



Phytochemical Evaluation and Antibacterial Activity of Fruit Extract of *Solanum surattense* Burm F. against Some Pathogenic Bacteria

Dhanalakshmi R*, JaffarIqbal J, Nausath Jabeen K,
Noorul Zaithun Hidhaya M, Meena K, Tamizharasan

Edayathangudy G.S. Pillay College of Pharmacy, Nagapattinam, Tamil Nadu, India

Abstract : The objective of this study was to evaluate phytochemical constituents and antibacterial activity of hydroalcoholic extract of *Solanum surattense* fruit against some gram-positive and gram-negative bacterial strains. The evaluation of antibacterial activity was carried out by using the disc diffusion method, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Ciprofloxacin was used as positive control. Hydroalcoholic extract of *Solanum surattense* fruit containing alkaloids, flavonoids, phenol, saponins, terpenoids, glycosides, sterols, proteins and tannins. Efficacy data analysis showed that hydroalcoholic extracts of fruit of *Solanum surattense* (1 mg/ml) inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* with mean diameters of inhibition zones being 24, 25, 28 and 30 mm respectively. On the other hand, minimum inhibitory concentration and minimum bactericidal concentration value of 0.062 and 0.25 mg/ml, 0.062 and 0.25 mg/ml, 0.312 and 0.125 mg/ml, 0.156 and 0.0312 mg/ml were recorded against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* respectively. Hydroalcoholic extracts of *Solanum surattense* fruit have potent antibacterial activity against the different tested bacterial strains. This activity supports their use in treatment of infections caused by such resistant bacteria.

Key words : *Solanum surattense*, phytochemical analysis, antibacterial activity, Inhibition zones, MIC and MBC.

Introduction

For ages nature has gifted us plenty of herbs and plants which form the main source of traditional medicines used to help in relief from illness and are still widely used all over the world¹. Medicinal plants have been used for centuries as remedies for human diseases as they contain components of therapeutic value. There are numerous plant natural products which have antifungal, antibacterial and antiprotozoal activities that could be used either systemically or locally². Since earliest times, many plants have been known to exert healing properties against human infections due to their content of secondary metabolites, which in more recent times have been found to act as antimicrobial agents against human pathogens³. Antibiotics are undeniably one of the

Dhanalakshmi R *et al* /International Journal of PharmTech Research, 2020,13(1): 79-87.

DOI= <http://dx.doi.org/10.20902/IJPTR.2019.130109>

most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products⁴. Antibiotic resistance has become a serious and widespread problem in developing countries, both in hospitals and the community, causing high mortality each year⁵. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants^{6,7}. Nevertheless, the discovery of new antibiotics is very expensive and time consuming, requiring about ten years to bring a new antibiotic to market. Therefore, the search for antibacterial substances derived from natural products, such as phytochemicals, has gained increasing importance alongside the discovery of new synthetic chemical compounds with antibiotic properties⁸. *Solanum surattense* (Solanaceae) is frequently used in Indian traditional medicine for curing various ailments such as respiratory diseases, gonorrhoea, rheumatism, fever and asthma⁹. *Solanum surattense* is widely spread throughout India in dry situations as abundant by road sides and wastelands. It is essentially a warm season crop grown mainly in tropical and sub-tropical regions¹⁰. The plant is useful in fever, cough, asthma and pain in chest, being used in the form of decoction or electuary. The fruit and leaf extract possess significant antihyperglycemic activity^{11,12,13}. The aim of this study was to screen the *in vitro* antibacterial activity of hydroalcoholic extract of fruit of *Solanum surattense* against some Gram-positive and Gram-negative bacterial strains.

Material and Methods

Collection and identification of plants materials:

The fruits of *Solanum surattense* were used for the study. It was collected from Tiruvarur district, Tamil Nadu during the month of March and April. The plant materials were taxonomically identified and authenticated by Dr. S. JOHN BRITTO., Director, The Rapinat Herbarium Centre for Molecular Systematic, St. Joseph's college (campus) Tiruchirappalli-620 002, Tamil Nadu, India. The plant was thoroughly washed in running tap water to remove soil particles and adhered debris and finally washed with distilled water. The fruits of the plant alone were segregated and dried under shade, pulverized by a mechanical grinder into fine powder. The powdered materials were stored in air tight polythene bags till use.

Preparation of extracts:

The powdered plant materials of *Solanum surattense* fruits were subjected to hydroalcoholic extraction in the ratio of water: ethanol as 30:70. The extraction was done by hot continuous percolation method in Soxhlet apparatus for 24 hrs. The extract was concentrated by using a rotary evaporator till dry powder was obtained.

Percentage yield for the hydroalcoholic extract of *Solanum surattense* fruits

The percentage yield for the hydroalcoholic extract of fruits of *Solanum surattense* was calculated with reference to air dried powder taken using the formula given below.

$$\text{Percentage yield} = \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant materials taken}} \times 100$$

Preliminary phytochemical screening:

Preliminary phytochemical screening was conducted on hydroalcoholic extract of fruits of *Solanum surattense* to determine the different phytochemical constituents present in the extracts¹⁴.

Thin-layer Chromatographic Studies (TLC)¹⁵:

Thin-layer chromatography was carried out on hydroalcoholic extract of fruits of *Solanum surattense* using a sheet of aluminium foil which is coated with a thin layer of adsorbent silica gel, which are commercially available 60 F254 (Merck). The plates were cut with scissors and marked with pencil about 2 cm from the bottom of the plate. TLC is performed on samples prepared with different solvents were spotted onto the TLC plate as a single spot with capillary tubes. TLC plates were viewed in UV chamber at both 254 nm and 365 nm. The movement of the analyte was expressed by its retention factor (Rf).

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Anti-bacterial activity of *Solanum surattense* fruit extracts

Bacterial strains

The antibacterial potency of plant extract was evaluated using four bacterial strains. Two strains of Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two strains of Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*). These microorganisms were collected from CSIR-National Chemical Laboratory, Dr.Homi Bhabha Road, Pune- 411 008, India.

Inoculums preparation:

Each bacterial strain was sub cultured overnight at 35°C in Mueller-Hilton agar slants. The bacterial growth was harvested using 5 ml of sterile saline water; its absorbance was adjusted at 580 nm and diluted to attain viable cell count of 10⁷ CFU/ml using spectrophotometer.

A. Disk diffusion method¹⁶:

The disk diffusion method is used to evaluate antimicrobial activity of the plant extract. The plant extract residues (1 mg) were re-dissolved in 0.5% DMSO (5ml DMSO make up to 100ml with water), sterilized through Millipore filter (0.22 µm) then loaded over sterile filter paper discs (8 mm in diameter) to obtain final concentration of 1 mg/ml. Ten ml of Mueller-Hilton agar medium was poured into sterile Petri dishes (as a basal layer) followed with 15 ml of seeded medium previously inoculated with bacterial suspension to attain 10⁷ CFU/ml of medium. Sterile filter paper discs loaded with plant extract concentration of (1 mg/ml) were placed on the top of Mueller-Hilton agar plates. Filter paper discs loaded with 30µg of ciprofloxacin was used as positive control. The plates were kept in the fridge at 5 °C for 2 h. to permit plant extracts diffusion then incubated at 35 °C for 24 h. The presence of inhibition zones was measured by Vernier calliper recorded and considered as indication for antibacterial activity.

B. Determination of MIC and MBC values¹⁷:

Using aseptic techniques, a single colony was transferred into a 100mL bottle of isosensitest broth capped and placed in incubator overnight at 35 °C. After 12–18 h of incubation, using aseptic preparation and the aid of a centrifuge, a clean sample of bacteria was prepared. The broth was spun down using a centrifuge set at 4000 rpm for 5min with appropriate aseptic precautions. The supernatant was discarded into an appropriately labelled contaminated waste beaker. The pellet was resuspended using 20mL of sterile normal saline and centrifuged again at 4000 rpm for 5min. This step was repeated until the supernatant was clear. The pellet was then suspended in 20mL of sterile normal saline, and was labelled as Bs. The optical density of the Bs was recorded at 500 nm, and serial dilutions were carried out with appropriate aseptic techniques until the optical density was in the range of 0.5–1.0. The actual number of colony-forming units was calculated from the viability graph. The dilution factor needed was calculated and the dilution was carried out to obtain a concentration of 5×10⁶ CFU/mL

Preparation of resazurin solution

The resazurin solution was prepared by dissolving a 270mg tablet in 40mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution.

Preparation of Microtitre plates

Microtitre plates were prepared under aseptic conditions. A germ-free 96 well plate was labelled. First row of the plate was poured with test materials (100 µl) in 10% (v/v) DMSO or sterile water. Remaining all wells were poured with 50 µL of nutrient broth or normal saline. To each well 10 µL of resazurin indicator

solution was added. 30 µl of 3.3x strength isosensitised broth was added individually to each well to ensure that the final volume was single strength of the nutrient broth. Finally, 10 µL of bacterial suspension (5×10^6 CFU/mL) was added to each well to attain a concentration of 5×10^6 CFU/mL. All plates were incubated at 37 °C for 18–24 h. Colour changes from purple to pink or colourless was noted. MIC Value were recorded from the lowest concentration at which colour changes. Different concentration of sample solution was streaked in petridish containing nutrient broth and incubated at 35°C for 24h. Lowest concentration that gave no visible growth was recorded as minimum bactericidal concentration (MBC).

Results

Percentage yield

The percentage yield for the hydroalcoholic extract of *Solanum surattense* fruit was calculated. Percentage yield was found to be 11.66 %w/w

Preliminary phytochemical screening

Preliminary phytochemical screening for the hydroalcoholic extract of *Solanum surattense* fruit was carried out and the results are tabulated in **Table 1**. The hydroalcoholic extract of *Solanum surattense* fruit containing alkaloids, flavonoids, phenol, saponins, terpenoids, glycosides, sterols, proteins and tannins.

Table 1: Phytochemical constituents of *Solanum surattense* fruit extract

S. No	Phytochemical constituents	Results
1	Alkaloids	(+)
2	Flavonoids	(+)
3	Phenol	(+)
4	Saponins	(+)
5	Terpenoids	(+)
6	Glycosides	(+)
7	Sterols	(+)
8	Proteins	(+)
9	Tannins	(+)

(+) = Present; (-) = absent

Thin layer chromatography

The results of thin layer chromatography profiling are summarized in Table 2 and illustrated in Fig2a – 2e. TLC Profiling of hydroalcoholic extract of *Solanum surattense* fruit in different solvent system confirms the presence of diverse group of phytochemicals.

Table 2: Rf values for various phytochemicals in *Solanum surattense* fruit extract

S. No	Phytochemical	Solvent system	Rf value
1	Alkaloids	n-butanol: acetic acid: water (4:1:3)	0.3846
2	Glycosides	Toluene: n-butanol (4:1)	0.6
3	Flavonoids	Ethyl acetate: Formic acid: acetic acid: water (100:11:11:27)	0.6285
4	Tannins	Butanol: acetic acid: Distilled water (4:1:5)	0.3538
5	Phenol	Chloroform: distilled water (27:3)	0.6769



Fig. 2a. Alkaloids



Fig. 2b. Glycoside



Fig. 2c. Flavonoids



Fig. 2d. Tannins



Fig. 2e. Phenol

Anti-bacterial activity of *Solanum surattense* fruit extracts

A. The disk diffusion method

Antibacterial activity of hydroalcoholic extracts of *Solanum surattense* fruit (1 mg/ml) was employed by disc diffusion method to evaluate their bacteriostatic and bactericidal properties. Effect of the effective plant extract was reported in Table 3 and illustrated in Fig. 3a and 3b. Efficacy data analysis showed that hydroalcoholic extracts of fruit of *Solanum surattense* (1 mg/ml) inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* with mean diameters of inhibition zones being 24, 25, 28 and 30 mm respectively.

Table 3: Anti-bacterial activity of *Solanum surattense* fruit extracts

Type of Bacterial strain	Inhibition zones (mm)		
	Standard Ciprofloxacin (30 µg)	<i>Solanum surattense</i> fruit extracts (1 mg/ml)	Solvent
<i>Staphylococcus aureus</i>	30	24	3
<i>Bacillus subtilis</i>	31	25	2
<i>Escherichia coli</i>	30	28	4
<i>Salmonella typhi</i>	33	30	2

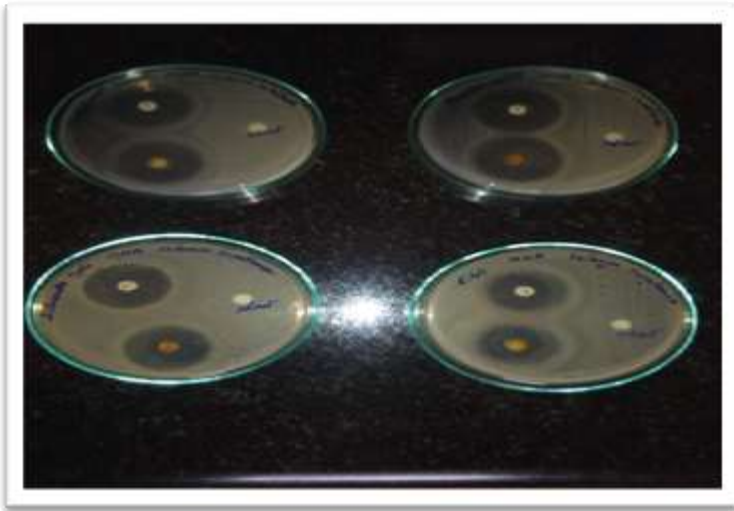


Fig.3a. Growth inhibition caused by *Solanum surattense* fruit extract

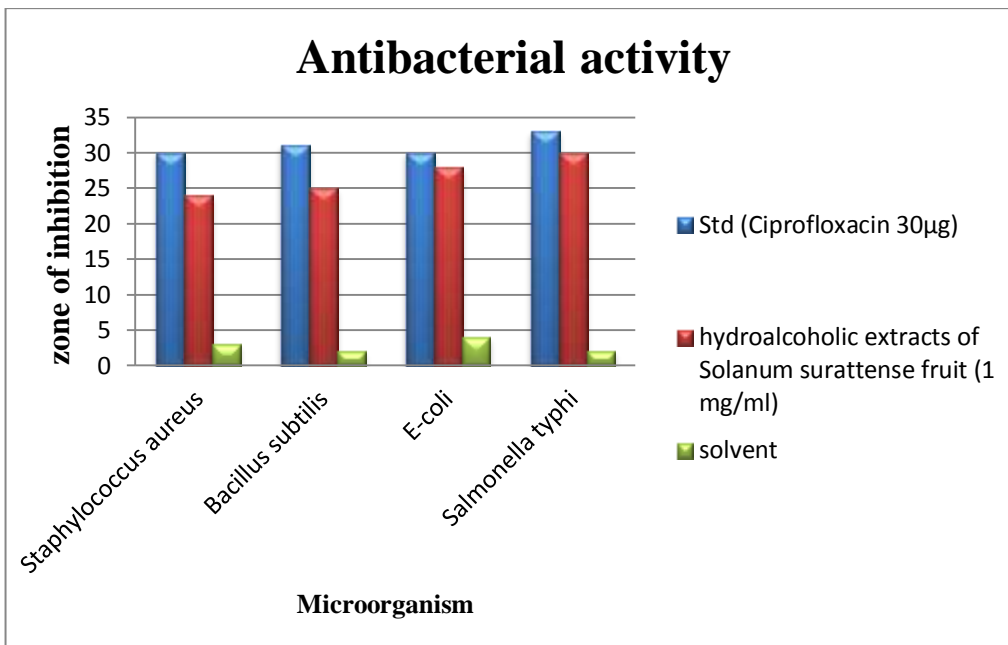


Fig.3b. Growth inhibition caused by *Solanum surattense* fruit extracts

Determination of MIC and MBC values

Minimum inhibitory concentration and minimum bactericidal concentration value have been determined with *Solanum surattense* fruit extracts (1 mg/ml)(Fig. 4). MIC and MBC value of 0.062 and 0.25 mg/ml, 0.062 and 0.25 mg/ml, 0.312 and 0.125 mg/ml and 0.156 and 0.0312 mg/ml were recorded against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* respectively (Table 4).

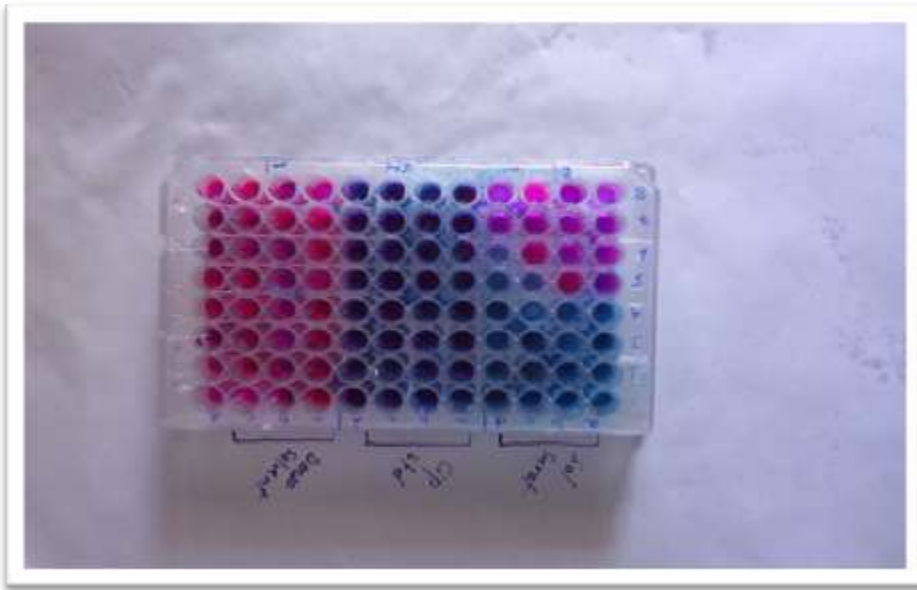


Fig 4: Determination of the MIC values of *Solanum surattense* fruit extract

Table 4: MIC and MBC values for the *Solanum surattense* fruit extracts

Type of Bacterial strain	Minimum Inhibitory Concentration (MIC)	Minimum bactericidal concentration (MBC)
<i>Staphylococcus aureus</i>	0.062 mg/ml	0.25mg/ml
<i>Bacillus subtilis</i>	0.062 mg/ml	0.25mg/ml
<i>Escherichia coli</i>	0.0312mg/ml	0.125mg/ml
<i>Salmonella typhi</i>	0.0156mg/ml	0.0312 mg/ml

Discussion

Research for new antibacterial agents has become a very important endeavour, especially in recent times, considering the escalating levels of antibiotic resistance among pathogenic bacteria. One of the efforts in this research is focused on the use of medicinal plants, which are widely available resources, less if no side effects, less expensive and have shown antimicrobial properties¹⁸. Preliminary phytochemical analyses revealed that hydroalcoholic extract of *Solanum surattense* fruit containing alkaloids, flavonoids, phenol, saponins, terpenoids, glycosides, sterols, proteins and tannins. These bioactive compounds have been reported to be used by plants for protection against bacterial and are responsible for antimicrobial activity^{19,20,21}. Thin layer chromatography is usually done for a better identification of the bioactive compounds. In the present study the TLC profiling of plant extract again revealed the presence of different metabolites such as alkaloids, flavonoids, glycosides, phenols and tannins. Agar well diffusion method and MIC value has been used by many investigators to find out the antimicrobial activity of the test compounds^{22,23,24}. Results of antimicrobial activity of the hydroalcoholic extracts of *Solanum surattense* fruit can suggest that *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* were most susceptible strains to the extract and showed strong antibacterial activity. Hence, experiments were conducted to determine their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against the most susceptible bacterial strains. In the present study the MIC value of the active plant extracts obtained in this study were lower than the MBC values suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration.

Conclusion

The result of this study showed that hydroalcoholic extracts of *Solanum surattense* fruits have potential antimicrobial components that may be definitely used as a therapy against various disease by the pharmaceutical industries. However, further studies are needed, including toxicity evaluation and purification of active antibacterial constituents from *Solanum surattense* extracts looking toward a pharmaceutical use.

Acknowledgement

The authors express their deep sense of gratitude to Prof. Dr. D. Babu Ananth Principal, E.G.S. Pillay College of Pharmacy, Tamil Nadu and India for providing all the supports and encouragement in conducting the research work.

References

1. Al-Essa M.A, Al-Mehaidib A, Al-Gain S. Parental awareness of liver disease among children in Saudi Arabia. *Ann. Saudi Med.* 1998;18 (1):79–81.
2. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999; 12(4): 564-82.
3. Borges A.J, Saavedra M, Simoes, M. Insights on antimicrobial resistance, biofilms and the use of phytochemicals as new antimicrobial agents. *Curr. Med. Chem.* 2015;22 (21):2590–2614.
4. Sharma A. Antibacterial activity of ethanolic extracts of some arid zone plants. *Int. J. of Pharm. Tech. Res.* 2011; 3(1):283-286.
5. Gyles C. The growing problem of antimicrobial resistance. *Can Vet J* 2011; 52(8): 817-20.
6. Kim H, Park SW, Park JM, Moon KH, Lee CK. Screening and isolation of antibiotic resistance inhibitors from herb material Resistant Inhibition of 21 Korean plants. *Nat. Prod. Sci.* 1995; 1: 50 - 54.
7. Alagesabooopathi C. Antimicrobial Potential and Phytochemical Screening of *Andrographis affinis* Nees, An Endemic Medicinal Plant from India. *Int. J. of Pharma. and Pharmaceutical Sci.* 2011; 3 (2): 157- 159.
8. Mandal S.M, Roy A, Ghosh A.K, Hazra T.K, Basak A, Franco O.L. Challenges and future prospects of antibiotic therapy: from peptides to phages utilization. *Front. Pharmacol.* 2014; 5 (5): 1–12.
9. Nadkarani AK. *Indian Materia Medica* 1. Bombay: Popular Book Depot; 1954; 3: 1153–1158.
10. <http://vikaspedia.in/agriculture/crop-production/package-of-practices/medicinal-and-aromatic-plants/solanum-surattense-1>
11. Kar DM, Maharana L, Pattnaik S, Dash GK. Studies on hypoglycaemic activity of *Solanum xanthocarpum* Schard. And Wendl. fruit extract in rats. *J Ethnopharmacol.* 2006;108(2):251–256.
12. Gupta S, Mal M, Bhattacharya P. Evaluation of hypoglycemia potential of *Solanum xanthocarpum* (Solanaceae) fruits in normal and streptozotocin induced diabetic rats. *Eur Bull Drug Res.* 2005; 13:51–55.
13. Sridevi M, Senthil S, Pugalendi KV. Antihyperglycemic effect of *Solanum surattense* leaf-extract in streptozotocin induced diabetic rats. *J Pharmacol Toxicol.* 2007;2(7):621–629.
14. Harborne A.J. *Phytochemical methods A Guide to Modern Techniques of Plant Analysis.* 1998; 3rd Edn., Springer, New York, USA
15. Harborne JB. *Phytochemical methods: A Guide to Modern techniques of plants Analysis.* 1998; Chapman and Hall London, UK.
16. Mostafa A.A. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases; *Saudi Journal of Biological Sciences.* 2018; 25: 361–366.
17. Sarker S.D. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals/ *Methods.* 2007; 42: 321–324.
18. Rubens DM, Constantin OO, Moevi AA, Nathalie GK, Daouda T. Anti-Staphylococcus aureus activity of the aqueous extract and hexanic fraction of *Thonningia sanguinea* (Cote ivoire). *Int J Pharmacogn Phytochem Res* 2015; 7(2): 301-6.
19. Sukandar EY, Sunderam N, Fidrianny I. Activity of *Kaempferia pandurata* (Roxb.) rhizome ethanol extract against MRSA, MRCNS, MSSA, *Bacillus subtilis* and *Salmonella typhi*. *Pak J Biol Sci.* 2014; 17(1): 49-55.

20. Najafi S. Phytochemical screening and antibacterial activity of leaf extract of *Ziziphus mauritiana* Lam. *Int Res J Appl Basic Sci.* 2013; 4(11): 3274-6.
21. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. *Scientific World Journal.* 2013; 2013: 162750.
22. Arora DS, Kaur GJ. Antibacterial activity of some Indian medicinal plants. *J. Nat. Med.* 2007; 61:313–317.
23. GurudeebanS, Rajamanickam E, Ramanathan T, Satyavani K. Antimicrobial activity Of *Citrulluscolocynthis* in Gulf of Mannar. *Int. J. of Curr. Res.* 2010; 2: 078-081.
24. Pavithra PS, Janani VS, Charumathi KH, Indumathy R, Potala S, Verma RS. Antibacterial activity of the plant used in Indian herbal medicine. *Int. J. of green pharma.* 2010; 10: 22-28.
