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Research Article

PRELIMINARY STUDIES ON PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *Toddalia asiatica.L* Var Floribunda

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ABSTRACT

Medicinal plants acts as a raw material base for the elaboration of more complex semi-synthetic chemical compounds. India is a home to a variety of traditional medicine systems that relay to a very large extent on native plant species for their raw drug materials. Therefore, now there is a need to look back towards the traditional medicine which can serve as novel therapeutics. *Toddalia asiatica. L* Var Floribunda, has been in folklore use in India and China from 18th century. Since, this plant possess many medicinal properties, the present study was designed to evaluate the phytochemicals and the antimicrobial activity of the methanolic stem extract of *Toddalia asiatica.L.* The methanolic extract of the stem was subjected to microbiological tests to ascertain their antimicrobial activity against various microorganisms like *Staphylococcus aureus, Klebsiella pneumonia, E.coli, Proteus vulgaris, Pseudomonas aeruginosa, Bacillus anthracis* and *Bacillus subtilis* and fungi viz. *Fusarium oxysporum, Aspergillus flavus, Pencillium sp* and *Candida albicans.* The extract showed maximum activity against the microorganism. The results of the above study conclusively validate the phytochemical treasures and its anti microbial components indulged in *Toddalia asiatica L* Var Floribunda.

Keywords: *Toddalia asiatica. L* Var Floribunda, antimicrobial activity, methanolic extract, phytochemicals.

INTRODUCTION

Nature has been a source of medicinal agents for 1000's of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. Thus plants continues to plays a main role in traditional medicine system for health care. The abundance of plants on the earth's surfaces has led the increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents[1]. There is a growing interest in correlating phytochemical constituents of a plant with its pharmacological activity[2]. Development of microbial resistance to the available antibiotics has led scientists to investigate the new drug with antimicrobial activity. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action[3]. The bioactive compounds like alkaloids, flavonoids, tannins and phenolic compounds are the reason for the medicinal value of plants that produce a definite physiological action on the body[4].

MATERIALS AND METHODS

Plant materials

The plant material (stem) of *Toddalia asiatica* was collected at Kolli hills Namakkal *Distr*ict which is rich in wide variety of medicinal plants. The collected sample was identified and confirmed by BSI, Coimbatore. The stem part was air dried and powdered.

Preparation of the extracts for phytochemical analysis

10 grams pulverized material were dissolved in 100 ml of methanol and kept in a shaker for overnight. The obtained extracts were filtered with Whatmann No.4 filter paper and the filtrate was collected and used for analysis[5]. The antibacterial activity of the plant extract against organisms of interest was determined by the agar diffusion method[6]. The antimicrobial activity of the extract was determined by measuring the diameter of zone of inhibition exhibited by the extract around the well.

Preliminary Phytochemical analysis

Detection of Carbohydrates

A minimum amount of extracts were suspended in 5ml of distilled water. The suspension was subjected to the following chemical tests.

Molisch's test

The extracts were treated with 2-3 drops of 1% alcoholic alpha napthol and 2 ml of concentrated Sulphuric acid was added along the sides of the test tube. The formation of purple ring between two layers, which shows the presence of carbohydrates.

Fehling's test

The extracts were treated with Fehling's A and B solution and heated for few minutes. Formation of brick red precipitate shows the presence of reducing sugar.

Benedict's test

The extracts were treated with Benedict's reagent and heated for few minutes. Formation of red precipitate shows the presence of reducing sugar.

Detection of Glycosides

Minimum quantities of the extracts were hydrolyzed with hydrochloric acid for few minutes on a water bath and the hydrolyzate was subjected to the following tests.

Legal's test

To the hydrolyzate 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide. The pink color changes in to red show the presence of glycosides.

Detection of Proteins and Amino acids

A small quantity of extract was dissolved in few ml of water and they were subjected to following tests

Million's test

The extracts were treated with Million's reagent. The precipitate was formed with the extract, which shows the presence of proteins.

Ninhydrin test

The extracts were treated with Ninhydrin reagent. The purple colour was formed with extract, which shows the presence of proteins.

Biuret test

To the extracts equal volume of 5% sodium hydroxide solution and 1%copper sulphate solution was added. A violet colour formation indicates presence of amino acids.

Detection of Alkaloids

A small quantity of the extract was separately treated with few drops of dilute hydrochloric acid and filtered. The filtrate was used for the following tests. The minimum amount of extract was treated with Mayer's reagent. Cream color precipitates if obtained with the aqueous extracts, will indicate the presence of alkaloids. The minimum amount of extract was treated with dragendroff's reagent. Reddish brown precipitate, if obtained, will indicate the presence of alkaloids.

Detection of Flavonoids

5 ml of dilute ammonia solution were added to the extract followed by addition of concentrated sulphuric acid. A yellow coloration was observed and it indicates the presence of flavonoids. A small quantity of the extracts was dissolved in alcohol to that, magnesium metal and concentrated hydrochloric acid were added. Colour change shows the presence of flavanoids. Small quantities of the extracts were treated with sodium hydroxide solution. Formation of yellow colour indicates the presence of flavanoids.

Detection of Phytosterols

Small quantity of the extract was suspended in 5ml of chloroform separately. The above obtained chloroform solution was subjected to following test.

Libermann Burchard test

The above prepared chloroform solutions were treated with few drops of concentrated Sulphuric acid followed by 1ml of acetic anhydride solution. A bluish green colour solution obtained in chloroform extract shows the presence of phytosterols.

Detection of Tannins- Phenolic compounds

The extract is dissolved or suspended separately in minimum amount of water and filtered. The filtrate was subjected to the following tests. Water extract was treated with 15 % ferric chloride test solution. The resultant colour was noted. A blue colour indicates condensed tannins, a green colour indicated hydrolysable tannins.

Detection of Saponins

Foam test: Dilute 1 ml of extract with distilled water to 20 ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of saponins.

Anti microbial assay

The antibacterial activity of the plant extract against organisms of interest was determined by the agar diffusion method[6]. The activity was expressed in terms of the zone of inhibition against different concentrations of the extract around the well. Muller-Hinton agar was prepared and sterilized. The medium was poured three times onto the plates after intermittent solidification there by a 4 mm thickness agar was prepared. Using well cutter wells (6mm diameter) were prepared. In each step of well cutting, the well cutter was thoroughly wiped with alcohol and sterilized on direct flame. Lawn of different organisms was prepared using sterile swabs and labeled accordingly and kept for few minutes. 100 µg of the extracted sample and the corresponding antibiotics were loaded using a sterile micropipette in the respective well and the plates were labeled at the bottom. The plates were incubated at 37°C for 24 hours. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no bacterial growth around the well.

Antifungal assay

For the evaluation of antifungal effects, PDA medium was incubated with fungal cells. The plates were incubated for 3 days at 25 ^{0} C. Further processes were repeated as above mentioned.

RESULTS

The qualitative phytochemical results reveals that the methanolic stem extract of *Toddalia asiatica.L.* contains secondary metabolites like Alkaloids, Flavonoids, Saponins, Steroids, Glycosides, Tannins (TABLE 1). The methanolic extract of the plant shows the antibacterial and antifungal activity against various organisms and the zone of inhibition obtained was comparable with the standard antibiotics. The results for the *invitro* antibacterial activity was represented in TABLE 2 and in TABLE 3 the results for antifungal activity is represented. The study supports the view that several medicinal plants might be useful as antimicrobial agents. The result of present study reveals that the employed extract of plant exhibited potential antibacterial activity against the tested pathogens.

DISCUSSION

The plant products over synthetic compound in the treatment of diseases are needed, because it does not have a deleterious effect in higher plants and animals including man. 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. Therefore, now there is a need to look back towards the traditional medicine which can serve as novel therapeutics. The pharmacological value of secondary metabolites from the plants is increasing due to constant discovery of their potential roles in health care and lead chemicals for new drug development. Plant synthesized many compounds with complex molecular structures, as a result of secondary metabolism. Some of the compounds and their derivatives such as alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glycosides, terpenes and phenolic compounds have antimicrobial properties[7]. In the present study the notable activity was observed against all tested micro organisms. In an overview of the bioactivity data obtained from the current investigation, it can be highlighted that the tested extracts have potential to inhibit bacteria and fungi. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial drugs for the treatment of various bacterial and fungal infections. Determination of respective antimicrobial potential and toxicological evaluation of these extracts with the view to formulate novel chemotherapeutic agents to be used future is worth mentioning. As a therapeutic source details, standarised study is warranted to in order to exhibit *Toddalia asiatica. L* Var Floribunda as an effective medicinal plant in near future.

TABLE 1: Shows phytochemical (qualitative) analysis of stem extract of *Toddalia asiatica. L*

Phytochemicals	Tests	Result
Carbohydrates	Molish test Fehling's test Benedicts Test	+
Proteins & Amino acids	Millon's Test Ninhydrin Test Biuret Test	+
Flavonoids	Reaction (Color with NaOH) Con H_2SO_4	+
Alkaloids	Mayer's Test Dragondroff's Test	+
Tannins	Ferric chloride Test	+
Steroids, Phytosterols	Libermann burchard	+
Saponins	Foam test	+
Glycosides	Legals Test	+
Coumarins	Alkaline Test	+

Table 2: shows anti bacterial activity of stem extract of *Toddalia asiatica.l*

Name of the organism	Diameter of Zone Inhibition (mm)		
	Standard	Negative Control	*Toddalia asiatica*
Staphylococcus aureus	16	-	13
Klebsiella pneumonia	15	-	11
E.coli	9	-	6
Proteus vulgaris	12	-	10
Pseudomonas aeruginosa	8	-	7
Bacillus anthracis	15	-	15
Bacillus subtilis	16	-	14

Values are mean of three replicates; Standard: Amphicillin; Negative Control: Distilled water

TABLE 3: Shows anti-fungal activity of stem extract of *Toddalia asiatica.L*

Name of the organism	Diameter of Zone Inhibition (mm)	
	Standard	*Toddalia asiatica*
Fusarium oxysporum	8	7
Aspergillus flavus	6	7
Fusarium solani	12	7
Candida albicans	11	6

Values are mean of three replicates; Standard : Clotrimazole.

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