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# *IN VITRO* ANTIBACTERIAL ACTIVITY OF PLANTS AVAILABLE IN SEMIARID REGION OF RAJASTHAN - II

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Seventeen plants and their parts commonly available in the semi-arid region of Rajasthan were screened for their antibacterial activities against the test organisms *E. coli*. The results were compared with wide spectrum antibiotics, like tetracycline. Some of the plants studied like, *Polyalthia longifolia, Datura stramonium* and *Trigonella foenum-graceum* showed antibacterial activities even stronger than the tetracycline. So the use of these and other herbal plants is recommended as alternatives to the antibiotics.

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

## Introduction

Antimicrobial activities of various plants and their parts have been reported by many works[1, 2]. The use of alternative medical therapy has increased the interest of pharmacologists and herbalists over the past decade. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made contributions to human health and well being[3]. Studies have indicated that in plants there are secondary metabolites such as unsaturated long chain aldehydes, alkaloidal constituents, some essential oils and phenols. These compounds have potentially significant therapeutic application against human pathogens, including bacteria, fungi or viruses[4, 5]. In India, companies like Ajanta Pharmaceuticals, Dabur and The Himalaya Drug Company have launched several herbal remedies in the market[6].

## Material and Methods

The present study deals with screening of extracts from 17 different wild and native varieties of various plants found in the semi-arid region of Jaipur. 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 were selected on the basis of their easy availability in the semi-arid region of Rajasthan. The plants were *Bauhinia variegata, Azadirachta indica, Murraya koenigii, Polyalthia longifolia, Acacia nilotica, Ficus religiosa, Nerium oleander, Tamarindus indica, Allium sativum, Aloe barbadensis, Trachyspermum ammi, Cinnamomum tamala, Datura stramonium, Jatropha curcas, Trigonella foenum-graceum, Calotropis procera* and *Piper nigrum*. There were some plants which are neither cultivated nor found in wild semi-arid region of Rajasthan, i.e., *Piper nigrum, Cinnamomum tamala*. Plant materials of such species were purchased from grocery shops. *Escherichia coli* was the test bacterium, procured from microbiology laboratory of our institute and was maintained on nutrient agar medium (NAM). Tetracycline was used as control in the disc diffusion Method. Tetracycline, a broad-spectrum polypeptide antibiotic produced by the *Streptomyces* genus of Actinobacteria was used as control in disc diffusion method.

Desired plants 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. 6 mm (diameter) discs were punched from Whattman 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 gm), Beef Extract (3 gm), Sodium Chloride (3gm) and Peptone (5 gm) were

**Table 1.** Antimicrobial activities of various plant extracts.

| S.No. | Plant name | Plant Part Used | Average Diameter of Zone of Inhibition (mm) | Activity Index* |
|---|---|---|---|---|
| 1. | *Polyalthia longifolia* | Fresh leaves | 29.0 | 1.24 |
| 2. | *Datura stramonium* | Fresh leaves | 24.0 | 1.03 |
| 3. | *Trigonella foenum-graceum* | Fresh leaves | 23.5 | 1.01 |
| 4. | *Nerium oleander* | Fresh leaves | 21.5 | 0.92 |
| 5. | *Allium sativum* | Fresh bulbs | 20.5 | 0.88 |
| 6. | *Ficus religiosa* | Fresh leaves | 17.5 | 0.75 |
| 7. | *Azadirachta indica* | Fresh leaves | 17.0 | 0.73 |
| 8. | *Piper nigrum* | Dried fruits | 17.0 | 0.73 |
| 9. | *Cinnamomum tamala* | Dried leaves | 15.5 | 0.66 |
| 10. | *Tamarindus indica* | Dried seeds | 15.0 | 0.64 |
| 11. | *Jatropha curcas* | Fresh leaves | 14.5 | 0.62 |
| 12. | *Aloe barbadensis* | Fresh leaves | 14.0 | 0.60 |
| 13. | *Trachyspermum ammii* | Dried fruits | 14.0 | 0.60 |
| 14. | *Calotropis procera* | Fresh leaves | 13.5 | 0.58 |
| 15. | *Murraya koenigii* | Fresh leaves | 13.5 | 0.58 |
| 16. | *Bauhinia variegata* | Fresh leaves | 13.0 | 0.55 |
| 17. | *Acacia nilotica* | Bark | 10.0 | 0.43 |

*Average diameter of zone of inhibition for tetracycline (used as control) was 23.23mm.

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. The medium was autoclaved and left 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 (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:

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

**Results and Discussion**

Different ethanolic plant extracts were screened for their antimicrobial activities. All of them showed growth inhibitory activity against test bacteria *(E.coli)*. The zone of inhibitions for various extracts were compared with tetracycline used as control and their activity index was calculated (Table 1; Fig. 1).

Ethanolic extract of dried floral buds of *Polyalthia longifolia* showed maximum antibacterial activity (1.24). Its zone of inhibition was found even wider (29.0mm) than that of the tetracycline (23.23mm). Similarly ethanolic extract of *Datura stramonium* (24.0mm), *Trigonella foenum-graceum* (23.5mm), also showed better antimicrobial property than tetracycline against the test organism (*E.coli*). In all the cases the paper disc diameter was 0.6mm *Nerium oleander* (21.5mm),

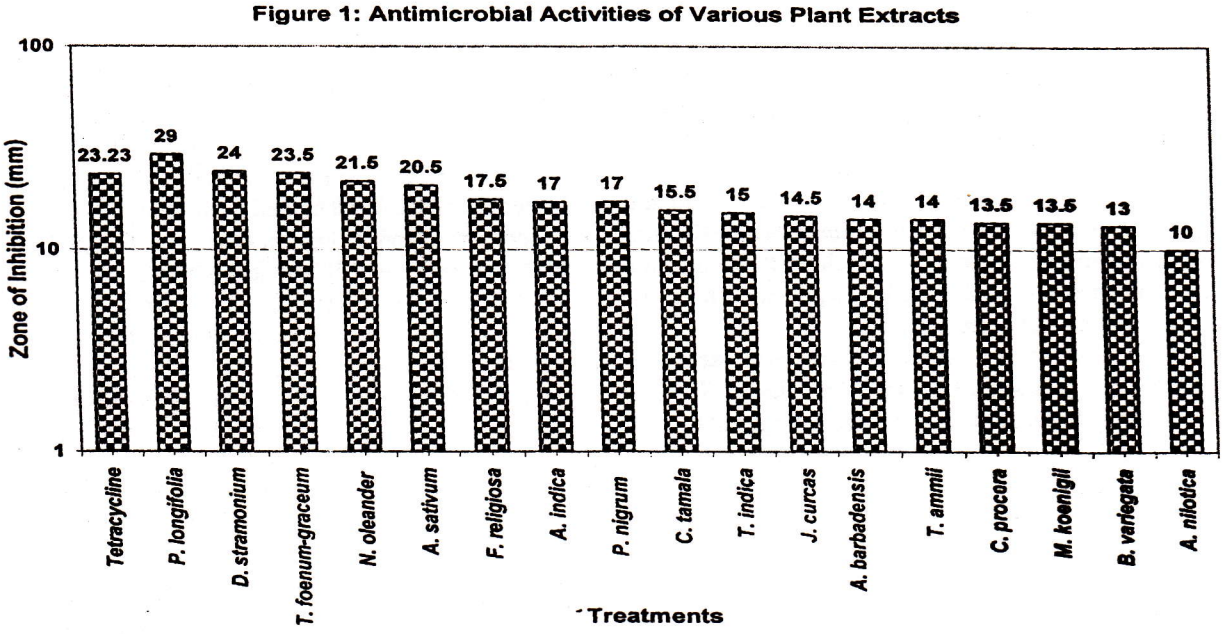


**Fig.1.** Antimicrobial activities of various plant extracts.

*Allium sativum* (20.5mm), showed antimicrobial activities moderately similar to tetracycline (23.23mm). Plants which showed 50 to 75% antimicrobial activities in comparision to tetracycline were *Ficus religiosa* (17.5mm), *Azadirachta indica* (17.0mm), *Piper nigrum* (17.0mm), *Cinnamomum tamala* (15.5 mm), *Tamarindus indica* (15.0 mm), *Jatropha curcas* (14.5mm), *Aloe barbadensis* (14.0mm), *Trachyspermum ammii* (14.0mm), *Calotropis procera* (13.5mm), *Murraya koenigii* (13.5mm) and *Bauhinia variegata* (13.0mm). Least antimicrobial activity was shown by bark extract of *Acacia nilotica* (diameter 10.0mm) with an activity index of 0.43.

Faizi *et al.*[7] carried out antimicrobial activity of various parts of *Polyalthia longifolia* var. *pendula* and isolated active principles from its leaves and berries. They found active antimicrobial agents in the methanolic extract with MIC values ranging between 7.8 and 500µg/ml. In our case ethanolic extract of *Polyalthia* showed maximum antimicrobial activity (1.24), even greater than the tetracycline. So it is strongly recommended that *Polyalthia* to be used for its antibacterial activity.

Chaudhry and Tariq[8] studied antibacterial potential of aqueous decoction of black pepper (*Piper nigrum L.*), bayleaf (*Laurus nobilis L.*), aniseed (*Pimpinella anisum L.*), and coriander (*Coriandum sativum L.*) against 176 bacterial isolates belonging to 12 different genera of bacterial population isolated from oral cavity of 200 individuals using disc diffusion technique. Overall aqueous decoction of black pepper was the most bacterial-toxic at the concentration of 10µl/disc. The aqueous decoction of coriander did not show any antibacterial effect against tested bacterial isolates. In present study also *Piper nigrum* showed moderate antibacterial activity (17mm) somewhat less than that of the tetracycline (23.23mm).

Arora and Kaur[9] studied aqueous extracts of ten medicinal plants for their antibacterial potential against some reference strains of human pathogenic bacteria. *Anethum graveolens*, *Elettaria cardamomum*, *Foeniculum vulgare*, *Trachyspermum ammi* and *Viola odorata* were found to be better/equally effective compared to standard antibiotics. They found *V. odorata* most effective antibacterial plant with minimum inhibitory concentration values ranging from 1 to 2%. Activity of *Trachyspermum ammii* was moderate (14.0mm) in the present study.

Eftekhar *et al.*[10] studied antibacterial activity of the methanol extracts of the aerial parts of the *Datura innoxia* and *Datura stramonium* was investigated. The extracts showed activity against Gram (+) bacteria in a dose dependent manner. They reported little or no antibacterial activity against *E. coli* and *Pseudomonas aeruginosa*. In the present study it was found that leaf extract from *Datura stramonium* (24mm) is even stronger than tetracycline (23.23mm). Thus it is recommended that *D. stramonium* can be used as an alternative to the antibiotics.

Various workers, Johnson and Vaughn[11], Skyrme[12], have reported antibacterial property of *Allium cepa*. Skyrme[12] reported inhibition against *E. coli* 10 times greater than that seen in *Lactobacillus casei* for the same garlic dose. In the present study it was found that antibacterial activity of bulb of *Allium sativum* (20.5mm) was moderately similar to tetracycline (23.23mm) and recommended it for further study as a potential alternative to tetracycline and other antibiotics.

Alzoreky and Nakahara[13] screened extracts of edible plants (26 species) from China, Japan, Thailand and Yemen for their antibacterial activity against bacteria including *E. coli* and *Bacillus cereus*. The minimum inhibitory concentrations (MICs) of extracts determined by the agar dilution method ranged from 165 to 2640 mg $l^{-1}$. The most sensitive microorganism to extracts from *Azadirachta indica*, *Cinnamomum cassia*, *Rumex nervosus*, *Ruta graveolens*, *Thymus serpyllum* and *Zingiber officinale* was *B. cereus*. *E. coli* was inhibited by *Cinnamomum cassia* extracts at the highest. Thakare[14] studied and investigated the antibacterial and feed additive potential of medicinal plants. Ethanol extracts of different medicinal plants including *Curcuma longa*, *Zingiber officinale*, *Piper nigrum* and *Cinnamomum cassia* were used against the common poultry pathogens including *E. coli*. Cinnamon extract exhibited antibacterial activity against *E. coli*. They recommended cinnamon as a substitute for antibiotics in the diet. Lower activities of *Azadirachta indica* (17.0mm) and *Cinnamomum tamala* (15.5 mm) in comparison to tetracycline (23.23mm) was found in the present studies. But they could be potential replacements for milder antibiotics, which need to be studied in the future.

Mothana *et al.*[15] reported good antibacterial activity by extracts from *Jatropha unicosta* against several Gram-positive and Gram-negative bacteria and against one yeast species using agar diffusion method. Alcoholic extract from leaves of *Jatropha curcas* in our case showed moderate antibacterial activity (14.5mm) in comparision to tetracycline.

Kareem *et al.*[16] studied the antimicrobial effect of ethanol, aqueous and chloroform extracts of leaf and latex of *Calotropis procera* on six bacteria namely, *E. coli, Staphylococcus aureus, S. albus, Streptococcus pyogenes, S. pneumoniae* and three fungi: *Aspergillus niger, A. flavus, Microsporium boulardii* and one yeast *Candida albicans* using agar well diffusion and paper disc methods. Their results revealed that ethanol was the best extractive solvent for antimicrobial properties of leaf and latex of *C. procera* followed in order by chloroform and aqueous. The ethanolic extracts of *C. procera* latex gave the widest zone of inhibition (14.1mm) against *E. coli* using agar well diffusion while 9.0 mm was recorded for the same organism in the disc plate method. The growth of six bacterial isolates was inhibited by the three extracts except *P. aeruginosa* and *S. pyogenes* that were not inhibited by the aqueous extracts of both leaf and latex of *C. procera*. They concluded that *C. procera* latex have strong inhibitory effect on the test organisms than *C. procera* leaf. The results, therefore, established a good support for the use of *C. procera* in traditional medicine. Result also support above data as 13.5mm wide zone of inhibition from the ethanolic leaf extract of *Calotropis procera* was found against *E.coli*. Different extraction mechanism will be employed in future study to check out its antibacterial and antimicrobial potential.

Cock[17] studied antibacterial activity of methanolic extracts from *Aloe barbadensis*. During the present investigation moderate antibacterial activity was observed from fresh leaf extract of *Aloe barbadensis* (14.0mm), with an activity index of 0.60. Antibacterial activity of *Acacia catechu* against 6 strains of *E. coli* causing gastrointestinal infections was reported by Supayang *et al.*[18]. Dried bark of *Acacia nilotica* in present case showed an activity index of 0.43, lowest among the plants selected for study.

Parekh *et al.*[19] evaluated the antibacterial activity and phytochemical analysis of bark of *Bauhinia variegata* L. the extraction was done in petroleum ether. The non-defatted plant material as well as the defatted plant material was individually extracted in different solvents with increasing polarity viz. 1,4-dioxan, acetone, methanol, dimethylformamide (DMF) and distilled water respectively. The extractive value of *B. variegata* L. for non-defatted extracts ranged from (0.7-13%) and for defatted extracts the extractive value ranged from (1-10.5%). The antibacterial activity of all the extracts (non-defatted and defatted) of *Bauhinia variegata* L. bark was determined by agar well diffusion method at three different concentrations *i.e.*, 10 mg/ml, 5 mg/ml and 2.5 mg/ml. The antibacterial activity of defatted extracts of *Bauhinia variegata* L. was more than those without defatting. Maximum activity was observed at highest concentration *i.e.* 10mg/ml. Defatted acetone and methanol extracts of *Bauhinia variegata* L. were most active as compared to other extracts against all the studied organisms. Petroleum ether extracts of *Bauhinia variegata* L. was inactive against all microorganism. In present study *Bauhinia variegata* did not show any significant activity (0.55) and found less antibacterial activity from the leaf extract of

*Bauhinia variegata* (13mm), among the 17 plants taken for study.

The present research work provides support to the work carried out by various workers and offers scientific basis for the centuries-old usage of extracts of these medicinal plants. These results further provide a basis for the isolation of compounds of biological interest from these medicinal plants.

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