Research Article

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Preliminary phytochemical screening and evaluation of antibacterial effect of *Amaranthus caudatus*

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ABSTRACT

Amaranthus caudatus is a medicinal plant belongs to the family Amarannthaceae. The present study was carried out to investigate the phytochemical constituents and assayed for antibacterial effect of this plant. In the phytochemical screening, different types of solvent like ethanol, chloroform, petroleum ether were taken which revealed that the leaves extract of *Amaranthus caudatus* contain flavonoids, terpenoids, steroids, cardiac glycosides, carbohydrates, tannins, saponins, proteins as secondary metabolites and chemical compounds. The microorganisms assayed for antibacterial activity were against three Gram positive bacteria *Listeria monocytogenes, Staphylococcus pyrogenes, Enterococcus faecalis* and three Gram negative bacteria *Salmonella enterica*, *Escherichia coli, Pseudomonas aeruginosa* The result showed that the leaf extract of *Amaranthus caudatus* can be used as a good herbal medicine.

Key words: *Amaranthus caudatus*, Leaf extract, Phytochemicals, Antibacterial.

INTRODUCTION

Antimicrobial resistance is a global problem that has created immense clinical problem in treatment of infectious diseases. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant (MDR) pathogens. Hospitals, worldwide are major contributors of the problem of antimicrobial resistance [1]. Hospital-acquired infections are expensive to control and extremely difficult to eradicate. In India, antimicrobial resistance has been reported in for the most predominant pathogenic microorganisms *Listeria monocytogenes, Staphylococcus pyrogenes, Enterococcus faecalis, Salmonella enterica, Escherichia coli, Pseudomonas aeruginosa.* Medicinal plants form an integral component of research in the pharmaceutical industry focusing on active screenings of natural products to develop better drugs against microbial infections [2]. Their medicinal power lies in phytochemical constituents that cause definite pharmacological action on human body. Some of the, most significant bioactive phytochemicals are alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds and many more [3]. Phytochemicals are mainly divided into two groups, which are primary and secondary constituents according to their activity in plant metabolism. Primary constituents contain common sugars, amino acids, proteins and chlorophyll while secondary constituents comprise of alkaloids, flavonoids, saponins, tannins, phenolic compounds and many more [4].

Amaranthus caudatus Linn. belongs to the family Amaranthaceae, occurs naturally in Southern Asia. *A. caudatus* is a small herbs, known for its high anti-oxidant, anti-hypercholesterolemic, anti-atherogenic, anti-arthritic and anti-microbial properties [5,6]. Various type of phytoconstituents such as Gallic acid (GA), Caffeic acid (CA), Rutin (RU), Ferulic acid (FA) and Quercetin (QU) present in *A. caudatus* are mainly responsible for their medicinal properties. In the present work it was investigated the antimicrobial activity of *A. Caudatus* leaf extract against various test pathogens.

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MATERIALS AND METHODS

Plant Materials

Fresh plant leaves of *Amaranthus caudatus* were collected from Chennai, Tamil Nadu. The leaves are thoroughly washed through tap water and dried under shade for 3-5 days. The dried leaves are ground to fine powder and stored in polythene bags for further use.

Preparation of plant extract

500 gm of dried powder of *Amaranthus caudatus* was weighed and added in 750ml of different solvents such as ethanol, chloroform and petroleum ether in separate round bottom flask for sample extraction. The extraction was conducted for each solvent for 7 days at room temperature with occasional shaking and stirring. At the end of the extraction the whole mixture then underwent a coarse filtration by a piece of clean, white cotton material followed by a fine filtration through Whatmann No.1 filter paper and the respective solvents were concentrated for phytochemical screening using rotary vacuum evaporator. The crude extracts were stored in airtight container and placed in refrigerator.

Qualitative Phytochemical Analysis

The ethanol, chloroform and petroleum ether extract of *Amaranthus caudatus* prepared to identify the presence of the phytocomponents [7-14].

Test for alkaloids

The small portion of extracts were stored separately with a few drops

of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal agents, such as Mayer's reagent (cream precipitate), Dragendorffs reagent (orange brown precipitate).

Test for cardiac glycosides

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxysugar characteristic of cardioids.

Test for flavonoids

5 ml of dilute ammonia solution were added to a portion plant extract followed by addition of concentrated H_2SO_4. A yellow coloration absorbed in extract indicated presence of flavonoids. The yellow coloration disappeared on standing.

Test for steroids

Two ml of acetic anhydride was added to 0.5 extract with 2ml H_2SO_4. The color changed from violet to blue or green in samples indicated presence of steroid.

Test for terpenoids

Five ml of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3ml), was carefully added to form a layer. A reddish brown coloration of the interface was formed indicated presence of terpenoids.

Test for saponins

About 1 ml of alcoholic and agrees extract was diluted with distilled water to 20ml and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated presence of saponin.

Test for protein

Million's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating.

Test for tannins

About 0.5 g of extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

Test for Carbohydrates:

A small amount of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

Antibacterial assay

Antibacterial activity spectrum of different solvent extract of *Amaranthus caudatus* was determined by agar well diffusion method according to National Commmittee for Clinical Laboratory Standards (NCCLS). For this three Gram positive bacteria such as *Listeria monocytogenes*, *Staphylococcus pyrogenes*, *Enterococcus faecalis* and three Gram negative *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa* were taken. Freshly cultured microbial suspensions in Mueller Hinton broth were standardized to a cell density of 1×10^7 CFU/ml. Antibacterial assay was carried out on Muller Hinton agar. About 25 ml of Nutrient agar medium was poured into each petri plate. Once the agar solidified, the plant extract were inoculated in the well (8 mm in diameter) on the plates at different concentrations. The plates were incubated at 37ºC for 24 h. The well containing the same volume of solvent served as control. After incubation, the diameters of the growth inhibition zones were measured in millimeters [2].

RESULTS

Table 1. Phytochemical constituents of leaf extract of *Amaranthus caudatus* in different extract

Phytochemical components	Ethanol	Chloroform	Petroleum ether
Alkaloids	-	-	-
Flavonoids	+	-	-
Tanins	+	-	-
Terpenoids	+	-	-
Saponins	+	+	+
Proteins	+	-	-
Carbohydrates	+	-	-
Steroids	+	-	-
Cardiac glycosides	-	+	-

+: Positive -: Negative

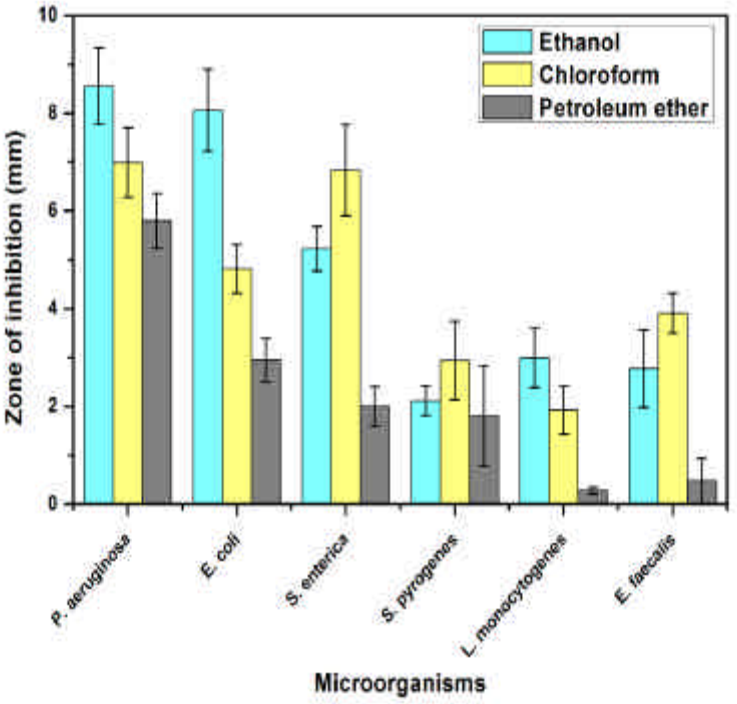

Figure 1. Graphical representation of antibacterial screening of different extract of *Amaranthus caudatus* against Test microoganisms

DISCUSSION

Table 1 shows the preliminary phytochemical screening of *Amaranthus caudatus* from different extract such as ethanol, chloroform and petroleum ether. The ethanolic extract shows that presence of flavonoids, terpenoids, steroids, carbohydrates, tannins, saponins, proteins and absence of alkaloids and cardiac glycosides. The chloform extract of the plant shows the positive result for saponins and cardiac glycosides and negative results for alkaloids, flavonoids, tannins, terpenoids, proteins, steroids and carbohydrates. The petroleum ether extract of this plant shows the positive result only for saponins whereas negative result for other phytochemicals. Hence the ethanolic extract shows the better result than choloroform and petroleum ether extract. The result implies that this plant is containing primary constituents like proteins, sugars and flavonoids, tannins, saponins, steroids as secondary metabolites which can be used in various pharmacological applications.

Three Gram positive bacteria *Listeria monocytogenes, Staphylococcus pyrogenes, Enterococcus faecalis* and three Gram negative bacteria *Salmonella enterica, Escherichia coli, Pseudomonas aeruginosa* were screened for antibacterial effect of *Amaranthus caudatus* (Fig 1.). Three different extract like ethanol, chloroform, petroleum ether were used for the extraction of phytochemicals. The ethanolic extract of this plant showed more effective against the test microorganism than the other extracts. The close spectrum of this screening revealed that the Gram negative bacteria have more zone of inhibition than the Gram positive. Among the Gram negative bacteria, *Pseudomonas aeruginosa* is more sensitive to the plant leaf extract. This can be explained by the structure of the cell wall of bacteria. The Gram positive bacteria is containing a thick peptidoglycan layer about 20-80 nm in their cell wall whereas Gram negative bacteria is having a thin peptidoglycan layer of 2-3 nm. Therefore the plant extract might not be penetrated easily in the cell wall of Gram positive bacteria compared to Gram negative bacteria.

Plant products are in the richest source of drugs. Traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs are directly or indirectly dependent on plants [15]. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Herbal medicine is still the mainstay of about 75 - 80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents [16]. The urge in research on new drugs from natural sources is now moving out of the herbalists shop, away from the core texts into the drug research laboratories [17]. Therefore there is need to concentrate on those natural herbal products which can be used as therapeutic agents.

CONCLUSION

The data presented here indicate that the dried leaves of *Amaranthus caudatus* were subjected in different solvents such as chloroform, ethanol and petroleum ether which revealed the presence of flavonoids, terpenoids, steroids, cardiac glycosides, carbohydrates, tannins, saponins, proteins as phytoconstituents. The antibacterial data showed that the plant extract in ethanol solvent showed more effective than others. So from the current study conclude that *Amaranthus caudatus* has the ethnopharmacological importance. Further work can be done to isolate, identify and characterize the structure of the bioactive compound responsible for pharmacological activity.

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