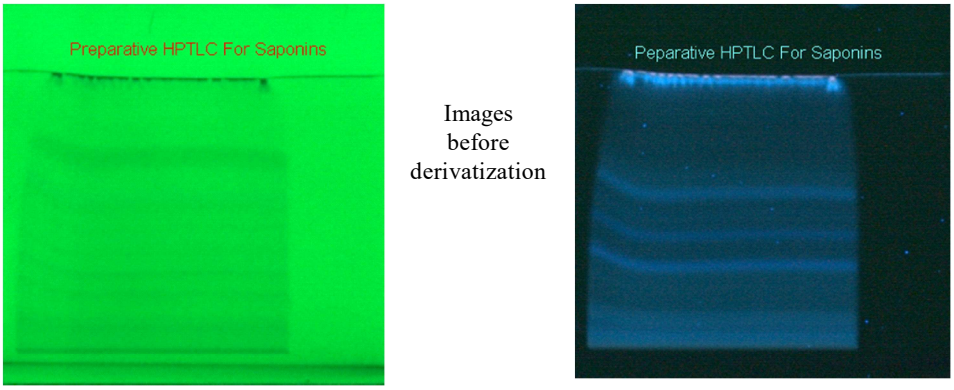
Fig. 3



W: Water extract,
A: Alcohol extract,
HA: Hydro-alcohol extract,
S: Saponin std

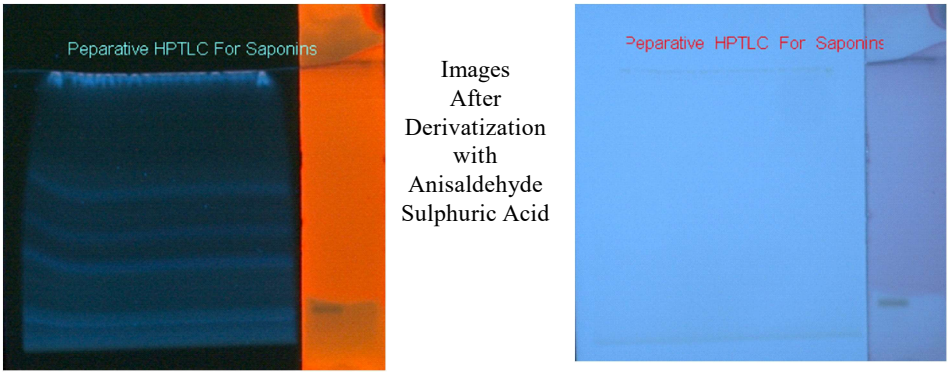
At 254 nm. At 366 nm.

Fig. 4. After derivitisation



Images before derivatization

Image@254nm Image@366nm



Images After Derivatization with Anisaldehyde Sulphuric Acid

Std.saponin Image@366nm Std.saponin Image@visible

Fig. 5

Preparative HPTLC

It was done in order to obtain saponin in substantial amounts for microbiological studies. The extracts were loaded on silica plate and saponin standard and oleanolic acid standard were made to run parallely. The plate was developed with anisaldehyde-sulfuric acid reagent. On observing these plates at 580nm, water extract and saponin extract of Sapindus trifoliatus showed bands which matched the saponin standard.

Elution of SAPONIN from silica plate

Cut the saponin fraction obtained into small pieces and dissove in 2ml methanol. Sonicate on a sonicator for 20mins. Filter the solution to remove silica. Concentrate the extract obtained on a boiling water bath. Reconstitute the dried extract in 1ml methanol. Repeat this procedure several times to obtain substantial amounts of saponin from plant extract.

Detection of activity of standard saponin and saponin from extract

In order to check for the activity of the saponin extract obtained against Malassezia furfur, disc diffusion method was used. Punch discs of 6mm from Whattman paper number 1. Sterilize them by autoclaving. Dip discs in saponin standard, saponin extract and methanol as control. Plate culture on Sabouraud's agar with corn oil and place discs over them. Incubate these plates at room temperature for 72 hr. Measure the zones of inhibition.

RESULTS AND DISCUSSION

Isolation and identification

The samples were streaked on rose bengal chloramphenicol agar with corn oil. The biochemical characters of the isolates were compared with the standard culture *Malassezia furfur* ATCC14521, CBS-1878 collected from IMTECH, Chandigarh. Colonies which were umbonate, smooth, soft, friable and cream in color were selected. Microscopically they were elongated, oval and had a broad bud base.

Minimum inhibitory concentration of extracts

MIC of different extracts of *Sapindus trifoliatus* was performed by tube macro dilution method, in a 5ml volume, using Saboraud's broth with corn oil as diluent. The results show that the cultures were inhibited at a concentration of 8% of water extract , 10% of hydro-alcohol extract and at 9% alcohol extract .

Separation of various components from the extracts of *Sapindus trifoliatus* by HPTLC

Since all the extracts of *Sapindus trifoliatus* plant were found to be effective in inhibiting standard strain of *Malassezia furfur*, an HPTLC was carried out to separate the components present in the plant extracts. Different extracts showed presence of varying number of components. Water extracts showed presence of eight components. Hydroalcohol extract showed presence of nine components. Alcohol extract showed presence of eleven components.

Identification of active component from extracts

This indicated that the plant extracts contained saponins which may be one of the active component present in the plant material.

Quatification of saponin from extracts

The amount of saponin present in the extracts was quantified using densitogram.

TLC scanner was used to scan the plates and quantify saponins present in the extracts.

The saponin content in the alcohol extract was found to be 0.7% and that of water extract was found to be 0.83%.no saponins were detected in hydro-alcohol extract.

Detection of activity of standard saponin and saponin from extract

Antibiotic susceptibility testing was done using disc diffusion for both saponin standard as well as saponin extracted from the alcohol extract. Standard strain of *Malassezia furfur* MTCC 14521 was spread on agar plate and sterile 6mm discs were dipped in saponin standard 50mcg/ml, saponin extract. The discs were placed on the agar plate. Since the saponin fraction was reconstituted in methanol, a disc dipped in methanol was kept as control.

The results obtained are tabulated in Table 1. Since saponin standard and saponin extract showed zone of inhibition, it is obvious that saponin is one of the components in the extract which inhibits *Malassezia furfur* and hence, will be effective for treatment of dandruff.

DISC	Zone of inhibition [mm]
Saponin standard	14
Saponin extract	12
Methanol control	No zone

Fig. 6

Table 1. Antimicrobial activity of extracts with isolates & Std. culture [mean Zone of inhibition]

Isolates	Water extractmm.	Hydro alcohol extract mm.	Alcohol extractmm.
1	21	11.5	20
2	18.3	12	16.4
3	20	11.3	18.5
4	21	11.8	19
5	20	19.1	19.2
6	19.5	12.4	15.5
7	16.6	16.5	11.3
8	18	12	14.5
9	18.2	11.5	12.7

CONCLUSION

Malassezia furfur was isolated from Dandruff samples. Out of 40 samples only 10 isolates were obtained. *Sapindus trifoliatus* extracts shows inhibition of *Malassezia furfur* and isolates . Water extract showed maximum inhibition followed by alcohol extract and hydro alcohol extract. The MIC value of water extract were low compared with alcohol, hydro alcoholic extract. & contact plate techniques shows the decrease in viable count in presence of extract .These extracts contains nine to eleven different components. The identification and characterization of these extracts shows presence of saponin is one of the active component of *Sapindus trifoliatus,* which inhibits *Malassezia furfur*. Water extract showed greater zone of inhibition as compared to alcohol extract as in water extract amount of saponin was more than other extracts. This implies that besides saponin in extracts contains some other components which may also be inhibits *Malassezia furfur.* Hence it is an excellent substitute for anti-dandruff shampoos as it is comparatively mild and is natural. *Sapindus trifoliatus* is an effective plant against treatment of dandruff.

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