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IN VITRO ANTIBACTERIAL ACTIVITY OF PLANTS AVAILABLE IN SEMIARID REGION OF RAJASTHAN - I

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In vitro antibacterial activity of 17 plants, available in semiarid region of Rajasthan (Jaipur), was studied against *E.coli* and the results were compared with tetracycline which was used as control. Some of the plants studied here like, *Syzygium aromaticum, Cassia fistula* and *Moringa oleifera* showed antibacterial activity even stronger than the wide spectrum antibiotics like tetracycline. So the use of these and other herbal plants is recommended as alternatives to antibiotics.

Keywords: Antibacterial activity; Antibiotics; Disc diffusion method; *E. coli;* Herbal drugs; Medicinal plant; Primary screening; Rajasthan; Semi-arid.

Introduction

Most of the drugs of the past were substances with a particular therapeutic action extracted from plants. More and more researchers found that food and their individual constituents perform similar fashion to modern drugs and sometimes better without the dreaded side effects. Throughout the world herbs and medicinal plants are used as the first medicines. Every culture on earth has relied on the vast variety of natural chemistry found in healing plants for their therapeutic properties[1]. From prehistoric times, herbal medicine is being used by various communities and civilization throughout the world to combat diseases[2]. Herbal formulation has reached widespread acceptability as therapeutic agents like anti-diabetics, antiarthritics, aphrodisiacs, hepatoprotective, cough remedies, memory enhancers and adaptogens[3].

Worldwide infectious diseases are the leading cause of death. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens[4]. It has been noted since long time that bacterial and fungal pathogens evolve numerous defense mechanisms against antimicrobial agents, and become resistant to old and newly produced drugs. Therefore, this increase in incidences of 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[5, 6]. There have been several incidences of new and re-emerging infectious disease and development of resistance towards antibiotics[7]. The present study deals with screening of alcoholic extracts collected from various plants or their parts available in the semi-arid region of Rajasthan for their antibacterial activities.

Material and Methods

The antimicrobial activity of 17 different plant species was studied with the help of disc diffusion method. These species which included both cultivated and wild plants normally available in the vicinity were selected for the study. The species were *Syzygium aromaticum, Cassia fistula, Moringa oleifera, Cuminum cyminum, Terminalia chebula, Elettaria cardamomum, Coriandrum sativum, Grevillea robusta, Croton oblongifolius, Nerium indicum, Foeniculum vulgare, Allium cepa, Curcuma longa, Epipremnum aureum (Money plant), Zingiber officinale, Cassia siamea* and *Carica papaya.* There were some plants which were neither cultivated nor found in wild semi-arid region of Rajasthan, i.e. *Elettaria cardamomum, Terminalia chebula, Foeniculum vulgare* and *Syzygium aromaticum.* Plant materials of such plants were purchased from grocery shops. *Escherichia coli (E.coli)* test bacterium was procured from microbiology laboratory of our Institute and was maintained on nutrient agar medium (NAM). Tetracycline, a broad-spectrum polypeptide antibiotic produced by the *Streptomyces* genus of Actinobacteria was used as control in disc diffusion method.

Desired plant parts were collected and washed in running tap water to remove dust. Four gm plant part was weighed, in each case, and was grinded in 10ml of 70% ethanol with the help of mortar pestle. The extract was then left for 24 hours in test tube. The extract was then centrifuged at 5000 rpm for 10 minutes. The residue was discarded and supernatant was taken for the antibacterial study. Six mm (diameter) discs were punched from Whattman's filter paper no.1 with the help of a punching machine. The prepared discs were autoclaved and left for 35 minutes after attaining a temperature of 121°C at 20 lbs pressure. After sterilization the discs were dipped in semidried supernatant plant extract obtained after centrifugation. To prepare 1lt nutrient agar medium (NAM) constituents like, Agar (18 gram), Beef Extract (3 gram), Sodium Chloride (3 gram) and Peptone (5 gram) were taken. All the above constituents except agar were dissolved in distilled water and the final volume was made upto 1000ml. The pH of the medium was set to 6.8 and then kept on hot plate for the proper dissolution of Agar. The medium was then transferred to conical flask and plugged with cotton and autoclaved for 20 minutes after attaining a temperature of 121°C and 20 lbs pressure. Autoclaved medium was poured in petriplates under aspetic conditions in a laminar air flow cabinet. Nine percent saline solution of NaCl was prepared and autoclaved. With the help of sterilized inoculating loop, a loop full of pure culture of *E.coli* was transferred into the test tube containing 10ml of the saline solution to prepare a saline suspension of bacteria under laminar air flow hood. The autoclaved filter paper discs were saturated with the semidried plant extract and air dried at room temperature in order to remove any residual solvent. The petri plates containing the NAM were inoculated with the test bacteria (*E.coli*) from the saline suspension. For that 0.2ml bacterial suspension was spreaded over the medium using glass spreader. Two discs with (containing extract) were then placed on the surface of a sterilized nutrient agar medium plate. A standard disc of tetracycline was used in each agar plate as control. The petriplates were placed in an incubator at 37° C for 24 hours to allow the diffusion of active compounds. The zone of inhibition or depressed growth was measured with the help of geometrical scale. Four to six discs were taken for every plant material and an average diameter of zone of inhibition was calculated. Activity index (A.I.) of every plant extract was calculated using following formula:

$$\text{Activity Index (A.I.)} = \frac{\text{Inhibition zone of the sample (mm)}}{\text{Inhibition zone of the standard (Tetracycline) (mm)}}$$

Result and Discussion

The 17 species taken for the antibacterial screening including both cultivated and wild plants were randomly selected, depending upon their availability. Different alcoholic extracts screened showed growth inhibitory activity against test bacteria *(E.coli)*. The inhibition zones of various plant extracts were compared among themselves. The inhibition zones of plant extracts were also compared with that of tetracycline and the activity index was calculated (Table 1; Fig. 1). Ethanolic extract of dried floral buds of *Syzygium aromaticum* or clove showed maximum antibacterial activity (29.5mm). This activity was found even better than the average activity of tetracycline (25.3mm). Similarly ethanolic extract of fresh leaves and fruits of *Cassia fistula* and *Moringa oleifera*, respectively also showed better antimicrobial property than tetracycline against the test organism (*E.coli*). Their diameters for zone of inhibition were 26.5mm and 25.5mm, respectively, with paper disc diameter of 0.6mm in all the cases. *Cuminum cyminum* (24.0mm), *Terminalia chebula* (23.0mm), *Elettaria cardamomum* (21.5mm), and *Coriandrum sativum* (20.0mm) showed antimicrobial activities moderately similar to tetracycline (25.3mm). Plants which showed 50% less activities in comparision to tetracycline were *Zingiber officinale* (12.5mm), *Epipremnum aureum (Money plant)* (12.5mm), *Cassia siamea* (11.0mm), *Carica papaya* (10.0mm). *Grevillea robusta* (17.5mm), *Croton oblongifolius* (15.0mm), *Nerium indicum* (14.5mm), *Foeniculum vulgare* (14.5mm), *Allium cepa* (14.5mm) and *Curcuma longa* (14.0mm) whereas showed activities slightly more than 50% to that of tetracycline.

Spices and herbs are used worldwide and their antimicrobial potential has been recognized since antiquity. Medicinal plants as a group comprises approximately 8000 species and account for around 50% of all the higher flowering plants species of India. Rajasthan-the largest state of India consists of arid and semi arid regions harboring lots of medicinal plants. Also there are several plants which are introduced in this region and are being cultivated for ornamental and other purposes but possess medicinal values[8].

Phongpaichit *et al.*[9] studied crude methanol extracts from leaves of *Cassia alata, Cassia fistula* and *Cassia tora* for their antifungal activities on three pathogenic fungi (*Microsporum gypseum, Trichophyton rubrum* and *Penicillium marneffei*). They found the extract of *C. fistula* the most potent inhibitor of *P. marneffei*. They also found all three *Cassia* leaf extracts effectively acting against *M. gypseum* conidial germination. Duraipandiyan *et al.*[10] evaluated the antimicrobial activity of 18 ethnomedicinal plant extracts against nine bacterial strains (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Ervinia sp, Proteus vulgaris*) and one fungal strain (*Candida albicans*). They found *Cassia auriculata* among the most

Table 1. Antimicrobial activity of various plant extracts.

S.No.	Plant name	Plant Part Used	Average Diameter of Zone of Inhibition (mm)	Activity Index*
1.	*Syzygium aromaticum*	Dried flower buds	29.5	1.17
2.	*Cassia fistula*	Fresh leaves	26.5	1.05
3.	*Moringa oleifera*	Fresh fruits	25.5	1.01
4.	*Cuminum cyminum*	Dried fruits	24.0	0.95
5.	*Terminalia chebula*	Dried seeds	23.0	0.91
6.	*Elettaria cardamomum*	Dried fruits	21.5	0.85
7.	*Coriandrum sativum*	Fresh leaves	20.0	0.79
8.	*Grevillea robusta*	Fresh leaves	17.5	0.69
9.	*Croton oblongifolius*	Fresh leaves	15.0	0.59
10.	*Nerium indicum*	Fresh leaves	14.5	0.57
11.	*Foeniculum vulgare*	Dried fruits	14.5	0.57
12.	*Allium cepa*	Fresh bulbs	14.5	0.57
13.	*Curcuma longa*	Dried rhizomes	14.0	0.55
14.	*Epipremnum aureum*	Fresh leaves	12.5	0.49
15.	*Zingiber officinale*	Fresh rhizomes	12.5	0.49
16.	*Cassia siamea*	Fresh leaves	11.0	0.43
17.	*Carica papaya*	Fresh leaves	10.0	0.39

*Note: For tetracycline, average diameter of zone of inhibition was 25.3mm.

active. Somchit *et al.*[11] tested crude ethanol and water extract of leaves and barks from *Cassia alata* against fungi, (*Aspergillus fumigatus* and *Microsporum canis*), yeast (*Candida albicans*) and bacteria (*Staphylococcus aereus* and *Escherichia coli*). They used water and ethanolic extracts of leaves of *C. alata* against *S. aureus*. The water extract exhibited higher antibacterial activity than the ethanol extract from leaves (inhibition zones of 11–14mm and 9–11 mm, respectively), but in their case *E. coli* showed resistance to all types of extracts. Based on their findings, they concluded that *C. alata* has antimicrobial activity, as potent as standard antimicrobial drugs against certain microorganisms. Samy and Ignacimuthu[12] studied 34 plant species belonging to 18 different families, selected on the basis of folklore medicinal reports practised by the tribal people of Western Ghats, India. They were assayed for antibacterial activity against *Escherichia coli, Klebsiella aerogenes, Proteus vulgaris,* and *Pseudomonas aerogenes* (Gram-negative bacteria) at 1000–5000 ppm using the disc diffusion method. Of these 16 plants showing antibacterial activity; *Cassia fistula* showed significant antibacterial activity against the tested bacteria. In present studies also leaf extract of *Cassia fistula* showed higher antimicrobial activity in comparision to tetracycline. Its inhibition zone was wider (26.5mm) than the tetracycline (25.3mm). Ethanolic extract of *Cassia siamea* also showed feeble antibacterial activity with an activity index of 0.43 only. Therefore, the use of *Cassia fistula* as antimicrobial agent in place of antibiotics is supported.

Emeruwa[13] reported that among various extracts (epicarp, endocarp, seeds and leaves) from *Carica papaya* except that of leaves produced very significant antibacterial activity on *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Shigella flexneri*. The minimum inhibitory concentration (MIC) of the substance was small (0.2-0.3 mg/ml) for gram-positive bacteria and large (1.5-4 mg/ml) for gram-negative bacteria. The substance was bactericidal and showed properties of a protein. Similarly the work carried out by Emeruwa[13] as in case of extract from leaves of *Carica papaya* least activity index (0.39) was observed, out of the total 17 plants studied.

Jabeen *et al.*[14] assayed the seed extracts of *Moringa oleifera* for the evaluation of antimicrobial activity against bacterial (*Pasturella multocida, Escherichia coli, Bacillus subtilis* and *Staphlocuccus aureus*) and fungal (*Fusarium solani* and *Rhizopus solani*) strains. They reported that the crude, supernatant, residue and dialyzed samples inhibited the growth of all microbes to various extents. The zones of growth inhibition showed

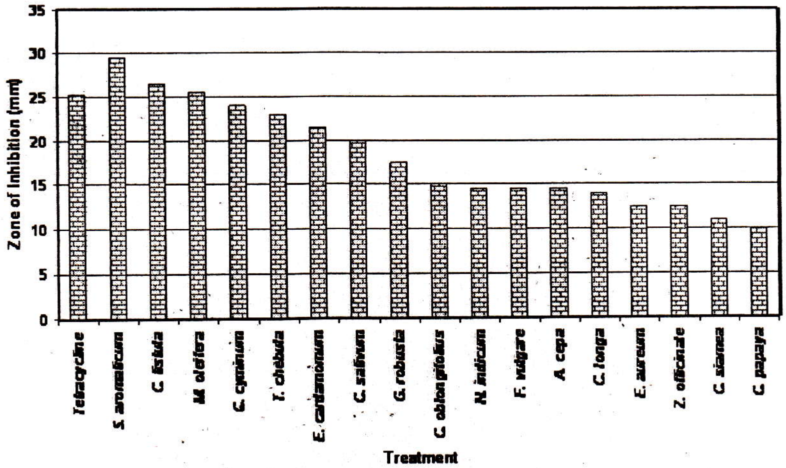

Fig.1. Antimicrobial activities of various plant extracts

greater sensitivity against the bacterial strains as compared to the fungal strains. Jamil *et al.*[15] reported antimicrobial properties of seed extract of *Moringa oleifera.* Present findings equally support the antimicrobial activity of *Moringa oleifera* fruit as it showed activity stronger than that of tetracycline with an average diameter of 25.5mm and an activity index of 1.01.

Lo Cantore *et al.*[16] studied antibacterial activity of essential oils extracted from the fruits of *Coriandrum sativum* and *Foeniculum vulgare* against *E. coli* and *Bacillus megaterium*, bacteria routinely used for comparison in the antimicrobial assays, and 27 phytopathogenic bacterial species and two mycopathogenic ones responsible for cultivated mushroom diseases. A significant antibacterial activity, as determined with the agar diffusion method, was shown by *C. sativum* essential oil whereas a much reduced effect was observed for *F. vulgare* oil. Gulfraz *et al.*[17] studied antimicrobial properties of essential oil obtained from the seed of *F. vulgare.* The antimicrobial activity of *F. vulgare* oil was assessed by disc diffusion as well as MIC method. Fennel oil showed inhibition against *Bacillus cereus*, *Bacillus magaterium*, *Bacillus pumilus*, *Bacillus substilis*, *E. coli*, *Klebsiella pneumonia*, *Micrococcus lutus*, *Pseudomonos pupida*, *Pseudomonos syringae*, and *Candida albicans* as compared to methanolic and ethanolic seed extracts. The lowest MIC values of fennel oil for *Candida albicans* (0.4% v/v), *Pseudomonos putida* (0.6% v/v) and *E. coli* (0.8% v/v) was obtained. It is observed that essential oil and seed extracts of *F. vulgare* exhibit different degree of antimicrobial activities depending on the doses applied. They recommended that fennel oil could be a source of pharmaceutical materials required for the preparation of new therapeutic and antimicrobial agents. The diameters of growth inhibition zone ranged from 14 to 31 mm (including the diameter of the disc that was 6 mm) with the highest inhibition zone values observed against *B. magaterium* (31 mm) and *B. substilis* (29 mm). In all, the extracts showed less activity than essential oil. However, some organisms showed resistance towards both oil and seed extracts. The essential oil showed greater or similar activity on Gram-positive and Gram-negative bacteria and *C. albicans* strains at a concentration of 100 µg/disk as compared to ofloxacin, (6 µg/disc) and miconazole nitrate (50 µg/disc) used as positive controls. The MIC values of 0.4% (v/v) were observed against *C. albicans*, 0.6% for *P. putida* and 0.8% for *E.coli.* It was observed that out of *Coriandrum* and *Foeniculum*, leaf extracts from the former showed activity slightly lesser than that of tetracycline, with an activity index of 0.79. An activity of around 50% (to that shown by the tetracycline) in case of *Foeniculum* dry fruit extract was also observed.

Malekzadeh[18] studied the effect of ether, alcoholic and water extracts of black myrobalan (*Teminalia chebula*) on *Helicobactor pylori* using an agar diffusion method. Water extracts of black myrobalan showed significant antibacterial activity and had a MIC and MBC (minimum bacteriocidal concentration) of 125 and 150 mg/l, respectively. The extract was active after autoclaving for 30 min at 121°C. Plant powder (incorporated in agar) gave higher MIC and MBC values (150 and 175 mg/l, respectively). Water extracts of the black myrobalan at a concentration of 1-2.5 mg/ml inhibited urease activity of *H. pylori.* The results showed that black myrobalan extracts contain a heat stable agent(s) with possible therapeutic potential. Other bacterial species were also inhibited by black myrobalan water extracts. *In vitro* antibacterial activity of extracts from the plants *T. chebula* and *O.*

sanctum by the disc diffusion technique was studied. The activity against *Salmonella* and *Shigella* organisms was shown by *T. chebula* but not by *O. sanctum*. *T. chebula* was found to be the most potent. Dry seed extract of *Terminalia* in the present studies showed antimicrobial activity at par (0.91) with that of the tetracycline. It can be further tested taking other antibiotics as control.

Alzoreky and Nakahara[19] screened extracts of 26 edible plants against *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *E. coli* and *Salmonella infantis*. Buffered methanol and acetone extracted inhibitory substances against tested bacteria from 16 plants, as revealed by the disc assay. The most sensitive microorganism to extracts from *Azadirachta indica*, *Cinnamomum cassia*, *Rumex nervosus*, *Ruta graveolens*, *Thymus serpyllum* and *Zingiber officinale* was *B. cereus*, with MIC of 165 to 660 mg l^{-1}. *E. coli* and *S. infantis* were only inhibited by *Cinnamomum cassia* extracts at the highest MIC (2640 mg l^{-1}).

Saeed and Tariq[20] investigated the potential of using aqueous infusion, decoction and essential oil of clove (*Syzygium aromaticum*) as natural antibacterial agents against 100 isolates belonging to 10 different species of Gram –ve bacilli including *E. coli*. The screening was performed by standard disc diffusion method. The aqueous infusion and decoction of clove exhibited maximum activity against *P. aeruginosa* with 10.43 mm and 10.86 mm mean diameter of zone of inhibitions, respectively. Essential oil of clove exhibited maximum activity against *V. cholerae* with 23.75 mm mean diameter of zone of inhibition.

Duraipandiyan *et al.*[10] evaluated the antimicrobial activity of 18 ethnomedicinal plant extracts against nine bacterial strains. They found activity of *Syzygium lineare* among the most active ones. In our study we found ethanolic extract of dried floral buds of *Syzygium aromaticum* showing maximum antibacterial activity against *E.coli* among all the seventeen plants selected. It showed maximum zone of inhibition (29.5mm) and activity index of 1.17 among all the 17 plants studied. Its effect was stronger than the tetracycline. Thus *Syzygium* can be used as an alternative to the antibiotics.

Alzoreky and Nakahara[19] screened extracts of edible plants (26 species) including *Zingiber officinale* against *Bacillus cereus* and found them effective. Azu *et al.*[21] studied the antibacterial activity of raw and aqueous extracts of *Allium cepa* and *Zingiber officinale* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which were common cause of nosocomial (hospital-acquired) and urinary tract infections using the cup-plate diffusion method. Their result showed that ethanolic extract of ginger gave the widest zone of inhibition against the two test organisms at the concentration of 0.8gml^{-1}. However, *Pseudomonas aeruginosa* was more sensitive to the extract of onion bulbs compared to *Staphylococcus aureus*. They also observed that the solvent of extraction and its varying concentrations affected the sensitivity of the two organisms to the plant materials. The MIC of ginger extracts on the test organisms ranged from 0.1gml^{-1} - 0.2gml^{-1}, which showed that ginger was more effective and produced marked inhibitory effect on the two test organisms compared to the onion extracts. *Zingiber* in present case didn't show the best result against *E.coli*. It showed a zone of inhibition of 12.5mm only. Though other extraction methods can be applied and will be considered in future study. Ethanolic extract of *Allium cepa* showed better antimicrobial activity in comparision to *Zingiber* with an activity index of 0.57 and an average diameter of zone of inhibition of 14.5mm. During the present investigation it was observed that ethanolic extracts of fresh leaves of *Grevillea robusta* showed better antimicrobial activity in comparision to *Curcuma, Zingiber* and *Foeniculum*. Similarly extract from dry fruits of *Cuminum cyminum* also showed good antibacterial activity with an activity index of 0.95, almost equal to tetracycline. Good antibacterial activity was shown by extract of *Elettaria cardamomum* (0.85) too.

Çýkrýkçý *et al.*[22] studied antibacterial and antifungal activity of Curcuminoids isolated from *Curcuma longa* and concluded that the extracts and curcumin didn't show any significant activity against the gram negative bacteria *E.coli* and *P.aeuroginosa*. Singh *et al.*[23] evaluated extracts of *Curcuma longa* rhizome for antibacterial activity against pathogenic strains of Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) bacteria. Essential oil was found to be most active and its activity was compared to standard antibiotics gentamycin, ampicillin, doxycycline and erythromycin in these strains. They suggested the use of essential oil from turmeric as a potential antiseptic in prevention and treatment of antibacterial infections. Dried rhizome of *Curcuma longa* in our case showed moderate activity with an activity index of 0.55 against *E.coli*. Ethanolic extract of fresh leaves taken from *Croton oblongifolius* and *Nerium indicum*, *Epipremnum aureum (Money plant)* also showed activities around 50% in comparision to tetracycline, with activities of 0.59, 0.57 and 0.49, respectively.

Due to increase in incidence of new and re-emerging infectious disease and development of resistance towards antibiotics the herbal extract should be promoted extensively[7]. Some of the plants studied here like, *Syzygium aromaticum, Cassia fistula, Moringa oleifera, Cuminum cyminum, Terminalia chebula* and *Elettaria cardamomum* have shown antibacterial activities even stronger than the wide spectrum antibiotics like tetracycline. So we support and recommend the use of these plants as medicinal herbs against bacteria and recommend carrying out different extraction methodology to recognize their maximum antibacterial potential.

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