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Studies on Antibacterial Activity and Phytochemical Analysis Of Solanum xanthocarpum Schrad and Wendl.

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Absrtact

The phytochemical study was performed to find out the active compounds presencefrom the extract of distinct parts like stem and leaf of *Solanumxanthocarpum* is represented in the results of the study showcased the presence of active compounds like alkaloids, flavonoids, saponins, tannins, glycosides, phenolic compounds, terpenoids and steroids in a wide ranging across the studied parts. Antibacterial activity was determined by well diffusion method on MHA medium. The sterile medium (20ml) was poured into Petri plates. The medium was allowed to cool in a sterile condition and plates were then inculcated with $1x10^5$cfu cultures of test bacteria. The concentration of bacterial cells in the suspension was adjusted to minimum of $1x10^5$cfu/ml in Muller Hinton broth solution. Agar well of 6mm diameter were made in the plates. The desired different concentrations of the extracts were added to each well into MHA plates already seeded with the standardized inoculums (5 × 105) of the test bacterial cells. All test plates were incubated at 37°C for 24h. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC.

Keywords Antibacterial activity, Zone of inhibition, Preliminary Phytochemical test, *Solanum xanthocarpum.*

INTRODUCTION

Solanum xanthocarpum is known as Indian night shade or yellow berried night shade plan [1]. The Common name is Kantakari, synonym Solanumsurattense and it belongs to family Solanaceae. It plays an important place among medicinal herbs,(especially, for the treatment of cough) especially in India since ancient times. The young branches are densely covered with star-shaped hairs. The zig-zag branches, and covered with yellow, sharp, shining prickles. The leaves are up to 10 cm in length, their midribs and other nerves with sharp yellow prickles. The flowers are purple in nature, about 2 long, found has small bunch opposite to the leaves. The fruits are glabrous, globular drooping berries, 1.5-2cm, yellow or pale with green veins. Kantakari is bitter and pungent in taste and has hot potency. It possesses light and dry attributes. Kantakari is useful in wide range of diseases. It is more commonly used in the diseases like bronchial asthma, cough, worms etc.

The microorganisms develop resistance against many antibiotics due to theindiscriminate use of antimicrobial drugs [2]**.** Antibiotics are sometimes associated with side effects (Cunha, 2001)whereas there are some advantages of using antimicrobial compounds of medicinal plants, some often fewer sideeffects, better patient tolerance, relatively less expensive,acceptance owing to use of right form antiquity and being renewable in nature [3]**.** An antimicrobial substance either kills microbes (micro-biocidal) or prevents the growth ofmicrobes (micro-biostatic).Clinical efficacy of many syntheticantibiotics is questioned emergence of multidrug pathogens. The failure of chemotherapeutics and antibioticsby pathogenic microbial infection lead toexplore the several medicinal plants for potentialantimicrobial activity a variety of phytochemical constituents in the planttissues which cast a definite physiological action on the human body and proved active.

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MATERIALS AND METHODS

PRELIMINARY TESTS

Gram staining

Bacterial smears of 16-18 hrs old cultures were made on clean grease free slides, heat fixed and stained as follows. The slide was flooded with crystal violet solution for a minute, drained and rinsed with water; followed by Grams iodine solution for one minute, drained and rinsed with water. Decolorized with ethyl alcohol for 30 Sec and later counterstained with safranin for one minute and observed under an oil immersion microscope.

Crystal violet

Crystal violet : 2.0 g in 20 ml 95% ethanol

Ammonium Oxalate : 8.0 g in 80ml distilled water.

Gram's iodine

Iodine : 1.0 g

Potassium Iodine : 2.0 g

Distilled Water : 300 ml

Acetone alcohol

95% ethanol : 70 ml

Distilled water : 30 ml

Gram's safranin

Safranin : 0.25 g

95% ethanol : 10 ml

Distilled Water : 100 ml

Motility test

The hanging drop technique was followed to observe the motility of the organism. Observation was made under the microscope.

Catalase test

A small amount of culture was placed over a clean slide. A drop of 3% hydrogen peroxide was placed over the culture

culture and observed for effervescene. The production of effervescence showed the ability to produce the enzyme catalase.

Oxidase test

The organism spotted on oxidase disc (HiMedia) the blue or purple colour change was observed within 10 seconds.

BIOCHEMICAL TESTS

Indole test

The culture was inoculated into indole medium and incubated at 37oC for 48 – 72 hours. About 0.2 – 0.3 ml of Kovac's reagent was then added to the test tube, shaken and allowed to stand. The formation of red ring on the surface of the broth confirmed the production of indole.

Methyl red test

Culture was inoculated with Methyl red – Vogesproskauer (MR-VP) broth and incubated for 48 – 72 hrs at 37oC. The appearance of a red colour on addition of methyl red solution was considered as positive.

Test for H2S production and glucose utilization

Culture was inoculated with Triple sugar iron agar slants and incubated at 37oC for 24 hrs. The change in colour of the medium from red to yellow indicated the production of acid from glucose. A blackening of the medium indicated production of H2S. Breaks in the medium showed production of gas from glucose.

Urease test

Culture was inoculated with urease mediumand incubatedat 37oC for 24 hrsUrea is a diamide of carbonic acid. Urease is the enzyme possessed by the bacterium which hydrolysis urea and releases ammonia and carbon-di-oxide, ammonia reacts in solution to form ammonium carbonate which is alkaline leading to increase in the pH. Phenol red indicator which was incorporated in the medium changes its colour from yellow to red in alkaline pH, thus indicating the presence of urease activity.

Antibacterial stability test

The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial profiles of the isolates. The nutrient broth was prepared and sterilized at 121°C at 15 min and inoculated the isolates then incubated at 37°C for 24 hrs. After incubation period the broth culture were swabbed into surface of the Mueller-Hinton agar plates and antibiotic discs were placed, then Plates were incubated at 37°C for 18 to 20 h. The zone of inhibition and resistance was measured, recorded and interpreted according to the recommendation of the disc manufacture's standard chart [4]

Preparation of plant extracts

The plant extracts were prepared using the modified method of **Alade&Irobi** [5]**.** Briefly, 10g of the dried powdered plant were soaked separately in 50 ml of methanol (98 %) and acetone (99 %), for 72 h. Then, each mixture was refluxed followed by agitation at 200 rpm for 1 h. The filtrates obtained were concentrated under vacuum at 40°C to obtain the dry extracts.

Preliminary phytochemical analysis test
Alkaloids (Oguyemi, 1979 [6])

(200 mg plant material in 10 ml methanol, filtered); a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayoros reagents/Wagneros reagent/Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids.

Flavonoids (Oguyemi, 1979 [6])

(200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + conc. HCl + magnesium ribbon pink-tomato red color indicated the presence of flavonoids.

Saponins (Ayoola et al., 2008 [7])

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigourously.and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously after which it was observed for the formation of an emulsion.

Tannins (Oguyemi, 1979)

(200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate + 2 ml FeCl3, blue-black precipitate indicated the presence of Tannins.

Glycosides (Borntrager's test):

Extract was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. A layer of pink, red or violet colour indicates the presence of glycosides.

Phenolic compounds (Venkatesan, 2009 [8])

Extract of the sample was treated with 5% ferric chloride test solution. The resultant colour was noted. A violet colour indicated the presence of hydrolysable tannin. Or into 1% solution of gelatine containing 10% sodium chloride in a beaker, 0.5 g of extract was added and shaken to dissolve. A white precipitate observed indicates the presence of tannins. Or into 10% lead acetate solution, 0.5 g of the extract was added and shaken to dissolve. A white precipitate observed indicates the presence of tannins and phenolic compounds.

Test for Terpenoids (Venkatesan, 2009)

Weigh about 0.5 g plant extract in separate test tubes with 2 ml of chloroform. And add concentrated Sulphuric acid carefully to form a layer. And observe for presence of reddish brown color interface was formed to show positive results for the presence of terpenoids.

Test for Steroids (Venkatesan,2009)

To the plant extract add 2 ml of acetic anhydride and add 0.5gm of ethanolic extract of each sample with 2 ml of Sulphuric acid .Observe for the color change from violet to blue or green in samples indicating the presence of steroids.

Test for proteins (Thenmozhi, 2011 [9])

Biuret test: To 1 ml of aqueous extract, 5-8 drops of 10% NaOH and two drops of 3% CuSO4 were added. Red or violet colour showed the presence of proteins.

ANTIBACTERIAL ACTIVITY ASSAY OF PLANT EXTRACT

Antibacterial activity was determined by well diffusion method on MHA medium. The sterile medium (20ml) was poured into Petri plates. The medium was allowed to cool in a sterile condition and plates were then inculcated with

a sterile condition and plates were then inculcated with $1x10^5$cfu cultures of test bacteria. The concentration of bacterial cells in the suspension was adjusted to minimum of $1x10^5$cfu/ml in Muller Hinton broth solution. Agar well of 6mm diameter were made in the plates. The desired different concentrations of the extracts were added to each well into MHA plates already seeded with the standardized inoculums (5 × 105) of the test bacterial cells. All test plates were incubated at 37°C for 24h. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC.

RESULTS AND DISCUSSION

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The phytochemical study was performed to find out the active compounds presence from the extract of distinct parts like stem and leaf of *Solanum xanthocarpum* is represented in **Table.1.** The results of the study showcased the presence of active compounds like alkaloids, flavonoids, saponins, tannins, glycosides, phenolic compounds, terpenoids and steroids in a wide ranging across the studied parts.

In flower extract of S. xanthocarpum using different solvents like acetone, chloroform, petroleum ether, ethanol and methanol against isolates wound-causing pathogens like *Staphylococcusaureus*, *Klebsiellapneumoniae*, *Streptococcus*spp, *Pseudomonas aeruginosa* and *Escherichia coli* were presented.

Acetone extract of flower showed maximum and more prominent inhibition against P2 (21.66±0.57735mm in 30mg/ml) P1(20.66±0.57735mm in 30mg/ml) sa1(18±1mm in 30mg/ml)sa2 (17.66±0,57735mm in 30mg/ml) **(Table.2)**.

In Petroleum ether extract of flower showed the highly inhibitory action against sa1 (29±1mm in 30mg/ml) E1(23.33±0.57735mm in 30mg/ml) P2(21.33±1.1547 mm in 30mg/ml)**(Table.3)**.

Ethanol extract of flower showed the highly inhibitory action against E2 (24.66±1.1547mm in 30mg/ml) E1(23.33±1.1547 mm in 30mg/ml) K2(21±1mm in 30mg/ml) **(Table.4)**.

Acetone extract of leaf showed maximum and more prominent inhibition against E1 and P2 (15.33±0.57735mm in 30mg/ml) sa1and K2(13.66±0.57735mm in 30mg/ml) **(Table.5)**.

Table.1: Preliminary Phytochemical analysis

Phytochemicals	Plant parts			
	Stem	Leaf	Flower	Fruit
Terponoids	+	+	+	+
Steroids	+	+	+	+
Saponin	+	+	+	+
Flavoniods	+	+	+	+
Phlobatanins	-	-	-	-
Tannin	-	+	+	+
Anthraquinon	-	-	-	-
Alkaloids	+	+	+	+
Phenol	+	-	+	-
Carbohydrate	-	-	-	-
Protein	+	+	+	+
Glycosides	+	+	+	+

Table.2: Antibacterial Activity Of *Solanum Xanthocarpum* Flower (Acetone Extract)

S.no	Isolate name	Inhibition Zone in mm Acetone extract (mg/ml)				
		6	15	22.5	30	Antibiotics
1.	Sa1	-	-	16.33 ±0.57735	18± 1	21.66667±1.52753
2.	Sa2	-	-	15±1	17.66±0.57735	19.33333±1.52753
3.	k1	-	-	12.33±0.57735	15±1	23.33333±1.52753
4.	K2	-	-	12.66±1.52753	15.33±0.57735	23.33333±1.52753
5.	St1	-	-	-	12±1	25±1
6.	St2	-	-	-	13.66±0.57735	25±1
7.	P1	-	-	18±1	20.66±0.57735	16±1
8.	P2	-	-	17±1	21.66±0.57735	14.66667±0.57735
9.	E1	-	9±1	14.66±1.52753	17±1	19±1
10.	E2	-	11±1	14.33±1.52753	17.66±0.57735	20.33333±0.57735

Values are expressed as mean ± SD

Table.3: Antibacterial Activity Of *Solanum Xanthocarpum* Flower (Petroleum Ether Extract)

S.no	Isolate name	Inhibition Zone in mm Petroleum ether extract (mg/ml)				
		6	15	22.5	30	A
1.	Sa1	-	-	-	29±1	18.33333±0.57735
2.	Sa2	-	-	-	11±1	18.33333±0.57735
3.	k1	-	-	12.66±1.52753	12.33±0.57735	22±1
4.	K2	-	-	11.33±0.57735	13.33±1.1547	22±1
5.	St1	-	-	-	11.66±0.57735	25.33333±1.52753
6.	St2	-	-	-	13.33±1.1547	25.33333±1.52753
7.	P1	10.66±1.1547	14.66±0.57735	17±1	20.66±0.57735	15±1
8.	P2	8.33±0.57735	14±1	16.33±1.1547	21.33±1.1547	15±1
9.	E1	-	14±1	21.33±1.1547	23.33±0.57735	21±1
10.	E2	-	10.33±0.57735	14.66±0.57735	20.66±0.57735	19±1

Values are expressed as mean ± SD

Table.4: Antibacterial Activity Of *Solanum Xanthocarpum* Flower (Ethanol Extract)

S.no	Isolate name	Inhibition Zone in mm Ethanol extract (mg/ml)				
		6	15	22.5	30	A
1.	Sa1	11.66±0.57735	12.33±0.57735	15.33±0.57735	17±1	19.33333±0.57735
2.	Sa2	9.66±0.57735	12.33±0.57735	14.66±0.57735	15±1	18±1
3.	k1	-	15.33±0.57735	18±1	18±1	23±1
4.	K2	-	12.33±0.57735	15±1	21±1	23±1
5.	St1	-	11.66±0.57735	12.33±0.57735	14.33±1.1547	24±1
6.	St2	-	11.66±0.57735	14.33±1.1547	16±1	27.33333±1.1547
7.	P1	-	11.66±0.57735	14.66±0.57735	17±1	16±1
8.	P2	-	13±1	13.66±0.57735	18±1	16±1
9.	E1	-	18±1	20.33±0.57735	23.33±1.1547	21.33333±1.52753
10.	E2	13.66±0.57735	20.33±0.57735	23.33±1.1547	24.66±1.1547	19.66667±0.57735

Values are expressed as mean ± SD

Table.5: Antibacterial Activity Of *Solanum Xanthocarpum* Leaf (Acetone Extract)

S.no	Isolate name	Inhibition Zone in mm Acetone extract (mg/ml)				
		6	15	22.5	30	A
1.	Sa1	-	10.33±0.57735	12.66±0.57735	13.66±0.57735	18.66667±0.57735
2.	Sa2	-	9.33±0.57735	13.66±1.1547	16±1	20±1
3.	K1	-	11.66±1.1547	11.33±0.57735	13.66±1.1547	20±1
4.	K2	-	9.33±0.57735	11.33±0.57735	13.66±0.57735	21.33333±0.57735
5.	St1	-	-	-	-	24±1
6.	St2	-	-	-	-	24±1
7.	P1	-	-	11.33±0.57735	16±1	17±1
8.	P2	-	13±1	14.33±0.57735	15.33±0.57735	14±1
9.	E1	-	-	14.66±0.57735	15.33±0.57735	24±1
10.	E2	-	-	-	11.33±0.57735	24±1

Values are expressed as mean ± SD

Table.6: Antibacterial Activity Of *Solanum Xanthocarpum* Leaf (Petroleum Ether Extract)

S.no	Isolate name	Inhibition Zone in mm Petroleum ether extract (mg/ml)				
		6	15	22.5	30	A
1.	Sa1	-	-	-	-	17.66667±0.57735
2.	Sa2	-	-	-	-	19±1
3.	k1	-	-	8.66±1.1547	13.33±0.57735	18±1
4.	K2	-	-	-	8.66±1.1547	21.66667±1.52753
5.	St1	-	-	-	-	24±1
6.	St2	-	-	-	-	24±1
7.	P1	-	-	-	-	16.66667±1.52753
8.	P2	-	-	-	9.66±0.57735	16.66667±1.52753
9.	E1	-	-	10.33±0.57735	12.33±0.57735	21±1
10.	E2	-	-	12.33±0.57735	14.66±1.1547	21±1

Values are expressed as mean ± SD

Table.7: Antibacterial Activity Of *Solanum Xanthocarpum* Stem (Acetone Extract)

s.no	Isolate name	Inhibition Zone in mm Acetone extract (mg/ml)				
		6	15	22.5	30	A
1.	Sa1	-	-	-	-	20.33333±0.57735
2.	Sa2	-	-	-	-	18±1
3.	k1	-	-	-	-	21±1
4.	K2	-	-	-	-	21±1
5.	St1	-	-	-	8.33333±1.52753	23.66667±1.52753
6.	St2	-	-	-	12.33333±1.52753	23.33333±0.57735
7.	P1		-	10.66667±0.57735	14±1	16±1
8.	P2	-	10.66667±1.52753	14±1	16.66667±1.52753	16±1
9.	E1	-	-	11±1	11.66667±1.52753	21±1
10.	E2	-	-	11±1	16±1	19±1

Values are expressed as mean ± SD

Table.8: Antibacterial Activity Of *Solanum Xanthocarpum* Stem (Chloroform Extract)

s.no	Isolate name	Inhibition Zone in mm Chloroform extract (mg/ml)				
		6	15	22.5	30	A
1.	Sa1	-	-	-	-	20.33333±1.52753
2.	Sa2	-	-	-	-	18±1
3.	k1	-	-	11.33333±1.52753	11.66667±0.57735	20.33333±1.52753
4.	K2	-	-	12±1	14±1	21±1
5.	St1	-	-	-	-	24±1
6.	St2	-	-	-	13±1	25.66667±1.52753
7.	P1		-	-	-	15.66667±1.52753
8.	P2	-	-	-	13±1.73205	15.66667±1.52753
9.	E1	-	-	-	-	21±1
10.	E2	-	-	-	-	21.33333±1.52753

Values are expressed as mean ± SD

Table.9: Antibacterial Activity Of *Solanum Xanthocarpum* Stem (Ethanol Extract)

S.no	Isolate name	Inhibition Zone in mm Ethanol extract (mg/ml)				
		6	15	22.5	30	A
1.	Sa1	12±1	12±1	18.66667±1.52753	19.33333±0.57735	20.66667±2.08167
2.	Sa2	8.33333±1.52753	15.66667±1.52753	15.66667±0.57735	21.33333±1.52753	17±1
3.	k1	-	16.33333±1.52753	19.66667±1.52753	21.33333±1.52753	22±1
4.	K2	-	16±1	20±1	24±1	22±1
5.	St1	-		11.66667±1.52753	13.33333±1.52753	25.66667±1.52753
6.	St2	-		16±1	16±1	25±1
7.	P1	12.66667±1.52753	15.33333±1.52753	17.66667±1.52753	21.66667±1.52753	17.33333±1.52753
8.	P2	8.33333±1.52753	12±1	16.66667±1.52753	17±1	17.33333±1.52753
9.	E1	-	16±1	16.66667±1.52753	20±1	21±1
10.	E2	11.33333±1.52753	15.33333±1.52753	17.66667±1.52753	20±1	18.66667±1.52753

Values are expressed as mean ± SD

Table.10: Antibacterial Activity Of *Solanum Xanthocarpum* Stem (Methnaol Extract)

s.no	Isolate name	Inhibition Zone in mm methanol extract (mg/ml)				
		6	15	22.5	30	A
1.	Sa1	-	16±1	16±1	20.66667±1.52753	19±1
2.	Sa2	-	13±1	14±1	21.33333±1.52753	19±1
3.	k1	-	10±1	16±1	19±1	23±1
4.	K2	10.33333 ±1.52753	10±1	14±1	21.33333±1.52753	23±1
5.	St1	-	13.33333±1.1547	16±1	21.33333±1.52753	25±1
6.	St2	10.33333 ±1.52753	14.33333±1.1547	14±1	19.66667±0.57735	25±1
7.	P1	15.33333±1.1547	18.66667±1.1547	19.66667±0.57735	20.33333±1.52753	15.66667±1.52753
8.	P2	12±1	16±1	18±1	22±1	15.66667±1.52753
9.	E1	15.33333±1.52753	17.33333±1.1547	16.66667±1.52753	22±1	19.66667±0.57735
10.	E2	12±1	16±1	16.66667±1.52753	20±1	19±1

Values are expressed as mean ± SD

Petroleum ether extract of leaf showed the highly inhibitory action against E2(14.66±1.1547mm in 30mg/ml) K1(13.33±0.57735mm in 30mg/ml)**(Table.6)**.

Acetone extract of stem showed maximum and more prominent inhibition against P2 (16.66667±1.52753mm in 30mg/ml) St2 (12.33333±1.52753mm in 30mg/ml)**(Table.7)**.

Chloroform extract of stem showed the highly inhibitory action against K2(14±1mm in 30mg/ml) P2 (13±1.73205mm in 30mg/ml) **(Table.8)**.

Ethanol extract of stem showed the highly inhibitory action against P1 (21.66667±1.5275mm in 30mg/ml) K1and Sa2 (21.33333±1.5275mm in 30mg/ml) E1(23.33±1.1547 mm in 30mg/ml) K2(21±1mm in 30mg/ml) **(Table.9)**.

Methanol extract of stem showed the highly inhibitory action against P2 and E1 (22±1mm in 30mg/ml) Sa2,K2 and Sa2 (21.33333±1.52753mm in 30mg/ml) **(Table.10)**.

The Solanum torvum shows more dissimilarity with other four species. Genetic variability among on the species is important to maintain since it represents the blue print for all the living things on earth. It is important to point out that the genetic variation occurs between the species due to the response the changing environmental conditions. Better understanding of distribution of genetic variation at the interspecific level would help identified superior genotypes for cultivar up gradation as well as to evolve strategies for the establishment of effective in-situ and ex-situ conservation programmes [10].

Solanum xanthocarpum is non toxic and safe for human use and is regarded as a valuable plant in both ayurvedic and modern drugs development areas for its versatile medicinal uses. By the present study, antimicrobial activity of leaf,fruit, flower and stem parts of the Solanum xanthocarpum was evaluated. From the above results it can be concluded that *S.xanthocarpum* extract have great potential as antimicrobial compound against tested organisms and that the can used in treatment of infection disease caused by resistant micro organisms. Further studies of other phytoactive compound well possibly lead to exploration of new method for therapeutic and industrial application [11].

CONCLUSION

The presence of phytochemical make the plant useful for treating different ailments and have a potential of potential of providing useful drugs of human use. The quatitative determination of pharmacognostic parameters will help for setting standards for crude drugs. The total ash is particulary important in evaluating the purity of drugs .The pharmacognostic constants for the parts of this plant .The diagnostic microscopic features and the numerical standareds reported in this work could be useful for the completion of a suitable monograph for it proper identification. The plant *S. xanthocarpum* activity against possesses good inhibition activity against the tested bacteria, which support their traditional use against infectious diseases. The presence of most general phytochemicals might be responsible for their therapeutic effects. Many more novel templates from this plant in future may serve for the production of synthetically improved therapeutuic agents.

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