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EVALUATION OF ANTIMICROBIAL EFFICACY OF *Solanum xanthocarpum* AGAINST PATHOGENICMICROORGANISMS IN ORAL CAVITY

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ABSTRACT

Dental caries and other gum and tooth issues have long been treated using herbal medicines. In the current microbiological investigation, medicinal plants *(Solanum xanthocarpum)* were tested for their ability to combat three pathogenic bacteria in the oral cavity (*Candida albicans*, *E. coli* and *Staphylococcus aureus*). *Solanum xanthocarpum* were used to make ethanolic extract concentrations (50 µg/ml, 100 µg/ml, 150 µg/ml, and 200 µg/ml).The agar well diffusion method was used to assess the antimicrobial effectiveness of the ethanolic extract concentrations of *Solanum xanthocarpum*, and the inhibition zone size was determined in millimeters. The acquired results demonstrated that the width of the zone of inhibition increased with an increase in extract content, and the antimicrobial activity of the *Solanum xanthocarpum* ethanolic extracts was detected. Antioxidant activity of extract was assessed by 1,1-diphenyl2- picrylhydrazyl (DPPH) and nitric oxide radical scavenging assays. The extract scavenged DPPH and Nitric oxide radical scavenging assay radicals dose dependently with an inhibitory concentration value of 45.66 and 7.37 µg/ml, respectively. The studied extracts from *Solanum xanthocarpum* was successful in combating the germs that cause tooth caries.

Keywords: *solanum xanthocarpum*, Agar well diffusion, DDPH, Nitric oxide scavenging assay.

INTRODUCTION

India is among the anciently diverse countries in biological resources. Here people trust on natural sources, have invaluable and uninterrupted practice of the use of medicinal plants and other natural resources for healthcare necessities (1). The knowledge of naturally occurring medicinal plants (drugs) was slowly acquired by humans on the basis of experience (2). Plants play important function in developing modern medicines as they contain active

phytochemical components. They have beneficial effects on the community by improving the health of human beings by treating many diseases for many years (3, 4). For such activity, the source of phytochemicals have commonly found in leaves, barks, roots, flowers, fruits and seeds of the plants (5). Sandhu and Heinrich (6) and Gupta *et al.* (7) has pointed out that many rural communities in developing countries depend on plant sources for their nutrient and scrounge, making household utilities as well as utilized them for fire, shadow and as herbal drugs. The plants usually possess antimicrobial substances for their own protection from microbial infection and deterioration; thatís why they are being used for the conservation and safety of food products (8-10). Ushimaru *et al.* (11) assessed the antimicrobial activity of aqueous and ethanol extracts of nine Nigerian species against four nutrient borne bacteria for checking their pharmacological activity in the direction of formulating new antiabscessed agents. Many analytical reports showed that Solanum plants are important source of large number of phytochemical compounds with substantial curative application against human pathogens (12). So they could be assessed as an alternate way to fight against bacterial diseases (13).

Black nightshade (Solanum nigrum L.) is a weed of amentaceous land, gardens, and soils rich in nitrogen and is broadly distributed in Pakistan (14). The fruit of S. nigrum contains many ingredients including fatty acid, tannins, cellulose, resins, dextrin, ash and moisture. Methanolic root extracts of S. nigrum showed antimicrobial and antifungal properties (15). It was also demonstrated that the S. nigrum extract acts as a larvicidal agent against five laboratory colonized strains of mosquito species (16). Fruits also contain tropeine, an alkaloid having mydriatic action, along with solanine (17). Four anti-cancer steroidal glycosides; solasonine, solamargine, diosgenin and solasodine were isolated from the immature S. nigrum fruits (18). Recent phytochemical analysis of S. nigrum fruit has resulted in the isolation of two novel disaccharides along with protein, fibre, carbohydrate, minerals like magnesium, phosphorus and vitamin C, B and folic acid (19).

Solanum xanthocarpum is also recognized as Indian night-shade or yellow berried nightshade plant. It is well versed in India and Pakistan; often in wastage places, on roadsides and in open spaces as well. Its fruit contains carpesterol, glucose, galactose, potassium chloride, a number of steroidal compounds and alkaloids mainly in the form of glycoalkaloids. The flavanoids quercitrin and apigenin glycosides were the major chemical constituents present in the fruits of *S. xanthocarpum* (20, 21). Many therapeutic activities of the fruits of this plant have been reported. Itsí being used for itching and fever reduces adipose tissues as well as seminal ejaculation (22, 23). The aqueous and organic solvent extracts of different parts of the plant demonstrated that all the extracts had very strong biological inhibition effects (24). Methanolic extracts of *S. xanthocarpum* and Datura metel exhibited highly significant antifungal activity against different species of pathogenic *S. aureus* (25). Okram *et al.* (26) also investigated the strong inhibitory effects on the radiated growth of *Candida albicans, E. coli* and *Staphylococcus aureus*.

The main objective of this study was to take into account the important aspect of antibacterial potential of fruits of *Solanum xanthocarpum*. Along with antifungal activity and the main reason for choosing the fruits of

these plants among the diversity are the reality that local people often employ these two important fruits as folk medicines for various infections. It is thus essential to assess the medicinal plants scientifically for various complaints that were made against the traditional medicine in the past.

EXPERIMENTAL

Collection of plant materials

The plant species namely *Solanum xanthocarpum* fruit was collected by in and around Suntharakkottai, Mannargudi Taluk, Thiruvarur District, Tamil Nadu, India.

Preparation of the Ethanol extract

The fresh plant materials were washed with running tap water and shade dried. The fruit were crushed to coarsely powdered. These coarse powders (25g) were then subjected to successive extraction in 250ml of ethanol solvent by using cold percolation method. The collected extracts were stored and then used for further analysis. The DMSO (Dimethyl sufloxide) is act as dissolved solvents for these extracts.

Phytochemical screening of *Solanum xanthocarpum*

Preliminary phytochemicals Qualitative analysis of ethanol extracts of the *Solanum xanthocarpum* were performed to detect the presence of plants' secondary metabolites of biological importance such as alkaloids, flavonoids, terpenoids, steroids, carbohydrates, proteins, fats, saponins, etc. All the experiments were carried out by using the standard methods mentioned by (27). To check for carbohydrates: 1 ml of Molisch's reagent, 1 ml of plant extract, and a few drops of concentrated sulfuric acid were added to 2 ml of plant extract. The presence of carbohydrates is indicated by the presence of a purple or reddish tint (28).Tannins: To 2 ml of 5% ferric chloride, 1 ml of plant extract was added. Tannins can be detected by the creation of dark blue or greenish black colour (29). Saponins: In a graduated cylinder, 2 ml of plant extract and 2 ml of distilled water were combined and shaken for 15 minutes lengthwise, Saponins are present when a layer of foam 1 cm thick forms (30). Flavonoids: 1 ml of 2N sodium hydroxide was added to 2 ml of plant extract. Yellow colouring is present, indicating that (31). Alkaloids: 2 ml of strong hydrochloric acid were added to 2 ml of plant extract. The Mayer's reagent was then added in a few drops. Alkaloids can be detected by the presence of white or green precipitate (32). Glycosides: 3 ml of chloroform and 10% ammonia solution were mixed with 2 ml of plant extract to produce glycosides. The development of a pink tint denotes the presence of glycosides (33). Terpenoids: 2 ml of chloroform and concentrated sulphuric acid were carefully added to 0.5 ml of extract to study terpenoids.Terpenoids are present when the contact develops a reddish-brown colour (34). Phenols: 2 ml of distilled water and a few drops of 10% ferric chloride were added to 1 ml of the extract. The formation of a blue or green hue suggests the presence of phenols (35). Phytosterols are steroids: The presence of steroids is shown by the emergence of a brown ring around 1 ml of plant extract, whereas the presence of phytosterols is indicated by the appearance of a bluish brown ring around the same amount of chloroform (36). Coumarins: 3 ml of 10% NaOH and 2 ml of sample were added. The presence of coumarins is indicated by the appearance of a yellow hue (37).

Scavenging activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical

The effect of extracts on DPPH radical was estimated using the method of LiyanaPathiranan *et al.* (2005). About 0.1 ml of DPPH-methanol solution (0.135 mM) was mixed with 1.0 ml of different concentrations of extract xxx. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Rutin and Butylated hydroxyl toluene (BHT) were used as standard drugs. The percentage of free radical scavenging was calculated according to the following equation: % scavenging = 100–(Abs sample–Abs blank)/Abs Control × 100. Ab = Absorbance of blank As = Absorbance of sample

Nitric oxide radical scavenging activity

In this assay, a solution of SNP (10 mM) in phosphate buffered saline (PBS, pH 7.4) was mixed with different concentration of extract. The mixture was incubated at 37°C for 60 min in the light. Half the quantity of the aliquots was taken and mixed with an equal quantity of Griess reagent, and the mixture was incubated at 25°C for 30 min in the dark. The absorbance of pink chromophore generated during diazotization of nitrite ions with sulfanilamide and subsequent 46 coupling with naphthyl ethylene diamine dihydrochloride (NED) was read at 546 nm against a blank (Bajpai *et al,* 2013). All tests were performed in triplicate. Ascorbic acid was used as the standard reference compound in the various concentration. The percent inhibition activity was calculated by the same formula as used for determination of DPPH radical scavenging activity.

Determination of antimicrobial activity

Microorganisms and culture

A total of three microorganisms were kindly provided by the Department of Microbiology, S.T.E.T Women's College, Tamil Nadu, India. They are Staphylococcus aureus, Escherichia coli, and candida albicuns. The strains were cultured at 37 °C on plate count agar (PCA) medium.

An agar-well diffusion method was employed for determination of antibacterial activities (NCCLS, 1999). The freezedried extract samples of spices and herbs were dissolved in phosphate buffered saline (PBS, pH 7.0–7.2) to the final concentration of 100 mg/mL and sterilized by filtration through 0.22 µm sterilizing Millipore express filter (Millex-GP, Bedford, OH). All bacterial and fungal were suspended in sterile water and diluted to 106 CFU/mL. The suspension (100 µL) was spread onto the surface of PCA medium. Wells (4.6 mm in diameter) were cut from the agar with a sterile borer and 60 µL extract solutions were delivered into them. Negative controls were prepared using PBS solution. Penicillin G (960 µg/well) and gentamicin (600 µg/well) were used as positive reference standards to determine the sensitivity of each microbial species tested. The inoculated plates were incubated at 35 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters. All tests were performed in triplicate.

RESULT AND DISCUSSION

Qualitative Phytochemical

Phytochemistry is the branch of science which deals with the study of the chemicals derived from plants. The plant chemicals fall under two categories- primary and secondary metabolites. Like all other organisms, plants

require sugars, proteins, fats and vitamins for basic body functioning. Such chemicals are together known as primary metabolites. Plants produce several other compounds which are intermediate products of metabolic pathways and they are known as secondary metabolites. Phytochemical constituents of *Solanum xanthocarpum* like alkaloids, Saponins, tannins, Terpenoids, amino acids, Protein, saponins, Coumarins, flavonoids were analysed qualitatively and reported in **(Table 1)** The phytochemical screening showed in all cases the absent of Steroids, Glycosides, Carbohydrates.

Table 1: Phytochemical Screening of *Solanum xanthocarpum*

S.No	Constituents	Ethanol
1.	Steroids	_
2.	Terpenoids	+
3.	Alkaloids	+
4.	Flavonoids	+
5.	Tannins	+
6.	Glycosides	_
7.	Carbohydrates	_
8.	Protein and amino acids	+
9.	Saponins	+
10.	Coumarins	+

Antioxidant activity of *Solanum xanthocarpum*

The assay involving scavenging of DPPH free radicals was developed by Blois, and it has been routinely used to evaluate free radical scavenging activity of various types of samples including plant extracts. The assay is considered as a valid, accurate, easy, and economical, and the results are reproducible. DPPH is a stable and nitrogen-centered organic free radical and has a strong absorption at 515-520 nm in alcoholic solution, and it does not need special methods for its generation as in case of ABTS radicals which needs to be generated before assay. Substances capable of donating hydrogen atom (often termed antioxidants) will reduce the purple-colored DPPH to DPPHH which has yellow-colored (38). In the present study, we screened the potential of various concentrations of plant seed ethanolic extract of *Solanum xanthocarpum* to scavenge DPPH radicals. We monitored bleaching of color of DPPH radical solution in the presence of varying concentrations of extract at 517 nm. The extract scavenged DPPH radicals in a dose-dependent manner with an IC50 value of 45.66 µg/ml. Scavenging of radicals was >50% at extract 55 concentration of 50 µg/ml and higher. At 200 µg/ml concentration, extract scavenged DPPH radicals to >90% (Fig. 1) Ascorbic acid scavenged DPPH radicals more efficiently (IC50 value of 8.89µg/ml) than that of ethanol extract of *Solanum xanthocarpum*. Although the scavenging of DPPH radicals by extract was less when compared to ascorbic acid, it is evident that the extract contains antioxidant

principles possessing hydrogen-donating property which could act as free radical scavengers. In earlier studies, (39) observed dose dependent scavenging of DPPH radicals by fruit extract and leaf extract, respectively. Archana and Jocob (Archana *et al.*, 2015) found dosedependent scavenging of DPPH radicals by root extract with an IC50 value of 52 µg/ml. In a recent study by Rajakumari *et al.*,2016 (40), the ethyl acetate extract of fruit was shown to exhibit scavenging of DPPH radicals **(Table 2,3 and Figure 1,2)**.

Table 2: DPPH radical scavenging activity extract of vitamin c

S.No	Concentration µg/ml	DPPH	Standard Vitamin C
1.	50 µg	13.43 ± 2.11	27.21 ± 1.56
2.	100 µg	26.50 ± 6.04	41.75 ±3.67
3.	150 µg	42.49 ± 5.21	62.19 ± 6.19
4.	200 µg	59.13 ± 4.77	75.89 ±2.77
5.	250 µg	75.89 ±2.99	83.83 ±6.03

Figure 1: Antioxidant DPPH radical scavenging activity

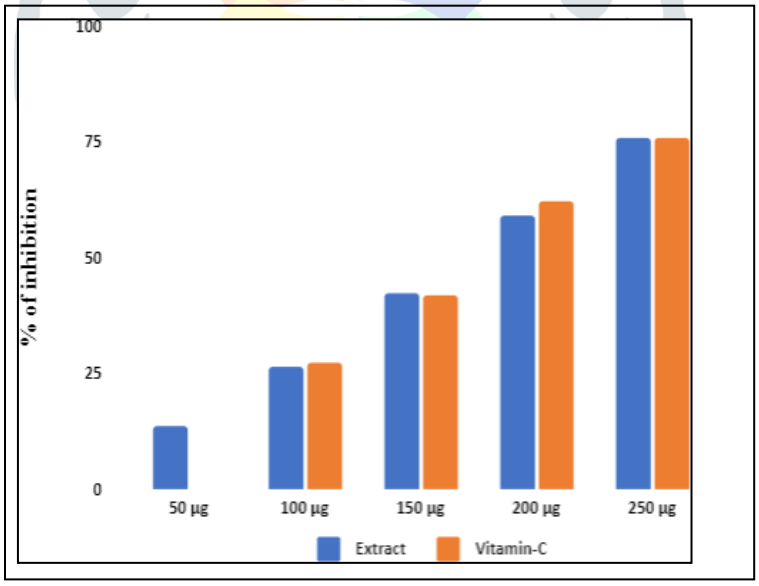
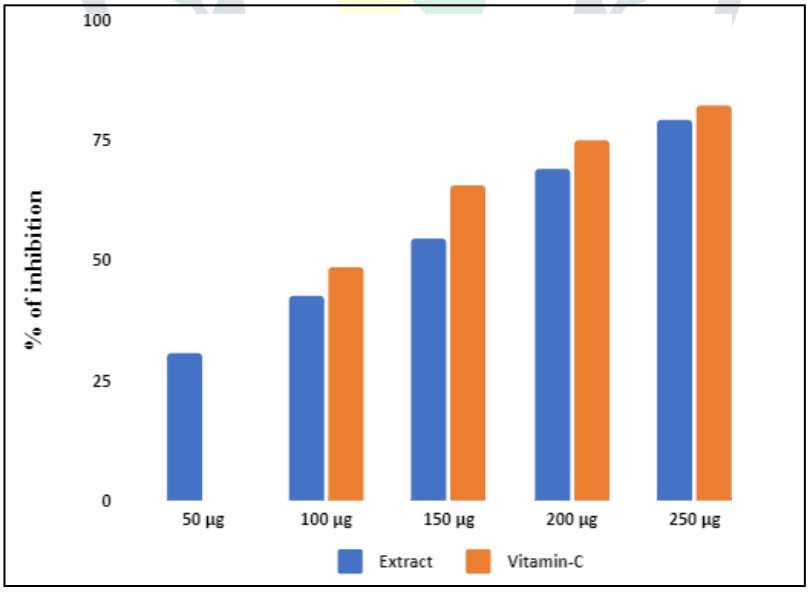


Table 3: Nitric oxide radical scavenging activity of vitamin c

S.No	Concentration µg/ml	NORS	Standard Vitamin C
1.	50 µg	30.70 ± 1.56	48.67 ± 3.77
2.	100 µg	42.79 ± 3.67	65.64 ±10.34
3.	150 µg	54.54 ± 6.19	75.33 ± 7.05
4.	200 µg	68.98 ± 2.77	82.37 ± 6.85
5.	250 µg	79.37 ± 6.03	92.36 ± 3.96

Figure 2: Antioxidant nitric oxide radical scavenging activity



Antibacterial activity of extract of *Solanum xanthocarpum*

Antibacterial activity can be determined by various methods such as disk diffusion, agar well diffusion, and broth dilution. Among these, agar well diffusion method is one of the widely used in vitro assays for determining

antibacterial activity of plant extracts (41). In the present study, we determined antibacterial activity of extract of *S. virginianum* by agar well diffusion assay. The presence of zone of inhibition around the well was considered positive for antibacterial activity. The extract was effective in inhibiting all test bacteria but to a varied extent. Marked and least inhibitory activity was observed against *Staphylococcus aureus* and *E. coli*, respectively. Among Gram -positive bacteria, *Staphylococcus aureus* was inhibited to higher extent while others were inhibited to more or less similar extent. In case of Gram-negative bacteria, *Staphylococcus aureus* and *E. coli* were susceptible to highest and least extent, respectively. Inhibitory activity displayed by standard antibiotic was higher than that of extract. DMSO did not cause inhibition of any of the test bacteria (Table 3, Figure 5, Plate1). Overall, Gram-positive bacteria exhibited marked susceptibility to extract as well as antibiotic when compared to Gram-negative bacteria. The presence of an outer membrane in Gram-negative bacteria might be responsible for the low susceptibility as it might have acted as a 51 additional barrier for the entry of extract/antibiotic. In a previous study, (42) showed concentration-dependent inhibitory activity of leaf extract against a panel of bacteria (43).

Antifungal activity of extract of *Solanum xanthocarpum*

Fungi are ubiquitous and are responsible for causing a number of diseases in crops leading to huge economic losses in severe cases. Management of fungal diseases by chemical method suffers from several drawbacks such as high cost, environmental pollution, adverse effects on humans, and emergence of resistant pathogens. This alarming situation triggered immense interest in scientific community to search alternates for controlling fungal diseases. Plants and plantbased formulations have shown promising antifungal activity (44). In the present study, we evaluated antifungal potential of extract of *S. Xanthocarpum* by poisoned food technique, and the result is shown in (Figure 3) the extract was effective in inhibiting fungal growth as evidenced by a considerable reduction in 52 the colony growth on plates poisoned with extract. Extract inhibited the mycelial growth of all fungi to >40%. Among fungi, marked susceptibility was recorded against *Candida albicans* (61.29% inhibition). Was inhibited to less extent (43.47% inhibition), It has been shown that various solvent extracts of fruit exhibited antifungal activity. Ethanol extract of leaf was more active in inhibiting *Candida albicans* when compared to aqueous extracts. Seed extracts were able to inhibit *C.albicans* dose dependently. The study carried out by Mamta *et al.*,. showed the potential of flower, stem, and fruit extract to inhibit *A. niger* and *A. flavus*. The study by Mamta *et al*.,2016. showed dose-dependent inhibition of *C. albicans* by ethanol extract of seed, whereas other extracts did not show any inhibitory activity.

Table 3: Antimicrobial activity of *solanum xanthocarpum*

S.No	Name of Species	1 mg/ml	1.5 mg/ml	2 mg/ml	Amoxicillin mg/ml
1.	***S. aureus***	11	11.5	14.5	16.5
2.	***E.coli***	9.5	10	14	19
3.	***C. albicans***	8	9	13	16

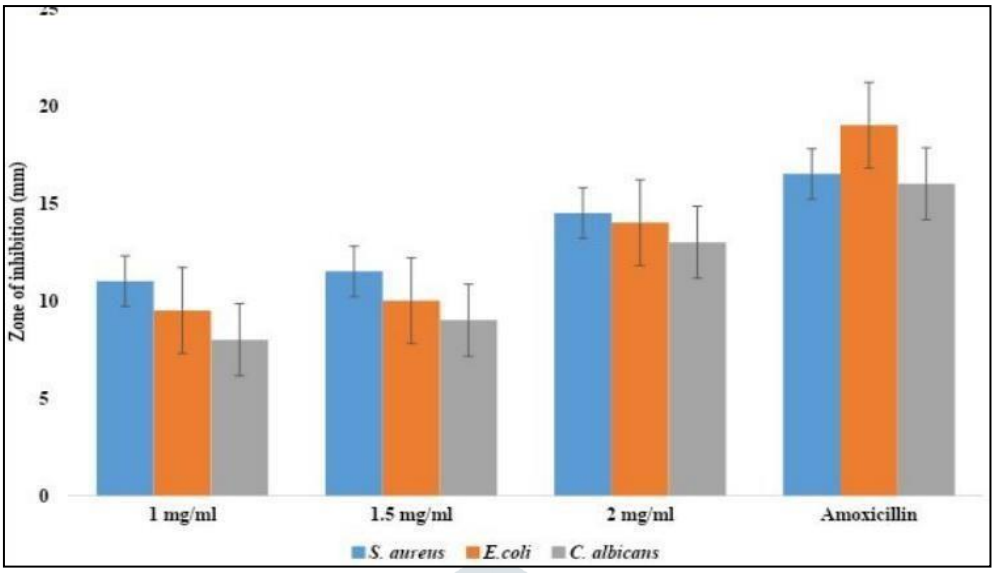
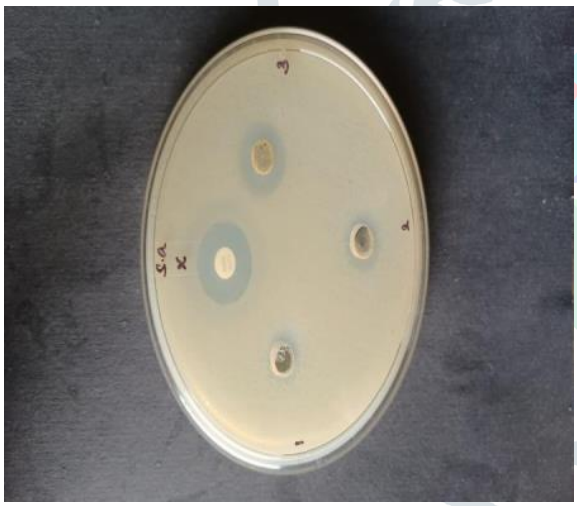
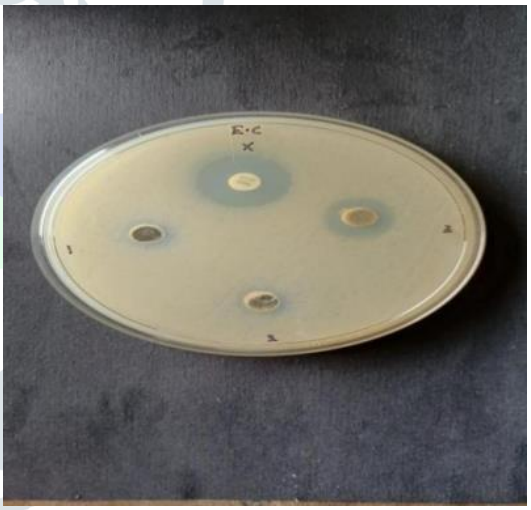


Figure 3: Antimicrobial activity of *Solanum xanthocarpum*

Plate 1: Antimicrobial activity of ethanol extract of *Solanum xanthocarpum*



Staphylococcus aureus



E.coli

Candida albicans

CONCLUSION

Ayurveda, is one of the official systems of medicine in India and is also widely practiced in many other countries. In last few years lots of research has been undertaken on medicinal properties, possible mechanisms and relevant information on popular herbs mentioned in traditional text as well as used by Vaidyas. India is blessed with largest and richest floral diversity in the world. Modern chemistry played an important role in a new era for the study and use of natural products. Elucidation studies enhance the secondary metabolite production as compare to their accumulation capacity of mother plant.

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