International Journal of Applied Research 2016; 2(8): 388-393

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(8): 388-393
www.allresearchjournal.com
Received: 29-06-2016
Accepted: 30-07-2016

Shubha KS
DOS in Microbiology,
University of Mysore, Mysore.

Lakshmidevi N
DOS in Microbiology,
University of Mysore, Mysore.

Correspondence
Shubha KS
DOS in Microbiology,
University of Mysore, Mysore.

Antibacterial activity of different solvent extracts of *Solanum xanthocarpum* Sch & Wend (root) against pathogens isolated from diabetic foot ulcer

Shubha KS and Lakshmidevi N

Abstract
In the current study, aqueous and solvent extracts of *S. xanthocarpum* root against some clinical bacterial isolates viz., *Proteus, E. coli, Klebsiella, Pseudomonas*, *Enterobacter* and *Staphylococcus aureus* recorded varied interesting results. Aqueous extract, solvent extracts were tested by well diffusion, disc diffusion and MIC methods. DMSO and Kanamysin served as negative and positive control respectively. In well diffusion, aqueous extracts was found ineffective against the pathogenic bacteria, rather than Kanamycin exhibited the zone of inhibition against *E. coli, Klebsiella* and *S. aureus.* Disc diffusion recorded the inhibitory property of the chloroform and Ethanolic extracts on all organisms tested, however, *Pseudomonas and S. aureus* exhibited wide susceptibility. MIC showed concentration dependent gradient decreasing in the level of inhibition against all the organisms. Ethanol and ethyl acetate extract were proved to be most effective though Pseudomonas and *S. aureus* were found resistant against the highest concentration.

Keywords: Antibacterial, *S. xantocarpum*, diabetic

1. Introduction
Bacterial infection in diabetic patients in general and pathogenic bacteria of diabetic foot ulcers in particular is found to be resistant to some synthetic drugs, however, an alternative treatment against bacterial foot infection needs attention. With this backdrop, the alternative means was the use of plants and its products. This has a long history that has started with folk medicine over the years which has been included in the traditional medicine too. Since the ancient times, many plants and plant species have been reported to have pharmacological properties as they are known to possess many different kinds of secondary metabolites *viz*., glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes, which can therefore be exploited to treat the disease causing pathogens (Hema *et al*., 2012) [8].
Clinical microbial samples are found to be resistant to many antibiotics and multi drug resistance (MDR) is becoming one of the global issues in the resent years. This is one of the important aspects especially in the developing countries as cross infections of these resistance strains are becoming the major cause for the mortality (Salar and Suchitra, 2009; Hema *et al*., 2012) [15, 12].
Considering the advantages and with the folklore medicinal importance along with the expensiveness of synthetic drugs with their various side effects, the research for the alternative drug derived from plants or its products are of greater concern in the recent years. Researchers have extended their thoughts to design the drug derived from the phytomedicines and biologically active ingredient that are isolated from plant species that are exploited in herbal medicine with marked therapeutic value (Britto *et al*., 2012; Sen and Batra, 2012) [5, 16].

2. Materials and Methods
3. Plant material collection
Fresh healthy root 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.

4. 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.

5. Preparation of aqueous extract

Fifty grams of root 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.

6. 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 (Khan *et al*., 1988) [10]. 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.

7. Antibacterial activity by agar well diffusion method

Antibacterial activity by agar well diffusion method was carried out as per the methods of Gulnaz and Savitha, (2013) [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 µl 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 µg (in the concentration of 0.5and 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.

8. Antibacterial activity by disc diffusion method

The different solvent extracts were tested for antibacterial activity by disc diffusion method (Bauer *et al*., 1966; NCCLS, 2001) [3, 11]. Bacterial suspension of 100 µl 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 µg concentration). The plates were kept at 4 °C for 30 min for the diffusion of the plant extracts (Brantner and Grein, 1994) [4]. 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 µg) served as positive control and 10% Dimethyl sulfoxide (DMSO) serve as negative control.

9. 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 (Brantner and Grein, 1994) [4]. Inhibition of bacterial growth was determined by measuring the absorbance at 465 nm using UV-visible Spectophotometer (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.

Percent growth inhibition= $[(A_{control} - A_{test}) A_{control}$

10. Results

11. Well diffusion

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

Solvent extract	mg/ml	*E. coli*	*Proteus*	*Klebsiella*	*Pseudomonas*	*Enterobacter*	*S.aureus*
Aqueous	0.5	00	00	00	00	00	00
	0.7	00	00	00	00	00	00
Ethanol	0.5	00	00	00	00	00	00
	0.7	00	00	00	00	00	11.3±0.8[a]
Ethyl acetate	0.5	8.6±0.3[b]	00	00	00	00	00
	0.7	12.3±0.8[a]	9.6±0.3[a]	11.0±0.5[a]	00	8.3±0.3[a]	00
Pet ether	0.5	00	00	00	00	00	00
	0.7	00	00	8.0±0.5[b]	00	8.0±0.5[a]	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 ($P \leq 0.05$)

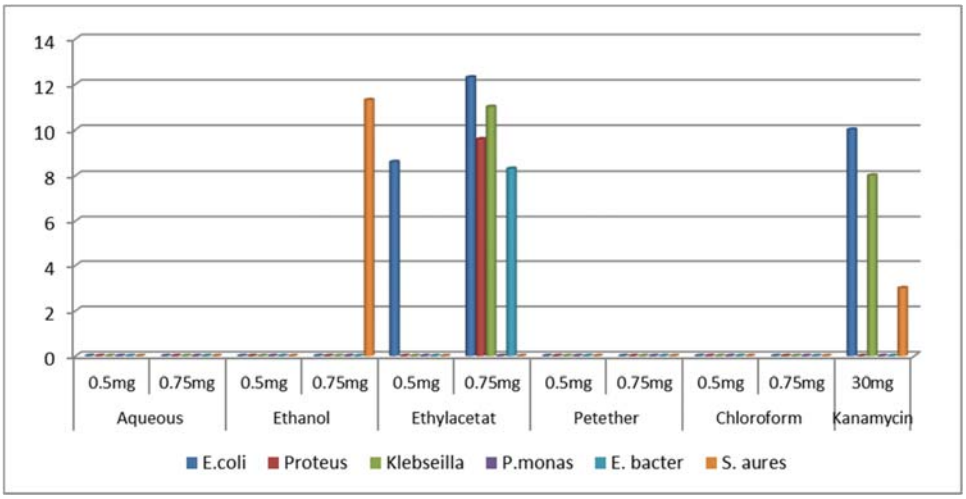

Fig 1a: Antibacterial activity of *S. xantocarpum* root by well method

E. coli *Klebsilla* *S. aureus*

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

E. coli *Proteus* *Klebsiella* *Enterobacter* *S. aureus*

Fig 1c: Ethyl acetate extract of *S. xantocarpum* (Root) and *S. aureus* for Ethanol extract by well method.

In the present study, results of inhibition zone in the well diffusion method using the solvent extracts of *S. xantocarpum* root *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, Fig 1b).

The aqueous extract of *S. xantocarpum* root was not found to be effective as there was no inhibition zone encountered. Similarly solvent extracts of *S. xantocarpum* root namely pet ether, chloroform, and ethanol showed nil inhibition zones except for ethyl acetate at 0.5 mg/ml concentration inhibited *E. coli*, where the inhibition zone measured was 8.6 mm (Table 1, Fig 1a).

Among the 0.75 mg/ml of *S. xantocarpum* root, pet ether did not show any zone of inhibition, however chloroform against *Klebsiella* (8 mm) and *Enterobacter* (8 mm), ethyl acetate against *E. coli* (12.3), *Proteus* (9.6), *Klebsiella* (11) and *Enterobacter* (8.3) and ethanol against *S. aureus* (11.3) (Table-1, Fig-1c.).

12. Disc method

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

Solvent extract	Mg	*E. coli*	*Proteus*	*Klebsiella*	*Pseudomonas*	*Enterobacter*	*S.aureus*
Aqueous	0.01	00	00	00	00	00	00
	0.02	00	00	00	00	00	00
Ethanol	0.01	00	00	00	00	00	00
	0.02	00	00	00	00	00	5.3±0.33[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	6.0±0.57[b]	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≤0.05)

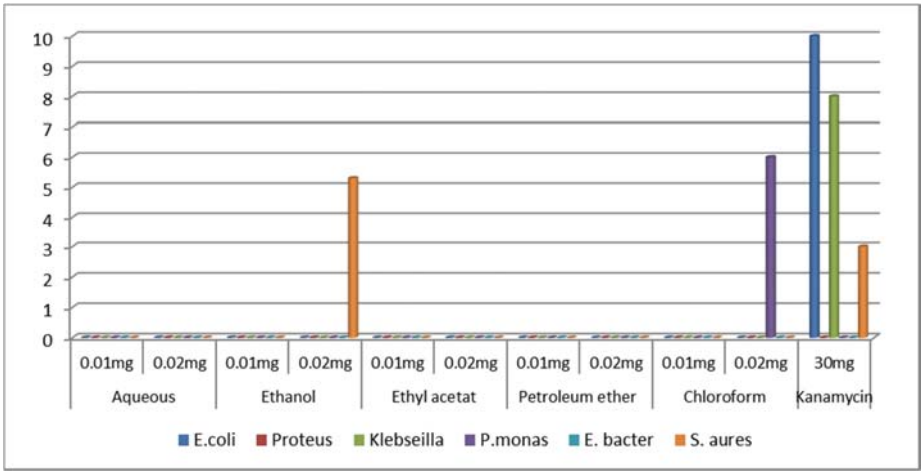

Fig 2a: Antibacterial activity of *S. xantocarpum* root by Disc method.

In the present study, the inhibition zone in the well diffusion method using the solvent extracts of root *viz*., pet ether, chloroform, ethyl acetate, ethanol and aqueous extract against control showed effective inhibition zone against six bacteria (*E. coli*, *Proteus*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, *S. aureus*) tested compared with the standard antibiotic drug 30 mg Kanamycin. The standard control shown inhibition for E. *coli*, *Klebsiella* and *S. aureus* by 10 mm, 8 mm and 3 mm respectively. *Proteus, Pseudomonas*, *Enterobacter* has not shown inhibited by the standard drug (Kanamycin) (Table 2, Figs.2a).

The antibacterial activity of different solvent extracts of *S. xantocarpum* root against the isolated pathogenic bacteria has not shown any inhibition at 0.01 mg/ml concentration. Similarly the aqueous extract also did not show any inhibition zone against standard.

At 0.02 mg/ml concentration pet ether, ethyl acetate did not show any activity. But chloroform has exhibited inhibition zone against *Pseudomonas* (6.0 mm) followed by ethanol 5.3 mm in *S. aureus*. But rest of the extracts alcohol, ethyl acetate has not shown any inhibition zone against the pathogenic bacteria. Test organism exhibited a vast degree of resistance to aqueous extract (Table 2, Fig 2a).

13. Minimum Inhibitory Concentration (MIC)

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

Organism		*E. coli*	*Proteus*	*Klebsilla*	*Pseudomonas*	*Enterobacter*	*S.aureus*
S. xanthocarpam. root	Alcohol	00	00	00	00	00	0.75
	Ethylacetate	0.25	0.5	0.75	00	0.75	00
Kanamycin		0.125	00	0.125	00	00	00

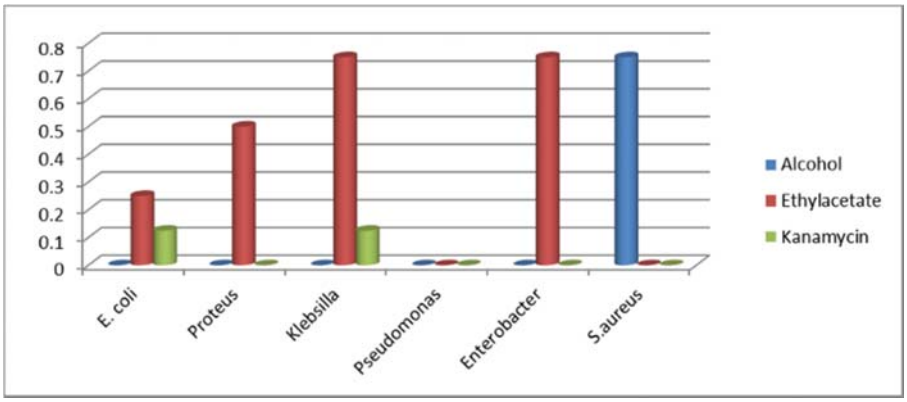

Fig 3a: Minimum inhibitory concentrations (MIC) of *S. xanthocarpum* root of alcohol and ethyl acetate extract against pathogens at different concentration (mg/ml).

The MIC was carried out for only alcohol and ethyl acetate extracts as these had given very effective results against the foot ulcers pathogens. The alcohol extract of *S. xanthocarpum* root has inhibited only *S. aureus* at 0.75 mg/ml concentration. Ethyl acetate extract of *S. xanthocarpum* root has inhibited *E. coli* (0.25 mg/ml), *Proteus* (0.5 mg/ml), *Klebsiella* (0.75 mg/ml) *and Enterobacter* (0.75 mg/ml) concentration (Table 3, Fig 3a).

14. Discussion for agar well diffusion method

Antibacterial activity of the root of *S. xantocarpum* 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 Oloyede *et al*. (2012) [13] 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. pnuemonia*, and *P. vulgaris.*

Aqueous extracts did not show the zone of inhibition and found that it was ineffective against the pathogenic bacteria. Similarly Sen and Batra (2012) [16] 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, Thomas (2012) [18] 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.

15. 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 (Aladesanmi *et al*., 1986; Al-Saleh *et al*., 1997; Singh and Nath, 1999; Quarenghi *et al*., 2000; Torrenegra *et al*., 2007) [1, 2, 17, 14, 20].

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 Toroglu *et al*. (2012) [19]. Root of *S. xantocarpum* at 0.1 mg and 0.2 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 (Toroglu *et al*., 2012; Dahikar *et al*., 2011) [19, 6].

16. Discussion for MIC

The MIC of the ethanol and ethyl acetate extract inhibiting concentration value of the plant extracts are shown in Tables. Of the entire tested organism, *S. aureus* was inhibited by ethanol extract of *S. xanthocarpum* root at 0.75 mg/ml; whereas Ethanolic extract of *P. guineense* and *O. gratissimum* also exhibited a concentration dependent gradient decreasing in the level of inhibition against the *E. coli and S. aureus* (Obinna *et al*., 2009) [12].

The ethyl acetate extracts of *S. xanthocarpam* root, has exhibited a range of MIC against the test organisms in the present study. *Pseudomonas* and *S. aureus* were found resistant against the highest concentration of *S. xanthocarpam* root. The results were found similar to *Hypericum roeperanum* ethyl acetate against *E. coli*, *Klebsiella*, *Enterobacter* and *S. aureus* findings of Kamga *et al*. (2012) [9].

17. Conclusion

Bacterial isolates were subjected for antimicrobial activity against the plant extract. The aqueous and different solvents extracts *viz*., alcohol, ethyl acetate, pet ether and chloroform were screened for antimicrobial property by performing well diffusion and disc diffusion assays techniques. Here both alcoholic and ethyl acetate extracts was found to be promising. These extracts were further subjected to MIC. Ethanol and ethyl acetate extract were proved to be most effective though Pseudomonas and S. aureus were found resistant against the highest concentration.

18. References

1. Aladesanmi AJ, Safowora A, Leary JD. Preliminary biological and phytochemical investigation of two Nigerian medicinal plants. International Journal of Crude Drug Research. 1986; (24):147-153.
2. Al-Saleh FS, Gamal AY, EL-Din Abbar JA, Saeed NA. Phytochemical and biological studies of medicinal plants in Bahrain: Family chenopodiaceae part 2.

International Journal of Pharmacogenesis. 1997; (35):38-42.
3. Bauer AW, Kirby WMM, Sheriss JC, Turck M. Antibiotic susceptibility testing by standard for anti-microbial susceptibility testing. 16th Informational Supplement. M100-S16. Wayne, PA, USA: CLSI, 1966.
4. Brantner A, Grein E. Antibacterial activity of plant extracts used externally in traditional medicine. J. Ethnopharm. 1994; 44(1):35-40.
5. Britto AJD, Sebastian SR, Sujin RM. Antibacterial activity of selected species of Lamiaceae against human pathogens. Indian Journal of Natural Products and Resources 2012; (3):334-342.
6. Dahikar SD, Bhutada SAT, ambekar DH, Vibhute SK, Kasture SB. *In-vitro* antibacterial efficacy of solvent extracts of leaves of *Bauhinia racemosa* Lam. (Caesalpiniaceae) against enteric bacterial pathogens.. International Journal of Pharmaceutical Sciences and Drug Research. 2011; (3):31-34.
7. Gulnaz AR, Savitha G. Evaluation of antimicrobial activity of leaf and stem extracts of Sidda medicinal plant Sida cordata. International Journal of Medicine and Pharmaceutical Sciences. 2013; 3(3):39-50.
8. Hema TA, Shiny M, Parvathy J. Atimicrobial activity of leaves of Azima tetracantha against clinical pathogens. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; (4):317-319.
9. Kamga PB, Mouthe GLT, Wache BO, Beng VP. Antibacterial activity of the ethyl acetate extract of Hupericum roeperanum Schimp. Ex A. Rich. (Guttifereae) and their secondary metabolites. *Pharmacologia* 2012; (3):632-636.
10. Khan NH, Nur-E MSA, Kamal, Rahman M. Antibacterial activity of *Euphorbia thymifolia*. Linn. Indian Journal of Medicine and Research. 1988; (87):395-397.
11. NCCLS-National Committee for Clinical Laboratory Standards Performance Standards for an-microbial susceptibility testing: eleventh informational supplement. Document M100-S11, National Committee for Clinical Laboratory Standards, Wayne, PA, USA, 2001.
12. Obinna CN, Nwodo SC, Olayinka OA, Chinwe OL, Kehinde OO. Antibacterial effects of *Ocimum gratissimum* and *Piper guinunse* on *Escherichia coli* and *Staphylococcus aureus*. African Journal of Food Science. 2009; (3):77-81.
13. Oloyede OB, Salau AK, Akeusola RT, Ganiyu OT, Azeez L, Ogunbode SM. Phytochemical content, Radical Scavenging and Antibacterial properties of aqueous extract of *Jatropa curcas* Lin leaces. Fountain Journal of Natural and Applied Sciences. 2012; 1(1):41-48.
14. Quarenghi MV, Tereschuk MC, Baigori MD, Abdala LR. Antimicrobial activity of flowers from Anthemis cotula. Fitoterapsia. 2000; (71):710-712.
15. Salar RK, Suchitra. Evaluation of antimicrobial potential of different extracts of *Solanum xanthocarpum* Schrad. and Wendl.. African Journal of Microbiology Research. 2009; (3):97-100.
16. Sen A, Batra. Evaluation of antimicrobial activity of different solvent extracts of medicinal plants: *Melia Azedarach* L. International Journal of Current Pharmaceutical Research. 2012; (4):67-73.
17. Singh RK, Nath G. Antimicrobial activity of *Elaeocarpus sphaericus*. Phytotherapy Research 1999; (13):448-450.
18. Thomas J Webster. Antimicrobial applications of nanotechnology methods and literature. International Journal of Nanomedicine. 2012; (7):2767-2781.
19. Toroglu S, Keskin D, Vural C, Kertmen M, Cenet M. Comparision of ntimicrobial activity of Echinops viscosus Subsp. Bithynicus and E. Microcephalus Leaves and flowers extracts from Turkey. International Journal of Agriculture and Biology. 2012; (14):637-640.
20. Torrenegra RD, Kocabae EE, Bedir E, Sukan FV, Ozek T, Baser KHC. Antimicrobial activities of methanol extracts and essential oils of Rosmarinus officinalis, depending on location and seasonl variation. Food. Che. 2007; (100):553-559.