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Evaluation of Antibacterial and Antifungal Activities of Leaf and Seed Extracts of *Croton Tiglium* Plant against Skin Disease Causing Microbes

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Abstract: *Medicinal plants have been used to treat human diseases from the time immemorial. Skin disorders can also be cured using herbal formulations. This study was carried out with an objective to investigate the antibacterial and antifungal potentials of leaf and seed extracts of Croton tiglium plant. The aim of the study was to assesses the antimicrobial activity and to determine the zone of inhibition of leaf and seed extracts of Croton tighlium for potential antimicrobial activity against skin disease causing microbes using agar well diffusion method. The antibacterial and antifungal activities of extracts of Croton tiglium were tested against four bacterial and three fungal strains. Bacterial strains were Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa and fungal strains were Candida albicans, Trichophyton rubrum and Microsporum canis. Zone of inhibition of extracts were compared with that of standards. The results showed remarkable inhibition of bacterial and fungal growth against tested organisms. The phytochemical analysis was also performed which showed the presence active components like phenol, flavonoids saponins, steroids etc. in the extracts. The antimicrobial activity of leaf and seed extracts of the plant due to presence of these phytochemicals. The study supports the folkloric use of Croton tiglium plant against skin disease causing microbes.*

Keywords: *Croton tiglium, antibacterial, antifungal, phytochemical analysis.*

1. INTRODUCTION

The whole human body is covered by the protective covering called skin[1]. Our skin serves many functions like thermoregulation, absorption, secretion etc. But the most important function of skin is protection. As a primary interface between the body and the external environment, our skin provides the first line of defence against the injuries caused by microbes and chemical agents[2]. But there are several pathogens like bacteria, fungi that impact on our skin. The synthetic antibiotics that are used to treat skin infections give adverse effect on skin. Apart from this indiscriminate use of antibiotics results in gaining resistant by microbes against antibiotics [3]. So an alternative source is needed for the treatment of skin diseases. Medicinal plants and herbs have been used to treat skin disorders for a long time in traditional medicine[4]. Due to presence of active phytochemicals they show several activities like antimicrobial, antihelminthic etc[5]. In this study an attempt was made to evaluate antibacterial and antifungal activities of leaf and seed extracts of *Croton tiglium* plant. It is a wild plant and traditionally used in the treatment different disorders including skin infections.

2. MATERIALS AND METHODS

2.1. Collection of Sample

Leaves and seeds of *Croton tiglium* were collected from Jokai Botanical Garden, Dibrugarh District of Assam. Collected samples were washed, shade dried and grinded into powder. They were kept in air tight containers until use.

2.2. Extraction

Ground samples were extracted with water, ethanol, methanol and acetone with continuous shaking on a shaker for 72 hours. Following filtration of suspension through Whatman No.1 paper, the crude extracts were evaporated on water bath and residue was dissolved in DMSO (Dimethyl sulphoxide). Extracts were preserved at 4^{o}C in air tight bottle.

2.2.1. Test organisms

Authentic cultures of bacteria and fungi viz *Staphylococcus aureus (*MTCC *9542), Staphylococcus epidermidis (*MTCC *6810), Escherichia coli, Pseudomonas Aeruginosa (*MTCC 6458), *Candida albicans* (MTCC 4748), *Trichophyton rubrum (*MTCC7860*)* and *Microsporum canis (*MTCC *3270)* were obtained from "Microbial Type Culture collection and Gene Bank", IMTECH, Chandigarh, India.

Bacterial cultures were maintained on Nutrient Agar medium whereas fungal cultures were maintained on Sabouraud Dextrose Agar medium. Each inoculum was prepared by inoculating the stock culture into freshly prepared media. All bacterial strains were incubated for 24 hour at 37^{o}C and fugal strains were incubated at 27^{o}C for 48 hours. The test organisms were grown in respective broth media i.e.; Nutrient Broth for bacterial strains and Sabouraud Dextrose Broth for fungal strains.

2.3. Determination of Antimicrobial Activity

2.3.1 Antibacterial assay

Antibacterial activity of different extracts was determined by Agar well diffusion method on Muller Hinton Agar medium. Agar plates were inoculated with 100 µl of overnight grown bacterial culture which were adjusted to 0.5 McFarland turbidity standards. After inoculation, wells were made using sterile cork borer and extracts were dispersed into wells. Plates were incubated for 24 hours and zone of inhibition were measured[6]. Here DMSO and Chloramphenicol were taken as negative and positive controls respectively.

2.3.2. Antifungal assay

Sabouraud Dextrose Agar media was taken for antifungal assay. Agar plates were inoculated with fungal strains and wells were made. Test samples were dispersed into wells and kept in BOD incubator for 48 hours[7]. After that zones of inhibition were measured. Here DMSO was taken as negative control whereas Nystatin and ketoconazole were taken as positive controls.

2.4. Determination of Minimum Inhibitory Concentration (MIC):

MIC of the plant extracts was tested by the two fold dilution method. The test extracts was dissolved in DMSO to obtain 1000 µg/ml stock solution.0.5ml of stock solution was incorporated with 0.5ml of Muller Hinton Broth for bacterial strains and Sabouraud Dextrose Broth for fungi to get a concentration 0f 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml and 31.25µg/ml respectively. 50µl of standardized suspension of the test organism was transferred to each tube. The negative control tube was containing only test organism and the positive control tube was containing organism and standard antibiotics. The culture tubes were incubated in BOD incubator at 37^{O}Cfor 24 hours for bacterial cultures and at 28^{o}C for 48 hours for fungal strains. The lowest concentration which did not allow any visible growth of tested organism was taken as MIC.

2.5. Minimum Bactericidal Concentration and Minimum Fungicidal Concentration

All the tubes used in MIC study which did not show any growth of the bacteria and fungi after the incubation period were first diluted (1:4) in fresh Muller Hinton Broth for bacteria and Sabouraud Dextrose Broth for fungi and then sub cultured on to the surface of the freshly prepared Muller Hinton Agar (bacteria) and Sabouraud Dextrose Agar (fungi) plates. Then bacterial plates were incubated in BOD incubator at 37^{0}C for 24 hours and fungal plates were incubated at 28^{0}C for 48 hours. The MBC and MFC were recorded as the lowest concentration of the extract that did not permit any visible bacterial and fungal colony growth on the appropriate agar plate after the period of incubation[8].

2.6. Phytochemical Analysis

Phytochemical chemical screening of leaf and seed extracts of the plant was performed by using the methods of Sofowara (1993)[9].

3. RESULTS

Results obtained from the study showed that the leaf and seed extract of *Croton tiglium* possesses antibacterial and antifungal activities against the microorganisms tested. A total of seven

microorganisms which consists of four bacteria and three fungi were tested. When the leaf and seed extracts of the plant were assayed against the test organisms using Agar Well Diffusion Assay, the mean zone of inhibition obtained were between 8mm and 20mm. The negative control (DMSO) did not inhibit any of the microorganisms tested. MIC values of 31.25-250µg/ml were obtained for the leaf and seed extracts in the tests with bacterial agent while the range of 62.5-250µg/ml was recorded against the fungal strains.

The results of the MBC of leaf and seed extracts showed that they had MBC value ranged 250-500µg/ml. MFC of the extracts showed that the leaf and seed extracts had a MFC values ranged between 125-500µg/ml.

Table1. *Shows the diameter of zone of inhibition of leaf and seed extracts of Croton tiglium plant against tested bacteria. (Std*= Chlramphenicol)*

Test organisms	Zone of inhibition of *Croton tighlium* leaf extracts (in mm)					Zone of inhibition of *Croton tighlium* seed extracts (in mm)				
	Water	Ethanol	Methanol	Acetone	Std*	Water	Ethanol	Methanol	Acetone	Std*
Staphylococcus aureus	10.21 ±0.33	14.33 ±0.33	15.34 ±0.33	13.45 ±0.67	20.00 ±0.33	10.67 ±0.33	14.78 ±0.33	13.35 ±0.33	10.35 ±0.33	20.35 ±0.33
Staphylococcus epidermidis	8.26 ±0.67	17.78 ±0.33	14.89 ±0.67	15.78 ±0.33	19.35 ±0.33	11.33 ±0.33	13.87 ±0.33	13.67 ±0.33	11.67 ±0.67	18.68 ±0.33
Escherichia coli	10.21 ±0.33	16.35 ±0.33	14.25 ±0.67	11.25 ±0.33	18.00 ±.33	10.67 ±0.33	15.48 ±0.33	14.33 ±0.67	13.33 ±0.33	19.67 ±0.33
Pseudomonas aeruginosa	9.12 ±0.33	13.33 ±0.33	13.45 ±0.67	10.67 ±0.33	20.00 ±0.33	11.33 ±0.33	12.33 ±0.33	13.33 ±0.33	10.67 ±0.33	18.33 ±0.33

Table2. *Shows the diameter of zone of inhibition of leaf and seed extracts of Croton tiglium against tested fungi*

**Std=Nystatin for Candida albicans and Ketconazole for Trichophyton rubrum and Microsporum canis*

Test organisms	Zone of inhibition of *Croton tighlium* leaf extracts (in mm)					Zone of inhibition of *Croton tighlium* seed extract (in mm)				
	Water	Ethanol	Methanol	Acetone	Std*	Wate	Ethanol	Methanol	Acetone	Std*
Microsporum cani	8.21 ± 0.33	11.67 ±0.33	13.34 ±0.33	12.34 ±0.33	18.24 ±0.33	11.33 ±0.33	12.67 ±0.33	11.67 ±0.33	10.33 ±0.33	18.67 ±0.33
Trichophyton rubrum	8.67 ±0.33	12.34 ±0.33	14.34 ±0.67	11.67 ±0.33	19.35 ±0.33	11.00 ±0.33	13.67 ±0.33	12.67 ±0.33	10.67 ±33	18.33 ±0.33
Candida albicans	8.56 ±0.67	13.64 ±0.33	12.64 ±0.33	13.46 ±0.33	18.65 ±0.33	12.00 ±0.33	14.33 ±0.33	15.33 ±0.33	11.33 ±0.67	17.33 ±0.33

Table3. *Shows MIC values of leaf and seed extracts of Croton tiglium against tested bacteria*

Test organisms	MIC of *Croton tiglium* leaf extracts (in µg/ml)				MIC Of *Croton tighlium* seed extracts (in µg/ml)			
	water	Ethanol	Methanol	Acetone	Water	Ethanol	Methanol	Acetone
Staphylococcus aureus	250	62.5	62.5	125	250	62.5	62.5	125
Staphylococcus epidermidis	250	62.5	62.5	250	250	62.5	62.5	125
Escherichia coli	125	31.25	62.5	125	125	31.25	31.25	125
Pseudomonas aeruginosa	500	125	125	125	500	62.5	125	250

Table4. *Shows the values of MIC of leaf and seed extracts of Croton tighlium against tested fungi*

Test organisms	MIC values of leaf extracts of *Croton tighlium* (inµg/ml)				MIC values of *Croton tighlium* seed extracts (in µg/ml)			
	Water	Ethanol	Methanol	Acetone	Water	Ethanol	Methanol	Acetone
Candida albicans	250	62.5	62.5	250	250	62.5	62.5	250
Microsporum canis	250	125	125	250	250	125	125	250
Trichophyton rubrum	250	125	125	250	250	125	125	250

Table5. *Shows the MBC values of leaf and seed extracts of Croton tighlium against tested bacteria*

Test organisms	MBC values of leaf extracts of *Croton tighlium* (in µg/ml)				MBC values of seed extracts of *Croton tighlium* (in µg/ml)			
	Water	Ethanol	Methanol	Acetone	Water	Ethanol	Methanol	Acetone
Staphylococcus aureus	500	125	125	250	500	125	125	250
Staphylococcus epidermidis	500	125	125	250	500	125	125	500
Escherichia coli	250	62.5	62.5	125	250	62.5	62.5	125
Pseudomonas aeruginosa	500	250	250	500	500	125	125	500

Table6. *Shows the MFC values of leaf and seed extracts of Croton tiglium against tested fungi*

Test organisms	MFC values of leaf extracts of *Croton tiglium*				MFC values of seed extracts of *croton tiglium*			
	Water	Ethanol	Methanol	Acetone	Water	Ethanol	Methanol	Acetone
Candida albicans	500	125	125	250	500	125	125	250
Microsporum Canis	500	250	250	500	250	125	125	500
Trichophyton rubrum	500	250	250	250	500	125	125	500

Table7. *Shows the presence of phytochemicals in the leaf and seed extracts of Croton tighlium prepared in different solvents*

Phytochemicals	Leaf extracts of *Croton tiglium*				Seed extracts of *Croton tiglium*			
	water	ethanol	Methanol	Acetone	Water	Ethanol	Methanol	Acetone
Phenol	+	+	+	-	+	+	+	-
Flavonoid	+	+	+	-	+	+	+	-
Alkaloid	-	+	+	-	-	+	+	-
Saponin	+	-	-	+	+	-	-	+
Tannin	-	+	+	-	-	+	+	+
Glycosides	+	+	+	-	+	+	+	-
Steroid	-	+	+	-	+	-	+	+

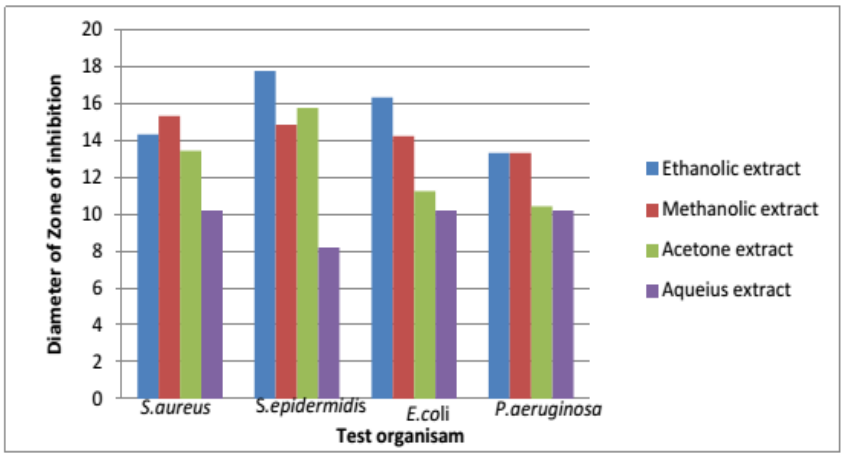

Fig1. *Shows diameter of zone of inhibition of different solvent extract made from leaves of Croton tighlium against tested bacteria*

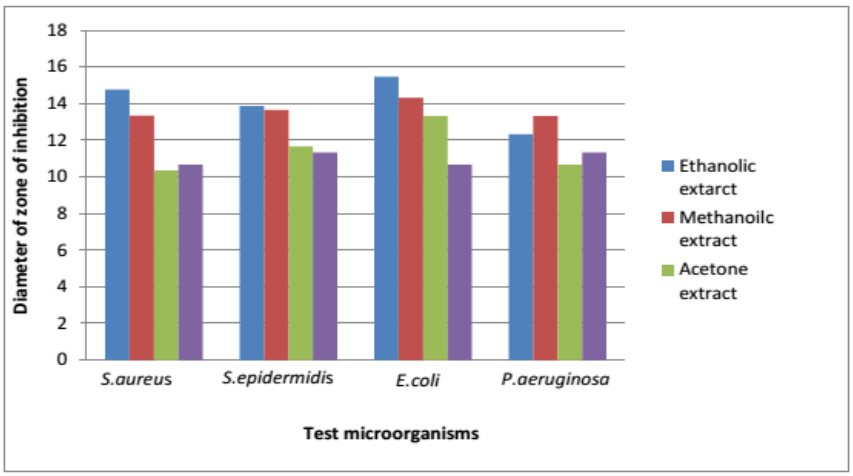

Fig2. *Shows diameter of zone of inhibition of different solvent extract made from seed extract of Croton tighlium against tested bacteria.*

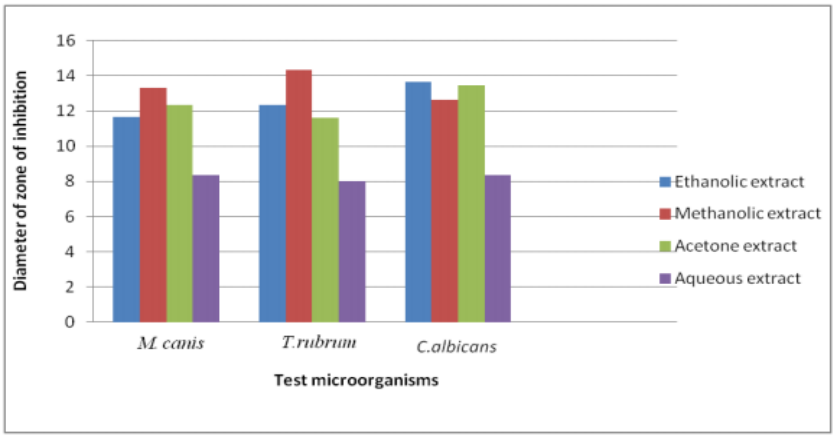

Fig3. *Shows the diameter of zone of inhibition of different solvent extracts made from leaves of Croton tighlium against tested fungi*

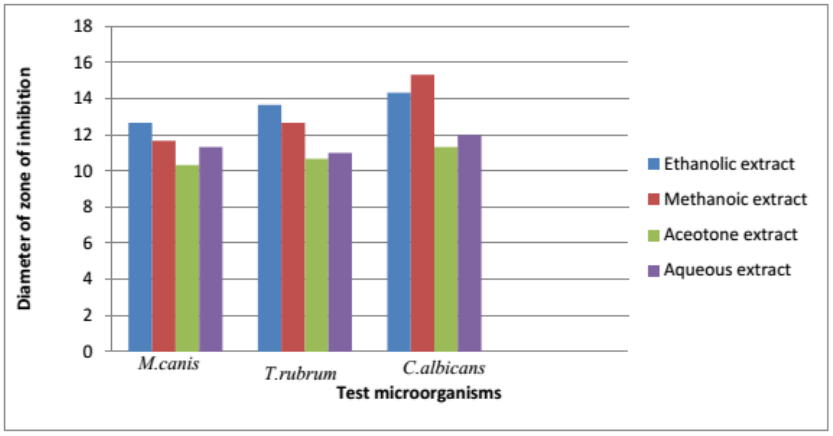

Fig4. *Shows the diameter of zone of inhibition of different solvent extracts prepared from seed extracts of Croton tighlium against tested fungi.*

4. Discussion and Conclusion

Antimicrobial assay of leaf and seed extracts of *Croton tighlium* plant gave positive results against tested microorganism. With MIC values between 31.25 and 500µg/ml alcoholic extracts of the plant showed highest zone of inhibition against microorganisms. Phytochemical analysis also revealed the presence of active phytocomponents like phenol, flavonoids, alkaloid, saponins, tannin, glycosides in leaf and seed extracts of the plant[10]. The presence of these phytochemical constituents in the extracts could be responsible for antimicrobial activities of *Croton tiglium*. Antimicrobial activity of different plant extracts on pathogenic bacteria was studied and reported by other worker[11].The values of medicinal plants lies in phytochemical constituents that cause definite pharmacological action on human body [12, 13].

This study confirmed that the leaf and seed extracts of *Croton tiglium* possesses antimicrobial activities against skin disease causing microbes. The antimicrobial activity of the plant may be attributed to various phytochemical constituents present in the crude extracts[14, 15]. It can be concluded that antimicrobial activity and its active components would be helpful nitrating skin disease.

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