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Research Article

Antimicrobial Activity of *Mallotus philippensis* and *Allophylus cobbe*

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ABSTRACT

The antimicrobial activity of the aqueous and methanolic extracts and their n-hexane, chloroform, n-butanol and water fractions were tested for antimicrobial activity against eleven microorganisms. The agar well diffusion method using Muller Hinton Agar was used for bacteria and SDA for fungi. The lyophilised cultures purchased from MTCC were revived and used for the study. The extracts were diluted to 500, 250, 100, 50, 25, 12.5 and 100, 50, 25, 12.5, 6.25 mg/ml using DMSO/ Tween 80 and 25 microlitres were transferred into pre-inoculated petri plates, incubated for 24hrs in case of bacterium and 48 hours for fungi and the zones of inhibition was measured. The extracts were subjected to phytochemical analysis as well as cute oral toxicity testing in rats at dose rate of 2000 mg/kg. From the results it is evident that the butanol, chloroform and water fractions of methanolic extract of *A. cobbe* and hexane fraction of *M. philippensis* showed maximum inhibition against *S. aureus, S. pyogenes* and *Cryptococcus.* There was no activity against any of the gram negative organisms tested. From the study, it could be concluded that the extracts of *A. cobbe* and *M. Philippensis* posses good antimicrobial property without any potent toxicities.

Keywords: *Allophylus cobbe*, *Mallotus philippensis*, Minimum Inhibitory concentration, *Cryptococcus, Staphylococcus aureus*

INTRODUCTION

Medicinal plants have long been used for treatment of ailments and such traditional knowledge is the back bone of discovery of many new drugs and novel molecules. Even though the pharmaceutical industry is behind use of chemicals for therapy of ailments, some interest is put back on use of natural substances for therapy of diseases[1]. A number of plants have been screened for their medicinal properties and the purified products have become a part of therapy. In traditional medicine, the crude extracts are used for the therapy and form a lead for the synthesis of newer drugs[2].

Continuous evolution of new pathogens and re-emergence of existing and eradicated pathogens along with the problem of resistance to the pharmaceuticals lead to the intensification of development of newer drug with different mechanism of action [2]. The emergence of these pathogens has made the therapy of diseases like tuberculosis and HIV cumbersome. Recently there are several reports of plants with antimicrobial properties. These phytochemicals may act on sites other than those that are commonly targeted and reduce the chance of drug resistance[3].

Allophylus cobbe L. belongs to the family sapindaceae, is a herb that grows in the hills of Wayanad district of Kerala. The plant has been shown to have wound healing, anti-diarrhoeal, oxytocic, antifeedant and antimicrobial properties[4]. *Mallotus philippensis* (Lam.) Muell. Arg (Euphorbiaceae) are shrubs or small trees which grow on mountain slopes or valleys, limestone hills or river valleys and forests at an altitude of 300–1600 m in Asia and Australia. Different parts of the plant have been used in traditional medicine. Kamala, a red powder consisting of glandular hairs from plant capsule has been used as anthelmintic and cathartic in traditional medicine and an orange dye for silk. Kamala is commonly administered in its crude form for the elimination of intestinal worms and also for skin irritation, ringworm, and freckles The fruit of the plant is purgative for animals[5,6].

The study forms a screening procedure of *A. Cobbe*.and *M. philippensis* for its antimicrobial property

MATERIALS AND METHODS

Preparation of plant material

The plant material was collected from the hills of Wayanad and identified at MSSRF, Kalpetta. The leaves were separated, dried under shade, pulverized and extracted using methanol and water. The extraction with methanol was done in a soxhlet extraction apparatus whereas the aqueous extraction was done as a decoction. The extracts were dried using a rotary vacuum evaporator and stored under refrigeration.

Fractionation of the methanolic extract

The methanolic extract was fractionated using various solvents based on their increasing strength of polarity. The extract was first mixed with 10 volumes of n-Hexane,

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Anti-microbial activity

	Allophylus cobbe								*Mallotus philippensis*							
Extract	Phenolics	Alakloids	Steroids	Glycosides	Tannins	Terpenes	Saponins	Flavonoids	Phenolics	Alakloids	Steroids	Glycosides	Tannins	Terpenes	Saponins	Flavanoids
Aqueous	+	-	+	-	+	+	+	+	+	-	-	-	+	-	+	+
Methanolic	+	-	-	+	+	+	+	-	-	-	-	-	+	-	+	+
Hexane fraction	-	+	-	-	-	-	-	-	+	-	+	-	+	+	-	+
Chloroform fraction	-	+	-	+	+	-	-	+	+	-	-	-	+	-	-	-
Butanol fraction	+	-	-	+	+	+	+	+	+	-	-	-	+	+	-	+
Water fraction	+	-	-	-	+	+	+	+	+	-	-	-	+	+	-	+

followed by the mixing of insoluble part with Chloroform. The left over residue was then mixed with 1:1 n- butanol and water and the fractions were separated using a separating funnel. The fractions were then dried using a vacuum evaporator and stored under refrigerated conditions till use.

Phytochemical analysis

The extract as well as the fractions was analyzed qualitatively for various phytochemical constituents[7]

Antimicrobial assay

The extracts as well as the fractions were diluted using 10% DMSO/ tween 80 solutions to get serial dilutions of 500, 250, 100, 50, 25, 12.5 and 100, 50, 25, 12.5, 6.25 mg/ml of the solution respectively. In all the tests 10% DMSO/ tween 80 solutions were kept as negative control. Octadisc (Himedia) containing Amoxicillin 10 mcg, Tetracycline 30 mcg,Co- Trimoxazole 25 mcg, Ciprofloxacin 5 mcg, Gentamycin 10 mcg, Erythromycin 15 mcg, Chloramphenicol 30 mcg and Cefalexin 30 mcg were used as positive control.

Antimicrobial assay was done in Muller Hinton Agar plates. Microbial cultures with 0.5 McFarland standard turbidity equivalents were prepared as suspensions in nutrient broth. The suspensions were further diluted to 1:10 to get a concentration of 106 CFU/ml. About 0.2 ml of the diluted inoculums was applied directly to the plate and spread using the sterile L shaped spreader. Wells were bored into the agar using a sterile 6 mm well borer. The wells were then filled with 25 µl of the DMSO/ tween 80 diluted extracts in different concentrations and incubated at 37^0 C for 24 hrs. Inhibition zones were measured and recorded as the mean diameter (mm) of complete growth inhibition. The tests were done in triplicates[8].

Assessment of Minimum Inhibitory concentration for antibacterial activity

The test was performed in microtitre plates. 100 µl of MH broth (for bacteria) was mixed with equal volume of the extract. The dose range between the last concentration that showed antibacterial property and the first concentration that did not show inhibition were divided equally into 5 dilutions. These dilutions were used for calculating MIC. 100µl of the inoculums specimens were added to each well and incubated for 24 hrs at 37° C[8].

Antifungal Assay

The inoculums was prepared as described earlier and the plates were made using SDA. The extract was well as the negative controls were added as described in the antibacterial assay. After incubation for 48 hrs at 28° C, the plates were examined for the presence of growth/ inhibition of growth and the diameter of zone of inhibition was measured in mm. Octadisc containing Amphotericin B 100 units, Clotrimazole 10 mcg, Fluconazole 25 mcg, Itraconazole 10 mcg, Ketoconazole 10 mcg and Nystatin 100 units were used as positive control. The tests were done in triplicates[9].

Minimum Inhibitory Concentration for antifungal activity

Minimum inhibitory concentration determination was done by a serial dilution plate technique where solutions of the reconstituted extracts at 0.01- 3mg/ml of the media (based on the results of agar dilution technique) were added into the pre- sterilized and pre- cooled SDA, the media poured and allowed to set. The plates were then inoculated with the test fungi and incubated at 28^0C for 2-7 days. Control plates which contained no extract were also prepared along with the extract. The MIC of each plant was determined after incubation, being the lowest concentration with no visible growth[10].

RESULTS

Phytochemical constituents

Table 1: Phytochemical constituents of different extracts and fractions

Phenolic compounds, tannins and flavonoids are the major compounds present in almost all the extracts and fractions. Steroids were absent in extracts of *A. Cobbe* whereas alkaloids and glycosides were absent in *M. philippensis.*

Maximum activity for both the extracts was seen against *Streptococcus pyogenes* and *Staphylococcus aureus*. The minimum inhibitory concentrations was 6.25 mg/ml in butanol, chloroform and water fractions of methanolic extract of *A. cobbe* and hexane fraction of *M. philippensis* against *Cryptococcus* species. The hexane and chloroform

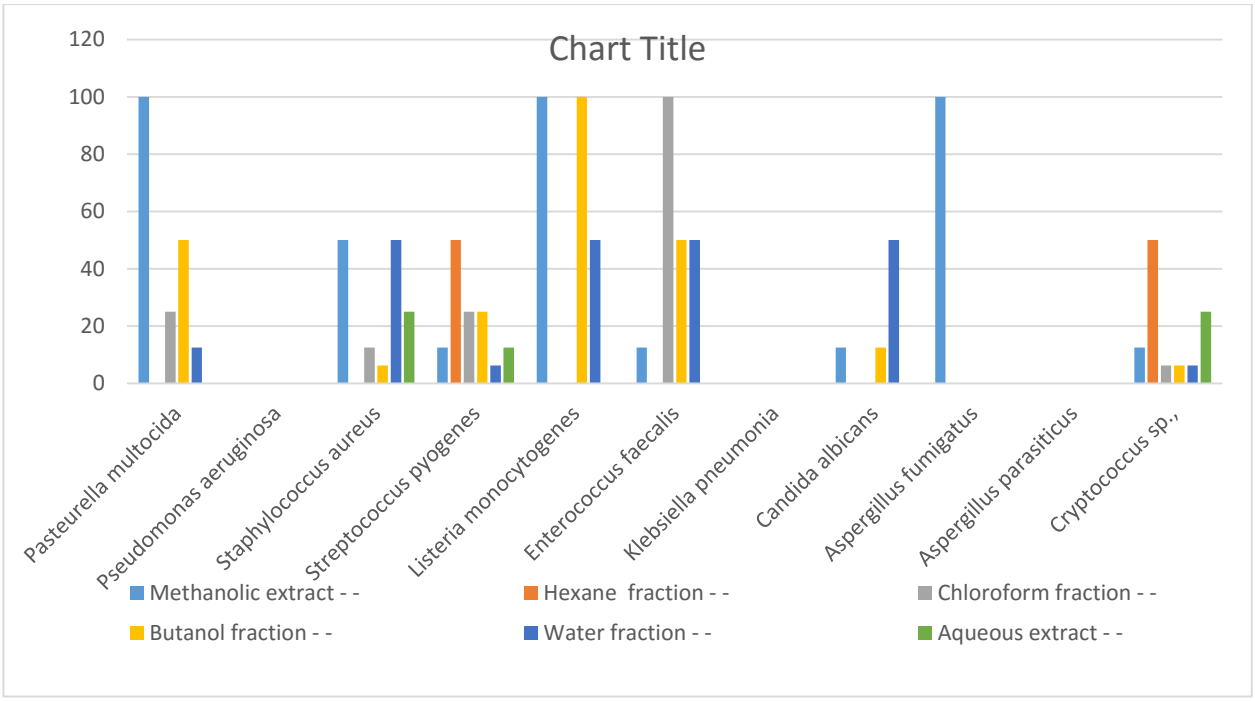

Figure 1: Minimum inhibitory concentration of the extracts and fractions of *A. Cobbe* against different pathogens (mg/ ml).

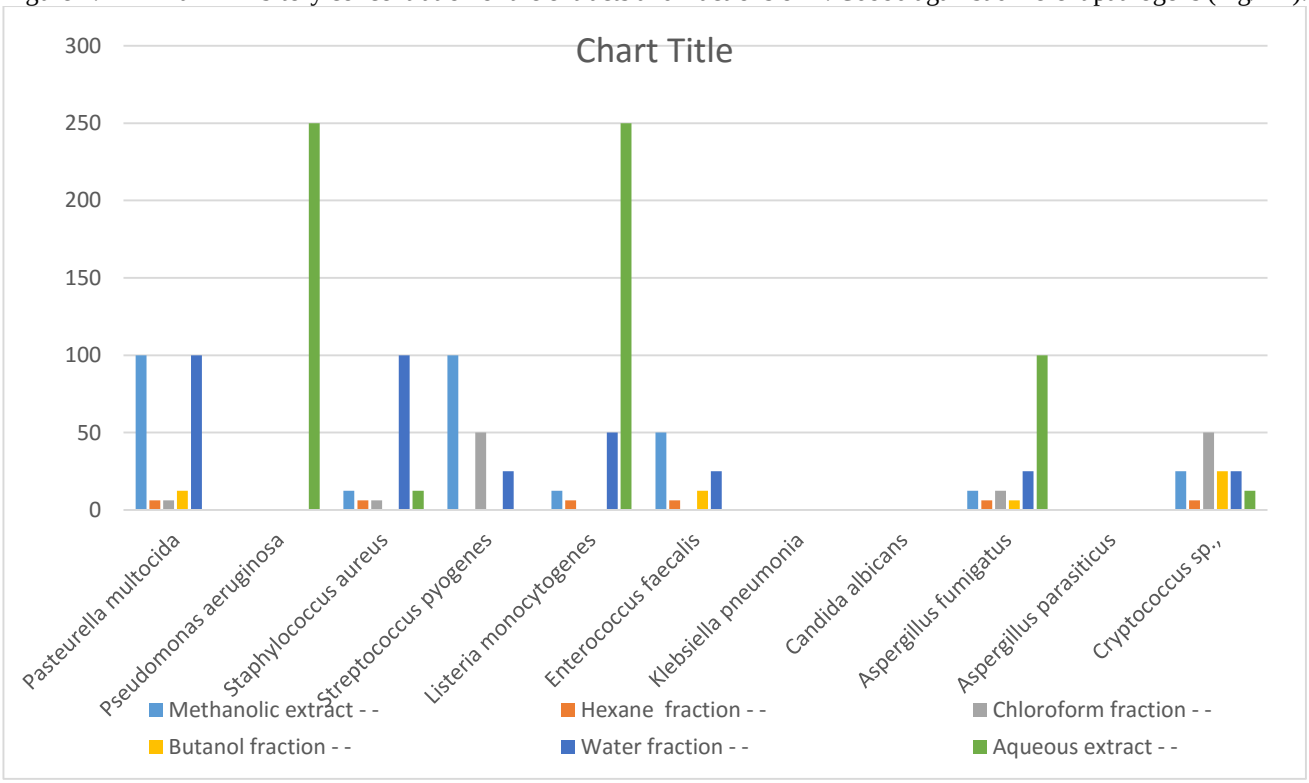

Figure 2: Minimum inhibitory concentration of the extracts and fractions of *M. Philipensis* against different pathogens (mg/ ml).

fraction of *M. philippensis* showed the same MIC against *Pasturella multocida, S. aureus* and *A. fumigatus.* There was very little effect of all the extracts on *C. albicans.* There was no effect against *Enterococcus, E. coli, Klebsiella* etc. which was very distinct from overgrowth of these bacterium above the wells.

Acute Oral toxicity

No toxicity or clinical signs were noticed during the entire period of observation.

DISCUSSION

A number of studies are being carried out in different parts of the world on the effect of phytochemicals against microbes. The ever rising problem of antibiotic resistance

has necessitated the development of a new molecule with a different mechanism of action. Screening of phytochemicals and crude extracts from plants provide a lead for development of a new novel antibiotic.

From the results of the study, it is evident that the extracts have got effect only on Gram positive bacteria and *Cryptococcus*. Since there is no activity against the gram negative organisms, the action of these extracts may be on the cell wall of the bacterium and the difference in their structure can be attributed to their selective action. Earlier studies states that a crude extract having less than 8mg/ml of MIC can be considered noteworthy to be a potential agent for antimicrobial activity[11]

Phytochemical screening of the potent extracts revealed the presence of tannins, terpenes and flavonoids. The antibacterial effect of tannins has already been substantiated in many plants[12,13]. The effects of saponins, tepernes and flavonoids on different bacteria and their mechanism has been understood[14,15,16]. The presence of these phytochemicals in our extract can be the reason for the potent antibacterial and antifungal property of these extracts.

Further investigations into the mechanism of action and the isolation of a molecule should be undertaken to elucidate the potential of these extracts.

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