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Qualitative Evaluation of Phytochemicals and Antifungal Activity of "*Solanum Xanthocarpum*" (Schrad. &Wendl.)

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Abstract: *Solanum xanthocarpum is a well-known medicinal herb in Asia, belongs to family Solanaceae. The Solanum Xanthocarpum is reported to have good medicinal values in traditional system of medicines. This present study deals with phytochemical investigations of different parts viz., leaf, stem, root, fruit and flowers of Solanum xanthocarpum, which were collected from three different agro-climatic zones of Chhattisgarh, India. Qualitative phytochemical test indicated that plant contains alkaloids, flavonoids, tannins, steroids and saponins as well as reducing sugars. Antifungal activity of methanol extract of different parts plant of Solanum xnathocarpum were assayed against Aspergillus niger. Screening for Anti-fungal activity is carried out by Agar diffusion method. However, the agar diffusion method can be used for determination of MIC values. Inhibition zones into MIC values were found in 20µg, 60 µg and 90 µg of FT1, ST2 and FL2 respectively. Thus Solanum xanthocarpum could be considered as a potential source of natural antifungal possesses good economic as well as medicinal importance.*

Keywords: *Solanum xanthocarpum, phytochemical, antifunal activity and minimum inhibitory concentration (MIC)*

I. Introduction

Solanum xanthocarpum (Kantkari) is an important medicinal plant belonging to Solanaceae family. It is a very spiny diffuse herb up to 1.2m tall, commonly found throughout India [**1**]. The herb is wildly distributed throughout the Asia. But now this herb is listed amongst endangered species in many areas in the countries. Plants also have been used in ethano-pharmacy for various diseases such as hypertension, eczema and diarrhea for centuries and today their scientific validation was provided by the identification and isolation of bioactive phytochemicals [**2**].

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like fruits, leaves, flowers, seeds [**3**], etc i.e., any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers [**4**].

It is used to cure various diseases like asthma, rheumatism, cough, gonorrhea, sore throat and catarrhal fever. Carbohydrate, proteins, vitamin C. anthocyanin and solasodine were reported in *Solanum xanthocarpum.* Aqueous and alcoholic extract of this plant showed hypotensive effect, antiviral and contraceptive properties [**5**]. Solanum xanthocarpum is valuable medicinal plants. Due to their medicinal and economical values this plant was selected for their qualitative chemical analysis and antifungal assay (MIC).

Natural products have been extensively used for the last few years ago as a source for the search of antifungal agents. This was done on the basis of fact that drugs developed from plant products are safe and reliable when compared with synthetic drugs which are toxic and costly for the general masses. Therefore, there is an extensive need to explore new effective and therapeutically active anti fungal activity from natural sources such as plants.

Solanum xanthocarpum has profound use in Ayurveda and folkore medicine. It is supposed that the plant has solasonine and solasomargine, sapogenins and solasodine which are responsible for medicinal effect. The whole plant extracts of Solanum xanthocarpum have a larvicidal defect, hypoglycemic activity and bronchitis.

II. Materials and Methods

2.1 Plant Material Collection

The medicinal plants *Solanum xanthocarpum* were collected from three different agro-climatic zones such as Raipur (1), Surguja(2) and Jagdalpur(3) of Chhattisgarh (fig 1), India. The plant materials were identified by experienced Botanist Name and designation of Pt. Ravishankar Shukla University, Chhatisgarh.

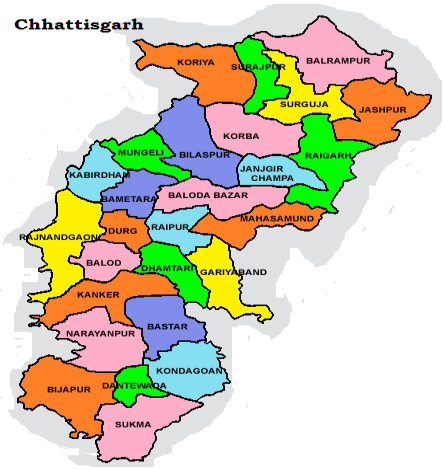

Fig 1: Chhatisgarh Map

Fig 2: Solanum xanthocarpum plant

Plant materials (fig 2) were washed under running tape water and then with distilled water. Then all three regions plant parts such as leaves (L1, L2 & L3), stems (S1, S2 & S3), flowers (FL1, FL2 & FL3), fruits(FR1, FR2 & FR3) and roots(R1, R2 & R3) were separated from whole plant (fig 3). All separated plant parts were dried under shade, pulverized into fine powder with the help of mortar and pastel and finally stored in airtight bottle before use.

Fig 3: leaf, stem, flowers, fruits and roots of *Solanum xanthocarpum*

2.2 Extraction of Photochemical

50 g of each powdered samples were extracted with methanol (1L) using a soxhlet extractor. Extraction procedure was repeated for three times and pooled together for each sample separately. Pooled extracts were filtered though whatman No 1 filter paper and concentrated by evaporating the solvents under reduced pressure in rotary evaporator.

2.3 Qualitative Estimation of Phytochemicals

The filtrate obtained from the *Solanum xanthocarpum* was subjected to preliminary screening to analysis phytochemicals. Presence or absence of different secondary metabolites in the sample extracts using the method described by Harbore (1996) [6].

2.3.1 Test for Alkaloids

Mayers test: To a few ml of filtrate, two drops of mayers reagent were added though the wall of the test tube. A white or creamy prepcipitate indicates the test as positive. Wagner's Test: Few drops of Wagner's

reagent were added into 2 to 3 ml extract. Formation of reddish brown precipitate indicates the presence of alkaloids [7].

2.3.2 Test for Flavonoids

NaOH Tests: 2-3 ml. of extract and few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids [8].

Pew's Tests: Zinc powder was added into 2-3 ml. extract, followed by drop wise addition of con. HCl. Formation of purple red or cherry colour indicates the presence of flavonoids [9].

2.3.3 Test for Glycosides:

Glycosides test: 1 ml water was added into the small amount of extract and shaked well. Then aqueous solution of NaOH was added. The appearance of yellow colour indicates the presence of glycosides [10].

Keller-Killani Test: Glacial acetic acid was added into 2 ml. extract and one drop 5% $FeCl_3$ and conc. H_2SO_4. Reddish brown colour appears at the junction of the two liquid layers and the upper layer of bluish green indicates the presence of glycosides [7].

2.3.4 Test for Tannins and Phenolic Compounds

Ferric Chloride Tests: 0.5 ml of $FeCl_3$ (w/v) solution was added into 2 ml of test solution, formation of an intense colour indicates the presence of phenols .

Lead acetate test: Few drops of 10% lead acetate solution were added into 5 ml of extract. Formation of yellow or red precipitate indicates the presence of tannins [10].

2.3.5 Test for Sterols and Triterpenoids

Salkowski test: 1 ml of extract was mixed with 2ml of chloroform. About 3ml of Conc. H_2SO_4 was added carefully from the side of the test tube. Reddish Brown colouration at the interface indicated the presence of titerpenoids [7].

H_2SO_4 Test: To 1ml of extract 6-7 drops of concentrated H_2SO_4 was added from the side wall of the test tube. The appearance of red colour indicated the presence of steroids.

2.3.6 Test for Carbohydrates

Molisch's Test: 2 drops of Molisch's regent was added into 1 ml of extract, and 2 ml of concentrate H_2SO_4 was added carefully into above solution. Formation of violet ring at the junction indicates the presence of carbohydrates [7].

Fehling test: 1ml of filterate is boiled on water bath with 1ml each of fehling solution A and B. A red ppt indicates the presence of sugar.

Benedicts test: To 1ml of filtrate, 1ml of Benedict reagent is added and heated on a boiling water bath for 2 minute. Red ppt indicates the presence of sugar.

2.3.7 Test for Proteins and Amino Acids

Ninhydrin test: Crude extract when boiled with 2ml of 0.2% solution of ninhydrin, Violet colour appeared suggesting the presence of aminoacid and proteins.

Xanthoprotein test: In 2 ml of extract, 3 drops of nitric acid were added by the side of the test tube followed by addition of 40% NaOH. Appearance of yellow colour indicates the presence of proteins and free amino acids.

2.3.8 Test for Saponins

Foam Test: The extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of saponins [7].

2.4 Antifungal Activity and MFC Minimum Fungicidal Concentration

Screening for Anti-fungal activity is carried out by agar diffusion assay method. All plant extracts were tested against two pathogenic fungi: *Aspergillus niger* MTCC 282 and *Aspergillus flavus* MTCC 277. They were procured from IMTECH, Chandigarh, India. Fungal strains were grown and maintained on Sabouraud Dextrose Agar (SDA) medium. A loopful of the bacteria from the stock culture was aseptically transferred to sterile SD broth medium. The organisms were allowed to grow for 24-36 h. harvested and used in the experiment. For anti fungal activity standard size of fungal inoculum ($1x\ 10^7$ CFU/ml) was used. The antifungal study was carried out by incorporating into molten SDA plates. The plates were allowed to solidify and wells of 8 mm diameter were bored in each agar plate. 80µl of each extracts were loaded into the well and methanol

solvent was served as negative control. All plates were incubated at 28°C for 24-48 hr. The zones of inhibition of individual plant extracts were determined.

Minimum fungicidal Concentration was determined against *Aspergillus niger* which were showed highest zone of inhibition compared to *Aspergillus flavus*. Sample was dessicated and dissolved in methanol. The final conc. was 0.1 mg/1000µl. SDA media was prepared and autoclaved. The fungal inoculum of *Aspergillus niger* was inoculated onto the media plated. Then wells were bored in the agar media plate and 10µl, 20µl, 30µl, 40µl, 50µl, 60µl, 70µl, 80µl, 90µl and 100µl of the prepared sample were loaded into the wells. The plates were then incubated at 28°C for 48 hrs. Zone of inhibition was measured for each test sample.

2.5 Thin Layer Chromatography (Tlc)

Thin layer chromatography was performed on glass sheet coated with a thin layer of Silica gel G from Hi-media as an adsorbent material. Slurry was air dried followed by oven drying for 1hr at 100°C. Each extract of samples were loaded onto the silica plate and placed in solvent system, Chloroform: methanol (9:1) serves as mobile phase. The chromatograph was developed by ascending technique. Solvent front was measured and visualized under visible light and UV light. Qualitative evaluation was done by determining the Rf value of individual compounds.

III. Results and Discussion

3.1 Physiochemical Investigation

The qualitative phytochemical screening tabulated in the Table-1 shows the presence of alkaloids, flavonoids, glycosides, tannins, phenols, sterols, triterpenes, carbohydrates, proteins and saponins in methanol extracts of all five parts i.e. leaf, stem, flower, fruit and root of the three different regions *Solanum xanthocarpum.* It has been reported that ethanolic extract of aerial part, fruit and root contains carbohydrate, saponin, flavanoid, phenols, tannins, phytosterol and triterpernoids as phytochemical constituents [11]. Phytochemicals are abundantly present in fruit extracts of all sources i.e. FT1, FT2 and FT3 compared to other part (Table 1). Phenolics compounds have anti-oxidative, anti-diabetic, anticarcinogenic, anti-inflammatory properties [12]. Saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss etc. It is a bioactive antibacterial agent of plants [13]. Tannins have general antimicrobial and antioxidant activities [14]. Plant steroids have cardiotonic activity, possess insecticidal and antimicrobial properties. It is generally used in herbal medicines and cosmetic products [15]. Apart from these tannins contribute property of astringency i.e. faster the healing of wounds and inflamed mucous membrane [16]. *Solanum xanthocarpum* fruit pericarp is very effective compared to other part such as stems, leaves, flowers because most parts of secondary metabolites are present in fruit [15]. It has reported that solasodine, an alkaloidal constituent of the plant has antiandrogenic activity [17].

Table 1. Phyto chemical constituents present in different extracts of the whole plant of *Solanum xanthocarpum*.

Name of Test	L1	L2	L3	S1	S2	S3	FL1	FL2	FL3	FT1	FT2	FT3	RT1	RT2	RT3
Alkaloid 1. Mayers Test	++	++	+++	+	+++	++	++	+++	++	+++	+++	+++	+++	++	++
2. Wagners	+	+++	+++	+	+++	++	++	+++	++	+++	+++	+++	+++	++	++
Flavonoid 1. NaoH	+++	++	+++	+	+++	++	+++	+++	++	+++	+++	+++	+++	++	++
2. Zinc Dust	-	-	-	-	-	-	+++	+++	+++	-	-	-	-	-	-
Glycoside 1. Glycoside	+++	+	++++	+	+++	++	+++	+++	++	+++	+++	+++	+++	+	+
2. Killerkillani	+++	+	+++	+	+++	++	+++	+++	++	++	+++	+++	+++	++	+
Tanins & Phenolic 1. Lead acetate	+++	+	++	+	+++	++	+++	+++	+++	+++	+++	+++	+++	++	++
2. Fecl3	+++	+	++	+	+++	++	+++	+++	+++	+++	+++	+++	+++	++	++
Sterols & Triterpenoid 1. Salkowaski	+++	+++	++	+	+++	++	+	+++	++	++	++	+++	+++	+++	+++
2. H2So4	+++	+++	++	+	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	++
Carbohydrate 1. Molisch	-	-	-	-	-	-	-	-	-				+++	++	+
2. Benedict	-	-	-	-	-	-	-	-	-	++	++	++	++	++	Trace
3. Fehling	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Protein 1. Ninhydrin	-	-	-	+	++	++	+	+++	+	-	-	-	+++	+++	+++
2. anthoprotein	+++	++	+++	+	+++	++	+++	++	+	+++	++	++	+++	++	+
Saponin	+	-	++	-	++	++	+++	++	++	+++	+++	+++	+	+	+

+ Slightly present, ++ moderately present, +++ Highly present, - absent, trace

Table 2 shows that methanolic extracts of different plant parts of *Solanum xanthocarpum* (SX) displayed antifungal activity against *Aspergillus niger* a fungal pathogen. FT1, fruit extract of SX collected from

Raipur, Chhattisgarh has shown highest antifungal activity. The minimum inhibitory concentration (MIC) was obtained at 20µg/ml of methanolic extract of FT1 of SX. 60µg/ml and 90µg/ml MIC were obtained from ST1 and FL2 respectively of methanolic extracts. No inhibitory effect was found in leaf and root extracts of the SX. Antifungal activity of various parts of extracts SX were studied by measuring the zone of inhibition formed around the well and the results are given in Fig 4. From this present study the methanolic extracts of SX showed highest sensitivity to *Aspergillus niger* and less sensitivity to *Asperfillus flavus*. Further MIC was carried out with Aspergillus niger and the samples (L3, FL2, FT1, RT1 and ST1) which has showed positive antifungal activity. It has reported that hexanic extract of leaf of SX has effective against *C.albicans.* Mimimum growth inhibition was found in 100µg/ml concentration of hexanic extract of SX [18].

Table 2: The zone of inhibition was calculated for different extracts at different concentrations.

Samples	ZONE OF INHIBITION (in mm)										
	-ve control	10µg	20µg	30µg	40µg	50µg	60µg	70µg	80µg	90µg	100µg
L3	10	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
FL2	10	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	16	17
FT1	10	NZ	13	16	19	19	20	20	22	24	27
RT1	10	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
ST1	10	NZ	NZ	NZ	NZ	NZ	12	12	13	15	16

L=Leaf; FT=Fruit; FL=Flower; RT=Root; ST=Stem.

This antifungal activity may be due to the presence of glycosides, phenolic compounds, flavonoids and tannins in the extracts of *Solanum xanthocarpum*. Therefore, this work can be an indication that it's potential as a drug that can be used against these micro organisms antifungal activity. On the other hand leaf and root extracts did not depict any antifungal effect against any of the above mentioned microorganisms in the present investigation However, methanolic extract of fruit at the concentration of 20 µg/mL showed antifungal activity i.e. against *A.niger* which is in line with that of work already, therefore in relation to this disease these finding agree with those of work done. The aqueous extracts displayed no antifungal activity against one *A.niger* fungi which is almost similar to the work already reported [18]. Methanol extract due to the presence of (alkaloids, glycosiede, saponins and tannins) showed the maximum antifungal activity which is somewhat comparable to that of standard antifungal activity.

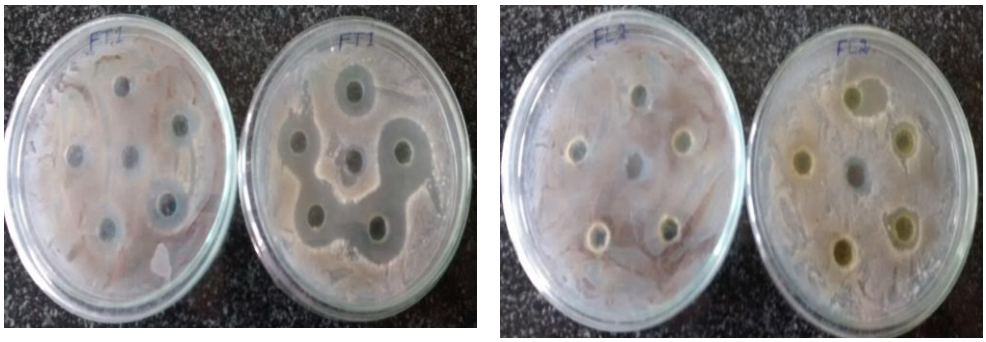

Fig 4: Antifungal assay of FT1 & FL2 against *Aspergillus niger*.

IV. Conclusion

Solanum xanthocarpum plant parts (i.e Fruit, Stem, Leaves &Flowers) have different types of medicinal properties. When compare to the other parts of the plant. These secondary metabolites are alkaloids, glycosides, saponin, lignin, phenol,tannins, Sterols etc. These secondary metabolites have anti-bacterial, anti-viral, anti-fever, anti-diabetes, anti-cancerous activities etc. Therefore it can be strongly involved in medicinal plant categories. Solanum xanthocarpum fruit has intense antifungal activity at low concentration.

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