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Study of Antibacterial Efficacy of Methanolic and Aqueous Leaf Extracts of *Scoparia dulcis* on Some Human Pathogenic Bacteria

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A B S T R A C T

Scoparia dulcis is a well known traditional medicinal plant used in various indigenous systems of medicine. It is widely distributed throughout India. The present study provides phytochemical and antimicrobial details of the methanolic and aqueous leaf extracts of *Scoparia dulcis* against clinically important human pathogens viz. *Staphylococcus aureus, Proteus mirabilis, Salmonella typhi, Vibrio cholerae and Bacillus subtilis*. The phytochemical analysis carried out revealed the presence of flavanoids, glycosides, alkaloids, tannins, steroids and many other metabolites and the absence saponins. Minimum inhibitory concentration (MIC) assay was determined for the extract. The methanolic and aqueous extract showed toxicity against all the bacteria, *V. Chlolerae* and *P.mirabilis* being highly susceptible with a zone of inhibition of 4 mm at 10mg/ml and 2 mm at 10mg/ml respectively in agar diffusion method. The broth dilution method showed more pronounced antimicrobial activity through 100% inhibition for all the pathogens in the range of 1-32mg/mL concentration. The MIC for *S.typhi* and *B.subtilis* in methanolic solution was 32mg/mL, for *P.mirabilis* 16mg/mL, for *S.aureus* 8mg/mL and *V.cholerae* 1mg/mL. The MIC for *S.aureus* and *V.cholerae* in aqueous solution was 16mg/mL, for *P.mirabilis* 4mg/mL, *S. typhi* 8mg/mL and for *B.subitlis* 32mg/mL.

Introduction

Chemical materials obtained from plants have recently become of great interest due to their versatile usefulness (Jose and Sinha, 2016). Medicinal plants are the richest bio-resources of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.*, 2008). The use of simple extracts of plant parts and phytochemicals, of known antimicrobial properties, can be of great significance in the curative treatments. Due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases, there have been increasing numbers of multiple resistances in human pathogenic microorganisms in recent years (Davies, 1994). This has compelled scientists to search for new antimicrobial substances from various sources like the medicinal plants (I.Wu.M.W., Duncan and Okunji. 1999). Secondary metabolites from higher plants serve as defence agents against invading micro-organisms (Balandrin *et al.*, 1985). Medicinal properties of plants are due to the active chemical constituents present in different parts of the plant (Palombo, 2006).

Five common human pathogenic bacteria under consideration are *Salmonella typhi*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus subtilis* and *Vibrio cholera.* *S. typhi* is causative agent of typhoid (Wain *et al.*, 2015). *P. mirabilis* is a Gram negative, facultatively anaerobic rod-shaped bacterium. It shows urease activity and swarming motility. *P.mirabilis* causes almost all of *Proteus* infections in humans. It is widely distributed in soil and water and is a causative agent of diseases like urethitis, prostatitia, and pneumonia etc., (Burall *et al*., 2004). *Vibrio cholerae* is a gram- negative comma-shaped bacterium. Some strains of *V. cholerae* cause the disease cholera. ("Laboratory Methods for the Diagnosis of *Vibrio cholerae*"). *Staphylococcus aureus* is a gram positive coccal bacteriam that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract and on the skin. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis and food poisoning (Bowersox, 1999). *Bacillus subtilis*, known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans. *B. subtilis* is only known to cause disease in patients who are weak in immunity. (Ryan; Ray, eds (2004)).

Scoparia dulcis is an annual erect herb distributed throughout tropical and subtropical regions of India, America, Brazil, West Indies, and Myanmar (Mukherjee, 2003). The whole plant is used for ailments like diarrhea, stomach-ache, kidney stones, kidney problems, and fever (Jain and Srivastava, 2005). Earlier studies also suggested that methanol extract of *Scoparia dulcis* has hypoglycemic activities and is used for the treatment of Diabetes Mellitus (Mishra *et al*.,). Many more therapeutic potentialities of *S.dulcis* have to be determined as it is an easily available and highly potential medicinal plant. Therefore, present study was undertaken to test the antibacterial activity of *S. dulcis* against some common human pathogenic bacteria.

Materials and Methods

Collection of plant material: The fresh and tender leaves of *S.dulcis* were collected, dried in a shade under room temperature for six days and then by using electric grinder it was crushed into coarse powdery substance. The coarse powdery substance was dried again and was then sieved to get fine powder using the fine plastic sieve, which was then stored in an air tight bottle in the laboratory until required (Sushmita Choudhury *et al*.,).

Extract Preparation

50 g of *S.dulcis* leaf powder was extracted by soxhlet using methanol and water separately. The extract obtained was filtered, concentrated after drying in rotary flash evaporator maintained at 45^{o}c., percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies.

Phytochemical analyses: Freshly prepared extract of the powdered leaves were subjected to phytochemical analyses to find the presence of the following phyto constituents such as flavanoids, alkaloids, carbohydrates, glycosides, polysaccharides, tannins, saponins, steroids, proteins, lipids, oils by standard methods (Trease and Evans, 2002; Sofowara, 2008).

Anti-bacterial analyses

Test Microorganisms: The organisms namely *Staphylococcus aureus*, *Salmonella typhi, Vibrio cholera*, *Bacillus subtilis* and

Proteus *mirabilis* used during the present experiment were procured from Institute of Microbial Technology (IMTECH), *Chandigarh, India.*

Concentrations screened: 25 µg, 50 µg, 100 µg, 250 µg, 500 µg, 1000 µg for agar disc diffusion method and for broth dilution method 1mg/ml, 2mg/ml, 4mg/ml, 8mg/ml, 16mg/ml and 32mg/ml.

Agar diffusion method

Media Used: Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate (25µg, 50µg, 100µg, 250µg, 500µg,and 1000µg) Each plate was inoculated with 18h old cultures (100µL, 104 cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of samples. The control wells were filled with Ciprofloxacin along with solvent. All the plates were incubated at 37°C for 24 h and the diameter of inhibition zones were noted (Threlfall *et al.*, 1999).

Broth dilution method

Media Used: Peptone-10 g, NaCl-10g and Yeast extract 5g, in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The tubes containing above media were prepared, autoclaved and respective concentrations of the sample were added. Each tube was inoculated with 18 h old cultures (100 µl, 10^4 cfu). A control tube with inoculums and without any sample was prepared along with a sterile media tube as blank. All the tubes were incubated at 37ºC on a shaker with 140 rpm for 24 h and the growth was measured at 660 nm (Walker, 2000). The % of inhibition was calculated by using the formula below.

$$\% \text{ Inhbition} = 100 - \left[\frac{\text{OD of culture with sample (Test)}}{\text{OD of culture without sample (Control)}} \times 100 \right]$$

Results and Discussion

Investigation on Physicochemical analysis of leaves of diverse medicinal plants has been studied by many workers that exposed the presence of phytochemicals like carbohydrates, glycosides, alkaloids, tannins etc., in them (Ayoola *et al.*, 2008, Jigna Parekh, and Sumitra Chanda, 2006). The results of the evaluation of phytochemical screening of methanolic extracts of *Scoparia dulcis* revealed the presence of carbohydrates, glycosides, polysaccharides, proteins, steroids, alkaloids, triterpenoids, tannins, lipids, oils, and flavanoids and the absence of saponins. These constituents are responsible for the curative nature of *Scoparia dulcis* against ,diarrhea, stomach-ache, kidney stones, kidney problems, and fever, hypoglycemic activities and Diabetes Mellitus (Mishra *et al*., Palombo, 2006) etc. which could make the plant useful for treating different diseases and having a potential of providing valuable and safe drugs which will be beneficial for human utilization.

Antibacterial assay

The antibacterial efficacy of the extracts of *S. dulcis* leaves was quantitatively measured on

the basis of inhibition zone (in mm) and the results are shown in Table 2 following the agar disc diffusion method, and minimum inhibitory concentration by broth dilution method. Antibacterial activity of Ciprofloxacin was also tested against the same pathogenic bacteria in agar well diffusion method (Table -3 and figure-2) to compare antibacterial efficacy of *S. dulcis* leaf extracts.

Agar diffusion method

In the present investigation the methanolic and aqueous extracts of *S.dulcis* were found to be effective against all the pathogens. When the above pathogens were screened by agar disc diffusion method the zone of inhibition (ZOI) observed for the methanolic extract was in the range 2-4mm at 10 mg/mL concentration of the extract.

Table.1 Proximate Phytochemical composition of Methanolic and aqueous extracts of *S.dulcis*

Phytochemicals	**Methanolic**	**Aqueous**
Carbohydrates	+	+
Glycosides	+	+
Polysaccharides	_	-
Proteins	+	+
Alkaloids	+	+
Steroids	+	+
Triterpenes	+	_
Flavanoids	+	+
Tannins	+	-
Lipid	+	+
Oils	+	-
Saponins	-	-

Table.2 Proximate Phytochemical composition of Methanolic and aqueous extracts of *S.dulcis*

concentration	*S. typhi*		*S. aureus*		*B. subtilis*		*V. cholerae*		*P. mirabilis*	
	Aq	*Met*	*Aq*	*Met*	*Aq*	*Met*	*Aq*	*Met*	*Aq*	*Met*
25µg	0	0	0	0	0	0	0	0	0	0
50µg	0	0	0	0	0	0	0	0	0	0
100µg	0	0	0	0	0	0	0	0	0	0
250µg	0	0	0	0	0	0	0	0	0	0
500µg	0	0	0	0	0	0	0	0	0	0
1000µg	0	0	0	0	0	0	0	4	0	2
MIC(mg/ml)	NF	NF	NF	NF	NF	NF	NF	1000	NF	1000

Table.3 MIC of Ciprofloxacin against the test organisms

Concentration	*S. typhi*	*S. aureus*	*B. subtilis*	*V. cholerae*	*P. mirabilis*
25µg	27	25	20	30	*
50µg	31	28	24	31	*
100µg	35	31	27	34	*
250µg	38	34	30	36	*
500µg	40	36	36	38	*
1000µg	*	*	*	*	*
MIC(mg/ml)	25	25	25	25	25

Fig.1 Zone of Inhibition of methanolic and aqueous leaf extracts of *S.dulcis* against different bacteria

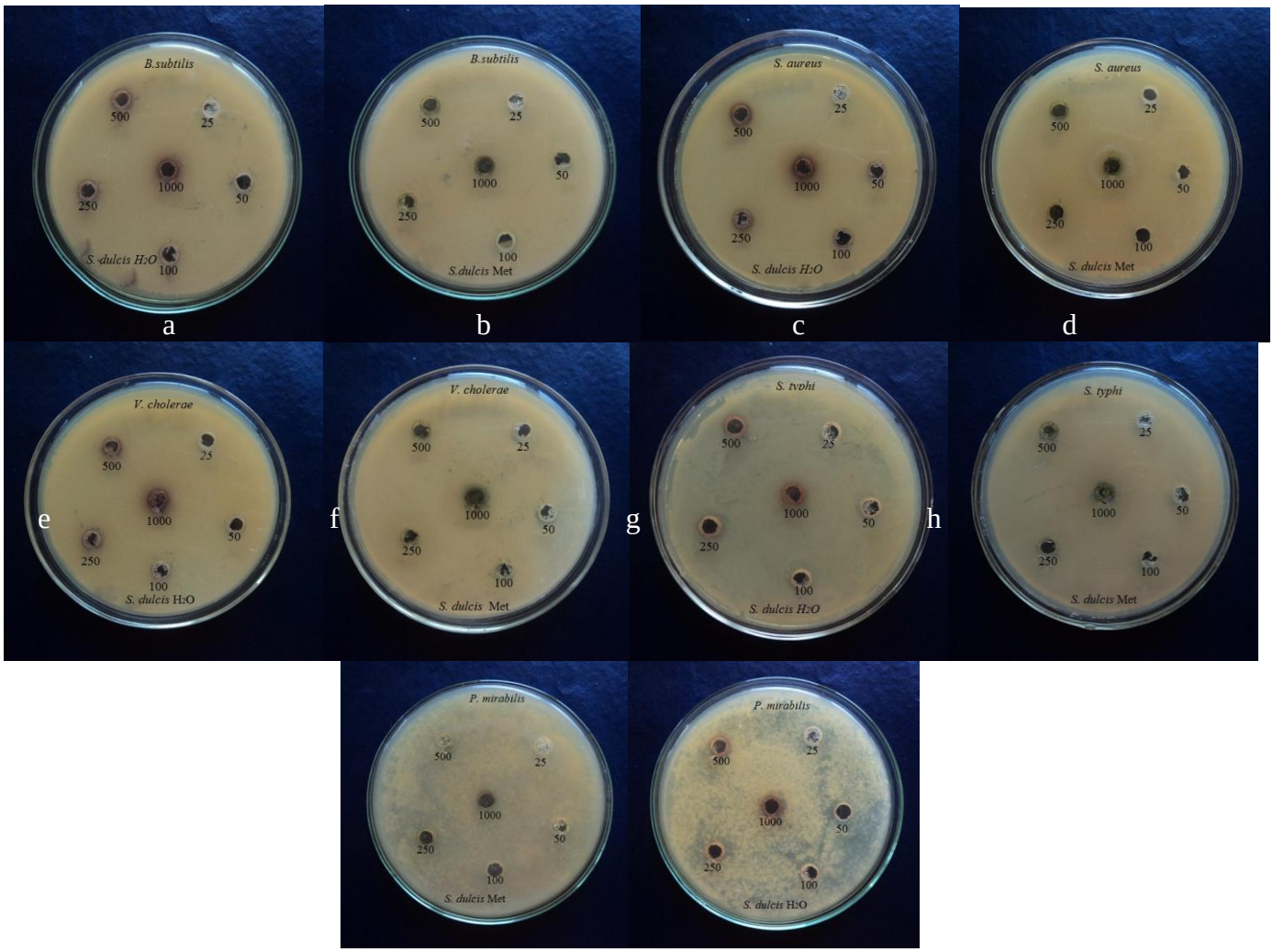


Fig.2 Zone of Inhibition of Ciprofloxacin against different bacteria

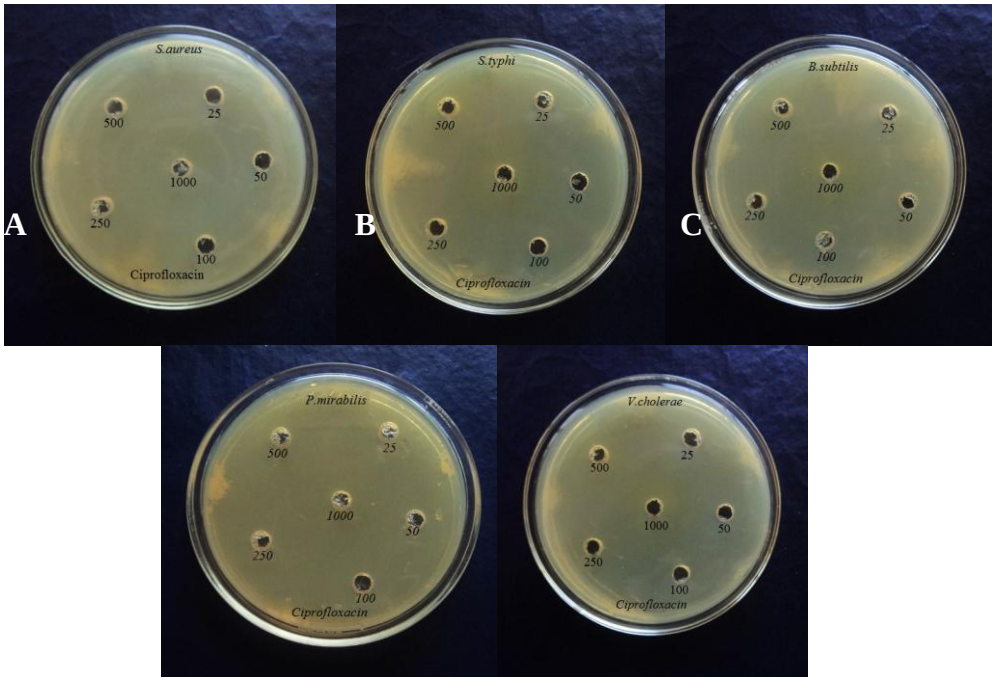


Fig.3 % Inhibition of *S. dulcis* leaf extract against S. aureus in broth dilution method

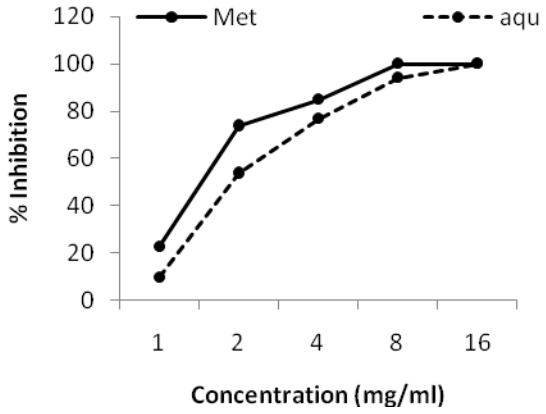


Fig.4 % Inhibition of *S. dulcis* leaf extract against *V.cholerae* in broth dilution method

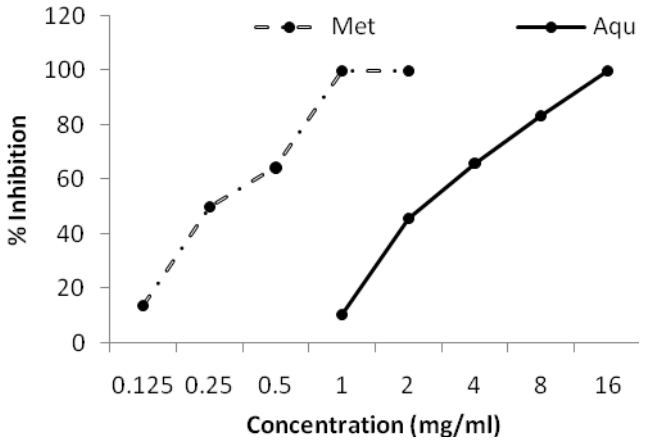


Fig.5 % Inhibition of *S. dulcis* leaf extract against *P.mirabilis* in broth dilution method

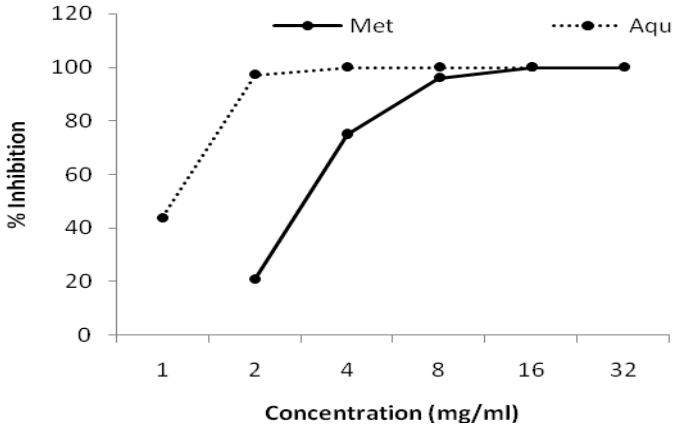


Fig.6 % Inhibition of *S. dulcis* leaf extract against *S.typhi* in broth dilution method.

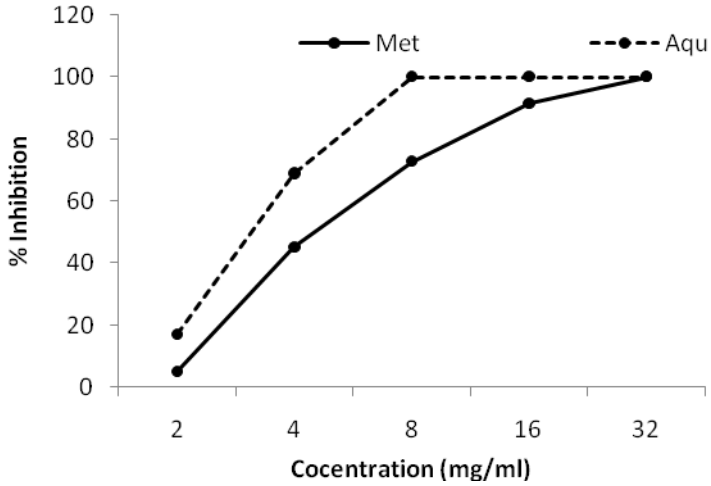
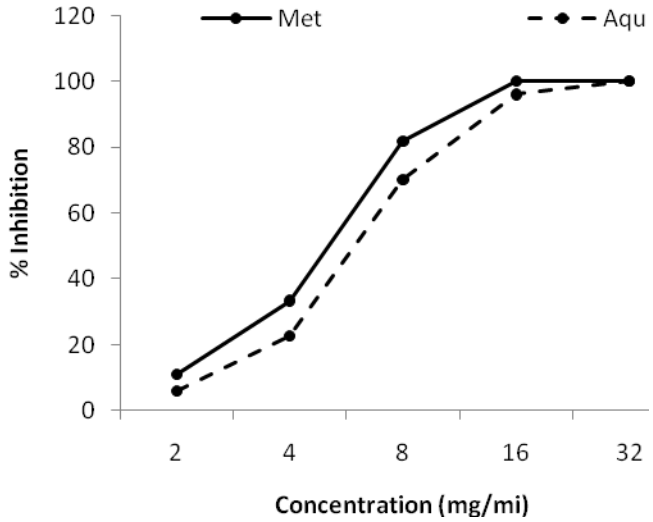


Fig.7 % Inhibition of *S. dulcis* leaf extract against *B.subitlis* in broth dilution method.



V. Cholerae was found to be highly susceptible as it showed an inhibition zone of 4mm at 1000µg concentration whereas *P.mirabilis* was comparatively less sensitive by showing 2mm ZOI at 10mg/mL concentration. *B.subtilis*, *S.typhi* and *S.aureus* did not show any zone of inhibition reflecting their insensitiveness towards the methanolic extract of the leaf. None of the test bacteria showed any zone of inhibition towards the aqueous leaf extract of the *S.dulcis* reflecting their insensitiveness towards the aqueous extract of the leaf.

Broth dilution method

The broth dilution method showed more pronounced antimicrobial activity through 100% inhibition for all the pathogens in the range of 1-32mg/mL concentration. The MIC for *S.typhi* and *B.subtilis* in methanolic solution was 32mg/mL (Fig. 1.h & b), for *P.mirabilis* 16mg/mL (Fig.1. i), for *S.aureus* 8mg/mL (Fig 1.d) and *V.cholerae* 1mg/mL (Fig.1.f) The MIC for *S.aureus* and *V.cholerae* in aqueous solution was 16mg/mL (Fig.1.c&e), for *P.mirabilis* 4mg/mL (Fig.1.j), *S. typhi* 8mg/mL (Fig.1.g) and for *B.subitlis* 32mg/mL (fig.1.a).

In conclusion, the present study suggests antibacterial property of methanolic and aqueous leaf extracts of *Scoparia dulcis*, which inhibits the growth of pathogenic bacteria *S. aureus*, *V. Cholerae*, *B. Subtilis* *S.typhi* and *P.mirabilis* causative agent food poisoning boils, abscesses, wound infection, cholera, pneumonia, toxic shock syndrome, typhoid fever and urethitis, cystitis, pylonephritis, prostatitis and pneumonia disease. It can be used as new drug for therapy, and it can be convincingly stated the methanolic and aqueous leaf extracts of *S.dulcis* are potential antibacterial agents and can be used to prepare new antibiotics to cure various ailments caused by bacteria.

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References

Ayoola, G.A., H.A.B. Coker, S.A. Adesegun, A.A. Adepoju-Bello, K. Obaweya1, E.C. Ezennia, T.O. Atangbayila. 2008. Phytochemical Screening and

Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in South-western Nigeria, *Trop. J. Pharmaceutical Res.*, 7(3): 1019-1024.

Balandrin, M.F., Kjocke, A.J., Wurtele, E. 1985. Natural plant chemicals: sources of industrial and mechanical materials. *Sci.*, 228: 1154- 116

Bowersox, John.1999. "Experimental Staph Vaccine Broadly Protective in Animal Studies". NIH. Archived from the original on 5 May 2007. Retrieved 28 July2007.

Davies. 1994. J. Inactivation of antibiotics and the dissemination of resistance genes. *Sci.*, 264: 375–382.

I.Wu.M. W., A.R. Duncan and C.O. Okunji. 1999. J. ASHA Press Alexandra V.A. pp. 457 – 462

Jain, S.K., Srivastava, S. 2005. Traditional uses of some Indian plants among the islanders of Indian Ocean. *Indian J. Tradit. Knowl.*, 4: 345–57.

Jigna Parekh, and Sumitra Chanda. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants, *African J. Biomed. Res.*, Vol. 10: 175 – 181.

Laboratory Methods for the Diagnosis of Vibrio cholerae. Retrieved 29 October 2013.*). Centre for Disease Control*.

Laurel, S., Burall, Janette, M., Harro, Xin Li, C. Virginia Lockatell, Stephanie, D. Himpsl, J. Richard Hebel, David E. Johnson, Harry, L.T. Mobley. 2004. Proteus mirabilis Genes That Contribute to Pathogenesis of Urinary Tract Infection: Identification of 25 Signature-Tagged Mutants Attenuated at Least 100-Fold. *Infect. Immunity*, p. 2922–2938.

Mishra, M.R., A. Mishra, D.K. Pradhan, A.K. Panda, R.K. Behera and S. Jha. Antidiabetic and Antioxidant Activity of *Scoparia dulcis* Linn.

Mukherjee, P.K.2003. Exploring botanicals in Indian system of medicine-Regulatory perspectives. *Clin Res Reg Aff*., 20: 249–64.

Ncube, N.S., Afolayan, A.J., Okoh, A.I. 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African J. Biotechnol.,* 7(12): 1797-1806.

Palombo, E.A. 2006. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. *Phytother. Res.*, 20(9): 717-724.

Ryan, K.J., Ray, C.G, eds. 2004. Sherris Medical Microbiology (4th ed.). McGraw Hill, ISBN 0-8385-8529-9.

Sofowora, A. 2008. Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicinal in Africa. 3rd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria. p. 134.

Sophy Jose and M.P. Sinha. 2016. Phytochemistry and antibacterial efficacy of *Schleichera oleosa* on some human pathogenic bacteria. J. The *Eco Scan Special issue*, Vol. IX: 00-00.

Sushmita Choudhury, Latika sharan and M.P. Sinha. antibacterial efficacy and phytochemistry of methanolic leaf extracts of mangifera indica linn.

Threlfall, E.J., Fisher, I.S.T., Ward, L., Tschape H. & Gerner-smidt, P. 1999. Harmonization of antibiotic susceptibility testing for *Salmonella:* Results of a study by 18 national reference laboratories within the European Union-funded Enter-Net group. *Microb. Drug Resist.*, 5: 195–199.

Trease and Evan. 2002. Qualitative Phytochemical Screening and In VitroAntimicrobial effects of Methanol

Stem Bark Extract of Ficus Thonningii (Moraceae).

Wain, J., Hendriksen, R.S., Mikoleit, M.L., Keddy, K.H., Ochiai, R.L. 2015. "Typhoid fever.". *Lancet*, 385(9973): 1136–45. doi:10.1016/s0140-6736(13)62708-7. PMID 25458731

Walker, R.D. 2000. Antimicrobial susceptibility testing and interpretation of results. *In:* Antimicrobial Therapy in Veterinary Medicine, Prescott J.F., Baggot J.D., Walker R.D., eds. Ames, IA, Iowa State University Press, 12–26.

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