Research Article [Bhagat & Kulkarni, 4(12): Dec., 2013]
CODEN (USA): IJPLCP **ISSN: 0976-7126**

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Phytochemical evaluation and *In vitro* Antimicrobial activity of *Jatropha glandulifera* Roxb. (Family Euphorbiaceae)

Rani B. Bhagat[1]* and D. K. Kulkarni[2]
1, Botany Department, Anantrao Pawar College, Pirangut Tal.-Mulshi, Pune, (MH) - India
2, BAIF Development Research Foundation,
Dr. Manibhai Desai Nagar, Warje, Pune, (MH) - India

Abstract

In vitro antibacterial activity of *J. glandulifera* Roxb. was tested against nine bacterial strains. The successive extraction of leaves and roots was carried out in solvents like petroleum ether, ethanol and methanol. Qualitative phytochemical analysis showed primary and secondary metabolites like- alkaloids, carbohydrates, fats, flavonoids, lignin, lipids, polyphenols, proteins, starch, saponins tannins. Microorganisms responsible for various infectious diseases especially diarrhoea and dysentery was screened. Zone of inhibition were measured and compared with standard values of antibiotics, tannic acid and quercetin. Maximum activity was recorded in order of methanol < ethanol < pet ether extracts. The extract of roots in methanol displayed potent antimicrobial activity against the major organisms causing diarrhoea, dysentery and other. Thus, the resultant anti-microbial activity may be due to presence of secondary metabolites like- tannins, flavonoids etc.

Key-Words: *J. glandulifera*, Phytochemical; HPTLC, Antimicrobial activity, MIC

Introduction

Jatropha species are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America [1-2]. *J. glandulifera* a small bushy shrub with yellowish-green flowers and glandular hairs in axils of petiole are commonly found all over India. In Sanskrit it is known as 'Nikumba' and in marathi 'Janglierand'. In ayurveda and ethno-medicine it is reported for various therapeutic actions like-analgesic properties, inflammation, in asthama and for bronchitis. It extensively reported as emmenagogue, as analgesic; in inflammation, asthma, bronchitis and used as antidote to scorpion stinge. The roots were reported to cure piles [3-6]. The viscid sap (latex) and leaf used in warts, tumors and for wound healing [7]. Ethanolic extract of aerial part was found to be effective against Ranikhet disease [8]. It was reported to be used as antirheumatic, antiparalytic, in chronic ulcers, ringworm, stomach disorders. Biological activity of aerial part was investigated for cancer and viral infections, while root and seed oil as purgative [9]. A new class of wound healing agents Alkannins and Shikonins (A/S) chiral-pairs of naturally occurring isohexenylnaphthazarins are reported from roots [10-11].

*** Corresponding Author**
E.mail: rb_botany@rediffmail.com
dilipkkulkarni@gmail.com

An investigation of coumarino-lignan, along with jatropholone-A and fraxetin from the roots were reported [12]. It was also found to be anticancerous against epidermoid carcinoma of nasopharynx in tissue culture and P 388 lymphatic leukemia in mice [13].
Thus, present work was designed to investigate the phytochemical analysis and antimicrobial properties of *J. glandulifera* by collecting the leaves and roots from different localities in Maharashtra.

Material and Methods

***Plant materials*:** Plant materials were collected from Sholapur districts of Maharashtra State. Their identity was confirmed using regional floras and a voucher specimen were deposited at the Agharkar Herbarium of Maharashtra Association (AHMA), at Agharkar Research Institute (ARI), Pune, India with voucher number AHMA-22484.

Extraction

Leaves and roots of well-matured plants were shade dried at room temperature, coarsely powdered and subjected to successive solvent extraction with petroleum ether (60-80°C), ethanol and methanol by cold maceration method. Extracts were concentrated for further studies at reduced temperature and pressure using rotary evaporator. Yields of petroleum ether, ethanol and methanol extracts were given in Table-2 respectively.

Phytochemical Analysis:

Qualitative Analysis:

Qualitative phytochemical analysis of the crude extract(s) was determined to detect the presence of plant secondary metabolites following standard methods- alkaloids, starch, flavonoids, glycosides, amino acids, carbohydrates, proteins, tannins, saponins, ketoses steroides, anthroquinones, mucilage, pentose sugar, reducing sugars [14-15].

Quantitative Estimation:

Quantitative physicochemical evaluation like- percentage of total ash, acid insoluble and water insoluble ash, and petroleum ether, ethanol, methanol and water soluble extractive were calculated as per Indian Pharmacopoeia, carbohydrate (%), crude fibre(%), starch (%),, protein (%) and nitrogen content (%), total alkaloids (%), total flavonoids (%), polyphenols, tannins (Table 3) were determined[14, 16].

High Performance thin layer chromatography (HPTLC) analysis

Powdered (1 g) material each of leaves and roots was extracted with 30 ml methanol thrice. The extract was concentrated under reduced temperature and pressure using rotary evaporator and yield 0.416 and 0.428 g. Known quantity of extract was dissolved in methanol and used as test solution for HPTLC. HPTLC of JGL-L was carried out on pre-coated silica gel plate (Merck 60 F 254) as stationary phase and PE: ACE (70:30) as mobile phase and JGL-R was carried out in PE: ACE (74:26). The extract was spotted using Camag Linomat IV spotter. These plates were sprayed with methanolic sulphuric acid and scanned at 254 and 366 nm using densitometer-TLC Scanner III, employing "CATS" software (Camag, Switzerland). Eight major spots in leaves 254 nm and seven at 366 nm at R_f 0.10, 0.21, 0.27, 0.39, 0.56, 0.59, 0.62, 0.91 and 0.05, 0.10, 0.20, 0.24, 0.27, 0.33, 0.41 respectively were recorded. While in root eight major spots at 254 and seven at 366 at R*f* 0.04, 0.08, 0.15, 0.22, 0.30, 0.35, 0.46, 0.53, 0.63, 0.86, 0.93, 0.99 and 0.07, 0.10, 0.16, 0.20, 0.33, 0.45, 0.52 respectively, were recorded.

Microbial cultures and growth conditions

Microorganisms

Microbial cultures used were obtained from Microbial Sciences Division of Agharkar Research Institute (ARI), Pune, and National Chemical Laboratory (NCL), Pune which includes- *Bacillus cereus* (+ ve) MCMB-817, *Staphylococcus aureus* (+ ve) MCMB-818, *Escherichia coli* (-ve) MCMB-813, *Enterococcus fecalis* (+ ve) MCMB-812, *Pseudomonas aeruginosa* (-ve) MCMB-816, *Shigella sonnei* (-ve) MCMB-815, *Klebsiella pneumoniae* (-ve) NCIM-5082, and *Shigella dysentrae*. (-ve) Microbial cultures were grown on Mueller-Hinton (MH) agar, Octo discs (O. D. 019), (HiMedia) at 37 °C for 12–14 h. They were maintained at 4 °C in the laboratory.

Antimicrobial assay

Bioassay:

Antimicrobial activity was screened using agar well diffusion technique [14]. An eighteen h culture of each microorganism was diluted in sterile saline with 10^5/mL colony forming unit (CFU) and used for assay. 100 µl of above culture was inoculated in plates containing Mueller-Hinton (MH) agar and spread evenly using sterile glass spreader. These plates were incubated at 37º C for 30 min. The wells were prepared by sterile cork borer with 6 mm diameter. All the plates were kept at 5°C for 30 min for diffusion. Each well was loaded with different concentrations of samples along with control (DMSO) and nalidixic acid (30 µg), Gentamycin (10 µg) and Tetracyclin (100 µg) was used as positive control. Plates were then incubated at 37°C for 24 h and zones of inhibition were recorded. All the experiments were done in Triplicate. The diameters of zones of inhibition (mm) are expressed as Means and Standard errors on Means.

(Table 4-5).

Minimum Inhibitory Concentration (MIC):

The MIC of the crude extracts was determined by broth dilution method. The highest concentrations of extracts were 50 mg/mL, dissolved in dimethyl sulphoxide (DMSO). 100 µl of logarithmic phase test culture was diluted in sterile physiological saline (0.9 % NaCl) having $1x10^5$ CFU under aseptic condition. Culture was inoculated in the Petri dishes containing MH agar and incubated at 37º C for 30 min. The different concentrations of extracts were added in the wells and observed to find out the MIC values for each organism after 24 h incubation. (Table 6).

Results and Discussion

The yield of the respective extracts and the results of the phytochemical screening are presented (Table 3). Extracts with their level of activity against the various organisms were given (Table 4-5). In present investigation six extracts of leaves and roots of JGO were screened for antimicrobial activity. *Bacillus* species are common microbes found in most natural environments including soil, water, plant and animal tissues. *Bacillus* species are regarded as having little pathogenic potential, which act as primary invaders or secondary infectious agents in a number of diseases and have been implicated in some cases of food poisoning[17].

In present investigation it was observed that *B. cereus* was the first most inhibited bacterial strain followed by *S. dysentrae* and *E. fecalis* with six plant extracts

completely inhibiting its growth. JGL-L–PE (6), EtOH (6) and MeOH (7) shows activity at 100 µg/ml and 200 µg/ml (values in brackets indicate number of extracts inhibiting the growth of bacteria) and are found to be promising against all organisms which are responsible for disease causing diarrhoea and dysentery. JGL-R-PE, EtOH, MeOH, inhibited the growth of all nine bacterial strains completely at 100 µg/ml and 200 µg/ml concentrations respectively.

Antimicrobial extracts from tested plants can be assumed to be useful in warding off infectious diseases and there is therefore a compelling reason to suppose that anti-infective agents could be active against human pathogens as was suggested by folkloric and historical accounts [18-19]. *S. sonnei* is the predominant species in industrialized countries [20-21]. Infections caused by *Pseudomonas aeruginosa* are among the most difficult to treat with conventional antibiotics [22]. The growth of *P. aeruginosa* was completely inhibited by all extracts at 100 µg//ml and 200 µg/ml concentrations. These plants may thus, be a source of drugs that could improve the treatment of infections caused by this organism. The root MeOH extracts were the most potent with activities comparable to that of Nalidixic acid, Gentamycin and Tetracyclin (Table-8). The common secondary metabolites like polyphenols, alkaloids and tannins are present in both extracts (Table-3). The MeOH extracts are richer in these metabolites than PE and EtOH, which may be due to the high polarity of MeOH, hence its ability to extract more components. It is noteworthy that the MeOH extract of the root showed potent broad-spectrum activity against all the microorganisms. The secondary metabolites are known to be biologically active. Tannins have been found to form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. Tannins have important roles such as stable and potent antioxidant[23] and for treating diarrhoea and dysentery[24]. The alkaloids the largest groups of chemical produced by plants have many biological activities. Therefore the antibacterial activities of extracts are expected. Flavonoids are phenolic structures containing one carbonyl group complexes with extra-cellular and soluble protein and with bacterial cell wall, exhibiting antibacterial activity [25]. Flavonoids have been reported to inhibit intestinal motility and secretion [26], they may presumably exert an antidiarrhoeal action [27]. It was confirmed by the comparison with quercetin and tannic acid (Table 7). It showed that antibacterial activity of *Jatropha* species was mainly due to presence of tannins. Previous work [2, 28-31] has shown that many *Jatropha* species possess antimicrobial activity, but this is the first report of antimicrobial activity of the extracts of *J. glandulifera* against bacteria causing diseases like diarrhoea, dysentery etc.

Conclusion

Study has revealed the presence of many secondary metabolites in the leaves and roots of *J. glandulifera*. The result also explains its ethno-botanical and indigenous use in a range of medicines. This probably explains the use of these plants in diverse infections since generations for treatment of various diseases as broad-spectrum antimicrobial agents. The antimicrobial activity of tannic acid and quercetin explains the role of secondary metabolites in inhibition. Based on these exploratory studies it is evident that it could be exploited for novel antibiotics against several microbial infections.

Acknowledgement

The authors are thankful to Director, ARI, Pune for facilities, Dr. K. Banerjee, President- MACS, Pune for laboratory facilities, Dr. P. K. Dhakephalkar for providing microbial cultures and Head, Botany Group, ARI for support.

References

1. Chopra R.N., Nayar S.L., Chopra I.C. (1956). Glossary of Indian Medicinal Plants PID, New Delhi 145.
2. Aiyelaagbe O.O., Adeniyi B.A., Fatunsisn O.F., Arimah B.D. (2007). *In vitro* Antimicrobial activity and phytochemical analysis of *Jatropha curcas* roots Int J Pharmacol 3(1): 106-10.
3. Kirtikar K.R., Basu B.D. (1984). Indian Medicinal Plants Vol III Beshen Singh Mahendra Pal Singh, Dehera Dun. 2241-47.
4. Desai G.V. (1927): The Materia Medica & Therapeutics of Indian Medicinal plants p.117.
5. Sawant, S.Y. (1974). Maharashtratil Divya Vanaushadi P.133
6. Bhagat R.B., Kulkarni D.K. (2011). Antiinflammatory activity of *Jatropha gossypifolia* L. leaves in albino mice and Wistar rats J Sci Indust Res 70: 289-92.
7. Daniel M. Medicinal Plants-Chemistry and Properties 2006; 95-96.
8. Babbar O.P., Joshi M.N., Madan A.R. (1982). Evaluation of plants for antiviral activity Ind J Med Res 76: 54.
9. Akhtar Husain; Virmani O.P., Popli S.P., Misra L.N., Gupta M.M., Srivastava G.N., et al. (1992). Dictionary of Indian Medicinal plants, *CSIR, New Delhi 263-64.*

10. Papageorgiou V.P. (1980). Naturally occurring isohexenylnaphthazarin pigments: a new class of drugs. Planta Med 38: 193–203.
11. Papageorgiou V. P, Assimopoulou A.N., Ballis A.C. (2008). Alkannins and shikonins: a new class of wound healing agents. Curr Med Chem 15(30):3248-3267.
12. Parthasarathy M.R., Saradhi K.P. (1984). A coumarino-lignan from *Jatropha glandulifera* Phytochem 23: 867-69.
13. Bhukuni D.S., Dhar M.L., Dhar M.M., Dhawan B.N., Gupta B., Srimal R.C. (1971). Screening of Indian plants for biological activity Part III. Ind Exp Biol 9: 91.
14. Bhagat R.B., Kulkarni D.K. (2010). *In vitro* Antimicrobial Activity, Lipid Content and Physico -Chemical Analysis of Non-Edible Oils Asi J Chem 9(22): 6884-90
15. Harborne J.B. (1973). Phytochemical Methods: A guide to Modern Techniques of plant Analysis. Chapman A ad Hal, London.
16. Sadasivam S., Manikam A. Biochemical Methods. (Rev. II Edi.) Pub. New Age International Publishers, New Delhi. 1996.
17. Turnbull P.C.B., Kramer J.M. (1996). *Bacillus*. In: A Barlows, Hausler, WJ Jr., Herrmann, KL, Isenberg, HD, Shadomy, H.J. Editors, Manuals of Clinical Microbiology (5th ed.), *American Society of Microbiology, Washington DC 345–55*.
18. Kirtikar K.R., Basu B.D. Indian Medicinal Plants, Vols. I and II. Lalit Mohan Basu, Allahabad, India. 1968.
19. Nadkarni K.M. (1976). Indian Materia Medica Vol. I Popular Prakashan, Bombay. 704-07.
20. Alcoba-Flórez J., Pérez-Roth1 E, González-Linares S, Méndez-Álvarez S. (2005). Outbreak of *Shigella sonnei* in a rural hotel in La Gomera, Canary Islands, Spain Int Microbiol (8): 133-36.
21. Preston M.A., Borczyk A.A. (1994). Genetic variability and molecular typing of *Shigella sonnei* strains isolated in Canada. J Clin Microbiol 32: 1427-30.
22. Levison W.E., Jawetz E. (1992). Medical Microbiology and Immunology, 2nd ed. Appleton and Lange, New York.
23. Trease G.E., Evans W.C. (1983). Text book of Pharmacognosy. 12th Edn., Balliere, Tridall, London 55-59.
24. Dharmananda S. Gallnuts and the uses of tannins in Chinese medicine: A paper Delivered at Institute for Traditional Medicine, Portland, Oregon. 2003.
25. Cowan M.M. (1999). Plant products as antimicrobial agents. Clin Microbiol Rev 12: 564-82.
26. Di Carlo G., Autore G., Izzo A.A., Maiolino P., Mascolo N., Viola P., et al. (1993). Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure-activity relationships. J Pharm Pharmacol 45 (12): 1054–59.
27. Rao V.S.N., Santos F.A., Sobreira T.T., Souza M.F., Melo C.L., Silveira E.R. (1997). Investigations on the gastroprotective and antidiarrhoeal properties of ternatin, a tetramethoxyflavone from *Egletes viscosa* Planta Med 63: 146-49.
28. Dekker T.G., Mathee E.F., Snyckers F.O. (1986**).** Studies of South African medicinal plants part 4, Jaherin, a new daphene diterpene with antimicrobial properties from *Jatropha zeyher* South Afr J Chem 40: 74-76.
29. Aiyelaagbe O.O. (2001). Antibacterial activity of *Jatropha multifida* roots. Fitoterapia, 72: 544-46.
30. Ogundare A.O. (2007). Antimicrobial effect of *Tithonia diversifolia* and *Jatropha gossypifolia* leaf extract Trends in Appli Sci Res 2 (2): 145-50.
31. Neogi U., Saumya R., Mishra R.K., Raju K.C. (2008). Lipid content and *in vitro* Antimicrobial activity of oil seeds of some Indian Medicinal Plants Curr Res Bacteriol 1(1): 1-6.

Table 1: Qualitative analysis of JGL-L and JGL-R

S/No.	Name of phytoconstituents	JGL-L	JGL – R
1	Carbohydrates	+	+
2	Proteins	+	+
3	Fats	+	+
4	Starch	+	+
5	Tannins	+	+
6	Polyphenols	+	+
7	Alkaloids	+	+
8	Flavonoids	+	+
9	Saponins	+	+
10	Steroids	_	-
11	Lignins	-	+
12	Glycosides	-	+

+Present, - Absent

Table 2: Percent Extractive values of leaves and roots of JGL

Name of the solvent	JGL-L	JGL-R
Hexane	1.709 ± 0.085	1.17 ± 0.026
Pet ether	1.437 ± 0.121	1.081 ± 0.039
Chloroform	2.345 ± 0.023	1.63 ± 0.036
Ethyl acetate	2.23 ± 0.109	1.29 ± 0.056
Acetone	2.258 ± 0.090	1.906 ± 0.054
Methanol	4.115 ± 0.045	4.39 ± 0.05
Ethanol	1.895 ± 0.027	2.314 ± 0.020
Water	4.564 ± 0.235	3.24 ± 0.057

Table 3 Quantitative estimation of phytoconstituent *in J. glandulifera*

S/No.	Name of phytoconstituents	JGL-L	JGL – R
1	Ash (%)	16.72 ± 0.13	8.16 ± 0.37
2	Acid insoluble ash (%)	8.01 ± 0.11	5.77 ± 0.34
3	Water insoluble ash (%)	5.77 ± 0.34	5.77 ± 0.34
4	Crude fiber (%)	0.82 ± 0.01	0.88 ± 0.02
5	Carbohydrates (%)	14.58 ± 14.58	23.42 ± 1.42
6	Proteins (%)	16.61 ± 0.03	2.23 ± 0.03
7	Nitrogen (%)	2.66 ± 0.01	0.47 ± 0.002
8	Starch (%)	2.21 ± 0.29	3.06 ± 0.50
9	Tannins (%)	3.19 ± 2.47	3.45 ± 2.46
10	Polyphenols (IC 50 µg)	41.68 ± 2.5	17.93 ± 1.59
11	Alkaloids (%)	1.22 ± 1.56	0.17 ± 1.2
12	Flavonoids (IC 50 µg)	12.02	16.56

L- Leaves R-Root

Table 4: Antimicrobial activity of petroleum ether, ethanol, and methanol extracts of JGL-L

NI-No Inhibition ND- Not Determined (Zone of inhibition in mm)									
Petroleum ether									
Conc. µg	*Bc*	*Sa*	*Ec*	*Ss*	*Efs*	*Pa*	*Kp*	*Sd*	*Sf*
100	3.9 ± 0.1	NI	NI	NI	5.4 ± 0.1	3.5 ± 0.2	NI	3.4 ± 0.03	NI
200	5.1 ± 0.1	NI	4.1 ± 0.1	NI	6.5 ± 0.1	5.0 ± 0.1	4.1 ±0.01	4.5 ± 0.03	NI
Ethanol									
100	4.0 ± 0.2	NI	NI	NI	5.1 ± 0.1	4.1 ± 0.1	4.1 ±0.01	3.5 ± 0.01	NI
200	6.0 ± 0.1	NI	3.1 ± 0.1	NI	6.4 ± 0.1	5.1 ± 0.1	5.2 ± 0.1	4.5 ± 0.03	NI
Methanol									
100	4.1 ± 0.2	NI	3.5 ± 0.1	NI	4.1 ± 0.1	NI	NI	3.4 ± 0.01	NI
200	5.3 ± 0.1	NI	4.5 ± 0.1	4.5 ± 0.1	5.0 ± 0.1	4.1 ± 0.1	5.1 ± 0.1	4.6 ± 0.01	NI

Table 5: Antimicrobial activity of Petroleum ether, Ethanol and Methanol extracts of JGL-R

NI-No Inhibition ND- Not Determined (Zone of inhibition in mm)									
Petroleum ether									
Conc. µg	*Bc*	*Sa*	*Ec*	*Ss*	*Efs*	*Pa*	*Kp*	*Sd*	*Sf*
100	9.2 ± 0.2	7.0 ± 0.2	NI	6.1 ± 0.1	6.5 ± 0.1	4.5 ± 0.2	NI	4.1 ± 0.03	NI
200	13.0 ± 0.3	9.1 ± 0.2	3.1 ± 0.1	8.4 ± 0.2	8.1 ± 0.1	5.5 ± 0.1	NI	4.7 ± 0.1	NI
Ethanol									
100	12.1 ± 0.1	8.2 ± 0.2	NI	6.1 ± 0.1	6.5 ± 0.1	4.5 ± 0.2	NI	4.1 ± 0.01	NI
200	15.0 ± 0.2	10.1± 0.1	3.1 ± 0.1	8.5 ± 0.1	8.1 ± 0.1	5.5 ± 0.1	4.6 ± 0.01	6.1 ± 0.01	NI
Methanol									
100	9.0 ± 0.2	7.1 ± 0.1	NI	NI	6.0 ± 0.2	4.4 ± 0.1	NI	3.5 ± 0.03	4.1 ± 0.1
200	11.1 ± 0.1	9.1 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	8.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.01	5.2 ± 0.1	5.1 ± 0.1

Bc-Bacillus cereus, Sa-Staphylococcus aureus, Ec-Escherichia coli Efs-Enterococcus fecalis, Pa-Pseudomonas aeruginosa, Ss-Shigella sonnei, Kp-Klebsiella pneumoniae, Sf- Streptococcus fecalis, Sd-Shigella dysentrae

Table 6: Minimum Inhibitory Concentration (MIC) of *J. glandulifera* (µg)

Name of Species	***Bc***	***Sa***	***Ec***	***Ss***	***E f***	***Sf***	***Pa***	***Kp***	***Sd***
JGL-L-PE	70	250	220	150	50	50	100	200	80
JGL-L-EtOH	50	230	150	ND	60	ND	80	100	90
JGL-L-MeOH	60	270	100	ND	70	ND	200	150	70
JGL-R-PE	10	10	220	100	50	50	200	350	100
JGL-R-EtOH	10	10	220	50	40	ND	90	130	70
JGL-R-MeOH	10	10	100	140	30	40	50	150	100

PE-Petroleum ether, EtOH-Ethanol, MeOH-Methanol

Table 7: Antimicrobial activity of standard Tannic acid and Quercetin

Tannic acid (Zone of inhibition in mm)									
Conc. µg	*Bc*	*Sa*	*Ec*	*Ss*	*Efs*	*Pa*	*Kp*	*Sd*	*Sf*
100	NI	6.0 ± 0.1	5.0 ± 0.1	3.5 ± 0.1	3.9 ± 0.1	7.0 ± 0.1	4.1 ± 0.1	4.6 ± 0.1	NI
200	NI	6.9 ± 0.1	5.5 ± 0.1	4.1 ± 0.1	5.0 ± 0.1	7.5 ± 0.1	6.5 ± 0.03	6.5 ± 0.03	NI
Quercetin									
100	5.1 ± 0.1	5.0 ± 0.1	4.0 ± 0.1	NI	NI	NI	NI	NI	NI
200	6.1 ± 0.1	6. ± 0.1	4.2 ± 0.2	NI	NI	NI	NI	NI	NI

Table 8: Antimicrobial activity against standard antibiotics (zone of inhibition in mm)

Std. antibiotic conc. (µg)	*Bc*	*Sa*	*Ec*	*Ss*	*Efs*	*Pa*	*Kp*	*Sd*	*Sf*
Nalidixic acid (30 µg)	15.7	15.8	12	15.6	9.8	9.0	14.4	14.0	15.8
Gentamycin (10 µg)	15.7	15	11	15.1	NI	10.9	14.8	10	15.8
Tetracyclin (100 µg)	15.8	15.7	14	8	NI	14.1	NI	5	14.5