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A Study of the Phytochemical Composition and Antibacterial activity of *Holostemma ada-kodien* Schultes.

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Abstract: The medicinal plant *Holostemma ada-kodien* Schultes is known for its rejuvenative potential. Preliminary phytochemical screening of stem and leaf samples of the herb showed positive test for flavonoids, hydrolysable tannins, phenols, saponins, sterols and terpanoids. The antimicrobial properties of leaf extracts namely aqueous, hydro alcoholic, ethanolic and methanolic were tested on both gram positive and gram negative bacteria. The plant exhibited significant antimicrobial potency, comparable to that of a standard antibiotic Gentamycin.
Key words:. Holostemma ada-kodien Schultes, Phytochemical analysis, antibacterial activity.

Introduction

Plants and their products were used in the treatment of infections for many centuries before scientific study of their use was made possible by the development of microbiology. Although a number of antibiotics were widely used in medicine, the search for anti-bacterial substances from plants will continue because better and safer drugs to combat Gram positive and Gram negative bacterial infections are still needed.

Holostemma ada kodien Schultes is an important medicinal plant belonging to family Asclepiadaceae and widely distributed in tropical forest in India [1,2]. The plant is used as antidiabetic [3], rejuvenative, aphrodisiac, expectorant, galactogogue, stimulant, and in ophthalmic disorders [4]. There is huge demand for this plant; more than 150 tonnes is required every year in south Indian pharmacies [5]. The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites.

The rise of antibiotic resistant microorganisms is one of the severe problems in health care systems of the world. Therefore, it is essential to find new compounds that have antimicrobial properties and it is worthwhile to screen plant species which have the above properties to synthesize new drugs[6]. Keeping all these in view the study was planned to analyse the phytochemistry and antibacterial activity of *Holostemma ada-kodien* Schultes.

Materials and Methods

The aerial parts of the plant *Holostemma ada kodien* Schultes was collected, identified and authenticated. The leaf and stem were dried separately under shade, powdered and stored in closed vessel for further use. The phytochemical analysis of the extract of *Holostemma ada-kodien* leaf and stem were carried out using the standard procedure[7].

Antimicrobial activity was checked using agar well diffusion method[8] . Two gram positive bacteria *Staphylococcus aureus (*MTCC 3160) and *Bacillus subtilis* (MTCC 3053) and three gram negative bacteria *Klebsiella pneumoniae (*MTCC 3384*),Salmonella enterica typhymurium (*MTCC 98) and *Escherichia coli (*MTCC 727*)* were used as the test organisms. The water extract of the leaf was prepared by dissolving 2gms of leaf powder in 20 ml of distilled water and extracted for 20 minutes under reflux. The supernatant was filtered and the filtrate was used. Similarly 50% hydro alcoholic, ethanolic and methanolic extracts were prepared. The plates were prepared by using Muller Hinton agar (Hi -Media). Eighteen hour old culture of test organisms in Nutrient Broth (Hi Media) was used as inoculumn. 150 µl each of the extract was used. Gentamycin disc (10mcg /disc) was used as the positive control and pure water, methanol, ethanol and 50% hydro alcohol were used as negative control. The diameter of zone of inhibition was measured after an incubation period of 24 hours at 37ºC.

Table 1: Preliminary phytochemical screening

Secondary metabolite	Leaf extract		Stem extract	
	Methanol	Water	Methanol	Water
Flavonoids	+	+	+	+
Glycosides	_	_	_	_
Phenols	+	+	+	+
Saponins	_	+	_	+
Sterols	+	_	+	_
Tannins	+	+	_	_
Terpanoids	+	_	+	_

(+ Presence, - Absence)

Table 2: Antibacterial activity of leaf extracts of *Holostemma ada-kodien* Schultes

Culture	Water extract	Inference	Methanol extract	Inference	Gentamycin (+ve control)	Inference
Staphylococcus aureus	-	Resistant	15mm	Sensitive	21mm	Sensitive
Bacillus subtilis	-	Resistant	14mm	Sensitive	26mm	Sensitive
Klebsiella pneumoniae	-	Resistant	-	Resistant	20mm	Sensitive
Salmonella typhymurium	-	Resistant	23mm	Sensitive	20mm	Sensitive
Escherichia coli	-	Resistant	-	Resistant	28mm	Sensitive

(- No zone of inhibition)

Table 3: Antibacterial activity of leaf extracts of *Holostemma ada-kodien* Schultes

Culture	Hydro -alcoholic extract	Inference	Ethanolic extract	Inference	Gentamycin (+ve control)	Inference
Staphylococcus aureus	15mm	Sensitive	18mm	Sensitive	21mm	Sensitive
Bacillus subtilis	12mm	Intermediate	14mm	Sensitive	26mm	Sensitive
Klebsiella pneumoniae	-	Resistant	-	Resistant	20mm	Sensitive
Salmonella typhymurium	13mm	Intermediate	-	Resistant	20mm	Sensitive
Escherichia coli	11mm	Intermediate	-	Resistant	28mm	Sensitive

(- No zone of inhibition)

Results and Discussion

The medicinal value of a plant lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [9]. The leaf extract shows the presence of flavonoids, phenols, saponins, sterols, tannins and terpanoids and the stem extract contains flavonoids, phenols, saponins, sterols and terpanoids. (Table-1).

The methanolic, ethanolic and hydroalcoholic extracts showed antibacterial activity against gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* (Table 2&3). The methanolic extract is showing greater activity than the antibiotic Gentamycin against the gram negative bacteria *Salmonella typhymurium.* The secondary metabolites present in the plant could be responsible for some of the observed antimicrobial activity. All the tested organisms are resistant to the water extract. No antibacterial activity was observed in negative controls. The findings of the study shows a new way in elucidating a potent anti-microbial agent from the leaf extract of *Holostemma ada-kodien.*

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