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Full Length Research Paper

Evaluation of antibacterial activity of Indian plant extracts against enterobacteriaceae pathogens in vitro

Parekh J and Chanda S*

Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot, 360 005, Gujarat, India.

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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 Scazzocchio et al., 2001). There are several rep-orts in the literature regarding the antimicrobial activity of crude extracts prepared form plants (El-Seedi et al., 2002; Rojas et al., 2003; Duraipandiyan 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 septice mias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs especially in debilitate 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 secon-dary invader of ulcers, pressure sores, etc. (Chees-brough, 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. commutate* 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*

^{*}Corresponding author. E-mail: sumitrachanda@yahoo.com.

 Table 1. Ethnobotanical information of some plant species screened.

| Botanical name | Vernacular | Habit | Voucher | Part(s) | Action/Therapeutic use |
|--|-------------------|----------|-------------|--------------|--|
| (family, genus, species) | name | · iabit | number | extracted | / total in morapeans acc |
| AMARANTHACEAE | T | | | | T |
| Celosia argentea L. | Lambadi | Herb | PSN645 | Whole | anpy, aphro, bl dis, dia, gon, infl, sor |
| ASCLEPIADACEAE | T | | T | | |
| Tylophora indica (Burm.f.) Merr. | Damnivel | Climber | PSN462 | Leaf | car, dip, em, expec, pur, stm, ath, bron, dia, dys, dyspep, fla, gou, ul, wo |
| ASTERACEAE | | | | | |
| Vernonia anthelmintica (L.) Willd. | Kalijiri | Herb | PSN415 | Whole | fev, ath, co, ul, sk, leucd, lep, dyspep, infl, ast, anth, exp, dmu, diu, stm, feb, gal, ton, pur |
| BALANITACEAE | | | | | |
| | | | | | alex, anal, anth, pur, verm, bo, bu, co, fra, |
| Balanites aegyptiaca (L.) Del. | Engoria | Shrub | PSN112 | Whole | leucd, sb, sk, sls |
| BIGNONACEAE | | | | | |
| Spathodea campanulata Beauv. | Kesudo | Tree | PSN563 | Aerial parts | Pur, sk |
| CAESALPINIACEAE | | | | | |
| Cassia fistula L. | Garmalo | Tree | PSN250 | Leaf | cat, em, feb, lax, pur, bil, bron, fev, rheu, rw |
| CHENOPODIACEAE | | | | | |
| | | | PSN654 | Leaf | aphro, car, diu, emmen, exp, pur, ton, con, eac, hac, infl, itc, para, sor, ul |
| Beta vulgaris L. | Beet | Herb | | | · |
| Spinacia oleracea L. | Palak ni Bhaji | Herb | - | Leaf | cat, feb, stm, infl |
| COMMELINACEAE | l | | | | T |
| Commelina benghalensis L. | Motishumliyu | Herb | PSN731 | Whole | diu, sti, dia, fev, lep |
| CONNARACEAE | ı | | | | |
| Rourea santaloides (Vahl.) | \ | l l =le | - | Root | ton, diab, rheu, sk |
| Wight &. Arnott CONVOLVULACEAE | Vardharo | Herb | | | |
| | Delline | I II- | DONIAGO | \A/I= = I = | anth anhar atm ath and tare |
| Cressa cretica L. | Paliyo | Herb | PSN496 | Whole | anth, aphro, stm, ath, con, ton |
| CRUCIFERAE | Λ - l l / Λ · · · | I II- | DONAG | 0 1 | |
| Lepidium sativum L. | Ashal/Aserio | Herb | PSN13 | Seed | antc |
| CUCURBITACEAE | I | . | 5011000 | | <u> </u> |
| Lagenaria vulgaris Seringe | Tumbada | Climber | PSN328 | Fruit | ton, pur |
| Momordica charantia 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 |
| CYPERACEAE | 0 | • | | | |
| Cyperus scarious R.Br. | Nagarmoth | Herb | PSN765 | Seed | aro, ast, dip, stm, dia |
| EHRETIACEAE | | | | | ,,,, |
| | | | | | anth, ast, diu, dmu, exp, pur, ton, co, dyspep, |
| Cordia dichotoma Forst. | Gunda | Tree | PSN472 | Leaf | fev, hac, jp, rw, sb, ul |
| EUPHORBIACEAE | | | <u>-</u> | _30. | ,, ,,,,,,,,,,,,,,,,,,,,,,,,,, |
| | | Shrub | PSN699 | Leaf | anth, aphro, car, cat, diu, gal, pur, ath, bron, co, con, drop, dyspep, fev, hac, infla, lep, lum, para, |
| Ricinus communis L. | Erado | | | | rheu, rw, sk |
| FABACEAE | | | DOM: | | |
| Arachis hypogaea L. | Magfali | Herb | PSN152 | Leaf | ast, adp, bron, con, fla |
| Canavalia gladiata DC. | Talvardi | Climber | PSN157 | Leaf | can |
| Vigna radiata L. | Mag | Herb | PSN235 | Whole | aphro, dig, feb, gal, ton, co, con, dia, dyspep, fev, fla, hae, infl, lep, pyr, sk |

Table 1. Contd.

| FUMARIAGEAE | | | | | |
|--|-----------------|----------|----------|-------------|--|
| FUMARIACEAE | Ι | | _ | Seed | dip, diu |
| Fumaria indica (Haussk.) | | | - | Seed | dip, diu |
| Pugsley. | Pitpopdo | Herb | | | |
| GUTTIFERAE | | ı | ı | | |
| Mesua ferrea Linn. | Nagkesar | Tree | - | Seed | aro, ast, col |
| LABIATAE | | | | | |
| Ocimum kilimanjaricum L. | Kapurtulsi | Herb | - | Whole | col, diu |
| LAURACEAE | | | | | |
| | | | - | Leaf | car, diu, dip, gal, sti, co, dyspep, fev, fla |
| Cinnamomum tamala Nees & Ebern. | Tamal patra | Tree | | | |
| LYTHRACEAE | | | | | |
| | | | | | anth, ast, em, feb, sed, sti, bil, bu, diab, hae, |
| Woodfordia fruticosa Kurz. | Dhawadi phool | Shrub | PSN303 | Flower | lep, sk |
| MALVACEAE | Dilawaai piiooi | Official | | | |
| WALVACEAE | | | PSN71 | Leaf | ast, col, ath, chl, co, dia, diab, dys, gon, haem, |
| Thespesia populnea (L.) Sol ex Correa. | Paras piplo | Tree | | | her, infl, lep, psor, rw, sca, ul, wo |
| | ι αιας ριριο | 1166 | | 1 | |
| MORACEAE | | | _ | Whole | abor, aphro, car, ton, bil, bo, dia, lep, sb, sk, ul, |
| Artagornus hotrophyllus Loro | Fanas | Troo | | | wo |
| Artocarpus hetrophyllus Lam. | | Tree | PSN705 | Leaf | - |
| Ficus elastica Roxb. | Rubber plant | Tree | | | |
| PIPERACEAE | <u> </u> | | | I | anth appro ant car cal lay ati ada ath bil |
| | | Climber | - | Root | anth, aphro, apt car, col, lax, sti, adp, ath, bil, bron, co, fev, gou, ins, infl, jaun, lep, leucd, lum, pil, tum |
| Piper longum L. | Piplimul | | | | |
| POACEAE | | | | | |
| | | Tree | PSN793 | Leaf | aphro, ast, col, diu, emmen, feb, lax, sti, ton, bil, bron, bu, co, dia, eac, fev, gon, jp, lep, lum, pil, |
| Bambusa arundinaceae (Retz.) | | | | | rw |
| Roxb. | Vans, bamboo | | | | |
| RUBIACEAE | | | | | |
| Gardenia resinifera Roth. | Dikamari | Tree | PSN351 | Gum exudate | car, fla, indi, sk |
| SAPOTACEAE | | | | | |
| | | Tree | PSN428 | Leaf | aphro, col, ton, bil, bron, lep, ul, urd |
| <i>Manilkara hexandra</i> (Roxb.) Dubard. | Rayan | | | | |
| VITACEAE | | | | | |
| Cissus quadrangularis L. | Hadsankar | Climber | PSN127 | Stem | anal, fra, mup, pil, tum, ul, wo |
| Cioda quadrangularis L. | <u> </u> | <u> </u> | <u> </u> | 1 | |

Key to abbreviations in Table 1.

| DISEASES | | | | | | | | | |
|----------------------|---------------------|----------------------|----------------------|---------------------|-------------------|-----------------|--|--|--|
| Α | С | dys - dysentry | Н | L | pil - piles | swe - swellings | | | |
| abs - abscesses | calc - calculi | dysame - | hac - headache | leucd - | pim - pimples | syp - syphilis | | | |
| adp - abdominal | can - cancer | dysanenorrhoea | hae - haemmorrhage | leucoderma | pneu - | Т | | | |
| pain | cd - cold | dyspep - dyspepsia | haem - haemorrhoids | leuch-leucorrhoea | pneumonia | toac - tooth | | | |
| aly - allergy | chl- cholera | E | her - hernia | lep – leprosy | psor- psoriasis | ache | | | |
| ame - amentia | chp - chest pain | eac-earache | hp - hydrophobia | lum - lumbago | psy - psycopathy | tum - tumors | | | |
| amen -amenorrhoea | co - cough | ecz - eczema | hys - hysteria | М | pyr - pyrexia | typh – typhoid | | | |
| anm - anaemia | con - constipation | elph - elephantiasis | I | mal - malaria | R | U | | | |
| ano - anorexia | D | epi - epilpsy | indi - indigestion | mig - migraine | rheu - | ul - ulcers | | | |
| arth - arthritis | deli -delirium | F | infl - inflammations | mum - mumps | rheumatism | urd - urinary | | | |
| ath - asthma | den fev - dengue | fat - fatigue | ins - insomnia | mup - muscular pain | rw - ringworm | disorders | | | |
| В | fever | fev - fever | itc - itch | N | S | V | | | |
| bil - biliousness | der - dermatitis | fla - flatulence | J | neu - neuralgia | sb - snake bite | vom - vomiting | | | |
| bl dis - blood | dia - diarrohea | fra - fracture | jaun - jaundice | 0 | sca - scabies | W | | | |
| diseases | diab - diabetes | G | jp - joint pain | ob - obesity | scia - sciatica | wo - wounds | | | |
| bo - boils | diph - diphtheria | gin - gingivitis | | P | sk - skin disease | wor - worms | | | |
| bron - bronchitis | drop - dropsy | gon - gonorrhoea | | para - paralysis | sls - sleeping | | | | |
| bu - burns | | gou - gout | | phg - pharyngitis | sickness | | | | |
| | | | | | smp - small pox | | | | |
| | | | | | sor - sores | | | | |
| | _ | | CINAL PROPERTIES | | | | | | |
| Α | antpy - antipyretic | В | D | emmen - | Р | sed - sedative | | | |
| abor - abortifacient | antsp - antiseptic | b.ton - brain tonic | dig - digestive | emmenagogue | pec - pectoral | sti - stimulant | | | |
| alex - alexipharmic | antsp - | С | dip - diphoretic | exp - expectorant | pur - purgative | Т | | | |
| anal - analgesic | antispasmodic | c.ton - cardiotonic | diu - diuretic | F | R | ton - tonic | | | |
| antd - antidote | aphro - | car - carminative | dmu - demulcent | feb - febrifuge | rub - rubefacient | | | | |
| anth - anthelmentic | aphrodisiac | cat - cathartic | E | G | S | V | | | |
| anthy - | apt - apetiser | col - coolant | em – emetic | gal - galactagogue | stm - stomachic | verm - | | | |
| antihypertensive | aro - aromatic | | | L | | vermifuge | | | |
| antpr - antiperiodic | ast - astringent | | | lax - laxative | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

(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 *Albizzia 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 | | Inhibition Of Zone (Mean ± SEM) | | | | | | |
|--|--------------------------|---------------------------------|------|----------------|----------------|------|-------|--|
| (family, genus,species) | Extract | Ea** | Ec** | Кр** | Pm** | Pv** | St** | |
| AMARANTHACEAE | | | | | | | | |
| | H ₂ 0 | - | - | 3±0.1 | - | - | - | |
| Celosia argentea L. | EtOH | - | - | 1±0.0 | - | - | - | |
| ASCLEPIADACEAE | • | | | | | | | |
| | H ₂ 0 | | | | | | | |
| | EtOH | _ | _ | | _ | _ | _ | |
| Tylophora indica (Burm.f.) Merr. | Lion | _ | _ | 3±0.0 | - | _ | _ | |
| ASTERACEAE | | | | | | | | |
| NOTERINGENE | H ₂ 0 | _ | _ | _ | - | _ | - | |
| Vernonia anthelmintica (L.) Willd. | EtOH | _ | - | 4±0.0 | _ | | - | |
| BALANITACEAE | Lion | <u>l</u> | | +±0.0 | | | | |
| BALANITACEAE | 11.0 | | | | | | 4.0.4 | |
| Balanites aegyptiaca (L.) Del. | H ₂ 0 EtOH | - | - | 2.5 ± 0.0 | - | - | 4±0.4 | |
| | EIOH | - 1 | | 2.5 ± 0.0 | - | - | - | |
| BIGNONACEAE | T | <u> </u> | | 1 | | | | |
| 0 4 4 | H ₂ 0 | - | | - | | | | |
| Spathodea campanulata Beauv. | MeOH | 3.0 ± 0.0 | - | 3±0.0 | - | - | - | |
| CAESALPINIACEAE | 1 | | | | | 1 1 | | |
| | H ₂ 0 | - | - | - | - | - | - | |
| Cassia fistula L. | MeOH | - | - | 3±0.0 | 2±0.0 | - | - | |
| CHENOPODIACEAE | | , , | | | | 1 | | |
| | H ₂ 0 | - | - | 1±0.6 | | | | |
| | EtOH | - | - | 1±0.0 | - | - | - | |
| Beta vulgaris L. | H ₂ 0 | - | - | - | - | - | - | |
| Spinacia oleracea L. | MeOH | - | - | 1±0.0 | 2±0.0 | - | - | |
| COMMELINACEAE | | | | | | | | |
| | H ₂ 0 | - | - | 1.5 ± 0.3 | - | - | - | |
| Commelina benghalensis L. | EtOH | - | - | 2±0.0 | - | - | - | |
| CONNARACEAE | | | | | | | | |
| | H ₂ 0 | - | | - | - | | | |
| Rourea santaloides (Vahl.) Wight &. Arnott | EtOH | $2.5 \pm .0$ | - | 6±0.0 | 0.2 ± 0.1 | - | - | |
| CONVOLVULACEAE | | | | | | | | |
| Cressa cretica L. | H ₂ 0 | _ | _ | _ | _ | _ | _ | |
| Cressa Cretica E. | EtOH | _ | _ | 04 ± 0.0 | _ | _ | _ | |
| COLICIEDAE | ElOH | - | | 04 ± 0.0 | | - | | |
| CRUCIFERAE | | + | | + | | | | |
| Lepidium sativum L. | H ₂ 0 | - | - | - | - | - | | |
| | MeOH | - | - | - | - | - | 3±0.6 | |
| CUCURBITACEAE | | | | | | | | |
| | H ₂ 0 | - | - | - | - | | | |
| Lagenaria vulgaris Seringe | MeOH | - | - | 7± 0.0 | 3 ± 0.6 | - | - | |
| | H ₂ 0 | - | - | - | - | - | - | |
| Momordica charantia L. | MeOH | 2±0.1 | - | 10.5 ± 0.3 | 1.25 ± 0.0 | - | - | |
| | H ₂ 0 | - | - | - | - | - | - | |
| Mukia maderaspatana (L.) M. Roem. | EtOH | - | - | 4± 0.0 | - | - | - | |
| CYPERACEAE | | | | | | | | |
| | H ₂ 0 | - | - | - | 1± 0.0 | - | - | |
| Cyperus scarious R.Br. | MeOH | 1± 0.0 | - | 4± 0.0 | 4.5± 0.30 | - | - | |

Table 2. Contd.

| EHRETIACEAE | | | | | | | |
|--|--------------------------|--------------------------------|-----------------------|---------------------------------|---------------------------------|--------------|---------------------------|
| | H ₂ 0 | _ | _ | _ | 1 ± 0.0 | _ | _ |
| Cordia dichotoma Forst. | EtOH | _ | _ | 4±0.0 | 1 ± 0.0 | _ | _ |
| EUPHORBIACEAE | Lion | | | 120.0 | 1 2 0.0 | | |
| Ricinus communis L. | H ₂ 0 | | | 1.7± 0.3 | 1.8 ± 0.2 | | |
| RICITUS COMMUNIS L. | MeOH | _ | - | 4.5± 0.3 | 1.0 ± 0.2 1.3 ± 0.3 | _ | _ |
| FABACEAE | WIGGIT | | | 1.02 0.0 | 1.0 ± 0.0 | | |
| Arachis hypogaea L. | H ₂ 0 | 1±0.6 | _ | 1±0.6 | - | _ | _ |
| Arachis hypogaea L. | EtOH | 1±0.0 | _ | 3±0.0 | _ | | _ |
| Occasionalis relativista DO | H ₂ 0 | | | 3±0.0 | | | |
| Canavalia gladiata DC. | EtOH | - | - | 1.00 | | | |
| | H ₂ 0 | - | - | 1±0.0 | - | - | - |
| Vigna radiata L. | | - | - | | 1 ± 0.0 | - | - |
| | EtOH | - | - | 3±0.6 | 1 ± 0.0 | - | - |
| FUMARIACEAE | 11.0 | | | | 4 . 0 0 | | |
| Fumaria indica (Haussk.) Pugsley. | H ₂ 0 EtOH | _ | - | 2±0.0 | 1 ± 0.0 | - | - |
| GUTTIFERAE | ElOIT | | <u> </u> | 2±0.0 | - | - | _ |
| OUT THE LINAL | H ₂ 0 | _ | _ | 4.5 ± 0.3 | 3.5 ± 0.3 | _ | _ |
| Mesua ferra Linn. | MeOH | 1±0.0 | - | 4.5 ± 0.3 19.5 ± 0.9 | 3.5 ± 0.3 22.5 ± 0.9 | 3±0.0 | _ |
| LABIATAE | | | | | | | |
| | H ₂ 0 | | | | | - | |
| | EtOH | - | - | - | - | 3.25 ± | - |
| Ocimum kilimanjaricum L. | | 1.5 ± 0.3 | 2.25 ± 0.1 | 4.5 ± 0.3 | 5 ± 0.6 | 0.1 | - |
| LAURACEAE | | | | | | | |
| | H ₂ 0 | - | - | - | 1 ± 0.0 | - | - |
| Cinnamomum tamala Nees & Ebern. | EtOH | - | - | 7±0.0 | 3.25 ± 0.01 | - | - |
| LYTHRACEAE | | | | | | | |
| | H ₂ O | 05.00 | 40.00 | 10 . 0 0 | 6 ± 0.06 | - 7 F . | 0.5.000 |
| Woodfordia fruticosa Kurz. | MeOH | 9.5 ± 0.3 7.5 ± 0.9 | 1.0 ± 0.0 14 ± 0.0 | 10 ± 0.0 19 ± 0.0 | 10 ± 0.06 | 7.5 ± 0.3 | 8.5 ± 0.03 4.25 ± 0.25 |
| MALVACEAE | | 7.10 = 0.10 | 0.0 | .0 _ 0.0 | | 0.0 | 0 _ 00 |
| | H ₂ 0 | _ | _ | 2±0.0 | | | |
| Thespesia populnea (L.) Sol ex Correa. | EtOH | - | - | 7±0.0 | 1 ± 0.6 | - | - |
| MORACEAE | | | | | | | |
| | H ₂ 0 | - | | | - | | |
| Artocarpus hetrophyllus Lam. | EtOH | 3.2± 0.2 | - | - | 3.5 ± 0.3 | | |
| | H ₂ 0 | - | - | - | - | - | - |
| Ficus elastica Roxb. | MeOH | - | - | 6±0.0 | 3 ± 0.0 | - | - |
| PIPERACEAE | | | 1 | | 1 | | 1 |
| Piper lengum I | H ₂ 0 EtOH | - | - | 6+0 0 - | - 5 + 0 6 | - | - 6±0.0 |
| Piper longum L. POACEAE | I EIUH | 4±0.0 | <u> </u> | 8±0.0 | 5 ± 0.6 | | 0±0.0 |
| FUAGEAE | ⊔. ∩ | | | | | | |
| Bambusa arundinaceae (Retz.) Roxb. | H ₂ 0 EtOH | _ | _ | - 4 ± 0.12 | _ | _ | _ |
| RUBIACEAE | LIOIT | | | 7 ± 0.12 | _ | | <u> </u> |
| NODINOLAL | H ₂ 0 | _ | _ | 13 ± 0.0 | 6 ± 0.6 | _ | _ |
| Gardenia resinifera Roth. | MeOH | _ | - | 13 ± 0.0 | 0 ± 0.0 | _ | - |
| SAPOTACEAE | 1 | 1 | | 1 | | • | <u> </u> |

Table 2. Contd.

| Manilkara hexandra (Roxb.) Dubard. VITACEAE | H₂0 MeOH | - 2± 0.0 | - 2± 0.0 | 2± 0.0 10± 0.0 | - 7± 0.0 | 2± 0.0 5± 0.0 | - |
|--|------------------|-------------|-------------|-------------------|-------------|------------------|---|
| | H ₂ 0 | - | - | - | - | - | - |
| Cissus quadrangularis L. | MeOH | - | • | - | 2±0.0 | - | - |

H20: 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 (108 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 fruitcosa* 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 mic-robes. Therefore, such screening experiments form a pri-mary platform for further phytochemical and pharmaco-logical studies that may open the possibility of finding new clinically effective antibacterial compounds.

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