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Efficacy of Antimicrobial and Antioxidant Activity of *Solanum xantocarpum* Whole Plant Hot Aqueous Extracts

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Present study was carried out to test the efficacy of hot whole plant aqueous extract (HAE) of *Solanum xanthocarpum* Schrad and Wendl fam. Solanaceae against some gram positive and gram negative bacteria and its antioxidant activity. Hot whole plant aqueous extract (HAE) showed the significant(p>.01) antibacterial activity against the *Bacillus subtilis, Staphylococus aureus, Escherichia coli*, *Pseudomonos aeruginosa* but no antifungal activity against *Candida albicans.* Tetracyclin and fluconazole was used as the standard antibiotic for bacteria and fungi. MIC of Hot whole plant aqueous extract (HAE) was calculated , MIC of *P.aeruginosa* found to be low 0.039mg/ml, .156 mg/ml for *Staphylococus aureus and Bacillus subtilis*, no MIC was to found against *Escherichia coli and* fungi *Candida albicans*. 2,2-diphenylhydrazyl free radical scavenging assay was use to determine the invitro antioxidant activity of Hot whole plant aqueous extract (HAE) of *Solanum xanthocarpum* for concentration 0.25mg/ml, 0.5mg/ml, 1.0mg/ml and 2mg/ml as compared to standard ascorbic acid and butylated hydroxyl toluene, resultshow the significant (p>0.1) antioxidant activity.

Keywords: *Solanum xantocarpum*, whole plant aqueous, antimicrobial.

It has been observed as a common practice to use the plants and its products to cure certain diseases by the different society. The use this traditional folk medicine are known as the ethonomedicine. The knowledge of ethonomedicine was transferred from generation to generation and all the common system like Ayurveda, Unani, Siddha, Nature care and even modern medicine is derived from the ehonomedicine[1]. *Solanum xanthocarpum Schrad and Wendl* family solanaceae commonly known as the Indian night shade or yellow berried night shade(English) and Kantkari (Sanskrit). It is spiney diffuse green perennial herb. *Solanum xanthocarpum* used in this study has profound use in Ayurveda as folkore medicine[2]. Solasonine is present in its different parts due to this SX show the pharmacological and medicinal value[3]extract prepared from different parts of SX contain vit C, anthocyanin and solasonin[4]. SX extract show the antibacterial[5], antifungal[6],Hypoglycemic[7], antifilariasis[8] and antioxidant[9] activity. phytochemical studies on the genus Solanum showed the presence of alkaloids (Maxwell *et al*, 1996), flavonoids (Kang *et al*, 1998), steroidal glycoside (Ripperger, 1995) and steroidal saponins (Zamilpa *et al* 2002). It is one of the members of the dashamula (ten roots) of the Ayurveda (Mohan *et al* ,2007).A glucoalkaloid termed solanocarpine is found in the fruits. A sterol known as carpesterol and solanocarpidine are also present. phenolic substance, diosgenin and sitosterol are present. Dry fruits contain traces of isochlorogenic, neochronogenic, chronogenic and caffeic acids. Solasodine, solasonine, solamargine and solamargine are present in fruits of Nepalese plant. Quercetin isolated together with apigenin and sitosterol. To validate the traditional medicin this present study was performed for the assay of antimicrobial and antioxidant activity of hot whole plant aqueous extract (HAE) of *Solanum*

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xanthocarpum Schrad and Wendl fam. Solanaceae.

MATERIALS AND METHODS

SX plant was collected from the month of feb from Mathura(India) and adjoining areas and was identified and authenticated by Dr. Anuradha Upadhye of Agharkar research institute Pune with voucher no. WP -32 . The plants was dried in shade and coarsely powdered. Powdered SX 200 gram used for hot aqueous extraction by soxlet apparatus at 100^{0}C for 8-10 hrs. The extracted solution was dried in rotator evaporator that result in dark tan coloured crystals, percentage yields was 24% w/v.

Phytochemical testing

Whole plant aqueous extract (HAE) of *Solanum xanthocarpum* were tested[10] for the presence of active phytochemicals such as alkaloids , carbohydrates, saponins glycoside , flavonoids, triterpenoids and proteins by standard protocol as described by (Debela,2002)

And the results are presented in table 1.

Microorganism

Two Gram positive bacteria (*Bacillus subtilis* MTCC-441 and *Staphlococcus aureus* MTCC-9760) and Two gram negative bacteria (*Escherichia coli* MTCC-1563 and *Pseudomonas aureginosa* MTCC-8076) was procured from Institute of Microbial technology Chandigarh along with one fungi Candida albicans for study the antimicrobial activity of HAE of *Solanum xanthocarpum*.

Antimicrobial assay

Anti microbial assay was performed by the disc diffusion method[11], Hi media chemical was procured for nutrient agar media preparation for antibacterial activity of *Bacillus subtilis*, *Staphlococcus aureus*, *Escherichia coli* and *Pseudomonas aureginosa* and potato dextrose agar media was used for *Candida albicans.* Fresh culture was inoculated in the 5 ml nutrient broth by scraping one to two loopfull growth , this nutrient broth is incubated at 37^{0}c for 8 hours, followed by centrifugation at 3000 rpm for 10 minutes that result in bacterial pellets isolation, this pellets was washed with normal saline and kept in 5 ml normal saline that was further adjusted to 5x10^6 CFU/ml by Mcfarland nephlometer. For the fungal spores candida albicans was inoculated in potao dextrose broth and incubated at 28^{0}c for 72 hours,10^4 spores/ml was taken for anti fungal activity.

Sterilized and dried filter paper disc was saturated with 10µl of HAE of *Solanum xanthocarpum* with 125mg/ml,250mg/ml, 500mg/ml and 1000mg/ml concentration. Each bacterial suspension of 0.5 ml was spread over the nutrient agar plate and filter paper disc having different HAE *Solanum xanthocarpumwas* placed in each plate along with tetracycline 10µg/disc for bacteria and fluconazole 10µg/disc for fungi as standard antibiotic as positive control and disc with distilled water as the negative control . All the culture plates was incubated at 37^{0}c for 24-48 hrs and result was observed by mapping the diameter of zone of inhibition along with the 5mm disc diameter . Each set was experiment was performed in triplet .

MIC was calculated by using UV-visible Spectrophotometer at 660-665nm. Fresh growth was inoculated in the nutrient broth media at 37^{o}c for 28 hrs and cell count was adjusted to 1x 10^8 cell/ml. 100 ml broth and 3.75mg/ml HAE SX stock was prepared. 1ml of stock was dissolved in 2 ml of broth and mix by vortex and then dilution was performed to the 10th test tube my mixing the 1ml of previous stock to the 1 ml of broth. In each test tube 0.5ml bacterial growth was added and incubated at 37^{0}c for 18 hrs and recorded the OD at 660-665nm. One tube containing the broth and Microorganism kept at 4^{0}c overnight as the standard and recorded the OD of it.

Antioxidant activity

Antioxidant activity of HAE *Solanum xanthocarpum* was performed invitro by free radical scavenging method[12] .2,2-diphenyl-1-picrylhydrazyl DPPH(Sigma), ascorbic acid(Merk) Butylated hydroxyl toluene (BHT) and other chemical of analytical grade was used. Different concentration of HAE *Solanum xanthocarpum*,0.25mg/10ml, 0.50mg/10ml, 1.0mg/10ml,and 2.0mg/10ml was prepared. 3 ml of DPPH solution was mixed with 0.1 ml of HAE *Solanum xanthocarpum* and this mixture was incubated in dark at 20^{0}C for 40 min. After incubation absorbance was measured at 517nm by UV-Vis Spectrophotometer water ethanol(1:1)was used as the blank. Scavenging activity of DPPH was calculated by

% Inhibition of DPPH =[(Ac-At)/Ac]x100 Where Ac is the absorbance of DPPH and At is the

absorbance of HAE *Solanum xanthocarpum*. BHT and ascorbic acid was used as the standards.

Statistical Analysis

Different data was analyzed statistically by the one way analysis of variance (ANOVA) using SPSS version 20.0 software and DMRT at p<.05 and .01 to determine significant differences among treatment means. Values are expressed as mean ± SEM.

RESULTS AND DISCUSSION

HAE of Solanum xanthocarpum was extracted by soxhlet and yield obtained was 23-27% with bright brown crystals . Phytochemical screening was performed by different statdard protocol and result show the presence of flavanoids,glycosides,oils, fats, tannins,phenolic compounds, alkaloids, carbohydrates, anthraquinones, Proteins,saponin and triterpenoids that was showed in table no. 1Gum and mucilage was absent.

HAE of SX show the significant anti bacterial activity (p<.01) against *P.aeruginosa, E.coli, S.aureus* and *Bacillus subtilis* as shown in the table no. 2. After 24hr incubation it was shown that *P.aeruginosa* was most sensitive followed by *Bacillus subtilis, S.aureus* and *E.Coli*. the inhibition show the dose dependent inhibition. Fig. 1. The dose 10 mg/disc show the most effective activity as comparison to 1.25 mg/disc. Tetracyclin 10mg/disc also show the significant antibacterial

Table 1. Qualitative Phytochemical Screening of HAE of *Solanum xanthocarpum*

Extract	Alkaloids			Glycosides	Tannins & Phenolics		Flavanoids		Proteins		Sterols		Triterpenoids	Carbohydrates	Fat and oils
	Mayer's test	Dragondroff's test	Wagner's test:	Legal's test	Gelatin test	Ferric chloride test	Shinoda test	Alkaline reagent test	Biuret test	Ninhydrin test	Salkowski test	Libermann-Buchard test	Salkowski test	Molisch's test	Saponification
HAE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2. Antibacterial activity of HAE of SX after 24 Hrs

Name of Bacteria	Zone of inhibition (mm)				
	1.25mg/Disc	2.5mg/Disc	5.0mg/Disc	10.0mg/Disc	Tetracyclin 10mg/disc
P.aeruginosa	6.73^{a}±0.39	8.62^{b}±0.33	12.19^{c}±0.28	19.88^{d}±0.66	15.74^{e}±0.57
E.coli	0.00±0	4.23^{b}±0.33	6.11^{c}±0.06	10.66^{d}±0.44	22.48^{e}±0.28
S.aureus	6.4^{a}±0.21	11.6^{b}±0.30	17.9^{c}±0.21	20.8^{d}±0.41	28.16^{e}±0.16
Bacillus subtilis	0.00±0	7.31^{b}±0.17	9.5^{c}± 0.28	12.5^{d}±0.76	30.33^{e}±0.88

Table 3. MIC of HAE of SX Against the Bacterial species

S. No.	Conc mg/ml	Absorbance *P.aeruginosa*	*E. coli*	*S.aureus*	*B. subtilis*
1	1.25	0.009	0.193	0.006	0.007
2	0.625	0.007	0.196	0.008	0.009
3	0.312	0.010	0.193	0.009	0.011
4	0.156	0.008	0.247	0.012	0.007
5	0.078	0.008	0.288	0.209	0.008
6	0.039	0.012	0.347	0.257	0.213
7	0.019	0.174	0.386	0.298	0.286
8	0.009	0.233	0.345	0.362	0.324
9	0.004	0.297	0.384	0.391	0.369
10	0.002	0.316	0.422	0.443	0.403

Table 4. Antioxidant activity of HAE of SX , Ascorbic acid and BHT using the DPPH free radical scavenging method

S.No.	Concentration (mg/ml)	HAE of SX	Ascorbic Acid	BHT
1	0.25	$16.30^{b} \pm 1.32$	$12.56^{a} \pm 0.28$	$19.72^{a} \pm 1.46$
2	0.50	$20.87^{b} \pm 1.05$	$24.31^{a} \pm 1.34$	$43.31^{a} \pm 1.01$
3	1.0	$28.67^{a} \pm 0.67$	$42.81^{ab} \pm 0.59$	$55.74^{a} \pm 1.73$
4	2.0	$51.35^{b} \pm 3.05$	$93.40^{a} \pm 0.15$	$75.88^{b} \pm 0.86$

Value show the mean ± SEM of triplet experiment. ANOVA followed by DMRT show the result are significant at $p<.01$. Same super script show the no significant difference between the value whereas different superscript represent the proportional difference at $p<.01$.

activity against all the test bacteria.After the 48 hr of incubation of all the test organism it was obserebed that zone og inhibition increased that infer the bacteriostatic and probably bacteriocidal activity when it was observed after 72 hrs of incubation. HAE of SX show no antifungal activity against the *Candida albicans*. This study was supported by the previous study that was carried out by other workers[13,14]. The anti bacterial activity is exhibited due to the saponin[15] it was already reported[16] that glycoside , phenolic compounds, Flavanoids and Tannin show the antibacterial activity that was present in Solanum trilobatum and screened against the two gram positive and two gram negative bacteria that was same as the our study for HAE of SX.

MIC of HAE of SX againes the different bacterial shown in table no. 3. MIC of *P.aeruginosa* found to be low 0.039mg/ml as compare to MIC of *B. Subtilis* and *S. aureus* . *E.coli* and Fungi *Candida albicans* had no MIC value.

Value show the mean ± SEM of triplet

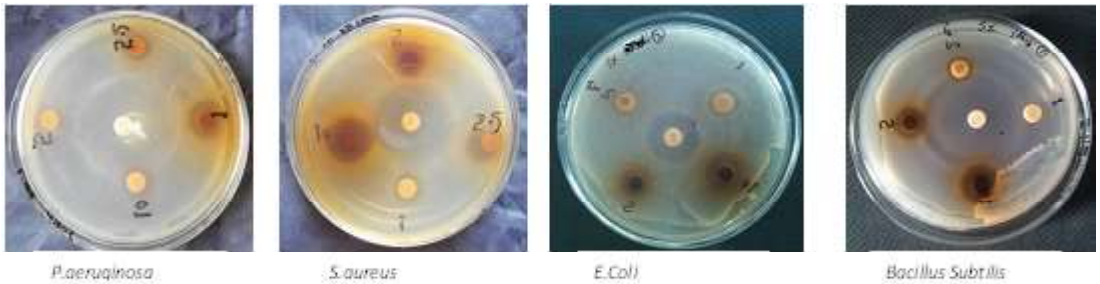

Fig.1. Antimicrobial properties of *Solanum xanthocarpum* hot aqueous extracts

experiment. ANOVA followed by DMRT show the result are significant at p<.01. Same super script show the no significant difference between the value whereas different superscript represent the proportional difference at p<.01.

Antioxidant activity with reference to percentage inhibition of free radical by the DPPH method was found to be less significant (p< .01) when compare with the BHT and ascorbic acid as shown in table no. 4. HAE of SX show the 16.30% free radical scavenging activity for dose 0.25mg/ 10ml as compare to ascorbic acid(12%) and BHT (19.4%). As the dose of HAE of Sx increases to 0.5mg , 1.0 mg and 2.0 mg per 10 ml the percentage inhibition of free radical also increases but less than the Ascorbic acid and BHT that show the dose dependent free radical scavenging activity but less significant than the ascorbic acid and BHT. Free radical can be developed as the peroxide, hydroxyl free radicle by the super oxide activity in the cells. Free radical can cause the cell wall breakdown, enzyme inhibition and nucleic acid mutation that lead to metabolic disorders. Anti oxidant can reduce the free radical formation, presence of Sterol carpesterol, alkaloids solasonine and solmargine in HAE of SX may be proven[17,18] as anti oxidant bioactive compound[19].

Value show the mean ± SEM of triplet experiment. ANOVA followed by DMRT show the result are significant at p<.01. Same super script show the no significant difference between the value whereas different superscript represent the proportional difference at p<.01.

CONCLUSION

The hot aqueous extract of *Solanum xanthocarpum* have the potential antibacterial activity that scientifically prove to SX as traditional medicin used against the bronchial asthma that was caused by *Staphylococus aureus* and these finding agree with the those work done already carried out.The bioactive compound present in it show the mild antioxidant activity that can be used in the soft herbal cosmetics so that it become less irritant . Further studies must be carried out to use the aqueous extract of SX against the some pathogenic bacteria and its use as the mild antioxidant in different herbal formulation.

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