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Biological Management of *Pseudomonas* Species in Cotton Crop Using Ethanomedicinal Flora of Sariska National Park, Rajasthan, India

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ABSTRACT: In vitro antibacterial activity of seventeen different medicinal plant extracts of Sariska National Park was tested against *Pseudomonas* species (*P. aeruginosa*, *P. syringae* and *P. viridiflava*) causing infectious diseases in cotton plant. Plant extracts of methanol introduced at 2 concentrations of 50 and 100 percent w/v used for testing. Results indicated that all 17 plants extracts show prominent effectiveness for control the pathogens. The extract of *Euphorbia nerifolia* stem latex was showed highest 1142.96 mm² inhibition annulus (IA) against *P. aeruginosa* at 100% concentration. The antibacterial activity highest found in extracts of *Desmodium gangeticum* resin (IA=3306.42 mm²), followed by *Terminalia bellerica* fruit (IA=2904.5 mm²) at 100% concentration against the *Pseudomonas syringae* pathogen and *Argemone mexicana* leaf extract was more effective to inhibit *P. viridiflava* pathogen exhibited 2176.02 mm² IA at 100% concentration. Pure methanol (100%) revealed the highest antibacterial properties concerning dilute form (50%). The present study concluded that the methanolic extracts of medicinal plants play a consequential role in eco-friendly disease management. Therefore, medicinal plant extracts can be used as antibacterial agents to replace synthetic bactericides.

KEYWORDS: Antibacterial, Ethanobotanical, *P. aeruginosa*, *P. syringae*, *P. viridiflava*, Sariska National Park

I. INTRODUCTION

Globally cotton is the most important fiber crop; a total of 34 million hectares of land cotton is grown in more than 50 countries economically in the tropical and temperate regions. For optimum production of the cotton crop, some environmental conditions are necessary such as adequate moisture with proper irrigation, dry and hot climatic condition, etc. These climatic conditions are found in some specific countries including India, Australia, USA, China, and the Middle East. A seedy coat of cotton expands into tubular fiber and is spun into yarn. In worldwide total 50 species of cotton in which four spp. are only cultivated. Among these 4 cultivated crops two of these tetraploids (*G. barbadense* and *G. hirsutum*), and two (*G. herbaceum* and *Gossypium arboreum*) are diploids. However, in the world more than 80 percent land of cotton crop. Also the 50 spp. acknowledge as dicotyledonous genus *Gossypium* (Malvaceae family). *Gossypium* genus has 45 diploids which are divided into 3 subgenera *Gossypium*, *Houzingenia*, and *Sturtia* according to geographical groups (Khadi *et al.*, 1970).

Various bacterial pathogens are the direct source of numerous diseases in several plant species (Lindeberget *et al.*, 2009; Martín-Sanz *et al.*, 2013). Among them, Pseudomonads are known as one of the pathogens to cause bacterial diseases associated with several infections. Species of *Pseudomonas* (Gram-negative bacteria) are generally aerobic bacilli measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm and due to presence of single polar flagellum it is motile. *Pseudomonas* genus having more than 140 spp., mostly are saprophytic (Iglewski, 1996). In addition, bacterial blight is a common disease of cotton caused by *Xanthomonas axonopodis* pathovar. The disease attacks the stem, leaf, and bolls of the cotton plant. On the stem it is known as a black arm; on the leaf as an angular leaf spot; and on the boll, as boll spot and boll rot. The trouble is disseminated by infected seeds. Generally the most noticeable symptom of the disorder form on the leaves, where spots occurred that is the appearance of water-soaked, at first dark green, bounded by small veins, and angular in form (Rofls, 1914). Thus, plant pathogens not only reduce crop yield with infections but also affects the economic value of the cotton plant.

The use of synthetic chemicals is dangerous to human health and the environment, demand increases the use of biologically active products such as essential oils, plant extracts, and medicinal plants are a natural source of antimicrobials to control microbial pathogens. Plants are an essential form for the production of novel CTA (Chemotherapeutic agents) (Agrawal *et al.*, 2012; Agrawal *et al.*, 2010; Soltania and Aliabadib, 2013). Mostly extracts of herbal plant parts have been utilized for their anti-bacterial activity.

Hills of Aravallis Sariska tiger reserve has its self-importance and certain features endowed with special biodiversity (Dular, 2015). Sariska National park (SNP) is located in the Alwar district of Rajasthan between the longitude (27°5' - 27°33'N and 76°17' - 76°34'E) latitude. Undulating area and around or on the hills *Anogeissus pendula* (Dhok) is the dominant species. Steep rocky areas growing species namely *Opuntia elatior* (Prickly pear), *Boswellia serrata* (Salar) and *Lannea coromandelica* (Garjan). *Zizyphus mauritiana* (Bordi), *Acacia catechu* (Khair), *Butea monosperma* (Dhak) and *Kydiacalycina* (Pulao) are grown in valleys (Meena, 2018). In Sariska Tiger Reserve approximately 403 cultivated indigenous plants and 86 families belonging to 271 genera. This also involves 4 plant species belongs to *Pteridophytes* under 3 genus and 3 families as well as one plant species relates to *Gymnosperm*. Sariska used total number of 110 spp. of plants which are belongs to 43 families and 88 genera as a medicinal healer of several disease like as diarrhea, jaundice, dysentery, fever, skin problems, rheumatism and diabetes etc. (Jain *et al.*, 2009). The aim of this study analysis the effectiveness of methanolic extracts of plants such as *Desmodium gangeticum* resin, *Butea monosperma* flower, *Azadirachta indica* leaf, *Phyllanthus niruri* leaf, *Tribulus terrestris* fruit, *Pongamia pinnata* leaf, *Eclipta prostrata* leaf, *Cassia fistula* leaf, *Nyctanthes arbor-tristis* leaf, *Argemone mexicana* leaf, *Bauhinia racemosa* leaf, *Terminalia bellerica* fruit, *Catharanthus roseus* leaf, *Euphorbia caducifolia* stem latex, *Euphorbia nerrifolia* stem latex, *Abrus precatorius* seeds and *Gloriosa superba* rhizome to control the cotton plant bacterial pathogen *Pseudomonas* species.

II. MATERIALS AND METHODS

Collection of Plant Sample

The infected plant materials (seed and fruit) were collected from the Thanagazi, Behror, and Mundawar areas of the Alwar district of Rajasthan from the farmer's field and ethnomedicinal plants were collected from Sariska National Park Area Alwar of Rajasthan.

Preparation of methanolic extracts

The collected seventeen Sariska medicinal plant parts were air dried in room temperature, followed by pounded into fine powder using grinder. By using Soxhlet extraction apparatus 5gm plant sample was extracted with 50ml methanol for 10 to 12 hours. Methanolic solution was filtered via Whatman No. 1 filter paper. Filtrate was concentrated by using rotary vacuum evaporator under reduced pressure at 90°C temperature. Sample can be stored at -18°C for further studies (Nahak and Sahu, 2017).

Test microorganism

Methanolic plant extracts examined antibacterial activity against *Pseudomonas aeruginosa*, *Pseudomonas syringae* and *Pseudomonas viridiflava* isolation from infected cotton plant parts.

Antibacterial screening of extracts

In vitro antibacterial activity of the crude methanolic extract of Sariska plants was studied through well diffusion method against isolated pathogen's (Perez *et al.*, 1990). In the bacteriological medium Mueller Hinton agar no. 2 (Hi Media, India) was used. The 100% concentration was prepared from crude methanol extract according to 10mg/ml with dimethyl-sulphoxide (DMSO). Further dilution of 50% conc. was made with DMSO (Soltania and Aliabadib, 2013). Both 100% and 50% concentration were used for the experiment. The Mueller Hinton agar was prepared and sterilized by autoclaving at 15 lbs pressure (121°C) for 15mins. After sterilized media preparation, Mueller Hinton agar plates were prepared in Laminar Air Flow. A standardized bacterial inoculum has 1.5 × 10⁸ CFU/mL, 0.5 McFarland was poured into sterile petri plates and allowed to stand for 30 second. After incubation, remaining non-adhere pathogenic bacteria culture was decanted. Further, in the nutrient agar plate wells of 6 mm diameter were prepared carefully. The extracts of plants (90 µl) were injected into the well. The desired temperature of 37°C agar plates was incubated. Determination of antimicrobial activity of the extract against pathogens was done by measuring inhibition diameter prepared around the each well. Inhibition zones of the each extract were compared with Dimethyl sulfoxide using as negative control. Inhibition annulus (IA) of each extract was measured (Smale and Keil, 1966; Thornberry, 1959; Jain and

Agrawal, 2011). $IA = \pi (R_1 - R_2) (R_1 + R_2)$ Here, R_1 is radius of inhibition zone + radius of filter paper disc, R_2 is radius of filter paper disc and π value is 3.14.

Table 1: Medicinal plant species selected for antibacterial activity

Plants	Local name	Family	Part used	Uses
<i>Desmodium gangeticum</i>	Salparni	<i>Papilionaceae</i>	Roots and seeds	postnatal complaints, diarrhoea, chronic fever, cough, vomiting, and asthma
<i>Butea monosperma</i>	Dhak	<i>Papilionaceae</i>	Seeds gum	Enhances Digestive System, Treats Hypertension, Combats Skin Infections
<i>Azadirachta indica</i>	Neem	<i>Meliaceae</i>	Leaves	Used neem as anti-diabetic, Anti-fungal, anti-viral, anti-bacterial, sedative and contraceptive.
<i>Phyllanthus niruri</i>	Stonebreaker	<i>Phyllanthaceae</i>	Whole plant	jaundice, gonorrhoea, frequent menstruation, and diabetes
<i>Tribulus terrestris</i>	Gokhru	<i>Zygophyllaceae</i>	Fruit	stone in urinary bladder
<i>Pongamia pinnata</i>	Karanj	<i>Fabaceae</i>	Tree	For migraine used leaf juice as a nasal drops. Treatment of dandruff in scalp applied seed powder.
<i>Eclipta prostrata</i>	Bhrangra	<i>Asteraceae</i>	Plant juice	jaundice and hair tonic
<i>Cassia fistula</i>	Amaltas	<i>Caesalpiniaceae</i>	Fruit pulp	treatment of inflammatory swellings and as a cleaning agent for ulcers and wounds
<i>Nyctanthes arbor-tristis</i>	Harsingar	<i>Nyctanthaceae</i>	Small tree	Leaves-bitter tonic, anti-inflammatory, antispasmodic, hypotensive, respiratory stimulant.
<i>Argemone mexicana</i>	Kateli	<i>Papaveraceae</i>	Root	chronic skin disease
<i>Bauhinia racemosa</i>	Bidi	<i>Caesalpiniaceae</i>	Stem bark	Weakness
<i>Terminalia belerica</i>	Bahera	<i>Combretaceae</i>	Fruit pulp unripe fruit	Purgative
<i>Catharanthus roseus</i>	Sadabaha	<i>Apocynaceae</i>	Leaf and stem	Anti-cancer activity
<i>Euphorbia caducifolia</i>	Danda-thor	<i>Euphorbiaceae</i>	Stem latex	treatment of bleeding wound, cutaneous eruption and other skin diseases
<i>Euphorbia nerrifolia</i>	Sehund	<i>Euphorbiaceae</i>	Stem latex	treatment of swelling, for purgation, cough, rhinitis
<i>Abrus precatorious</i>	Chirmi/Ratti	<i>Papilionaceae</i>	Seeds	nervous disorder
<i>Gloriosa superba</i>	Bachnag	<i>Colchicaceae</i>	Rhizome	Paste apply forehead and neck cure asthma in children



III. RESULTS AND DISCUSSION

Medicinal plants have several applications especially anti-microbial activity; therefore they can be used as therapeutic agent. Nowadays bacterial diseases are continuously increasing also increases in antibiotic resistance, which enhanced several disorders (Austin *et al.*, 1999). In higher plants found antimicrobial compounds which is useful in development of new medicines. Herbal based drugs have more effective to control bacterial diseases (Karthikeyan *et al.*, 2012).

All the data detailed in Table number 2, highest activity shown from methanol extract of *Desmodium gangeticum* resin (3306.42 mm²) and *Terminalia bellerica* fruit (2904.5 mm²) extracts at 100% conc. against the *Pseudomonas syringae* pathogen. *Butea monosperma* and *Eclipta prostrata* showed maximum antibacterial activity (2712.96 mm²) at 100% conc. against *Pseudomonas syringae* followed by *Desmodium gangeticum* resin (2348.72 mm²) at 50% conc., *Nyctanthes arbor-tristis* (2176.02 mm²) at 100% conc. against *Pseudomonas syringae*. The maximum IA against *P. aeruginosa* was displayed by *Euphorbia nerrifolia* stem latex extract with 1142.96 mm² thereafter *A. mexicana* leaf extract with 904.32 mm² at 100% concentration. The significant inhibition annulus 2176.02 mm², 1406.72 mm², 1271.1 mm² and 1142.96 mm² were recognized with pure methanolic extract of *A. mexicana* leaf, *D. gangeticum* resin, *A. precatorius* seeds, *C. roseus* leaf, *P. pinnata* leaf, *T. bellerica* fruit and *G. superba* rhizome against *P. viridiflavus* pathogen. 100% methanolic extract play important role as compared to 50% concentration.

Karthikeyan *et al.*, 2012 reported that the methanolic extract showed the highest antibacterial (24±2.3mm) against *S. mutants* thereafter extracts of *D. gangeticum* such as chloroform, aqueous and ethanol. Due to the presence of bio-active constituents such as sterols, essential oil, terpenoids and alkaloids, methanolic extracts consider as higher antibacterial properties which may be increased by the existence of methanol and higher potentiality extraction of methanol form a huge number of biological active compounds important for anti-microbial potential compared with chloroform, ethanol and aqueous extracts. Likewise Dave *et al.*, 2019 reported the methanolic extract of *Butea monosperma* exhibits maximum diameter of inhibition against various test microorganisms namely *B. cereus* (21±0.45mm), *B. subtilis* (22±0.5 mm) and *S. aureus* (17±0.5mm). Extracts of methanol showed better anti-bacterial activity towards the test pathogens in comparison to the other extracts like aqueous and acetone. Additionally, compared with acetone extract, the yield of methanol extract notably increased, which may be due to the highest solubility with *Butea monosperma* active compounds that responsible to the enhancement of anti-microbial activity. Hirapure and Pote, 2014 examined the antibacterial activity of *N. arbortristis* Linn. chloroform and ethanol extract of stem bark, roots bark and leaf according to inhibition zone of bacterial pathogens. Both chloroform and ethanol extracts exhibited various antibacterial potential against all the bacterial strains with the ranges of 3 to 13mm. The *A. precatorius* seeds contain steroids, flavonoids, alkaloids, lectins, anthocyanins, and fixed oils that is helpful to showing therapeutic potential especially antimicrobial property (Aswin *et al.*, 2022). A study conducted by Orozco-Nunnally *et al.*, 2021 reported that methanol extract of *A. mexicana* outer root and leaf possessed the strongest antimicrobial activity, with greatest effects against pathogenic bacteria. Latex of *Euphorbia* species is known for its use in traditional medicines. Several studies confirmed biological activity of *Euphorbia* extracts and pure compounds. These compounds could be used for the treatment of different diseases caused by different micro-organisms (Mishra and Parida, 2020).

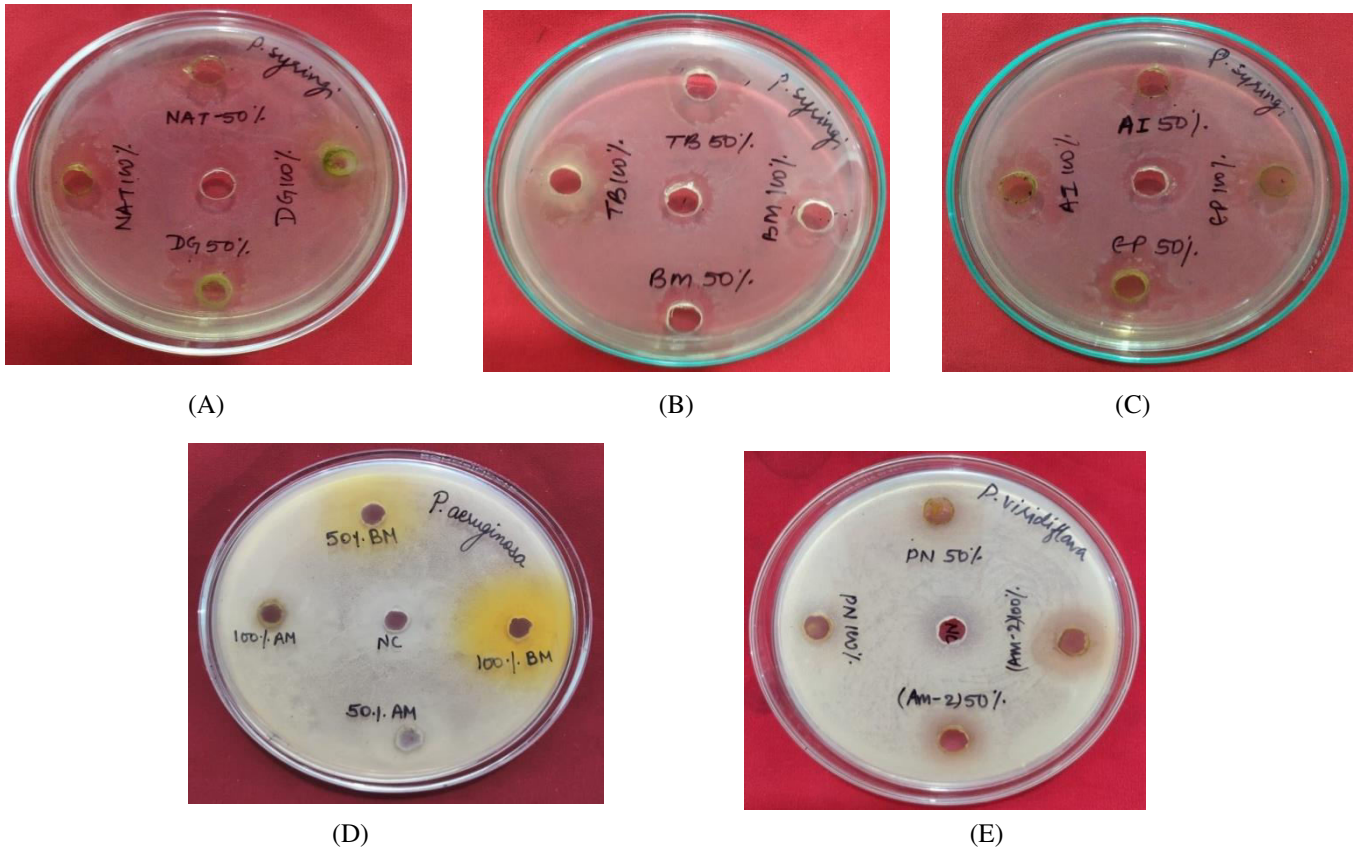


Figure 1: Maximum antibacterial activity of methanolic plant extract against *Pseudomonas spp.* (A) *Desmodium gangeticum* resin, *Nyctanthes arbor-tristis* leaf (B) *Terminalia bellerica* fruit (C) *Butea monosperma* flower (D) *Eclipta prostrata* leaf (E) *Argemone Mexicana* leaf

Table 2: *In vitro* evaluation of methanolic plant extracts for inhibition of *Pseudomonas spp.*

Methanol extract	Part used	Percent Concentration (w/v)					
		<i>Pseudomonas aeruginosa</i>		<i>Pseudomonas syringae</i>		<i>Pseudomonas viridiflava</i>	
		50% IA(mm ²)	100% IA(mm ²)	50% IA(mm ²)	100% IA(mm ²)	50% IA(mm ²)	100% IA(mm ²)
<i>Desmodium gangeticum</i>	Resin	Nil	593.46	2348.72	3306.42	690.8	1406.72
<i>Butea monosperma</i>	Flower	502.4	593.46	2009.6	2712.96	794.42	904.32
<i>Azadirachta indica</i>	Leaf	Nil	Nil	2009.6	2176.02	Nil	Nil
<i>Phyllanthus niruri</i>	Leaf	Nil	Nil	1271.1	1695.6	Nil	Nil

<i>Tribulusterrestris</i>	Fruit	Nil	Nil	1271.1	1548.02	794.42	904.32
<i>Pongamiapinnata</i>	Leaf	Nil	Nil	Nil	Nil	904.32	1142.96
<i>Ecliptaprostrata</i>	Leaf	Nil	Nil	2009.6	2712.96	741.82	794.42
<i>Cassia fistula</i>	Leaf	502.4	593.46	Nil	Nil	690.8	904.32
<i>Nyctanthes arbor-tristis</i>	Leaf	690.8	794.42	2009.6	2176.02	Nil	Nil
<i>Argemonemexicana</i>	Leaf	690.8	904.32	Nil	Nil	1476.58	2176.02
<i>Bauhiniaracemosa</i>	Leaf	Nil	Nil	Nil	Nil	741.82	848.58
<i>Terminalia bellerica</i>	Fruit	593.46	690.8	1695.6	2904.5	904.32	1142.96
<i>Catharanthroseous</i>	Leaf	Nil	Nil	1271.1	2009.6	Nil	1271.1
<i>Euphorbia caducifolia</i>	Stem latex	Nil	Nil	904.32	1849.46	Nil	690.8
<i>Euphorbia nerrifolia</i>	Stem latex	Nil	1142.96	1548.02	1849.46	904.32	1020.5
<i>Abrusprecatorious</i>	Seed	Nil	Nil	794.42	1271.1	1142.96	1406.72
<i>Gloriosasuperba</i>	Rhizome	Nil	690.8	794.42	904.32	690.8	1142.96

IV. CONCLUSION

Pseudomonas species cause mostly infection in cotton plants. The methanolic extracts of *Euphorbia nerrifolia* stem latex, *Desmodium gangeticum* resin and *Argemone Mexicana* leaf showed maximum inhibition activity towards *Pseudomonas aeruginosa*, *Pseudomonas syringae* and *Pseudomonas viridiflava* pathogens, respectively at 100% concentration. Other plant extracts also recognized as a good anti-bacterial agents. Overall, the results indicated that all 17 plants were prominent effective to inhibit bacterial pathogens. Hence, used Sariska plants extracts to inhibit harmful bacterial growth and their infection can be proved more effective in future due to being non-expensive, non-hazardous, and environmentally safe.

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