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PHYTOCHEMICAL ANALYSIS AND ISOLATION OF COMPOUNDS FROM METHANOLIC LEAF EXTRACT OF *AMARANTHUS SPINOSUS* AND ITS ANTIMICROBIAL EFFECT AGAINST *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

The crushed leaf of *Amaranthus spinosus* is used to treat skin infections and wound. The leaves are boiled without salt and consumed for 2-3 days to cure jaundice. Recent studied showed that *Amaranthus* leaves contain various health essential nutrients that exert anti-inflammatory action against skin infection. The aim of the study is to see the effect of isolated compounds from the methanolic leaf extract of *Amaranthus spinosus* against *Staphylococcus aureus*. In our study we found that the methanolic leaf extract of *Amaranthus spinosus* contained alkaloids, carbohydrate, glycosides, saponin, steroids and flavonoids. Antimicrobial activity of the crude extract showed an efficient activity against S. aureus. On performing the column chromatography of the leaf methanolic extract using hexane, mixture of hexane-methanol and methanol we obtained 17 fractions. The isolated compounds in Fraction 1 and Fraction 2 gave positive saponin test, Fraction 4 and 7 gave alkaloids positive test. Fraction 9, 12 and 15 showed the positive result of glycosides and steroids, Fraction 13 showed positive flavonoids test. Fraction 16 gave positive carbohydrate test. On performing the antimicrobial activity of these fractions against *Staphylococcus aureus* it was found that Fraction 4, 7, 12 and 15 showed antimicrobial activity against S. aureus whereas the rest of the fractions showed no activity at all.

KEYWORDS: *Amaranthus spinosus*, *Staphylococcus aureus*, Methanolic extract, Phytochemical constituents.

INTRODUCTION

Recent studied showed that Amaranthus leaves contain various health essential nutrients that exert anti-inflammatory action against skin infection. Due to the powerful astringent activity, these leaves act as a natural remedy to treat acne and other skin problem like eczema. Traditionally pastes of leaves are applied on the boils to treat it.

Humans are natural hosts for many bacterial species that colonize the skin as a normal flora. *Staphylococcus aureus* is infrequent resident flora, but they account for a wide variety of bacterial pyodermes. Predisposing factor to infection include minor trauma, pre-existing skin diseases, poor hygiene and rarely impaired hosts immunity. Therefore, this study has been aimed to see the effect of methanolic leaf extract on *Staphylococcus aureus*.

METHODOLOGY

Collection of the sample

Fresh *Amaranthus spinosus* plant was collected from various areas of Guwahati. The collected specimen was supplied to the dept. of Botany, Gauhati University for identification. The supplied herbarium specimen identified as *Amaranthus spinosus* L. Family Amaranthaceae Acc. No. 18132 dated 25.04.2016. GUBH dept. of Botany. The leaf part were separated from the whole plant and then shade dried. The dried plant leaves were ground in powdered form and stored in air tight container.

Extraction

10 gram of the powdered leaves sample was extracted in methanol in soxhlet apparatus. After extraction it was evaporated to dryness in a porcelain dish. Then it was stored at 4˚C for the phytochemical analysis.

Phytochemical Screening of Plant Extracts

Chemical tests for the screening and identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, proteins, amino acids, flavonoids, and tannins, in the medicinal plants under study were carried out in extracts by using standard procedure in.[11]

Column chromatography

The column was placed in a ring stand in a vertical position. A plug of the cotton was pushed down to the bottom of the column. The slurry of the silica gel was prepared with the solvent and poured gently in the column. Then the stop clock was opened to drain out some solvent. The sample was dissolved a small amount of the methanol solvent. The solvent was removed by placing it in a rotary evaporator at a low temperature. The dry powered was transferred to the top of the column through the funnel.

Continuously the column was filled with hexane and the stop cock was opened. The components of the extract run down the column forming separate band. The fractions were collected in different five conical flaks. Then the mixture of hexane and methanol in 9:1 ratio were added continuously and the fractions were collected in seven different conical flasks. After these methanol were added and in five conical flaks fractions were collected.

Antimicrobial activity of the fractions

After evaporation anti-microbial activity was seen by using disc diffusion method. The Muller Hinton Agar media was prepared and it was kept for solidifying. Then the test microorganisms were swab aseptically onto a Muller Hinton Agar plate. Swab was done in three dimension to ensure complete plate coverage. Each fraction were applied on the sterile filter paper discs and placed on Muller Hinton Agar plate. The plates were incubated at 37˚C. Antibacterial activity was evaluated by measuring diameter of the zone of inhibition.

RESULT

The result of the phytochemical test of methanol leaf extract of *Amaranthus spinosus* indicates that all tests for Alkaloids, Carbohydrate, Glycosides, Saponin, Steroids and for Flavonoids showed positive result. These six phytoconstituents are present in the methanol leaf extract. The result of phytochemical analysis showed negative for Protein and Tanin. The methanolic extract showed antimicrobial activity against Staphylococcus aureus. After 24 hours of incubation it showed 15mm zone of inhibition.

Fraction 1 and fraction 2 gave positive result for Saponin test and for other phytochemical test all are negative. Fraction 4 and fraction 7 showed Alkaloids positive result. Fraction 9, fraction 12 and fraction 15 gave Glycoside and Steroids positive results. Fraction 13 gave positive result for flavonoids. Fraction 16 showed the positive result for Carbohydrate. After 24 hours of incubation the effect of the fraction 4 was 12mm, fraction 7 and fraction 12 were 15mm and fraction 15 was 9mm against *Staphylococcus aureus*.

Figures of antimicrobial assay of plant extract and its fractions

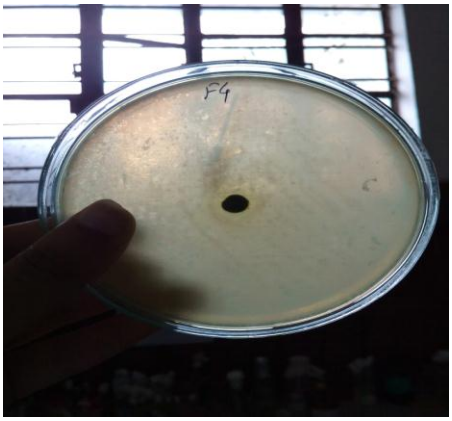

Fig. 1: The effect of fraction 4 on MHA media against *Staphylococcus aureus*.

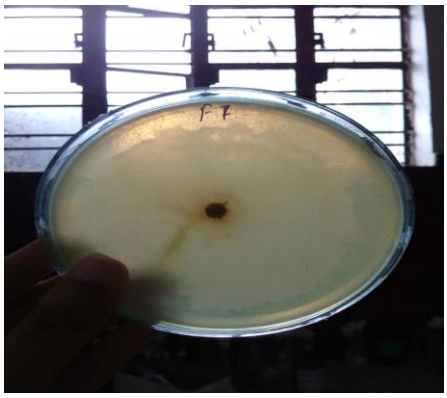

Fig. 2: The effect of fraction 7 on MHA media against *Staphylococcu aureus*.

Fig. 3: The effect of fraction 12 on MHA media against *Staphylococcus aureus*.

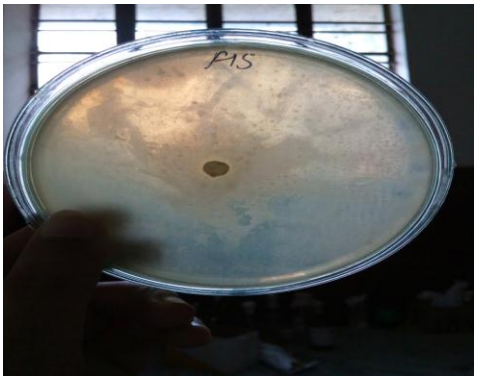

Fig. 4: The effect of fraction 15 on MHA media against *Staphylococcus aureus*.

DISCUSSIONS

The main objective of the study is the analysis of the phytochemical compound present in the leaves and its antimicrobial activity. Phytoconstituents were extracted using methanol as the solvent for its ability to extract majority of the polar compounds. The methanolic extract of leaves showed antimicrobial activity due to the presence of phytochemicals. Further separation through column chromatography showed 17 fractions. The leaf extract was found to contain phytochemicals like alkaloids, carbohydrate, glycosides, saponin, steroids etc.

It was found that fraction 1 and fraction 2 contained saponin, fraction 4 and fraction 7 contained alkaloids, fraction 9, fraction 12 and fraction 15 contained glycosides and steroids, and fraction 13 contain flavonoids, fraction 16 contain carbohydrate. Antimicrobial activity carried out from these fractions showed that Fraction 4, 7, 12 and 15 were active against S. aureus. From the phytochemical tests it was seen that fraction 4 and fraction 7 contained alkaloids and fraction 12 and fraction 15 contained glycosides and steroids. Therefore in our study we can conclude that the activity by these four fractions were possibly because of these compounds alkaloids, glycosides and steroids which were found to be present.

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