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Full Proceeding Paper

RECENT PHARMACOGNOSTICAL PHYTOCHEMICAL AND ANTIFUNGAL ANALYSIS OF *ABUTILON INDICUM (TAMIL - MANJAL THUTHI)* IN DISEASE OF RINGWORM INFECTION – RESEARCH REPORT

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ABSTRACT

Ringworm is a contagious infection at skin; it is caused by a fungus only The name of the fungus like *Trichiphyton rubrum* and *Microsporum canis*. Ringworm infection can affect the skin on our body, scalp, groin area, and feet also. The skin infection of ringworm is a skin disorder only it is especially among children and it may affect people of all ages also. Although its name suggests otherwise, it is caused by a fungus, not a worm. Antibiotics and other antimicrobial agents are effective in the prevention and treatment of ring worm, but they also cause undesirable side effects such as ecologic disturbance of oral and gut flora. Therefore, search for the anti fungal herbs could offer an effective alternative to antibiotic strategies for fungal infection disease like ring worm. The plant *Abutilon indicum was* screened for its Macroscopic, Microscopic, Physiochemical parameter, Florescence analysis, General and microchemical analysis for crude powder and Plant cell inclusions. Qualitative and Total microbial load showed that they all within limit. Extraction was carried out by using soxhlet apparatus. Ethanolic extract effective against list out the fungal like *Trichiophyton rubrum* and *Microsporum canis* by Disc diffusion method. The Ethanolic extract of *Abutilon indicum*., showed highest activity at minimum concentration. Thus from our findings, it was concluded that the bioactive principles present in the extracts may be responsible in the treatment of ringworm infection. Developing countries like India having the percentage of poor people more, to meet with the demand of the poor public, the *Abutilon indicum* may serve the purpose once the evaluation and detailed studies may over.

Keywords: *Abutilon indicum., Trichiophyto rubrum, Microsporum canis*,

INTRODUCTION

The word of ringworm with refers to fungal infections that are present on the skin. Some of these fungi produce round spots on the skin. The physical examination for this ring worm it is scrapped to collected from the infectious patient and tested by using the microscope., some times culture test also carry to identified. After this culture test only physician comes one conclusion. Successful treatment by the proper diagnosis of the disease.[1] The Latin name of Tinea is called as ringworm. The name of the ringworm is varied depending upon the affected parts of the body. Although the world is full of yeasts, molds, and fungi, only a few cause skin problems. Mainly the ringworm infection is caused by the following organisms like *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton interdigitale*, *Trichophyton mentagrophytes*, *Microsporum canis*, and *Epidermophyton floccosum.* These fungi mainly present in animal hair, nail and soil also. The ringworm infection fungi must be spread from one person to others, from animal to person , and fom the soil to a person. The ringworm affected the face and neck means the name is called as Tinea barbae, The ringworm affected the place of scalp means the name is called as Tinea capitis, The tinea capitis came the persons affect the hair loss problems also. The body surface area affected the ring worm diseases means the name is called as Tinea corporis. The ring worm produced the reddish brown colour or yellowish red colour of ring structure of batches on the body surface. Some patients affected the ring worm disease the place of face mean the name of the disease is called as Tinea faciei. Some of the patients affect the ringworm the place of hand is called as Tinea manus. The Athlete's foot may possible to affect the ring worm is called as Tinea pedis. Some time the ringworm produced the finger area of human body is called the name of Tinea unguinum.

MATERIALS AND METHODS

Plant material

The plant of *Abutilon indicum* was collected from Thirumalaisamudram 7km away from Thanjavur (Tamil Nadu) in the month of December 2010. The plants was identified by local people of that village and authenticated by Dr. N.Ravichandran, Asst. Professor, Drug Testing Laboratory, CARISM, SASTRA University Thanjavur, and the Voucher specimen is preserved in laboratory for future reference.

Chemicals

All the reagents used were of analytical grade obtained from S.D. fine chemicals, Ltd, and Hi Media, Mumbai.

Pharmacognostical Screening of Plants

Macroscopic Characters and Physiochemical Parameters of *Abutilon indicum* leaf and leaf powder: The Macroscopic evaluation was carried out for shape, size, color, odor, taste and fracture of the drug. Different physiochemical values such as Ash value, extractive values, loss on drying, foreign organic matter, Crude fiber content, were determined and reported on Table No:1

Fluorescence analysis study of *Abutilon indicum* leaves powder

Fluorescence analysis study of powdered drug material with different reagents was carried out to observe the color reactions, were determined and reported on Table No:2

General chemical and Micro chemical Tests

General chemical and Micro chemical tests of powdered drug material with different reagents were carried out to observe the colour reactions to identify the compounds were reported on table No: 3 [2]

Study of Plant cell inclusions

Plant cell inclusions study of powdered drug material with different reagents was carried out to observe the colour reactions, were determined and reported on Table No: 4 [2]

Preparation of Extract from *Abutilon indicum* leaf powder

The leaves were dried under shade, powdered and passed through 40meshes and stored in closed vessel for further use. The dried powder material (150g) was subjected to soxhelt extraction with Ethanol for continuous hot extraction for 24 hours. The extracts were concentrated under reduced pressure to obtain the extracts solid residues. The percentage value of extract was 29 (%w/w).

Screening of Thin layer Chromatography

TLC for Alkaloids

Stationary phase: Silicagel G

Mobile Phase: Butanol: Acetic acid: Water (4:5:1)

Detecting Reagent: Dragendorffs reagent

TLC for Terpenes

Stationary Phase: Silicagel G

Mobile Phase:Hexane: acetone (9:1)

Detecting Reagent: Iodine Champer

TLC for Saponins

Stationary Phase: SilicagelG

Mobile Phase: Chloroform: Methnol: Water (7:4:1)

Detecting Reagent: Iodine Champer

Phytochemical Evaluation of Ethanolic Leaf extract of *Abutilon indicum*, [3,4]

The Ethanolic Extract of *Abutilon indicum* (Leaf) was subjected to preliminary Phytochemical tests followed by the methods of Harbone (1998), and Trease and Evans (1983) and the phytoconstituents reported in table No: 6

Determination of Microbial load

The plant material obtained was subjected to microbial analysis. 1ml of sample is taken and added to 9ml of sterile distilled water for preparing the serial dilution. The samples in the flask were kept in a mechanical shaker for few minutes to obtain uniform suspension of microorganisms. The dilution is 1:10 or 10-1. From that 1ml of the 10-1 dilution is transferred to 9ml of sterilized distilled water. This is 1: 100 or 10-2. This procedure was repeated up to 10-7 dilution. 0.1 ml of serially diluted samples was inoculated in to the sterile plate containing Nutrient agar, Salmonella Shigella Agar (SSA) and Potato Dextrose Agar (PDA) Medium by spread plate method. Nutrient agar, and SSA plates were incubated at 37ºC for 24 hours and PDA plates were incubated at room temperature for 3-5 days. Bacterial and fungal colonies were counted using colony counter. *Salmonella, Shigella* and *E.coli* can be counted using SS Agar medium.

Determination of Minimum inhibitory concentration

Preparation of the Standard Solution

The stock solution was prepared by dissolving 5gm of the standard preparation of the given *Abutilon indicum* extract, which was accurately weighed. Five test tubes were taken and named as s1, s2, s3, s4 and s5. Dilutions of the stock solutions were prepared by various concentrations such as 10, 5, 2.5, 1.25 and 0.625 mg/ml were prepared and are labeled. The Test Organism: MTCC 3272 and MTCC 3270 were used as the test organism on MIC. UN inoculated culture medium is placed in one test as control.

Antifungal activity by Disc diffusion Method

Sabouraud dextrose agar medium was prepared and sterilized at 121°C for 15 minutes. The medium was poured on the sterile petriplates and allowed to solidify. After solidification sterile cotton swab and dip it into culture containing two fungal strains (MTCC 3272 and MTCC 3270) separately. Inoculate the organisms first in horizontal and then vertical direction for even distribution, using the swab and dry for 15 minutes. A sterile filter paper disc was dipped in four different concentrations (100mg, 50mg, 25mg and 12.5mg) of Ethanolic Extract of Abutilon indicum using sterile forceps. The disc was placed on the agar surface of the inoculated plate. The standard disc (Ketaconazole) as positive control was also placed on inoculated plate. The inoculated plate was incubated at 30°C for 3 days in an inverted position.

RESULTS

Macroscopic Characters of *Abutilon indicum (Linn)* Sweet leaf

T.S. of Abutilon leaf consists of midrib and lamina. The midrib consists of single row ovoid short cells the outer cell wall contains cuticle and three different types of trichomes (stellate type, uniseriate multicellular and multicellular multiseriate glandular trichome). The cortex is several cell rows parenchymatous cells in abaxial side of the midrib but in adaxial side the cortex cells are consists of 3-4 rows of angular collenchyma and 2-3 rows of parenchyma cells. The parenchyma cells are containing druce type of calcium oxalate crystals. The vascular bundle consist of phloem and xylem the xylem surrounded by phloem. Phloem cell are several rows with phloem fibres. Phloem parenchyma cell also contains druce type calcium oxalate crystals. Each rows of xylem cells are differentiated with xylem parenchyma. Xylem parenchyma cells containing, simple ovoid starch grains.

Fig. 1: *Abutilon indicum*(*Linn*) sweet leaf

Margin: Acuminate and toothed, Apex: Pointed, Base: Symmetrical, Venations: Reticulate, Taste: Sweet to characteristic,Odour: Odourless, Surface: Smooth on both the surfaces.

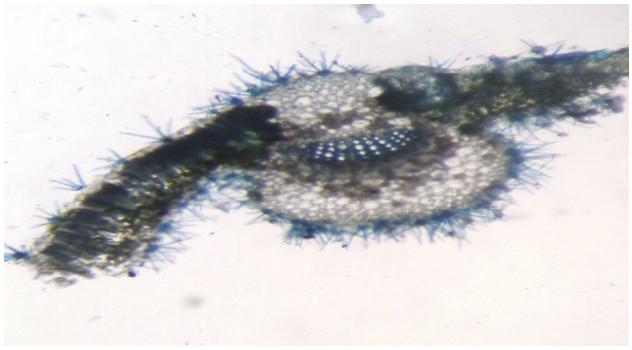

Fig. 2: T.S of *Abutilon indicum* leaf

Lamina

The T.S. of lamina consists of upper and lower epidermis the epidermis are single row with elongated cells and the outer wall of the cuticle. In abaxial side the stellate type of trichomes frequency is more than the adaxial. Mesophyl cell are 2 rows with elongated tangentially arranged chlorenchyma cells and some of the mesophyl cell contains druce type of calcium oxalate crystals. Spongy parenchyma tissue is made up of 3-4 rows of ovoid short parenchyma cells with intercellular space.

Physiochemical Parameters

The extractive value was highest in water and was recorded to be 15.2%w/w, and methanol soluble extractive value was about 10.4 %w/w .The different ash values and the different physiochemical parameters were screened and are presented in the table.

Table 1: Physiochemical Parameters of *Abutilon indicum (Linn) Swee*t Leaf Powder

S. No	Parameters	%W/W
1.	Hexane Soluble extractive	4%
2.	Pet ether Soluble extractive	3.2%
3.	Chloroform Soluble extractive	6.4%
4.	Acetone soluble extractive	4%
5.	Ethanol soluble extractive	12.8%
6.	Ethyl acetate soluble extractive	11.2%
7.	Methanol soluble extractive	10.4%
8.	Water soluble extractive	15.2%
9.	Foreign organic matter	3.5%
10.	Loss on drying	6%
11.	Crude fiber content	41%
12.	Total Ash	5%
13.	Acid insoluble ash	2%
14.	Sulphated ash	3.6%
15.	Water Soluble ash	2.5%

Table 2: Fluorescence analysis study of *Abutilon indicum* leaves powder

S. No	Sample	Colour in Day light	Colour in UV
1.	Powder	Pale Green	Green
2.	Powder + 0.1N Sodium Hydroxide	Green	Dark green
3.	Powder + Acetic anhydride	Pale green	Dark green
4.	Powder + 0.1N Hydrochloric acid	Pale gray	Dark green
5.	Powder + water	Slight yellowing green	Green

Table 3: General Chemical and Micro chemical tests for Leaf powder of *Abutilon indicum*

S. No	Test	Results
1.	Test with water /aqueous extract	+
2.	Test For Tannins	+
3.	Test for Anthra quinine	-
4.	Test for Mucilage	+
5.	Test for Carbohydrate	+
6.	Test for alkaloids	+

+ Present - absent

Table 4: Study of Plant cell inclusions

S. No	Test	Result	Colour
1.	Cellulose	+	Pale yellow
2.	Lignin	+	Deep blue
3.	Suberin	+	Deep yellow
4.	Chitin	+	Violet
5.	Starch	+	Blue
6.	Mucilage	+	Pink
7.	Proteins	+	Brick red
8.	Alkaloids	+	Reddish brown
9.	Tannins	+	Bluish black
10.	Calcium oxalate	+	Needle shaped crystals
11.	Calcium carbonate	+	Needle shaped crystals

+ Present- Absent

Table 5: screening of Thin layer Chromatography

S. No	Colour of Spot	Rf Value
1.	TLC for Alkaloids – ReddishBrown	0.6
2.	TLC for Terpenes – purple	0.7
3.	TLC for Saponins – Brown	0.9

Table 6: Preliminary phytochemical Analysis of Ethanolic Leaf Extract of *Abutilon indicum*[5]

S. No	Phytoconstituents	Ethanolic Extract
1.	Alkaloids	+
2.	Aminoacids	-
3.	Glycosides	-
4.	Carbohydrates	+
5.	Flavonoids	+
6.	Phenolic groups	+
7.	Resins/gums	+
8.	Saponins	+
9.	Steroids	-
10.	Tannins	+
11.	Terpenoids	+

+ = Present - = Absent

Table 7: Total Microbial load

S. No	Name of the Organisms	Plant sample of *Andrographis paniculata*	WHO Limit	Inference
1.	E.coli	Nil	102	Within limit
2.	*Salmonella sp.*	Nil	Absence	With in limit
3.	*Shigella sp.*	Nil	Absence	Within limit
4.	Total	2 x 102	107	Within limit
5.	Yeast &Mould	1 x 103	104	Within limit

Table 8: Determination of Minimum Inhibitory Concentration

Name of the strains	Minimum Inhibitory concentration (Mg/ml)
Trichophyton rubrum MTCC 3272	2.5
Microsporum canis	1.25

Table 9: Antifungal activity of *Abutilon indicum* against two fungal strains

Name of the strains	Ketaconazole (Positive Control)	*Abutilon indicum(Ethanolic extract)*			
		100mg	50mg	25mg	12.5mg
Trichophyton rubrum MTCC 3272	30 ± 0.05	30±0.05	28±0.02	24 ± 0.02	18 ± 0.01
Microsporum canis MTCC 3270	29 ± 0.05	28±0.05	25±0.02	23 ± 0.02	16± 0.01

DISCUSSIONS

Ringworm refers to a fungal infection that affects the scalp, feet, and nails. It is also called as Tinea. In ringworm a red ring appears on the infected person's skin. It is contagious diseases and may spread one person to another person with contact. Ringworm is caused by several different fungus organisms that belong to a group called Dermatophytes, Derma means skin and Phytes means organisms. The duration of ringworm for example scalp ringworm is 10-14 days and that of skin ringworm is 4-10 days. The Macroscopic[6] evaluation was carried out for shape, size, color, odor, taste and fracture of the drug. The Microscopic evaluation was performed the Transverse section of midrib and lamina region of the leaf, Physiochemical parameters including Extractive vales determined by according to polarity of solvents, the extractive value was highest in water and was recorded to be 29%w/w, and ethanol soluble extractive value was about 15.2%w/w .The different ash values[7](Total ash-5%, Acid insoluble ash 3.6%, Sulphated ash-4% and Water soluble ash – 2.5%) and the different physiochemical parameters were screened. Ethanolic extract of *Abutilon indicum* was performed by using Soxhelt apparatus. The percentage value of extract was 15.3%w/w, Florescence analysis, General and microchemical analysis[8] for crude powder and Plant cell inclusions. Qualitative and Total microbial load showed that they all within limit. Ethanolic extract effective against list out the fungal like *Trichiophyton rubrum* and *Microsporum canis*. The Ethanolic extract of *Abutilon indicum*[9-10] showed highest activity at minimum concentration. Thus from our findings, it was concluded that the bioactive principles present in the extracts may be responsible in the treatment of ringworm infection.

CONCLUSION

Ringworm is a contagious fungal skin infection. There are many types of fungus germs some can infect the skin, nails, and hair. A small area of infected skin after spread overall the body surface. It typically develops into a circular, red, inflamed patch of skin. Only one patch of infection occurs at some times other vice several patches also produced over the body, particularly if we catch the infection from handling an infected animal. Antibiotics and other antimicrobial agents are effective in the prevention and treatment of ring worm, but they also cause undesirable side effects such as ecologic disturbance of oral and gut flora. Therefore, search for the anti fungal herbs could offer an effective alternative to antibiotic strategies for fungal infection disease like ring worm. The plant was screened for its Macroscopic, Microscopic, Physiochemical parameter, Florescence analysis, General and microchemical analysis for crude powder and Plant cell inclusions. Qualitative and Total microbial load showed that they all within limit. Extraction was carried out by using soxhlet apparatus. Ethanolic extract effective against list out the fungal like *Trichiophyton rubrum* and *Microsporum canis*. The Ethanolic extract of *Abutilon indicum* showed highest activity at minimum concentration. Thus from our findings, it was concluded that the bioactive principles present in the extracts may be responsible in the treatment of ringworm infection. Developing countries like India having the percentage of poor people more, to meet with the demand of the poor public, the *Abutilon indicum* may serve the purpose once the evaluation and detailed studies may over.

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