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Antimicrobial Properties of the Leaves of *Saraca indica*

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Acetone, chloroform, methanol, petroleum ether and water extracts of leaves of *Saraca indica* (family-Caesalpiniaceae)were investigated for their antibacterial activity against standard strains of *Bacillus cereus*, *Bacillus subtilis* ,*Bacillus megaterium*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* by well diffusion method. Acetone, chloroform, methanol and petroleum ether extracts showed significant antibacterial activity against only Gram positive bacteria.Water extract is found active against *Bacillus subtilis* and *Bacillus megaterium*. Fresh leaves juice and acetone extracts showed highest antimicrobial activity against Gram positive microorganisms.Results were compared with standard antibiotics Amoxicillin-Am^{30}, Ciprofloxacin-Cf^{30}, Cotrimaxazole-Co^{25},Gentamicin-G^{50} and Tetracycline-T^{30} . The antibacterial activities of the Acetone, chloroform, methanol and petroleum ether extracts are discussed according to their phytochemical components.It is concluded that the *Saraca indica* may serve as valuable source of compounds with therapeutic potential.

Key words: Antibacterial activity, *Saraca indica*,antibiotics, Acetone, Chloroform, Methanol and Petroleum ether extracts.

Herbal medicine has such an extraordinary influence that numerous alternative medicine therapies treat their patients with Herbal remedies, Unani and Ayurveda. Approximately 25 percent of all prescription drugs are derived from trees, shrubs or herbs. Nature has bestowed our country with an enormous wealth of medicinal plants therefore India has often been referred to as the medicinal garden of the world. So stand the medicinal plants *Saraca asoca* as one of the foremost plants utilized from antiquity till to date (Pradhan,P.*et al.*,2009).

The diverse culture of our country is a rich source of traditional medicine, many of which are of plant origin. Scientific data of such plant derivatives could be of clinical use. Through out the world in current research scenario, the development of a new drug entity with promising efficacy and less toxicity for treatment of various dreadful diseases requires huge investment and longer period of clinical trials. Drugs from natural plants are considered to be a promising source to serve purposes. Among the diversity of most natural products, medicinal plants are still the most favorable for the scientist to explore a new drug. *Saraca indica* is the one of the most interesting medicinal plant as all part of this plant contain significant & beneficial chemical constituents, which helps in treating various diseases and disorders (Shelar,D.B.*et al.*,2010).

Plant materials have been used for the treatment of serious diseases throughout the world before the advent of modern clinical drugs (Hidayathula S.*et al.*,2011).The use of medicinal plants still plays an important role to cover the basic health needs in the developing countries

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(Shihabudeen HMS *et al.,*2010). Several top selling drugs of modern times such as Quinine, Artemisinin, Shikonin,etc.are obtained from plants (Hidayathula S. *et al.*,2011). Most of the phytochemicals, secondary metabolites of plants,are physiologically active(Shihabudeen HMS *et al.,* 2010).The plants are known to provide a rich source of botanical,anthelmintic,antibacterials, and insecticides (Acharya S.*et al.,*2011).

Plants are used as medicine since time immemorial. Drugs from plant sources are being used by about 80% of the world population. Herbal medicines have stood the test of time for their safety, efficacy, acceptability and lesser side effects (Kamboj, V. P.,*et al.,* 2000, Sannomiya, M.,*et al*, 2007).

Saraca indica belongs to the family Caesalpiniaceae.It is found in India, China, Ceylon and Malaysia. It occurs almost throughout India up-to an altitude of 750 m in the central and in the eastern Himalayas and Khasi, Garo and Lushai hills, wild in Chittagong, Bihar, Orissa, Konkan, Deccan, Mysore. It has become quitescarce in several localities and is reported to be threatened in North Eastern Region of India(Shilpakala Sainath R.*et al.,* 2009).

Saraca indica leaves and stem found to contain quercetin, quercetin-3-O-α-Lrhamnoside, kaempferol 3-O- α-Lrhamnoside, amyrin, ceryl alcohol and β-sitosterol (Pradhan, P.et.al.2009, Anonymous,2006, Sadhu, S. K.*et al.,*2007). The antidiabetic, oxytocic, anticancer, peptic ulcer, antimicrobial, antibacterial and antioxidant activities of the plant have been reported (Preethi, F. *et al.,* 2010, Satyavati, G. V.et.al. 1970, Sainath, R. S.*et al.,* 2009, Kaur, J. D.*et al.,*1980, Maruthappan, V.*et al.,*2010, Pal, S. C.*et al.,* 1985, Sandhu, J. K,*et al.,*. 2007)

MATERIAL AND METHODS

Selection of medicinal plant for this study

Saraca indica

Family

Caesalpiniaceae

Parts used

Leaf

Traditional uses

The plant is useful in dyspepsia, fever, burning sensation, colic, ulcers, menorrhagia, leucorrhoea, pimples etc. The bark,used for pharmaceutical preparations,is bitter, astringent, refrigerant, anthelmintic, styptic, stomachic, constipating, febrifuge and demulcent. Even the juice of the leaves, mixed with cumin seeds, is used for the treatment of stomachalgia (Srivastava G.N.*et al.,*1988).

Chemical constituents

It mainly contains glycosidic principles, non-phenolic, sapogenetic glycoside, sterols and aliphatic alcohols. catechol, (-) epicatechol and leucocyanidin has been isolated from pods and wood contains quercetin. In the powdered bark ash of Ashoka minerals like silica,sodium, potassium, phosphate, magnesium, iron, calcium, strontium and aluminium have been found (N. Indrani, K. *et al.,* 1984, D. J. Kaur, *et al.,* 1980**,** T. B. Middelkoop,*et al.,* 1985**).** The flowers contain fatty acids and gallic acid; apigenin-7-O-beta-Dglucoside, cyanidin-3, 5-diglucoside, kaempferol 3-O-beta-D-glucoside, pelargonidin-3, 5-diglucoside, quercetin and its 3-O-beta-D-glucoside and sitosterol. The bark yields alkanes,esters and primary alcohols. It gave n-octacosanol, tannin, catechin, (+)-catechol, (-)-epicatechin, (-)-epicatechol, leucocyanidin, leucopelargonidin, procyanidin derivatives,methyl- and ethylcholesterol derivatives. Quercetin and its 3-O-rhamnoside, kaempferol-3-O-α-L-rhamnoside, amyrin, ceryl alcohol and β-sitosterol have been isolated from leaves and stems (M. Behari, *et al.,*1977).It also contains flavonoids and sterols (L. Vijai, *et al.,* 1976, M. Behari,*et al.,* 1977).

Identification and Preservation of Plant materials

Fresh plant leaves were collected from the Nagpur area of India. The taxonomic identities of this plant was determined by the expertise of the Post Graduate Department of Botany of Rashtrasant Tukadoji Maharaj Nagpur University,Nagpur. Specimen was labeled, numbered and noted with date of collection, the locally and their medicinal uses and their approximate dosages of administration were recorded. Plant leaves were washed with 70% alcohol and then rinsed with sterilized distilled water, air dried and stored in airtight bottles at 4^{o}C for further use.

Preparation of crude extracts (Fresh juice)

Plant leaves were collected from around

Nagpur region in the month of August-September. Leaves were cleaned under running potable water and were washed with 70% alcohol and then rinsed with sterilized distilled water,cut into pieces and grounded in pestle and mortar (made up of dolerite stone) till homogenized mass was obtained. Homogenized mass was squeezed in 400 mesh nylon cloth (pore size 37 micron) to obtain crude extract. Crude extract was kept in sterilized glass bottle. All crude extract were prepared fresh and used before 2 hours. Cold extracts were prepared using individual fresh plant leaves.

Aqueous extraction

Ten grams of dried powder was extracted in 100 ml distilled water for 6 h at slow heat. Every 2 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000g for 15 min. The supernatant was collected. This process was repeated twice and after 6 h, the supernatant was concentrated to make the final volume one-fourth of the original volume (Shahidi Bonjar GH 2004). It was then autoclaved at 121 ºC and 15 lbs pressure and then stored at 4ºC.

Solvent extraction

Ten grams of dried powder was extracted with 100 ml of each solvent (acetone, chloroform, methanol and petroleum ether)and flasks were kept on a rotary shaker at 190-220 rpm for 24h. Thereafter, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Shahidi Bonjar, G.H. 2004). It was stored at 4^{o}C in airtight bottles for further studies.

Bacterial cultures

The microbial strains are identified strains and were obtained from the National Chemical Laboratory (NCL),Pune, India. The studied bacterial strains were *Bacillus cereus* NCIM2155, *Bacillus subtilis* NCIM2063,*Bacillus megaterium* NCIM2087, *Escherichia coli* NCIM2931, *Proteus vulgaris* NCIM2857 and *Pseudomonas aeruginosa* NCIM5029. *Staphylococcus aureus* MTCC96 this strain was procured from Institute of Microbial Technology (IMTECH), Chandigarh, India.They were sub-cultured on nutrient agar for every 15 days and maintained on nutrient agar slants at 4^{0}C,fresh inoculums were taken for test.

Media

Hi -Sensitivity test broth (M 486) and Hi-sensitivity test agar (M 485) were procured from Hi-media Mumbai,India. The media were prepared according to the instructions given.

Screening for the antimicrobial potential of the plant leaves extracts

The antimicrobial activity of different solvent extracts was evaluated by agar well diffusion(Perez C,et.al. 1990& Parekh,J.*et al.*,2007) using Hi-sensitivity test agar (M 485).

Preparation of inoculum

A loopful of culture was inoculated from the stock slant culture in 5 ml of Hi-sensitivity test broth and broth was incubated at 35±0.5^{0}C in incubator for 18-20 hrs.After incubation a loopful of actively growing culture was inoculated into 10 ml of Hi-sensitivity broth. Broth was incubated at 35±0.5^{0}C for 6-8 hours. This culture was used for the inoculation of Hi-sensitivity test agar plates.

Preparation of Hi-sensitivity test agar medium

Hi-sensitivity test agar medium was prepared as per instructions of manufacturer. Required amount of agar medium was melted and 25 ml of molten medium was distributed in test tubes (25x150 mm). Medium was autoclaved at 15 lb. for 20 min. After autoclaving, medium was maintained at 45-50^{0}C in constant temperature water bath.

Inoculation of medium with test organism

0.5 ml of 6-8 hours old test organism is transferred to petridish of 100mm size(Sterilized in oven at 180^{0}C for 1 hr.) using sterile micropipette. Hi-sensitivity test agar medium maintained at 45-50^{0}C was poured and mixed properly to ensure uniform distribution of organism with medium. Seeded plates are allowed to set at room temperature.

Preparation of agar well for fresh leaves juice

10 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made.A 100 µl crude extract(fresh leaves juice) was transferred by micropipette per well. Plates were immediately kept at 4^{0}C in refrigerator for 1 hr.for the diffusion of extract and then shifted to 35±0.5^{0}C in incubator. Zone of inhibition was measured after 24 hrs. of incubation by zone scale.

Preparation of agar wells for different solvent extracts

5 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made.

A 50 ul solvent extract was transferred by micropipette per well. Plates were immediately kept at 4^0C in refrigerator for 1 hr. and then shifted to $35^0C \pm 0.5^0$C in incubator. Zone of inhibition was measured after 24 hrs. of incubation.For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract.The control zones were sustracted from the test zones and the resulting zone diameter is obtained.

RESULTS

All the microorganisms responded differently to the various plant extracts and standard antibiotics.All the plant extracts and antibiotics tested showed some antimicrobial activity(Table-1).The fresh leaves juice was found to be active against only Gram positive bacteria.When we compared the activity of aqueous extract with fresh leaves juice,the fresh leaves juice is more active.The aqueous extract found to be active against *Bacillus subtilis* and *Bacillus megaterium*(Fig-3&Fig-2).

Acetone ,methanol and petroleum ether extracts are active against only Gram positive bacteria *Staphylococcus aureus*(Fig-4) , *Bacillus cereus*(Fig-1). *Bacillus subtilis*(Fig-3) and *Bacillus megaterium*(Fig-2).All the organisms are susceptible to Ciprofloxacin- Cf^{30}, Gentamicin-G^{50} ,and Tetracycline-T^{30} .*Proteus vulgaris* is found to be resistant to Amoxycilin Am^{30} *Pseudomonas aerugenosa* and *Bacillus cereus* found to be resistant to Cotrimaxozole Co^{25}.

DISCUSSION

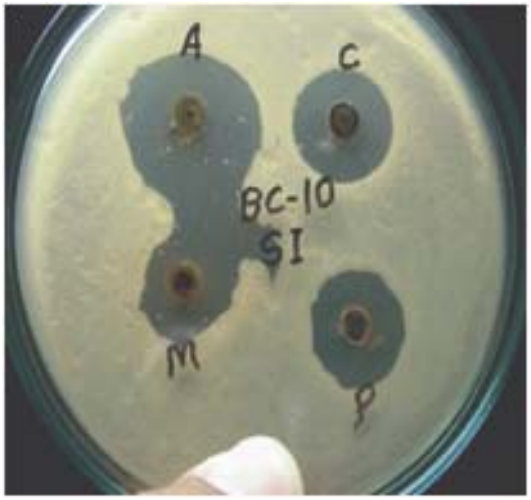
Acetone ,methanol ,chloroform and petroleum ether extracts are active against only Gram positive bacteria *Staphylococcus aureus* , *Bacillus cereus*,. *Bacillus subtilis* and *Bacillus megaterium*.All the Gram negative microorganisms ie. *Escherchia coli Proteus vulgaris* and *Pseudomonas aerugenosa* are found resistant to fresh juice of leaves and acetone ,methanol ,chloroform and petroleum ether extracts.Seetharaman *et al.,*and Sarojini Nayak *et al.,*found that the ethanolic extract of leaves was active against *Escherchia coli* and *Staphylococcus aureus.* Pal *et al.,*and Sarojini Nayak *et al.,* saw that the methanolic extract was active against *Bacillus subtilis*.Moreover, Sarojini Nayak *et al.,* found that the methanolic extract was more effective in cases of *Staphylococcus aureus* and *Escherichia coli*. Rajesh Dabur *et al.,*found that the petroleum ether extract is active against *Staphylococcus aureus* and *Proteus vulgaris.*Methanol extract is found active against *Pseudomonas aerugenosa, Staphylococcus aureus* and *Proteus vulgaris.* Chloroform extract is found inactive against *Pseudomonas*

Table 1. Results of antimicrobial activities of fresh leaves juice and solvent extracts of *Saraca indica* and compared with standard antibiotics

S. No.	Microorganisms	Zone of inhibition in mm										
		Leaves extracts						Standard antibiotics				
		FJ	WE	AE	ME	CE	PE	Am^{30}	Cf^{30}	Co^{25}	G^{50}	T^{30}
1.	*Escherchia coli*	-	-	-	-	-	-	32	29	24	17	22
2.	*Proteus vulgaris*	-	-	-	-	-	-	-	23	31	20	24
3.	*Pseudomonas aerugenosa*	-	-	-	-	-	-	14	36	-	34	22
4.	*Staphylococcus aureus*	28	-	21	18	14	15	31	23	20	16	17
5.	*Bacillus cereus*	25	-	24	15	18	17	15	27	-	23	24
6.	*Bacillus subtilis*	28	17	32	28	28	28	31	50	36	40	32
7.	*Bacillus megaterium*	23	10	24	21	18	20	29	46	24	23	33

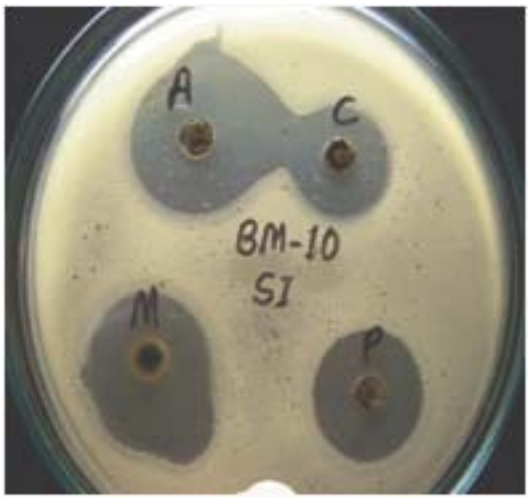
Key: FJ-Fresh juice of leaves; WE-Water extract; AE-Acetone extract; ME-Methanol extract;PE-Petroleumether; Am^{30}-Amoxycilin; Cf^{30} –Ciprofloxacin; Co^{25} –Cotrimaxazole; G^{50} -Gentamicin; Tetracycline-T^{30} . ND—Not determined; - Negative.

Antibacterial activity of different solvent extracts of leaves of *Saraca indica* (SI),
Zone of inhibition in millimetre (mm).



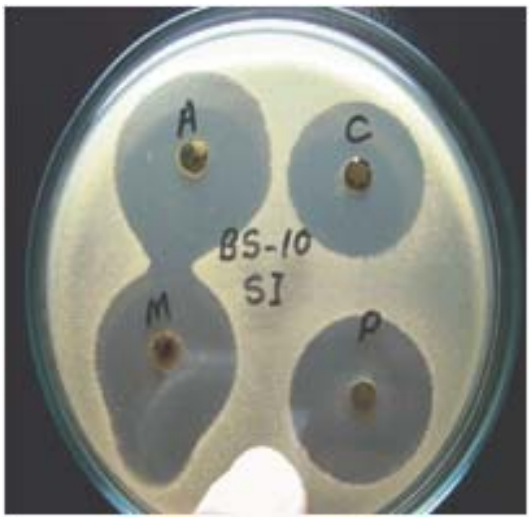
Activity against *Bacillus cereus*
Acetone extract (A)-24 mm
Chloroform extract (C)-18 mm
Methanol extract (M)-21 mm
Petroleum ether extract (P)-20 mm

Fig. 1:



Activity against *Bacillus megaterium*
Acetone extract (A)-24 mm
Chloroform extract (C)-18 mm
Methanol extract (M)-15 mm
Petroleum ether extract (P)-17 mm

Fig. 2:



Activity against *Bacillus cereus*
Acetone extract (A)-32 mm
Chloroform extract (C)-28 mm
Methanol extract (M)-28 mm
Petroleum ether extract (P)-28 mm

Fig. 3:

Activity against *Staphylococcus aureus*
Acetone extract (A)-21 mm
Chloroform extract (C)-14 mm
Methanol extract (M)-18 mm
Petroleum ether extract (P)-15 mm

Fig. 4:

aerugenosa, Staphylococcus aureus and *Proteus vulgaris.*They also found that water extract is more active against *Escherchia coli Proteus vulgaris, Pseudomonas aerugenosa, Staphylococcus aureus* and *Bacillus cereus.*

The fact that the aqueous extract is more active than methanol extract implies that the antimicrobial action may be due to the synergistic action of different chemical constituents, some of which probably are lost upon extraction with solvent (Bibitha B *et al.*,2002).

Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent (Marjorie,M.C.1999). Acetone extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity. The demonstration of antimicrobial activity by water extracts provides the scientific basis for the use of these plants in the traditional treatment of diseases, since most traditional medicine men use water as their solvent in which the decoctions are prepared.In our study fresh leaves juice and acetone extracts showed highest antimicrobial activity against Gram positive microorganisms.

Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava,J.*et al.,*1996, Reuben, K.D.,2008). Natural products either extract or pure compounds provide unlimited opportunities for the development of new drugs due to the availability of chemical diversity(Cos, P., *et al.,*2006). To overcome the problem of antibiotic resistance ethnic medicinal plants have been extensively studied as an alternative treatment for diseases due to their ability to produce a variety of compounds of known therapeutic properties (Harbone, S.B.*et al.,*1995& Kumar, B.,*et al.,*2007) and much attention has been paid to plant extracts and their biologically active compounds (Suresh,G.*et al.,*2010). The screening of natural products has been the source of innumerable therapeutic agents (Korosecchviz,J.I.*et al.,*1992) . Higher plants as a source for new potential drugs is still largely unexplored and only a small percentage of them has been subjected to phytochemical investigation and the fractions submitted to pharmacological screening is very low. Such screening of various natural organic compounds and identifying active agents is a need of the hour as due to successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay of later in drug development.

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REFERENCES

1. Acharya S,Dash G.K., Brahma D.K., Chhetree R.R. Preliminary phytochemical investigation and anthelmintic activity of Acacia suma (Roxb)barks .IRJP **2**(1): 136-141 (2011).
2. Anonymous,*Wealth of India ,A Dictionary* of *Indian Raw Materials and Industrial Products*,National Institute of Science Communication and information Resources, CSIR, New Delhi (2006).
3. Behari,M; C. K. Andhiwal, J. A. Ballantine. *Ind. J. Chem.,* **15**B: 765 (1977).
4. Bibitha B, Jisha VK, Salitha CV, *et al.,* Antibacterial activity of different plant extracts. *Indian J Microbiol* **42**(4): 361-363 (2002).
5. Cos, P., Vilietinck, A J., Vanden Berghe,D., and Maes, L., 2006. Anti-infective potential of natural products: how to develop a stronger *in vitro* proof-of concept. Journal of Ethanopharmacology,**106**: 290-302 (2006).
6. Dabur R., Gupta,A.; Mandal, T K; Singh* D. D.Singh, Bajpai#,V.; Gurav,A.M. Lavekar, G.S,; *AfricanJ.Traditional, Complementary and Alternative Medicines.* **4**(3): 313-318 (2007).
7. Harbone, S.B. and Baxer, H., 1995.Phytochemical dictionary. A handbook of bioactive compounds from plants.Taylor and Francis, London.
8. Hidayathula S.; Chandra K.K.; Chandrashekar K.R.; Phytochemical evaluation and antimicrobial activity of Pterospermum diversi folium blume. *Int J Pharma Pharma Sci.,* **3**(2):165-167 (2011).
9. Indrani N, K. Balasubramanian, *Leather Sci.,* **31**: 349 (1984).
10. Kamboj, V. P., *Current Science.,* **78**: 35-39 (2000).
11. Kaur, J. D., Misra, K., *J. Indian Chem. Soc.*, **57**: 1243 (1980).
12. Khare.C.P.; Indian Medicinal Plants. An Illustrated Dictionary. Springer Science, Springer-Verlag Berlin/Heidelberg (2007).

13. Korosecchviz, J. I., and Howe-Grant, M.,1992. Kirk-othmer Encyclopedia of Chemical Technology, 2: 893 632.
14. Kumar, B.,Vijaykumar, M., Govindarajan, R. and Pushpangadan, P., Ethnopharma-cological approaches to wound healing-Exploring medicinal plants of India. Journal of Ethnopharmacology, **114**: (2):103-113 (2007).
15. Marjorie M .C. Plant products as antimicrobial agents. *Clin Microbiol Rev*, **12**(4): 564-582 (1999).
16. Maruthappan, V., Shree, K. S., J. *Pharm. Res.*, **3**: 17-20 (2010).
17. Middelkoop, T.B.; R. P. Labadie, Z. Naturforsch B: *Anorg Chem, Org. Chem.,* **40**B: 855 (1985).
18. Nayak, S.; Sahoo, A.M., *Int. J. Pharm. Res. and Dev*. **3**(3): 160-163 (2011).
19. Pal, S. C, Maiti, A. P., Chatterjee, B. P., Nandy,A., *Indian J. Med. Res.*, **82**: 188-189 (1985).
20. Parekh J, Chanda S. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk J Biol* 31: 53-58, 2007.
21. Perez C, Paul M, Bazerque P. Antibiotic assay by agar well diffusion method. *Acta Bio Med Exp* 15: 113-115, 1990.
22. Pradhan, P.; L. Joseph; V.Gupta; R.Chulet; H.Arya; R.Verma;and A.Bajpai;Saraca asoca (Ashoka:A Review.*J.Chem.and Pharm. Res.* **1**(1): 62-71 (2009).
23. Preethi, F., Fernandes, J., Pricilla, K., *J. Pharm. Res.* **3**: 491-493 (2010).
24. Reuben, K.D., Abdulrahman, F.I., Akan,J.C., Usman, H., Sodipo, O.A. and Egwu, G.O., Phytochemical screening and *in vitro* antimicrobial investigation of the methanolic extract of *Croton zambesicus* Muell. ARG. stem bark. *Eur. J. Sci. Res.*,**23**: 134-140 (2008).
25. Sannomiya, M., Cardoso, C. R. P., Figueiredo,M. E., Rodrigues, C. M., dos Santos, L. C., dos Santos, F. V., Serpeloni, J. M., Colus, I. M. S.,Vilegas, W., Varanda, E. A., *J.Ethnopharmacol.*, **112**: 319-326 (2007).
26. Satyavati, G. V., Prasad, D. N., Sen, S. P.,*Indian J. Med. Res.* 1970, 58, 660-663.
27. Sainath, R. S., Prathiba, J., Malathi, R., *Eur. Rev. Med. Pharmacol. Sci.* **13**: 371-374 (2009).
28. Sadhu, S. K., Khatun, A., Phattanawasin, P.,Ohtsuki, T., Ishibash, M., *J. Nat. Med.*, **61**: 480-482 (2007).
29. Seetharam N, Sujeetha H, Jyothiswaran G, Barad A, Sharanbasappa G, Praveen S, Antibacterialactivity of Saraca Asoka Bark, *Ind. J. Pharm. Sci*., **65**(6): 658-659 (2003).
30. Shahidi Bonjar GH. Evaluation of antimicrobial properties of Iranian medicinal plants against *Micrococcus luteus, Serratia marcescens, Klebsiella pneumoniae* and *Bordetell bronchiseptica. Asian J Plant Sci* **3**: 82-86 (2004).
31. Shihabudeen HMS,Priscilla D H, Thirumurugan K.Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. *Int J Pharma Sci Res* **1**(10): 430-434 (2010).
32. Shilpakala S.R;Pratibha J.;Malathi R.Antimicrobial properties of the stem bark of Saraca indica (Caesalpiniaceae). *Europen review for Medical and Pharmacological Sciences*.,**13**: 371-374 (2009).
33. Shelar, D.B, P.J.Shirote, N.S.Naikwade. Anti-inflammatory activity and brine shrimps lethality test of Saraca Indica Linn leaves extract. *J. Pharm. Res.* **3**(8): 2004-2006 (2010).
34. Srivastava, J., Lambert, J. and Vietmeyer,N., Medicinal plants: An expanding role in development. World Bank Technical Paper, No. 320 (1996).
35. Srivastava G.N.Bagchi G.D. Srivastava A.K.Pharmacognocy of Ashoka stem bark and its adulterants. *Int J Crude Drug Res* **26**(2): 65 (1988).
36. Suresh, G., Ramesh, B., Kavitha, K., Ravichandran, N., R., Suresh, A., Gopalakrishnaan, V. and Vijaiyan Siva,G., Preliminary screening of antibacterial compounds from Palar river basin flora. *Journal of Phytology,* **2**(2): 24-29 (2010).
37. Vijai, L., J. S. Chauhan, *J. Ind. Chem. Soc.,* 53 (1976).