

Evaluation of Antibacterial Activity of Solanum Xantocarpum SCH & WEND (Fruit) Against Pathogens Isolated From Diabetic Foot Ulcer

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Type of Review: Peer reviewed.

DOI: <http://dx.doi.org/10.21013/jas.v4.n2.p5>

How to cite this paper:

K S, S., & N, L. (2016). Evaluation of Antibacterial Activity of Solanum Xantocarpum SCH & WEND (Fruit) Against Pathogens Isolated From Diabetic Foot Ulcer. *IRA-International Journal of Applied Sciences* (ISSN 2455-4499), 4(2), 246-258.
doi:<http://dx.doi.org/10.21013/jas.v4.n2.p5>

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ABSTRACT

In the present study efficacy of various solvent extracts of S. xantocarpum fruit was assed against six pathogenic bacteria Proteus, E. coli, Klebsiella, Pseudomonas, Enterobacter and S. aureus, isolated from diabetic foot ulcer. Among the different extracts ethyl acetate showed highest activity in terms of inhibitory zone against E. coli, where as in ethanol extract significant activity was seen against Enterobacter.

Key words: Pathogens, antimicrobial activity, *Solanum xantocarpum* fruit.

Introduction

The medicinal plants have been used to cure diseases since antiquity. Plants still constitute one of the major sources of drug in modern as well as traditional medicine throughout the world [1]. Over 25% of prescribed medicines in industrialized countries derived directly or indirectly from plants [2]. However, plants used in traditional medicines are still understudied, particularly in clinical microbiology [3]. In developing countries where medicines are quite expensive, investigation on antimicrobial activities from ethanomedicine plants may still be needed [4,5]. Several plants were known to possess antimicrobial properties in the literature. In the present study, we have selected *S. xantocarpum* fruit to claim to possess promising antimicrobial effects on the infected diabetic foot ulcer.

Materials and Methods**Plant material collection**

Fresh healthy fruit of *S. xantocarpum*. Sch & Wend were collected in and around of Mysore district, Karnataka, India. These were washed thoroughly 2-3 times with running tap water and once with sterile water, and then they were dried in shade. The taxonomic identification of these plant species was determined at National Ayurveda Dietetics Research Institute, Bangalore, Karnataka, India (*S. xantocarpum*. Sch & Wend. Voucher no. **RRCBI-3721** The plant material were powered to 100-120 mesh in an apex grinder (Apex constructions, London) and stored.

Test microorganisms

The test organisms used were isolated from diabetic foot ulcer *viz.*, *Proteus*, *E. coli*, *Klebsiella*, *Pseudomonas*, *Enterobacter* and *S. aureus*. The bacterial cultures were maintained on NAM respectively.

Preparation of aqueous extract

Fifty grams of fruit of *S. xantocarpum* were macerated with 100 ml sterile distilled water in a blender for 10 min. The macerate was first filtered through double layered muslin cloth and centrifuged at 4000 rpm for 30 min. The supernatant was filtered through Whatman No.1 filter paper and heat sterilized at 121°C for 30 min. The extracts were preserved aseptically in brown bottles at 4°C until further use.

Preparation of solvent extract

Twenty grams of the powered plant materials were loaded in the thimble of Soxhlet apparatus. It was fitted with appropriate size round bottom flask and plant material was extracted with 150 ml of pet ether (Merck, Darmstadt) by Soxhlet apparatus [6]. Constant heat was provided by Mantox heater for recycling the solvent. After complete extraction, the extract in the round bottom flask was transferred into sterile dry Petri plate and the solvent was evaporated. The sediment was scrapped off, and preserved at 4°C in airtight bottles until further use. Similar procedure was followed for other.

Antibacterial activity by agar well diffusion method

Antibacterial activity by agar well diffusion method was carried out as per the methods of [7], briefly the bacterial suspension was adjusted to 0.5 Mc Farland. About 15-20 ml of NAM was poured in the sterilized petridish and allowed to solidify. Bacterial suspension of 100 μ l was pipetted and spread using spreader for the bacterial lawn preparation. Well of 6 mm diameter and about 2 cm apart were punched in the culture medium by cork borer. For each well 500 and 750 μ g (in the concentration of 0.5 and 0.75 mg/ml) of plant extract were loaded. Sterile DMSO was used as negative control, Kanamycin served as positive control. Plates were kept at 4°C for 30 min for the diffusion of plant extract. Plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the inhibition zone after 24 h.

Antibacterial activity by disc diffusion method

The different solvent extracts were tested for antibacterial activity by disc diffusion method [8, 9]. Bacterial suspension of 100 μ l containing 2.0×10^6 CFU/ml was pipetted and spread using spreader for bacterial lawn preparation in the previously prepared sterilized solidified NAM plates. Sterile filter paper discs of 6 mm diameter were impregnated with 0.01 and 0.02 mg/ml of plant extract (10 and 20 μ g concentration). The plates were kept at 4°C for 30 min for the diffusion of the plant extracts [10]. The plates were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters. All the tests were performed in triplicates. Kanamycin (30 μ g) served as positive control and 10% Dimethyl sulfoxide (DMSO) serve as negative control.

Determination of minimum inhibitory concentration

The minimum inhibition concentration (MIC) was carried out by broth dilution method. 0.5 ml (0.5 Mc Farland) of bacterial suspension was inoculated aseptically 10-0.0195 mg/ml of plant extract was added, this tube was considered as stock solution (10^{-1}), and then this is serially diluted up to 10^{-8} dilution factor. After serial dilution, the tubes were tapped for uniform distribution of bacteria and plant extract. All the tubes were kept for incubation for 24 h at 37°C. Similar procedure was adapted for remaining test bacterial suspensions [10]. Inhibition of bacterial growth was determined by measuring the absorbance at 465 nm using UV-visible Spectrophotometer (Hitachi U-2000, Japan) against negative control. The minimum concentration of the organisms was determined as the MIC. The percentage of inhibition was calculated according to the formula.

$$\text{Percent growth inhibition} = \left[\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right] \times 100$$

Results

Well method

In the present study, results of inhibition zone in the well diffusion method using the solvent extract fruit of *S. xantocarpum* viz., pet ether, chloroform, ethyl acetate, ethanol and aqueous extract against control showed significant inhibition zone against six bacteria (*E. coli*, *Proteus*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, *S. aureus*) tested compared to the standard antibiotic drug 30 mg Kanamycin. The standard control exhibited inhibition of *E. coli*, *Klebsiella* and *S. aureus* by 10 mm, 8 mm and 3 mm respectively. *Proteus*, *Pseudomonas*, *Enterobacter* was not inhibited by the standard drug (Kanamycin) (Table 1, Figs.1, 1a and 1b).

Aqueous extract of *S. xantocarpum* fruit at 0.5 mg/ml concentration did not inhibited the test bacterial isolates, however ethyl acetate, the only solvent inhibited *E. coli*, the zone of inhibition was 11.3.

Aqueous fruit *S. xanthocarpum* extract at 0.75/ml mg concentration inhibited *Enterobacter* and *S. aureus* by 9.6 and 12.6 mm respectively. Whereas pet ether and chloroform did not show inhibition. Ethyl acetate showed highest inhibition zone for *E. coli* (14.3) least inhibition zone for *Klebsiella* (11.0) and *S. aureus* (11.0) and Ethanolic extract also exhibited the zone of inhibition of *E. coli* (11.0), *Proteus* (12.3 mm), *Pseudomonas* (9.6) and *Enterobacter* (18 mm). Highest inhibition zone for *Enterobacter* (18 mm) and least inhibition zone for *Pseudomonas* (9.6 mm) (Table 1, Fig.1b and 1c).

Disc method

The efficacy of various solvent extracts of *S. xanthocarpum* fruit against the isolated pathogenic bacteria has not shown any inhibition zone at 0.01 mg/ml concentration. The aqueous extract also did not exhibit any inhibition zone at this concentration against standard. Whereas in 0.02 mg/ml concentration, ethanol had exhibited zone of inhibition against *Klebsiella* and *S. aureus* with 3.6 mm and 8.0 mm respectively. But rest of the extracts viz., pet ether, chloroform, ethyl acetate has not showed any activity against bacteria. Aqueous extract has shown zone of inhibition against *Proteus* only with 6.0 mm (Table 2, Fig. 2a and 2b).

Minimum Inhibitory Concentration (MIC)

The MIC was carried out for only alcohol and ethyl acetate extracts as these had given very effective results against the foot ulcers pathogens. *Solanum xanthocarpum* fruit has shown the MIC of test organisms viz., *E. coli* (0.5 mg/ml), *Proteus* (0.5 mg/ml), *Pseudomonas* (0.75 mg/ml) and *Enterobacter* (0.125 mg/ml) concentration. (Table 3, Fig. 3a).

Ethyl acetate extract of *Solanum xanthocarpum* fruit has shown the MIC for *E. coli* (0.067 mg/ml) which is found to be most effective against *E. coli*, *Klebsiella* (0.75 mg/ml), *Pseudomonas* (0.25 mg/ml) and *S. aureus* (0.75 mg/ml) concentration.

Discussion for agar well diffusion method

Antibacterial activity of the fruit of *S. xanthocarpum* and were evaluated *in vitro* against six bacterial species. They were frequently found in diabetic foot ulcers. The aqueous and solvent extracts of the plant tested in the present study showed varied level of antibacterial activity. The results that were obtained show that the standard Kanamycin exhibited the zone inhibition against *E. coli*, *Klebsiella* and *S. aureus*. Similarly [11] have used amoxicillin as the standard and have recorded the zone of inhibition against *E. coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Yersinia*, *Enterocolitica*, *K. pneumonia*, and *P. vulgaris*. Concordantly [12] have reported that tetracycline that was taken as control have shown the inhibition zone against gram +ve bacteria i.e., *B. cereus* and Gram -ve bacteria *Enterobacter aerogenes*, *E. coli*, *Klebsiella pneumonia* and *Acromonas hydrophila*.

Aqueous extracts did not show the zone of inhibition and found that it was ineffective against the pathogenic bacteria. Similarly [13] have also reported that aqueous extracts were least effective against the *B. cereus*, *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, *A. niger*, *A. flavus*, *R. stolonifer*, *F. oxysporum*. However, [14] has reported that the ethanol and aqueous extracts of five Nigerian medicinal plants had broad spectrum of antimicrobial activity on Gram+ve and Gram -ve bacteria which responded to the inhibition activity of plant extract.

Discussion for disc method

An herbal remedy has been accompanied since a long time to the human kind and they continued to be the rich resource of therapeutic agent. It is anticipated that the active chemical component of the plants with the efficient antibacterial property is exploited for treating bacterial infections. Some of the

phytochemical preparation with more flavonoids content is reported by many researchers which exhibit antibacterial activity [15, 16, 17, 18, and 19].

In the present investigation, the disc diffusion method was performed for *E. coli*, *Proteus*, *Klebsiella*, *Pseudomonas*, *S. aureus* against plant extracts. The standard drug has shown the inhibitory property for *E. coli*, *Klebsiella*, *S. aureus* during the present investigation in concordance with [20]; where they have tried with vancomycin, erythromycin and nystatin. Presently, the fruit in aqueous and ethanolic extracts have inhibited *Proteus*, *Klebsiella*, *S. aureus*. [20] and [21] have also recorded the inhibition of *Proteus*, *Klebsiella*, *S. aureus*. Fruit of *S. xanthocarpum* at 1 mg and 2 mg has shown the inhibition property chloroform and Ethanolic extract respectively, where *Pseudomonas* and *S. aureus* inhibited to greater extent. Similar results have been recorded by many researchers [10, 21]. Similarly, the observation made by researchers with solvent extract of leaves of *Bauhinia recemosa*, leaf extract of *Moringa olifera*, seed, leaf, fruit, bark, steam of *Acacia arabica* (leaf), *Eucalyptus globules* (leaves), *Helicteres isora* (fruit), *Aegle marmelos* (leaves) *Azadirachta indica* (leaves) *Caesalpinia bonducella* (seed), *Margifera indica* (bark), *Anonna squamosa* (fruit), *Jatropha careus* (stem), *Delonie regia* (bark) [21, 22].

Discussion for MIC

The MIC of the ethanol and ethyl acetate extract inhibiting concentration value of the plant extracts are shown in Table 3. *Solanum xanthocarpum* fruit was found to be more effective in inhibiting *Enterobacter* at 0.75 mg/ml concentration, *Klebsiella* and *S. aureus* exhibited the resistance at 0.5 and 0.125 mg/ml concentration. Similar results were observed in the ethanol extract of *Ocimum sanctum*, *Aegle marmelos* and *Adhatoda vasica* [23].

The ethyl acetate extracts of *S. xanthocarpum* fruit has exhibited a range of MIC against the test organisms in the present study. *Solanum xanthocarpum* fruit exhibited more effectiveness in inhibiting *E. coli* at a very low concentration. The results were contrary to the findings of [24]. *Proteus* and *Enterobacter* were found resistant to the ethyl acetate extract of *S. xanthocarpum* fruit. This was also observed in the *Toona ciliate roemer* by [25] against *Klebsiella* and *S. aureus* organisms.

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Table 1: Zone of inhibitory activity (in millimeter) of different solvent extracts of *S. xantocarpum* fruit against the test bacteria well method (0.5 and 0.75mg).

Solvent extract	mg	<i>E. coli</i>	<i>Proteus</i>	<i>Klebsiella</i>	<i>Pseudomonas</i>	<i>Enterobacter</i>	<i>S.aureus</i>
Aqueous	0.5	00	00	00	00	00	00
	0.7	00	00	00	00	9.6±0.3 ^a	12.6±0.3 ^a
Ethanol	0.5	00	00	00	00	00	00
	0.7	11.0±0.5 ^a	12.3±0.8 ^a	00	9.6±0.8 ^a	18.0±0.5 ^a	00
Ethyl acetate	0.5	11.3±0.8 ^a	00	00	00	00	00
	0.7	14.3±0.5 ^a	00	11.0±0.5 ^a	11.6±0.8 ^a	00	11.0±0.5 ^a
Pet ether	0.5	00	00	00	00	00	00
	0.7	00	00	00	00	00	00
Chloroform	0.5	00	00	00	00	00	00
	0.7	00	00	00	00	00	00
Kanamycin	30	10.0±0.0 ^a	00	8.0±0.0 ^a	00	00	3.0±0.0 ^a

0.5 and 0.75 mg/ml concentration of the extract used

Values are means of three independent replicates

Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD ($P \leq 0.05$)

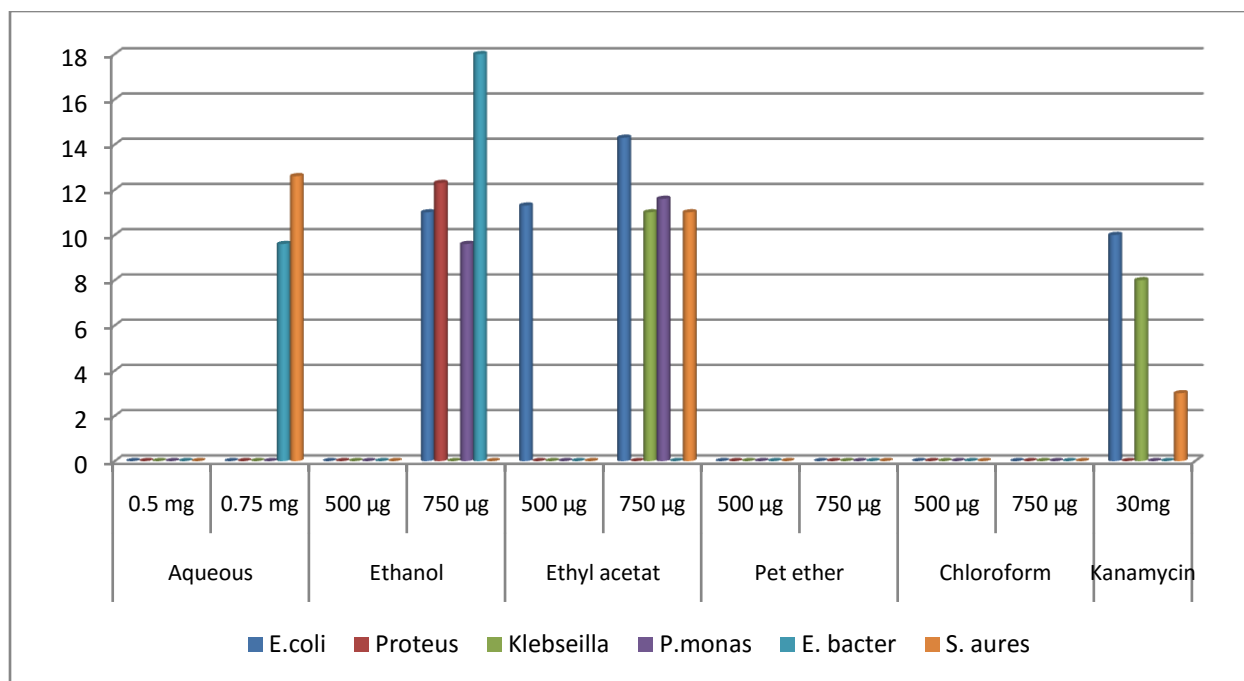


Fig 1: Antibacterial activity of *S. xantocarpum* fruit by well method

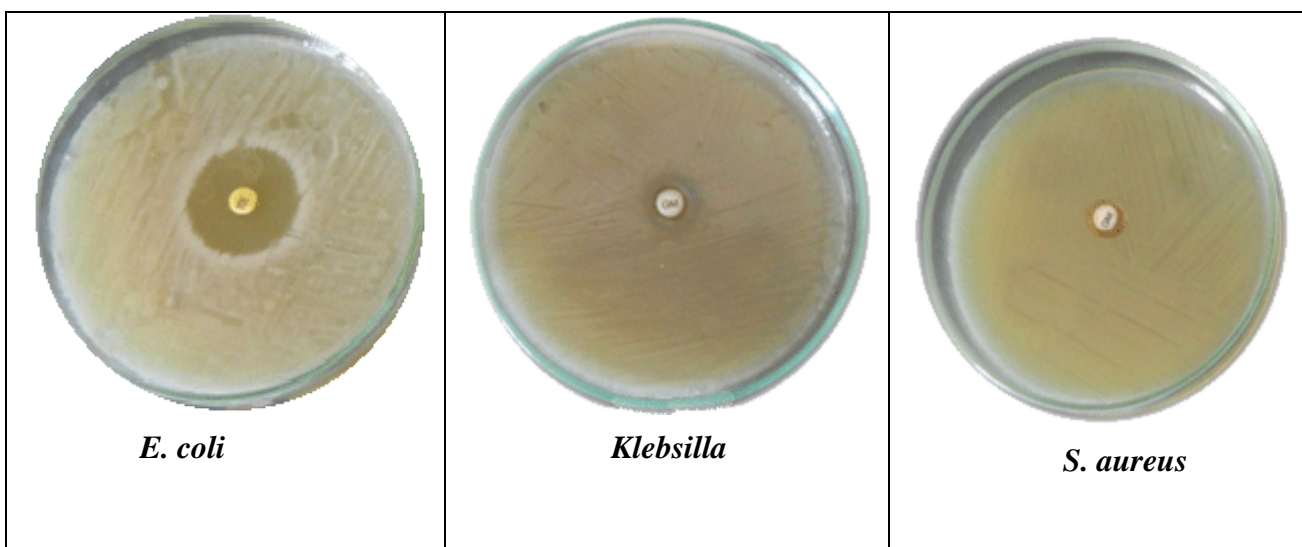


Figure 1a: Antibacterial activity of the standard antibiotic Kanamycine 30 mg / disc against the test bacteria (a) *E. coli* (b) *Klebsilla* (c) *S. aureus*.

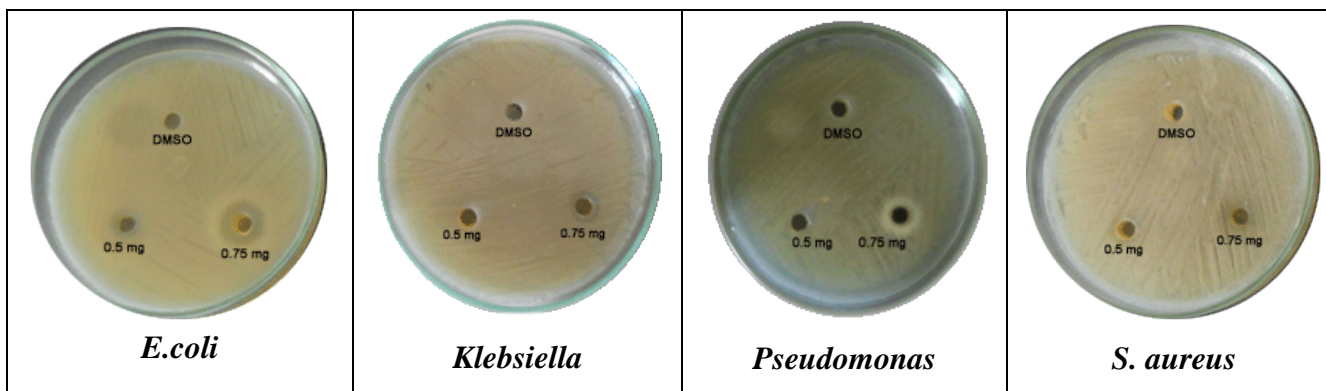


Figure 1b: Ethyl acetate extract of *S. xantocarpum* (Fruit) by well method.

Table 2: Zone of inhibitory activity (in millimeter) of different solvent extracts of *S. xantocarpum* fruit against the test bacteria well method (0.1 and 0.2mg).

Solvent extract	mg	<i>E. coli</i>	<i>Proteus</i>	<i>Klebsiella</i>	<i>Pseudomonas</i>	<i>Enterobacter</i>	<i>S.aureus</i>
Aqueous	0.01	00	00	00	00	00	00
	0.02	00	6.0±0.57 ^a	00	00	00	00
Ethanol	0.01	00	00	00	00	00	00
	0.02	00	00	3.6±0.33 ^b	00	00	8.0±0.57 ^a
Ethyl acetate	0.01	00	00	00	00	00	00
	0.02	00	00	00	00	00	00
Pet ether	0.01	00	00	00	00	00	00
	0.02	00	00	00	00	00	00
Chloroform	0.01	00	00	00	00	00	00
	0.02	00	00	00		00	00
Kanamycin	30	10.0±0.0 ^a	00	8.0±0.0 ^a	00	00	3.0±0.0 ^a

0.01 and 0.02 mg/ml concentration of the extract used

Values are means of three independent replicates

Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD ($P \leq 0.05$)

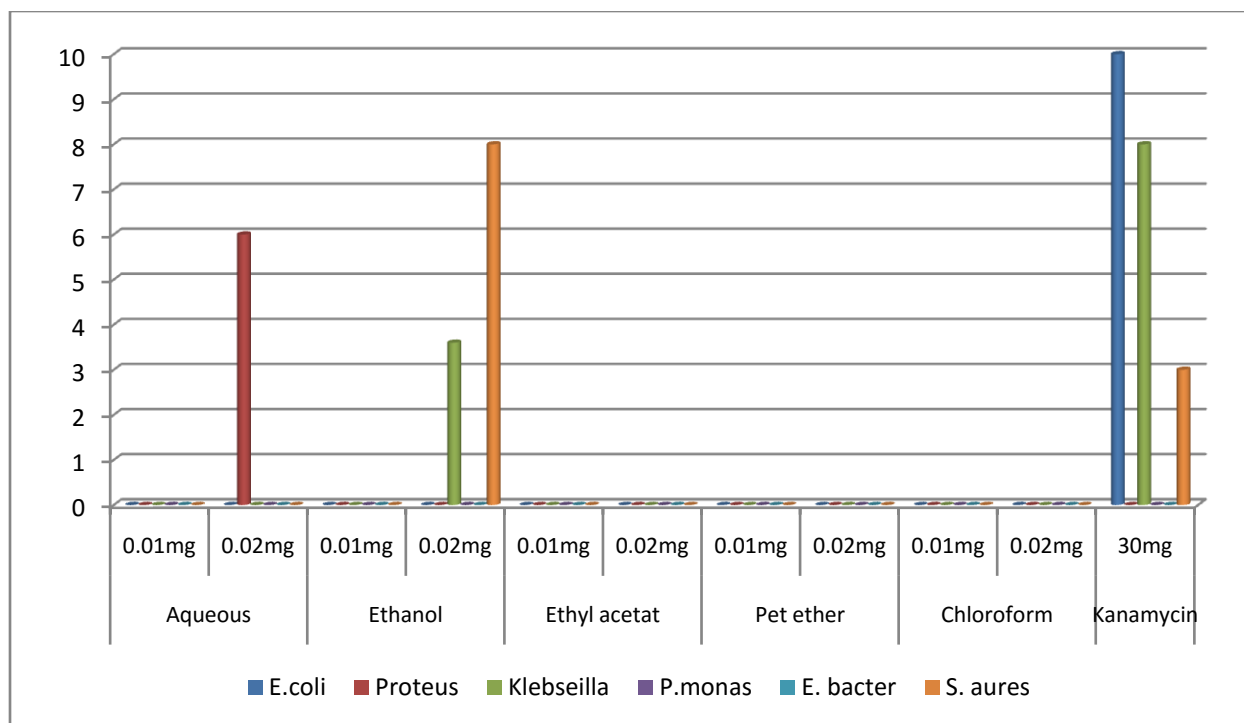


Fig 2a: Antibacterial activity of *S. xantocarpum* fruit by disc method.

Figure 2b: Alcoholic extract of *S. xanthocarpum* (Fruit) by well method

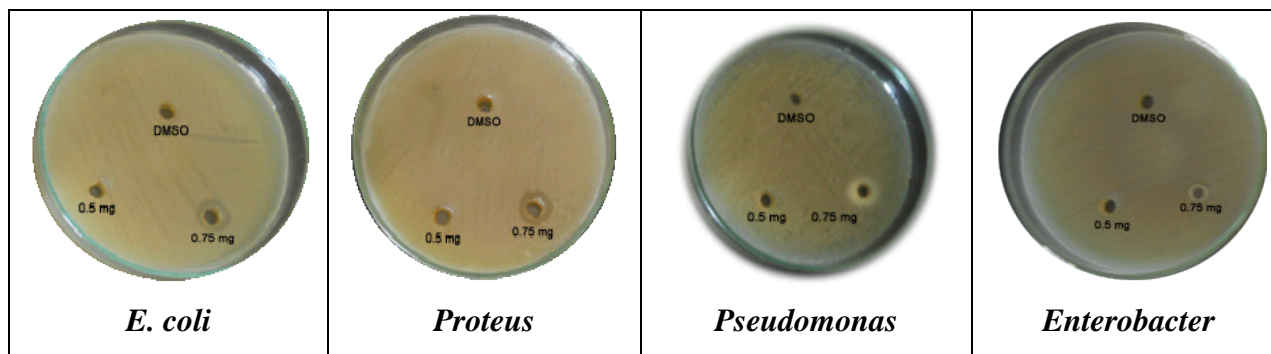


Table 3: Minimum Inhibitory Concentration (MIC) of *S. xanthocarpum* fruit of alcohol and ethyl acetate extract using broth dilution method against pathogens at different concentration (mg/ml).

Organism		<i>E. coli</i>	<i>Proteus</i>	<i>Klebsilla</i>	<i>Pseudomonas</i>	<i>Enterobacter</i>	<i>S.aureus</i>
<i>S.xanthocarpam.</i> fruit	Alcohol	0.5	0.5	0.5	00	0.75	0.125
	Ethylacetate	0.67	00	0.75	0.5	00	0.5
Kanamycin		0.125	00	0.125	00	00	00

Fig 3a: Minimum inhibitory concentrations (MIC) of *S. xanthocarpum* fruit of alcohol and ethyl acetate extract against pathogens at different concentration (mg/ml).

