ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. IX: 797-802: 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

PHYTOCHEMISTRY AND ANTIBACTERIAL EFFICACY OF *SCHLEICHERA OLEOSA* ON SOME HUMAN PATHOGENIC BACTERIA

Sophy Jose and M. P. Sinha

KEYWORDS

Schleichera oleosa
Pathogens
Antibacterial activity
Extracts

Proceedings of National Conference on
Harmony with Nature in Context of
Resource Conservation and Climate Change
(HARMONY - 2016)
October 22 - 24, 2016, Hazaribag,
organized by
Department of Zoology, Botany, Biotechnology & Geology
Vinoba Bhave University,
Hazaribag (Jharkhand) 825301
in association with
National Environmentalists Association, India
www.neaindia.org

The Ecoscan
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

SOPHY JOSE* AND M. P. SINHA
Department of Zoology,
Ranchi University, Ranchi - 834 008, Jharkhand, INDIA
e-mail: josesophy35@gmail.com

ABSTRACT

Schleichera oleosa is a well known traditional medicinal plant used in various indigenous systems of medicine. It is widely distributed throughout India. The present study was undertaken to investigate phytochemical and antimicrobial details of the methanolic and aqueous leaf extract of *Schleichera oleosa* 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, saponins and steroids and many other metabolites. Minimum inhibitory concentration (MIC) assay was determined for the extract. The methanolic and aqueous extract showed toxicity against all the five bacteria under consideration, *S.typhi* and *V. Chlolerae* being highly susceptible with a zone of inhibition of 5mm and 3mm at 10mg/ml in agar diffusion method. The broth dilution method showed more prominent antimicrobial activity through 100% inhibition for all the pathogens in the range of 1-32mg/mL concentration. The MIC in the methanolic solution for *S.typhi* was 16mg/mL, for *P.mirabilis* 4mg/mL, for *S.aureus* and *B.subtilis* 8mg/mL and for *V.cholerae* 1mg/ml The MIC in the aqueous solution for *S. aureus*, *V.cholerae* and *B.subtilis* was *16mg/mL ,* for *P.mirabilis* 32mg/ml *and for S.typhi* 8mg/mL.

***Corresponding author**

INTRODUCTION

People have been using medicinal plants since time immemorial as a source of relief from illnesses (Thomson, 1978). Though there are around 250, 000-500, 000 plant species, very few plants have been investigated phytochemically (Mahesh and Satish, 2008). Antimicrobials obtained from plants have enormous therapeutic efficacy and drugs from higher plants occupy very important place in modern medicine (Girish and Satish, 2008). Over the past twenty years, there has been immense attention in the research of natural materials as sources of new antibacterial agents (Werner *et al*., 1999, Samy *et al*., 2000.). Reports of tests on extracts from different traditional medicinal plants have shown the effectiveness of traditional herbs against microorganisms. Therefore, plants are strong basis for modern medicine to attain new principles (Evans, Banso and Samuel, 2002).

Communicable diseases are one of the main reasons of ill health in developing nations of the world and a major agent of them is pathogenic bacteria. (Solanki, 2010, Kumar *et al.,* 2013). Five common pathogenic bacteria *Salmonella typhi*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus subtilis* and *Vibrio cholera* have been studied by several workers. *S. typhi* is causative agent of typhoid (Wain, *et al*., 2015). *P. mirabilis* is a Gram-negative, facultative anaerobic, rod-shaped bacterium. It shows swarming motility in large numbers and urease activity. *P.mirabilis* causes 90% of all *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. (Laurel *et al.,* 2004). *Vibrio cholerae* is a Gram-negative, comma-shaped bacterium. Some strains of *V. cholerae* causes the disease cholera [Laboratory Methods for the Diagnosis of *Vibrio cholerae*"]. *Staphy lococcus aureus* is a gram-positive coccal bacterium that is a member of the Firmicutes, and is commonly found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction. 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. *Skin infections are the most common. They can look like pimples or boils* impetigo, boils, cellulitis, folliculitis, carbuncles, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia and sepsis. [Bowersox, John. (27 May 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 severely immuno compromised patients. (Ryan *et al.,* eds. 2004).

Many therapeutic potentialities of higher plants are still unexplored. *Schleichera oleosa*, is commonly called in Hindi as *'Kusum'* and belongs to the family of *Sapindaceae*. Its leaf, seed, oil and bark are used for curing itch, pain in the back and loins, promotes hair growth, treats rheumatism, head ache, skin diseases, malarial fever and is prophylactic against cholera [Palanuvej , Vipunngeun , 2008].

But scientific antibacterial efficacy of *S.oleosa* leaf extract has not been explored yet. Therefore, present study was undertaken to test the antibacterial activity of

S.oleosa leaf extract as an attempt to establish credibility, awareness, and scientific data to support the therapeutic use of methanolic and aqueous extracts of *Schleichera oleosa* leaves as an alternative to conventionally used pharmaceutical drugs for the treatment of ailments against a few clinically important multi-drug resistant pathogens including *Staphylococcus aureus*, *Salmonella typhi, Proteus mirabilis*, *Bacillus subtilis* and *Vibrio cholerae*.

MATERIALS AND METHODS

Collection of plant material

The fresh and tender leaves were collected, dried in a shade under room temperature for six days and then crushed into coarse powdery substance by using electric grinder. 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.

Extract preparation

50 g of *S.oleosa* 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 45ºC, percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies. (Ahmad, and Sharma, 2001).

Phytochemical analyses

Freshly prepared extracts of the powdered leaves were subjected to phytochemical analyses to find the presence of the phyto constituents such as flavanoids, alkaloids, carbohydrates, glycosides, polysaccharides, tannins, saponins, steroids, proteins, lipids and 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.

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 37oC 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 37oC for 24h 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 samples were added. Each tube was inoculated with 18 h old cultures (100 ìl, 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

Physicochemical analysis of leaves of various medicinal plants has been reported by various workers from time to time. The phytochemical screening of *C. papaya (*Ayoola *et al.,* 2008) showed the presence of flavonoids terpenoids, saponins and tannins. Phytochemical studies done on *Ammannia baccifera, Bauhinia variegate, Piper nigrum* etc., revealed the presence of tannins, saponins and alkaloids, steroids etc,. (Jigna Parekh, and Sumitra Chanda, 2006). *Sansevieria roxburghrana* revealed the presence of carbohydrates, saponin, flavonoids, phenols, alkaloids, anthocyanin and â-cyanin, glycosides, proteins and phytosterols (Deepa Philip *et al.,* 2011). The powdered leaf of Terminalia catappa showed the presence of secondary metabolites like alkaloids, reducing sugars, saponins, tannins, resins and steroids in ethanol soluble fraction. (Muhammad and Mudi, 2011). Preliminary phytochemical studies carried out on the crude methanol and aqueous extracts of the leaves of *Syzygium cumini* revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins (Gowri and Vasantha, 2008). The phytochemical analysis carried out on Scoparia dulcis revealed the presence of

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

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

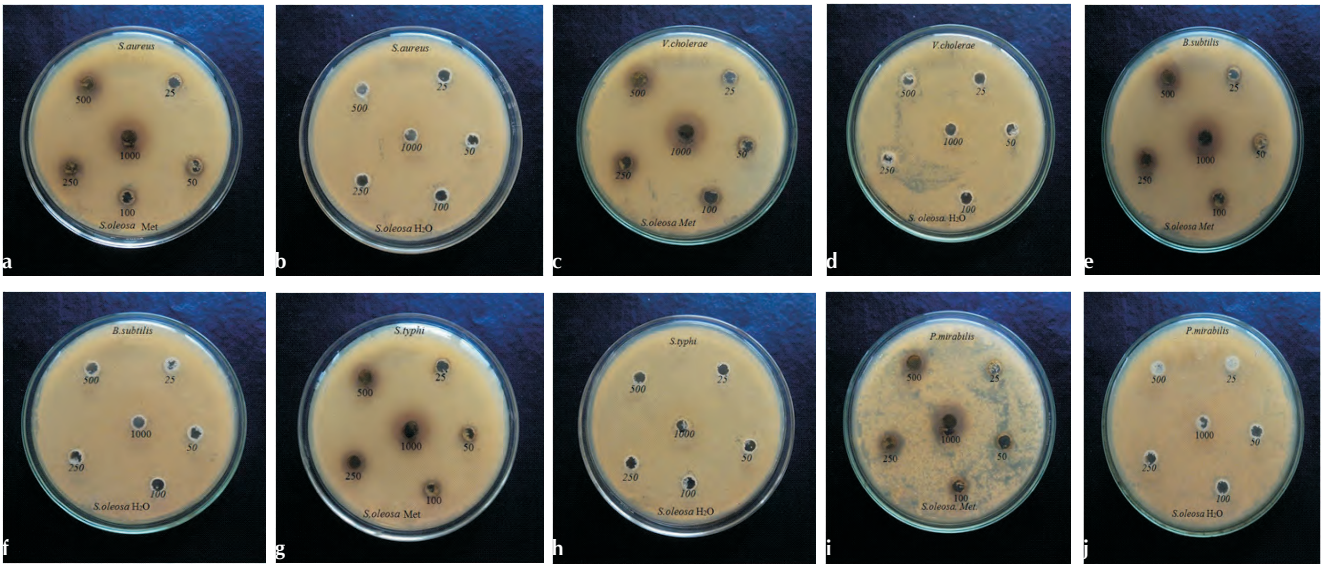

Figure 1: Zone of Inhibition of methanolic and aqueous leaf extracts of *S. oleosa* against different bacteria

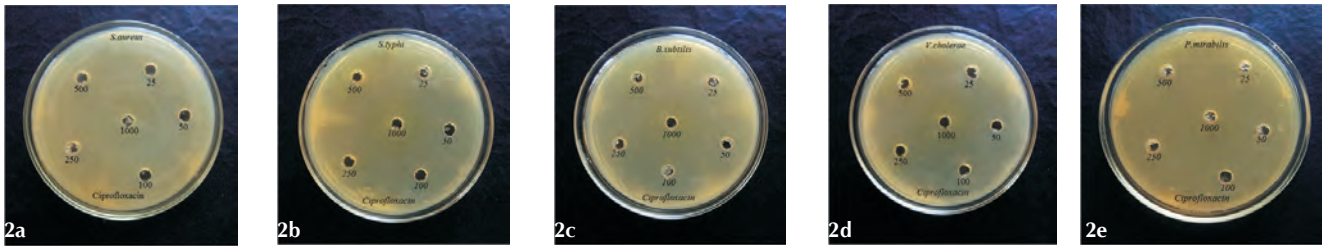

Figure 2: Zone of Inhibition of Ciprofloxacin against different bacteria.

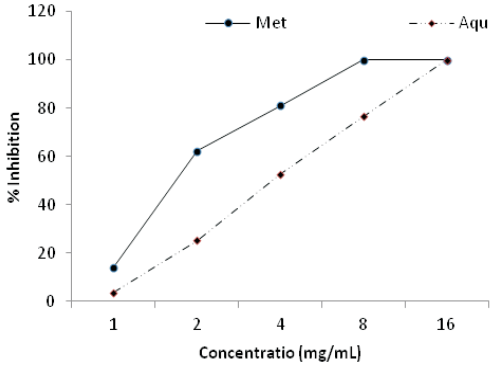

Figure 3: % Inhibition of *S. Oleosa* leaf extract against *S. aureus* in broth dilution method

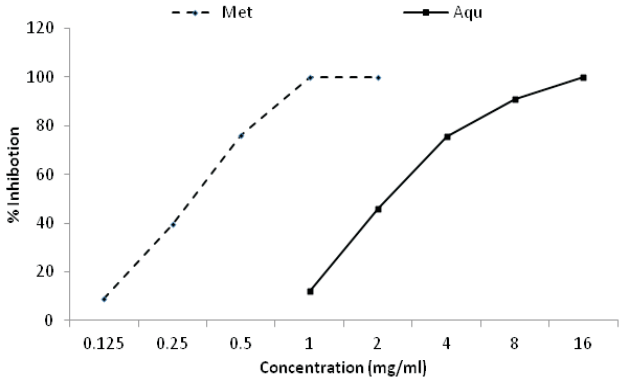

Figure 4: % Inhibition of *S. Oleosa* leaf extract against *V. cholerae* in broth dilution method

Table 2: Zone of inhibition (in mm) of aqueous and methanolic leaf extract of *Schelichera oleosa*

concentration	*S. typhi*		*S. aureus*		*B. subtilis*		*V. cholerae*		*P. mirabilis*	
	Aq	*Met*	*Aq*	*Met*	*Aq*	*Met*	*Aq*	*Met*	*Aq*	*Met*
25¼g	0	0	0	0	0	0	0	0	0	0
50¼g	0	0	0	0	0	0	0	0	0	0
100¼g	0	0	0	0	0	0	0	0	0	0
250¼g	0	0	0	0	0	0	0	0	0	0
500¼g	0	0	0	0	0	0	0	0	0	0
1000¼g	0	0	0	0	0	0	0	5	0	3
MIC(mg/ml)	NF	NF	NF	NF	NF	NF	NF	1000	NF	1000

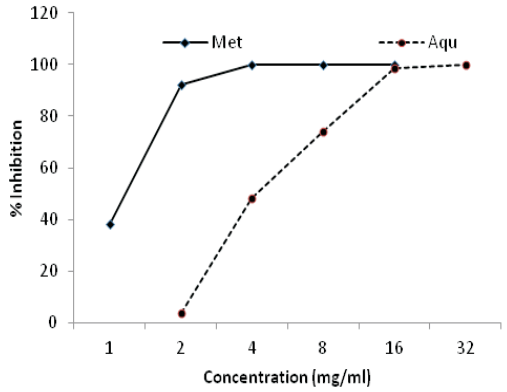

Figure 5: % Inhibition of *S. Oleosa* leaf extract against *P. mirabilis* in broth dilution method

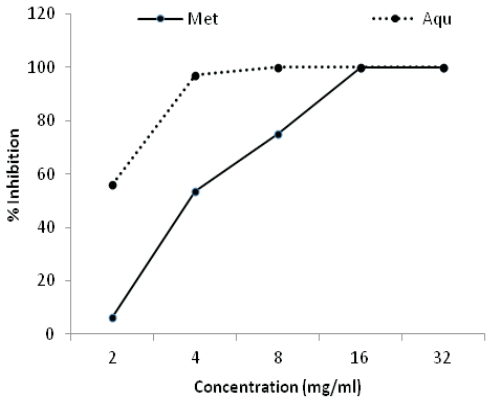

Figure 6: % Inhibition of *S. Oleosa* leaf extract against *S. typhi* in broth dilution method

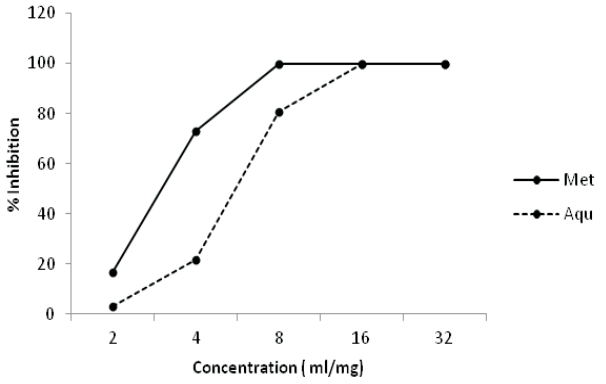

Figure 7: % Inhibition of *S. Oleosa* leaf extract against *B. Subitlis* in broth dilution method

Table 3: MIC of Ciprofloxacin against the test organisms

concentration	*S. typhi*	*S. aureus*	*B. subtilis*	*V. cholerae*	*P. mirabilis*
25¼g	27	25	20	30	*
50¼g	31	28	24	31	*
100¼g	35	31	27	34	*
250¼g	38	34	30	36	*
500¼g	40	36	36	38	*
1000¼g	*	*	*	*	*
MIC(mg/ml)	25	25	25	25	25

flavanoids, glycosides, alkaloids, tannins, steroids and many other metabolites (Sophy Jose and M. P. Sinha, 2016). The results of the evaluation of phytochemical screening of methanolic extracts of *Scleichera oleosa* represented in table 1 revealed the presence of carbohydrates, glycosides, polysaccharides, proteins, steroids, alkaloids, triterpenoids, tannins, lipids, oils, saponins and flavanoids . These constituents are responsible for the curative nature of *Schelichera oleosa* against itching, head ache, malaria, skin diseases etc. which could make the plant useful for treating different ailments and having a potential of providing useful and safe drugs and drug leads for human use. (Danish *et al*., 2010, Gupta, 2003).

Antibacterial assay

The antibacterial efficacy of the extracts of *S. oleosa* leaves was quantitatively assessed 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. oleosa* leaf extracts.

Agar diffusion method

In the present investigation the methanolic and aqueous extracts 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 2-5mm at 10 mg/mL concentration of the extract. *V. Cholerae* was found to be highly susceptible as it showed an inhibition zone of 5mm at 1000μg concentration whereas *P.mirabilis* was comparatively less sensitive by showing 3mm 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. Oleosa* reflecting their insensitiveness towards the aqueous extract of the leaf.

Broth dilution method

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

ACKNOWLEDGEMENT

The authors acknowledge the facilities provided by the Department of Zoology, Ranchi University, Ranchi.

REFERENCES

Thomson, W. A. R. 1978. Medicines from the Earth. *Maidenhead, United Kingdom, McGraw-Hill Book*. **158:** 353-357.

Mahesh, B. and Satish, S. 2008. Antimicrobial Activity of Some Important Medicinal Plants Against Plant and Human Pathogens. *World J. Agricultural Sciences*. **4(S):** 839-843.

Girish, H. V. and Satish, S. 2008. Antibacterial Activity of Important Medicinal Plants on Human Pathogenic Bacteria-a Comparative Analysis. *J. World Applied Sciences*. **5(3):** 267-271.

Werner, F., Okemo, P. and Ansorg, R. 1999. Antibacterial activity of East African Medicinalplants. *J. Ethnopharmacol*. **60:** 79-84.

Evans, C. E., Banso, A. and Samuel, O. A. 2002. Efficacy of some nupe medicinal plants against *Salmonella typhi*: an in vitro study. *J Ethnopharmacol*. **80:** 21-24.

Solanki, R. 2010. Some medicinal plants with antibacterial activity, In *Int. J. Comprehen. Pharma*. **4(10):** 1-4.

Kumar, A., Dandapat, S., Kumar, M. and Sinha, M. P. 2013. Antipathogenic efficacy and haemolytic activity of *Calotropis procera* leaves. *In World J. Zool*. **8(4):** 366-370.

Wain, J., Hendriksen, R. S., Mikoleit, M. L., Keddy, K. H. and Ochiai, R. L. 2015. "Typhoid fever". *Lancet* 385 (9973):1136-45.doi:10.1016/ s0140-6736 (13)62708-7. PMID 25458731(21March 2015)

Laurel, S., Burall,1 † Janette M. Harro,1 † Xin Li,1 C. Virginia Lockatell, 2 Stephanie, D. Himpsl,1 J. Richard Hebel, 3 David, E. Johnson, 2, 4 and Harry, L. T. Mobley1 2004. Proteus mirabilis Genes That Contribute to Pathogenesis of Urinary Tract Infection: Identification of 25 Signature-Tagged Mutants Attenuated at Least 100-Fold. Infection and immunity, May 2004, pp. 2922-2938.

Laboratory Methods for the Diagnosis of *"Vibrio cholerae"* 2013. Centre for disease control**.** *Retrieved* 29 October 2013.

Bowersox, John (27 May 1999). "Experimental Staph Vaccine Broadly Protective in Animal Studies". NIH. Archived from the original on 5 May 2007. Retrieved 28 July 2007.

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

Palanuvej, C. and Vipunngeun, N. 2008. Fatty acid constituents of *Schleichera oleosa* (Lour) Oken seed oil. *J. Health Res*. **22:** 203-2.

Ahmad, R. V. and Sharma, R. K. 2001. Evaluation of drug for standardization. Proceedings of WHO training cum- workshop, Pharmaceutical lab for Indian medicine, Ministry of health and family welfare, Govt. of India, Ghaziabad.

Danish Iqbal, Raju, K. Pawar and Rajeev, Kr. Sharma 2010. Physico-chemical Standardization of *Butea Monosperma* (Lam.) Kuntze (Palasha): An Ayurvedic Drug International *J. Pharmaceutical Quality Assurance*. **2(1):** 49-51.

Gupta, A. K. 2003. Quality standards of Indian medicinal plants. *Indian Council of Medical Research, India* Vol-I.

Trease, G. E. and Evans, W. C. 2002. Pharmacognosy. 15th Ed. Saunders Publishers, London. p. 42.1

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.

Threlfall, E. J., Fisher, I. S. T., Ward, L., Tschape, H. and 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.

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. pp. 12-26.

Ogbulie, J. N., Ogueke, C. C. and Okorondu, S. 2004. Antibacterial properties of *A cordifola, M. flurum, U. chaeme B. pinnatum, C.albidem* and *A. cilata* on some hospital isolates. *Niger. J. Microbiol*. **18(1-2):** 249-255.

Borokini, T. I. and Omotayo, F. O. 2012. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. *J. Med. Plants Res*. **6(7):** 1106-1118.

Sushmita Choudhury, Latika sharan1 and M. P. Sinha. Antibacterial efficacy and phytochemistry of methanolic leaf extracts of *Mangifera indica* linn. *J Environmental Sciences*. **1:** 419-423.

Alkizim, F. O., Matheka, D., Abdulrahman, F. K. and Muriithi, A. 2012. Inhibitory effect of *Mangifera indica* on gastrointestinal motility. *Med. Chem. Drug Discovery*. **2(1):** 9-16.

Ayoola, G. A., Coker, H. A. B., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya1, K., Ezennia, E. C. and Atangbayila, T. O. 2008. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in South-western Nigeria, *Tropical J. Pharmaceutical Research, September* 2008; **7(3):** 1019-1024.

Jigna Parekh and Sumitra Chanda Antibacterial and phytochemical studies on twelve species of Indian medicinal plants, *African J. Biomedical Research*. **10:** 175-181.

Deepa Philip, P. K., Kaleena, K., Valivittan and Girish Kumar, C. P. 2011. Phytochemical screening and antimicrobial activity of *Sansevieria roxburghiana* Schult. And Schult. *F. Middle-East J. Scientific Research* **10(4):** 512-518.

Sophy Jose and Sinha, M. P. 2016. A comparative study of antibacterial efficacy of Methanolic and aqueous leaf extracts of *Scoparia dulcis* on some human pathogenic bacteria.

Muhammad, A. and Mudi, S. Y. 2011. Phytochemical Screening and Antimicrobial Activities of *Terminalia catappa*, Leaf Extracts. J Biokemistri 0795-8080 Vol. 23, No. **1:** 35-39.

Shyamala Gowri, S. and Vasantha, K. 2010. Phytochemical Screening and Antibacterial Activity of *Syzygium cumini (L.) (Myrtaceae*) Leaves Extracts. *International J. Pharm Tech Research* Vol.2, No.2, pp1569-1573.