

Full Length Research Paper

# ***In vitro* screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae**

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Thirty four medicinal plants, belonging to twenty eight different families, were screened for potential antibacterial activity against six bacterial strains belonging to Enterobacteriaceae, viz. *Enterobacter aerogenes* ATCC13048, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* NCIM2719, *Proteus mirabilis* NCIM 2241, *Proteus vulgaris* NCTC8313, and *Salmonella typhimurium* ATCC23564. Antibacterial activity of aqueous and alcoholic extracts was tested by the agar disc diffusion and agar well diffusion methods. The ethanol/methanol extracts were more active than aqueous extracts for all the plants studied. The most susceptible bacterium was *K. pneumoniae*, while the most resistant bacteria were *S. typhimurium* and *E. coli*. From the screening experiment, *Woodfordia fruticosa* Kurz. showed best antibacterial activity. Hence, this plant may be used further to isolate and evaluate the therapeutic antimicrobials.

**Key words:** Medicinal plants, antibacterial activity, aqueous extracts, alcoholic extracts, Enterobacteriaceae.

## INTRODUCTION

Infectious diseases are the leading cause of death worldwide. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow et al., 2003). Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and resistance to old and newly produced drugs is on the rise. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio 1996; Scazzocchio et al., 2001). There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants (El-Seedi et al., 2002; Rojas et al., 2003; Duraipandiyani et al., 2006; Parekh and Chanda, 2007a).

Risk factors for nosocomial Enterobacter infections include the prior use of antimicrobial agents, a prolonged hospital stay, a serious underlying illness, and immunosuppression. From a clinical point of view, *Klebsiella*

*pneumoniae* is the most important member of the *Klebsiella* genus of Enterobacteriaceae and it is emerging as an important cause of neonatal nosocomial infection (Gupta et al., 1993). *Escherichia coli* causes septicemia and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs especially in debilitated and immunodeficient patients (Black, 1996). Infection caused by *Salmonella typhimurium* is a serious public health problem in developing countries and represents a constant concern for the food industry (Mastroeni, 2002). *Proteus mirabilis* causes wound infections and urinary tract infections in the elderly and young males often following catheterization or cystoscopy, and it is a secondary invader of ulcers, pressure sores, etc. (Cheesbrough, 2000).

There are various reports in the literature regarding characterization of medicinal plant extracts that may inhibit the above mentioned bacteria. For example, the antibacterial potential of *Mesua ferrea* Linn. flowers has been reported (Mazumder et al. 2004), and organic solvent extracts of *P. commutata* showed inhibitory activity against *E. coli*, *Enterobacter aerogenes* and *K. pneumoniae* (Ilhan et al., 2006). The methanol extract of *Phyllanthus amarus* inhibited *E. coli* and *S. typhimurium*

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Table 1. Ethnobotanical information of some plant species screened.

Botanical name (family, genus, species)	Vernacular name	Habit	Voucher number	Part(s) extracted	Action/Therapeutic use
<b>AMARANTHACEAE</b>					
<i>Celosia argentea</i> L.	Lambadi	Herb	PSN645	Whole	anpy, aphro, bl dis, dia, gon, infl, sor
<b>ASCLEPIADACEAE</b>					
<i>Tylophora indica</i> (Burm.f.) Merr.	Damnivel	Climber	PSN462	Leaf	car, dip, em, expec, pur, stm, ath, bron, dia, dys, dyssep, fla, gou, ul, wo
<b>ASTERACEAE</b>					
<i>Vernonia anthelmintica</i> (L.) Willd.	Kalijiri	Herb	PSN415	Whole	fev, ath, co, ul, sk, leucd, lep, dyssep, infl, ast, anth, exp, dm, diu, stm, feb, gal, ton, pur
<b>BALANITACEAE</b>					
<i>Balanites aegyptiaca</i> (L.) Del.	Engoria	Shrub	PSN112	Whole	alex, anal, anth, pur, verm, bo, bu, co, fra, leucd, sb, sk, sls
<b>BIGNONACEAE</b>					
<i>Spathodea campanulata</i> Beauv.	Kesudo	Tree	PSN563	Aerial parts	Pur, sk
<b>CAESALPINIACEAE</b>					
<i>Cassia fistula</i> L.	Garmalo	Tree	PSN250	Leaf	cat, em, feb, lax, pur, bil, bron, fev, rheu, rw
<b>CHENOPODIACEAE</b>					
<i>Beta vulgaris</i> L.	Beet	Herb	PSN654	Leaf	aphro, car, diu, emmen, exp, pur, ton, con, eac, hac, infl, itc, para, sor, ul
<i>Spinacia oleracea</i> L.	Palak ni Bhaji	Herb	-	Leaf	cat, feb, stm, infl
<b>COMMELINACEAE</b>					
<i>Commelina benghalensis</i> L.	Motishumliyu	Herb	PSN731	Whole	diu, sti, dia, fev, lep
<b>CONNARACEAE</b>					
<i>Rourea santaloides</i> (Vahl.) Wight & Arnott	Vardharo	Herb	-	Root	ton, diab, rheu, sk
<b>CONVOLVULACEAE</b>					
<i>Cressa cretica</i> L.	Paliyo	Herb	PSN496	Whole	anth, aphro, stm, ath, con, ton
<b>CRUCIFERAE</b>					
<i>Lepidium sativum</i> L.	Ashal/Aserio	Herb	PSN13	Seed	antc
<b>CUCURBITACEAE</b>					
<i>Lagenaria vulgaris</i> Seringe	Tumbada	Climber	PSN328	Fruit	ton, pur
<i>Momordica charantia</i> L.	Karela	Climber	PSN333	Fruit	anth, lax, sed, bron, co, elph, pil, ul
<i>Mukia maderaspatana</i> (L.) M.Roem.	Chadakachima	Climber	PSN335	Aerial parts	exp, sti
<b>CYPERACEAE</b>					
<i>Cyperus scarious</i> R.Br.	Nagarmoth	Herb	PSN765	Seed	aro, ast, dip, stm, dia
<b>EHRETIACEAE</b>					
<i>Cordia dichotoma</i> Forst.	Gunda	Tree	PSN472	Leaf	anth, ast, diu, dm, exp, pur, ton, co, dyssep, fev, hac, jp, rw, sb, ul
<b>EUPHORBIACEAE</b>					
<i>Ricinus communis</i> L.	Erado	Shrub	PSN699	Leaf	anth, aphro, car, cat, diu, gal, pur, ath, bron, co, con, drop, dyssep, fev, hac, infla, lep, lum, para, rheu, rw, sk
<b>FABACEAE</b>					
<i>Arachis hypogaea</i> L.	Magfali	Herb	PSN152	Leaf	ast, adp, bron, con, fla
<i>Canavalia gladiata</i> DC.	Talvardi	Climber	PSN157	Leaf	can
<i>Vigna radiata</i> L.	Mag	Herb	PSN235	Whole	aphro, dig, feb, gal, ton, co, con, dia, dyssep, fev, fla, hae, infl, lep, pyr, sk

Table 1. Contd.

<b>FUMARIACEAE</b>					
<i>Fumaria indica</i> (Hausk.) Pugsley.	Pitpopdo	Herb	-	Seed	dip, diu
<b>GUTTIFERAE</b>					
<i>Mesua ferrea</i> Linn.	Nagkesar	Tree	-	Seed	aro, ast, col
<b>LABIATAE</b>					
<i>Ocimum kilimanjaricum</i> L.	Kapurtulsi	Herb	-	Whole	col, diu
<b>LAURACEAE</b>					
<i>Cinnamomum tamala</i> Nees & Ebern.	Tamal patra	Tree	-	Leaf	car, diu, dip, gal, sti, co, dyspep, fev, fla
<b>LYTHRACEAE</b>					
<i>Woodfordia fruticosa</i> Kurz.	Dhawadi phool	Shrub	PSN303	Flower	anth, ast, em, feb, sed, sti, bil, bu, diab, hae, lep, sk
<b>MALVACEAE</b>					
<i>Thespesia populnea</i> (L.) Sol ex Correa.	Paras piplo	Tree	PSN71	Leaf	ast, col, ath, chl, co, dia, diab, dys, gon, haem, her, infl, lep, psor, rw, sca, ul, wo
<b>MORACEAE</b>					
<i>Artocarpus heterophyllus</i> Lam.	Fanas	Tree	-	Whole	abor, aphro, car, ton, bil, bo, dia, lep, sb, sk, ul, wo
<i>Ficus elastica</i> Roxb.	Rubber plant	Tree	PSN705	Leaf	-
<b>PIPERACEAE</b>					
<i>Piper longum</i> L.	Piplimul	Climber	-	Root	anth, aphro, apt car, col, lax, sti, adp, ath, bil, bron, co, fev, gou, ins, infl, jaun, lep, leucd, lum, pil, tum
<b>POACEAE</b>					
<i>Bambusa arundinaceae</i> (Retz.) Roxb.	Vans, bamboo	Tree	PSN793	Leaf	aphro, ast, col, diu, emmen, feb, lax, sti, ton, bil, bron, bu, co, dia, eac, fev, gon, jp, lep, lum, pil, rw
<b>RUBIACEAE</b>					
<i>Gardenia resinifera</i> Roth.	Dikamari	Tree	PSN351	Gum exudate	car, fla, indi, sk
<b>SAPOTACEAE</b>					
<i>Manilkara hexandra</i> (Roxb.) Dubard.	Rayan	Tree	PSN428	Leaf	aphro, col, ton, bil, bron, lep, ul, urd
<b>VITACEAE</b>					
<i>Cissus quadrangularis</i> L.	Hadsankar	Climber	PSN127	Stem	anal, fra, mup, pil, tum, ul, wo

**Key to abbreviations in Table 1.**

DISEASES						
<p><b>A</b></p> <p>abs - abscesses adp - abdominal pain aly - allergy ame - amentia amen - amenorrhoea anm - anaemia ano - anorexia arth - arthritis ath - asthma</p> <p><b>B</b></p> <p>bil - biliousness bl dis - blood diseases bo - boils bron - bronchitis bu - burns</p>	<p><b>C</b></p> <p>calc - calculi can - cancer cd - cold chl- cholera chp - chest pain co - cough con - constipation</p> <p><b>D</b></p> <p>deli -delirium den fev - dengue fever der - dermatitis dia - diarrhoea diab - diabetes diph - diphtheria drop - dropsy</p>	<p>dys - dysentery dysame - dysanenorrhoea dyspep - dyspepsia</p> <p><b>E</b></p> <p>eac-earache ecz - eczema elph - elephantiasis epi - epilepsy</p> <p><b>F</b></p> <p>fat - fatigue fev - fever fla - flatulence fra - fracture</p> <p><b>G</b></p> <p>gin - gingivitis gon - gonorrhoea gou - gout</p>	<p><b>H</b></p> <p>hac - headache hae - haemorrhage haem - haemorrhoids her - hernia hp - hydrophobia hys - hysteria</p> <p><b>I</b></p> <p>indi - indigestion infl - inflammations ins - insomnia itc - itch</p> <p><b>J</b></p> <p>jaun - jaundice jp - joint pain</p>	<p><b>L</b></p> <p>leucd - leucoderma leuch-leucorrhoea lep – leprosy lum - lumbago</p> <p><b>M</b></p> <p>mal - malaria mig - migraine mum - mumps mup - muscular pain</p> <p><b>N</b></p> <p>neu - neuralgia</p> <p><b>O</b></p> <p>ob - obesity</p> <p><b>P</b></p> <p>para - paralysis phg - pharyngitis</p>	<p>pil - piles pim - pimples pneu - pneumonia psor- psoriasis psy - psychopathy pyr - pyrexia</p> <p><b>R</b></p> <p>rheu - rheumatism rw - ringworm</p> <p><b>S</b></p> <p>sb - snake bite sca - scabies scia - sciatica sk - skin disease sls - sleeping sickness smp - small pox sor - sores</p>	<p>swe - swellings syp - syphilis</p> <p><b>T</b></p> <p>toac - tooth ache tum - tumors typh – typhoid</p> <p><b>U</b></p> <p>ul - ulcers urd - urinary disorders</p> <p><b>V</b></p> <p>vom - vomiting</p> <p><b>W</b></p> <p>wo - wounds wor - worms</p>
MEDICINAL PROPERTIES						
<p><b>A</b></p> <p>abor - abortifacient alex - alexipharmic anal - analgesic antd - antidote anth - anthelmintic anthy - antihypertensive antpr - antiperiodic</p>	<p>antpy - antipyretic antsp - antiseptic antsp - antispasmodic aphro - aphrodisiac apt - apetiser aro - aromatic ast - astringent</p>	<p><b>B</b></p> <p>b.ton - brain tonic</p> <p><b>C</b></p> <p>c.ton - cardiotonic car - carminative cat - cathartic col - coolant</p>	<p><b>D</b></p> <p>dig - digestive dip - diphoretic diu - diuretic dmu - demulcent</p> <p><b>E</b></p> <p>em – emetic</p>	<p>emmen - emmenagogue exp - expectorant</p> <p><b>F</b></p> <p>feb - febrifuge</p> <p><b>G</b></p> <p>gal - galactagogue</p> <p><b>L</b></p> <p>lax - laxative</p>	<p><b>P</b></p> <p>pec - pectoral pur - purgative</p> <p><b>R</b></p> <p>rub - rubefacient</p> <p><b>S</b></p> <p>stm - stomachic</p>	<p>sed - sedative sti - stimulant</p> <p><b>T</b></p> <p>ton - tonic</p> <p><b>V</b></p> <p>verm - vermifuge</p>

(Mazumder et al., 2006), while the flower heads and leaves of *Setaria italica* showed strong inhibition against *S. typhimurium*, *Proteus vulgaris* and *P. mirabilis* (Basile et al., 2006). Dabur et al. (2007) studied antibacterial activity of some Indian medicinal plants that could inhibit various bacterial strains, including *E. coli*, *S. typhimurium* and *P. vulgaris*. Various solvent extracts of *Aegle marmelos*, *Lawsonia inermis* and *Albizia libbeck* showed good antibacterial activity against *E. coli* and *P. vulgaris* (Sudharameshwari and Radices, 2007), whereas *Emilia coccinea* inhibited *S. typhimurium* and *E. coli* (Teke et al., 2007). Thus, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action.

Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Cos et al., 2006). Therefore, in the present study, 34 Indian plant species were screened for their antimicrobial potential against selected members of the Enterobacteriaceae.

## MATERIALS AND METHODS

### Ethno-medical information and plant collection

Fresh plant or plant parts were collected randomly from the semi arid region of Rajkot Gujarat, India. The taxonomic identities of plants were confirmed by Dr. P.S. Nagar and Dr. N.K. Thakrar, Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India with the help of Flora of Bombay. The ethno-medical information is reported in Table 1. Fresh plant material were washed with tap water, air dried and then homogenized to a fine powder and stored in air-tight bottles.

### Plant extraction

For aqueous extraction, 10 g of air-dried powder was mixed with distilled water and boiled on slow heat for 2 h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 10 min. The supernatant was collected. This procedure was repeated twice more. After 6 h, the supernatant, collected at an interval of every 2 h, was pooled and concentrated to make the final volume one-fourth of the original volume. It was then autoclaved (121°C, 15 lbs pressure) and stored at 4°C. For solvent extraction, 10 g of air-dried powder was mixed with 100 ml of organic solvent (methanol

**Table 2.** Screening of some plant species for potential antimicrobial activity

Botanical name (family, genus, species)	Extract	Inhibition Of Zone (Mean $\pm$ SEM)					
		<i>Ea</i> **	<i>Ec</i> **	<i>Kp</i> **	<i>Pm</i> **	<i>Pv</i> **	<i>St</i> **
<b>AMARANTHACEAE</b>							
<i>Celosia argentea</i> L.	H <sub>2</sub> O	-	-	3 $\pm$ 0.1	-	-	-
	EtOH	-	-	1 $\pm$ 0.0	-	-	-
<b>ASCLEPIADACEAE</b>							
<i>Tylophora indica</i> (Burm.f.) Merr.	H <sub>2</sub> O	-	-	-	-	-	-
	EtOH	-	-	3 $\pm$ 0.0	-	-	-
<b>ASTERACEAE</b>							
<i>Vernonia anthelmintica</i> (L.) Willd.	H <sub>2</sub> O	-	-	-	-	-	-
	EtOH	-	-	4 $\pm$ 0.0	-	-	-
<b>BALANITACEAE</b>							
<i>Balanites aegyptiaca</i> (L.) Del.	H <sub>2</sub> O	-	-	-	-	-	4 $\pm$ 0.4
	EtOH	-	-	2.5 $\pm$ 0.0	-	-	-
<b>BIGNONACEAE</b>							
<i>Spathodea campanulata</i> Beauv.	H <sub>2</sub> O MeOH	- 3.0 $\pm$ 0.0	-	- 3 $\pm$ 0.0	-	-	-
<b>CAESALPINIACEAE</b>							
<i>Cassia fistula</i> L.	H <sub>2</sub> O	-	-	-	-	-	-
	MeOH	-	-	3 $\pm$ 0.0	2 $\pm$ 0.0	-	-
<b>CHENOPODIACEAE</b>							
<i>Beta vulgaris</i> L. <i>Spinacia oleracea</i> L.	H <sub>2</sub> O	-	-	1 $\pm$ 0.6	-	-	-
	EtOH	-	-	1 $\pm$ 0.0	-	-	-
	H <sub>2</sub> O	-	-	-	-	-	-
	MeOH	-	-	1 $\pm$ 0.0	2 $\pm$ 0.0	-	-
<b>COMMELINACEAE</b>							
<i>Commelina benghalensis</i> L.	H <sub>2</sub> O	-	-	1.5 $\pm$ 0.3	-	-	-
	EtOH	-	-	2 $\pm$ 0.0	-	-	-
<b>CONNARACEAE</b>							
<i>Rourea santaloides</i> (Vahl.) Wight & Arnott	H <sub>2</sub> O	-	-	-	-	-	-
	EtOH	2.5 $\pm$ .0	-	6 $\pm$ 0.0	0.2 $\pm$ 0.1	-	-
<b>CONVOLVULACEAE</b>							
<i>Cressa cretica</i> L.	H <sub>2</sub> O	-	-	-	-	-	-
	EtOH	-	-	04 $\pm$ 0.0	-	-	-
<b>CRUCIFERAE</b>							
<i>Lepidium sativum</i> L.	H <sub>2</sub> O	-	-	-	-	-	-
	MeOH	-	-	-	-	-	3 $\pm$ 0.6
<b>CUCURBITACEAE</b>							
<i>Lagenaria vulgaris</i> Seringe	H <sub>2</sub> O	-	-	-	-	-	-
	MeOH	-	-	7 $\pm$ 0.0	3 $\pm$ 0.6	-	-
<i>Momordica charantia</i> L.	H <sub>2</sub> O	-	-	-	-	-	-
	MeOH	2 $\pm$ 0.1	-	10.5 $\pm$ 0.3	1.25 $\pm$ 0.0	-	-
<i>Mukia maderaspatana</i> (L.) M. Roem.	H <sub>2</sub> O	-	-	-	-	-	-
	EtOH	-	-	4 $\pm$ 0.0	-	-	-
<b>CYPERACEAE</b>							
<i>Cyperus scarious</i> R.Br.	H <sub>2</sub> O	-	-	-	1 $\pm$ 0.0	-	-
	MeOH	1 $\pm$ 0.0	-	4 $\pm$ 0.0	4.5 $\pm$ 0.30	-	-

Table 2. Contd.

<b>EHRETIACEAE</b>							
<i>Cordia dichotoma</i> Forst.	H <sub>2</sub> O	-	-	-	1 ± 0.0	-	-
	EtOH	-	-	4 ± 0.0	1 ± 0.3	-	-
<b>EUPHORBIACEAE</b>							
<i>Ricinus communis</i> L.	H <sub>2</sub> O	-	-	1.7 ± 0.3	1.8 ± 0.2	-	-
	MeOH	-	-	4.5 ± 0.3	1.3 ± 0.3	-	-
<b>FABACEAE</b>							
<i>Arachis hypogaea</i> L.	H <sub>2</sub> O	1 ± 0.6	-	1 ± 0.6	-	-	-
	EtOH	-	-	3 ± 0.0	-	-	-
<i>Canavalia gladiata</i> DC.	H <sub>2</sub> O	-	-	-	-	-	-
	EtOH	-	-	1 ± 0.0	-	-	-
<i>Vigna radiata</i> L.	H <sub>2</sub> O	-	-	-	1 ± 0.0	-	-
	EtOH	-	-	3 ± 0.6	1 ± 0.0	-	-
<b>FUMARIACEAE</b>							
<i>Fumaria indica</i> (Hausk.) Pugsley.	H <sub>2</sub> O	-	-	-	1 ± 0.0	-	-
	EtOH	-	-	2 ± 0.0	-	-	-
<b>GUTTIFERAE</b>							
<i>Mesua ferra</i> Linn.	H <sub>2</sub> O	-	-	4.5 ± 0.3	3.5 ± 0.3	-	-
	MeOH	1 ± 0.0	-	19.5 ± 0.9	22.5 ± 0.9	3 ± 0.0	-
<b>LABIATAE</b>							
<i>Ocimum kilimanjaricum</i> L.	H <sub>2</sub> O	-	-	-	-	-	-
	EtOH	1.5 ± 0.3	2.25 ± 0.1	4.5 ± 0.3	5 ± 0.6	3.25 ± 0.1	-
<b>LAURACEAE</b>							
<i>Cinnamomum tamala</i> Nees & Ebern.	H <sub>2</sub> O	-	-	-	1 ± 0.0	-	-
	EtOH	-	-	7 ± 0.0	3.25 ± 0.01	-	-
<b>LYTHRACEAE</b>							
<i>Woodfordia fruticosa</i> Kurz.	H <sub>2</sub> O	-	-	-	-	-	-
	MeOH	9.5 ± 0.3 7.5 ± 0.9	1.0 ± 0.0 14 ± 0.0	10 ± 0.0 19 ± 0.0	6 ± 0.06 10 ± 0.06	7.5 ± 0.3	8.5 ± 0.03 4.25 ± 0.25
<b>MALVACEAE</b>							
<i>Thespesia populnea</i> (L.) Sol ex Correa.	H <sub>2</sub> O	-	-	2 ± 0.0	-	-	-
	EtOH	-	-	7 ± 0.0	1 ± 0.6	-	-
<b>MORACEAE</b>							
<i>Artocarpus hetrophyllus</i> Lam.	H <sub>2</sub> O	-	-	-	-	-	-
	EtOH	3.2 ± 0.2	-	-	3.5 ± 0.3	-	-
<i>Ficus elastica</i> Roxb.	H <sub>2</sub> O	-	-	-	-	-	-
	MeOH	-	-	6 ± 0.0	3 ± 0.0	-	-
<b>PIPERACEAE</b>							
<i>Piper longum</i> L.	H <sub>2</sub> O	-	-	-	-	-	-
	EtOH	4 ± 0.0	-	8 ± 0.0	5 ± 0.6	-	6 ± 0.0
<b>POACEAE</b>							
<i>Bambusa arundinaceae</i> (Retz.) Roxb.	H <sub>2</sub> O	-	-	-	-	-	-
	EtOH	-	-	4 ± 0.12	-	-	-
<b>RUBIACEAE</b>							
<i>Gardenia resinifera</i> Roth.	H <sub>2</sub> O	-	-	13 ± 0.0	6 ± 0.6	-	-
	MeOH	-	-	-	-	-	-
<b>SAPOTACEAE</b>							

Table 2. Contd.

<i>Manilkara hexandra</i> (Roxb.) Dubard.	H <sub>2</sub> O	-	-	2± 0.0	-	2± 0.0	
	MeOH	2± 0.0	2± 0.0	10± 0.0	7± 0.0	5± 0.0	-
<b>VITACEAE</b>							
<i>Cissus quadrangularis</i> L.	H <sub>2</sub> O	-	-	-	-	-	-
	MeOH	-	-	-	2 ± 0.0	-	-

H<sub>2</sub>O: aqueous extract, EtOH: ethanol extract, MeOH: methanol extract; #values are the mean of inhibition zone diameter and subtracted from the control; - means no activity. Ea: *Enterobacter aerogenes*, Ec: *Escherichia coli*, Kp: *Klebsiella pneumoniae*, Pm: *Proteus mirabilis*, Pv: *Proteus vulgaris*, St: *Salmonella typhimurium*;

or ethanol) in a conical flask, plugged with cotton and then kept on a rotary shaker at 190 - 220 rpm for 24 h. After 24 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 *g* for 10 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume, and stored at 4 °C in air-tight bottles.

### Microorganisms

The microbial strains used in this study were obtained from the National Chemical Laboratory (NCL), Pune, India. The studied bacterial strains comprised: *E. aerogenes* ATCC13048, *E. coli* ATCC25922, *K. pneumoniae* NCIM2719, *P. mirabilis* NCIM 2241, *P. vulgaris* NCTC8313 and *S. typhimurium* ATCC23564. Microorganisms were maintained at 4°C on nutrient agar slants.

### Antibacterial activity

The antibacterial assay was performed by two methods. The agar disc diffusion method (Bauer et al., 1966; Parekh and Chanda, 2006) was used for aqueous extracts and the agar well diffusion method (Perez et al., 1990; Nair and Chanda, 2005) was used for solvent extracts. The media (Mueller Hinton Agar No.2), along with the inoculum (10<sup>8</sup> cfu/ml), was poured into the Petri plate (Hi-Media). For the agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated with 100 µl of the test compound, allowed to dry and then placed on the upper layer of the seeded agar plate. For the agar well diffusion method, a well was prepared in the plates with a cup-borer (0.85 cm) and 100 µl of the test compound was pipetted directly into the well. The plates were incubated overnight at 37°C. Antibacterial activity was determined by measuring the diameter of the zone of inhibition surrounding bacterial growth. For each bacterial strain, controls were included that comprised pure solvents instead of the extract (Parekh and Chanda, 2007b). The control zones were subtracted from the test zones and the resulting zone diameter is shown in the Table 2. The experiments were repeated three times and the mean values are presented with ± Standard Deviation (SD).

## RESULTS AND DISCUSSION

Since ancient times, plants have been a veritable source of drugs. However, man tends to ignore the importance of herbal medicine. Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects. As a result, some natural products have been approved as new antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance. Conti-

nued further exploration of plant-derived antimicrobials is needed today.

Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent, but we found in this study that plant extracts prepared with methanol and ethanol as solvents provided more consistent antimicrobial activity, as also reported earlier (Allero and Afolayan, 2006; Parekh and Chanda, 2007b). The antibacterial activity of the 34 Indian plants against seven members of Enterobacteriaceae are shown in Table 2. None of the aqueous extracts (except one or two) produced zones of inhibition in the Kirby- Bauer analysis. This might have resulted from the lack of solubility of the active constituents in aqueous solutions. Alternatively, active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed (Taylor et al., 2001).

Alcoholic plant extracts, on the other hand, showed some activity. Maximum antibacterial activity was shown by *Mesua ferra*, but it was active only against *P. mirabilis* (23 mm) and *K. pneumoniae* (20 mm), while *Woodfordia fruticosa* showed activity against all six members investigated, maximum activity being against *K. pneumoniae* (19 mm). *K. pneumoniae* was the most susceptible bacterium followed by *P. mirabilis*, while the most resistant bacteria were *S. typhimurium* and *E. coli*. Amongst *Proteus* species, *P. mirabilis* was susceptible, while *P. vulgaris* was resistant. Earlier work from this laboratory reported the inhibitory activity of some medicinal plant extracts on the studied members of Enterobacteriaceae (Nair and Chanda, 2007; Parekh and Chanda, 2007c).

Recently, multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs that are commonly used in the treatment of infectious diseases, making it a global growing problem. There is an urgent need to develop new antimicrobial drugs for the treatment of infectious diseases from medicinal plants, which may be less toxic to humans and possibly with a novel mechanism of action. There are numerous examples of antimicrobials of plant origin that have an enormous therapeutic potential (Parekh and Chanda, 2007d).

From the screening experiment, *Woodfordia fruticosa* Kurz. Showed best antibacterial activity; and hence this

plant can be further subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation. The potential of *W. fruticosa* has already been reported (Parekh and Chanda, 2007d; Das et al., 2007). The potential for developing antimicrobial drugs from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Therefore, such screening experiments form a primary platform for further phytochemical and pharmacological studies that may open the possibility of finding new clinically effective antibacterial compounds.

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