

Biocide potentialities of different plant methanolic extracts against crown gall bacteria viz *Agrobacterium tumefaciens*

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ABSTRACT

In this contemporary investigation fifty different plants used in traditional Indian medicine were examined against crown gall disease causing phytopathogen i.e. *Agrobacterium tumefaciens* using agar well diffusion method. The antimicrobial activities of fifty plant methanolic extracts demonstrated various inhibition effects on *A. tumefaciens*. Except the following six plants such as *A. ilicifolius*, *A. officinalis*, *C. sativum*, *P. rubra*, *R. communis*, and *T. pumila* were not exhibited the antimicrobial activity against *A. tumefaciens*. Among the selected fifty plants 88% of plants given remarkable bioactivity where as only the 12% of plants did not give antimicrobial activity against *A. tumefaciens*.

Key words: *Agrobacterium tumefaciens*, Crown gall disease, Indian medicinal plants, Soxhlet extraction, Bioactivity, Well diffusion method.

INTRODUCTION

A. tumefaciens is the causal agent of Crown Gall disease (the formation of tumors) in over 140 species of dicots. It is a rod shaped Gram Negative soil bacterium. *A. tumefaciens* is an alpha proteobacterium of the family Rhizobiaceae, which includes the nitrogen fixing legume symbionts. Unlike the nitrogen fixing symbionts, tumor producing *Agrobacterium* are parasitic and do not benefit the plant. The wide variety of plants affected by *Agrobacterium* makes it of great concern to the agriculture industry¹. Economically, *A. tumefaciens* is a serious pathogen of grape vines, stone fruits, nut trees, sugar beets, horse radish and rhubarb.

The plants that possess therapeutic properties on the animal or plant body are generally designated as medicinal plants. A medicinal plant

is any plant which, in one or more of its organ contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of new drugs. The plants which ecologically synthesis and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils, minerals and vitamins, they possess medicinal properties. Higher and aromatics plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts². Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases³. The use of phytochemicals as natural antimicrobial agents commonly called "biocides" is gaining popularity⁴. There is growing interest in correlating phytochemical constituents of plant with its pharmacological activity⁵. The main advantage of natural agents that they do not enhance the

antibiotic resistance, a phenomenon commonly encountered with the long-term use of synthetic antibiotics. It has been reported that the higher plants have shown to be a potential source for the new antimicrobial agents⁶. The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used. Antimicrobials therefore, may have a significant clinical value in treatment of resistant microbial strains⁷. In particular, the antimicrobial activities of plant oils and extracts have formed the basis of many applications including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies⁸. According to the above information the authors selected and collected fifty different medicinal plants from coastal region of Andhra Pradesh. They were extracted by using soxhlet apparatus and have been evaluated antibacterial activity against *A. tumefaciens*.

MATERIAL AND METHODS

Plant material and preparation of the extracts

The plant materials of fifty plant species (Table 1) were collected from different places at Visakhapatnam. The collected plants were identified and authenticated by Professor M. Venkaiah, Department of Botany, Andhra University, Visakhapatnam, Andhrapradesh. Voucher specimens have been deposited at the Herbarium of our department. The selected parts of different medicinal plants were cut into small pieces and shade dried at room temperature for fifteen days, finely powdered plant materials were successively extracted with methanol using soxhlet apparatus. The different extracts obtained were subsequently concentrated under reduced pressure to get their corresponding residues. The extracts were screened for antimicrobial activity using the method described under the section.

Microorganism employed

The plant extracts were assayed for antimicrobial activity against one reference bacterial strain *Agrobacterium tumefaciens* (MTCC 2250) obtained from Microbial Type Culture Collection & Gene Bank (MTCC), Chandigarh. And a strain isolate from soil and identified with biochemical examination was used in this present research. The bacteria was grown in the nutrient broth at 37°C

and maintained on nutrient agar slants at 4°C until used.

Antimicrobial analysis

The Methanolic extracts of fifty different plant extracts were screened for antimicrobial activity by agar well diffusion method⁹ with cork borer of size 6.0mm. The overnight cultures grown in nutrient broth was used for inoculation of nutrient agar plates. An aliquot (0.02ml) of inoculums was introduced to molten nutrient agar and poured on petri dish by pour plate technique. After solidification the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05ml of methanolic extracts of fifty different plant extracts were introduced serially after successful completion of one plant analysis. Incubation period of 24-48hours at 37p C was maintained for observation of antimicrobial activity of plant extracts. The antimicrobial activity was evaluated by qualifying zones of inhibition of bacterial growth surrounding the plant extracts. The complete antimicrobial analysis was carried out under strict aseptic conditions and results were represented in Table 2.

RESULTS AND DISCUSSION

The antimicrobial screening of fifty different methanolic plant extracts were reported in table 2. The antimicrobial activities of different medicinal plants (Table 1) methanolic extracts were studied in this investigation. Among the selected fifty plants forty four plants given remarkable bioactivity where as only the six plants *A. ilcifolius*, *A. officinalis*, *C. sativum*, *P. rubrum*, *R. communis* and *T. pumila* were not exhibited antimicrobial activity against *A. tumefaciens*. The antimicrobial activity was observed by forty four plants with variable concentrations, not with the same concentrations. Based on this criterion plants can be divided as A, B and C and called them as highest activity plants, moderate activity, and least activity plants respectively. The plants given activity up to 50mg/ml, 55-100mg/ml, and 110-300mg/ml concentrations were treated as group A, B and C respectively. The plants *B. montana*, *P. pterophorus*, *S. dulcis*, *T. chebula* and *W. somnifera*, were given bioactivity up to 50mg/ml concentration. Among them the highest activity was exhibited by *S. dulcis* at 15mg/ml concentration

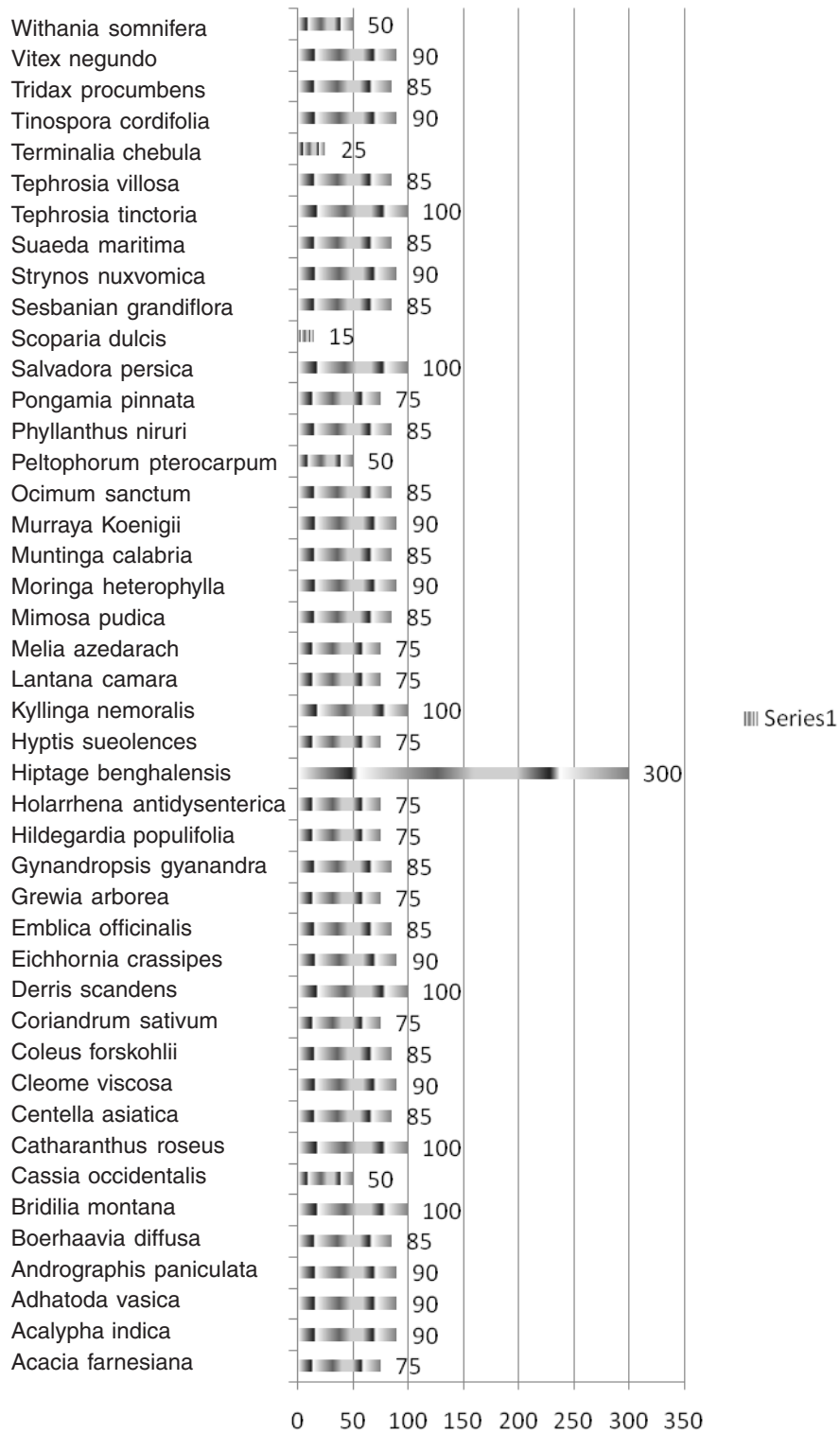
Table 1: List of investigated medicinal plants

Botanical Name	Parts used	Uses / Ailments treated
<i>Acacia farnesiana</i>	Bark, roots	Astringent, Demulcent, Poultice, Stomachic.
<i>Acalypha indica</i>	Aerial parts	Skin diseases, Ulcers Bronchitis, Head ache, Snake bite
<i>Acanthus ilicifolius</i>	Leaf extract	Relieve rheumatism
<i>Adenocalymma alliaceum</i>	Leaves	Astringent,
<i>Adhatoda vasica</i>	Leaves, whole plant	Cough chronic bronchitis, rheumatism, asthma and asthma.
<i>Andrographis paniculata</i>	Whole plant, leaves	Anti-biotic, anti-viral, anti-parasitic and immune system stimulant.
<i>Avicennia officinalis</i>	Seed	Relieving ulcers
<i>Boerhaavia diffusa</i>	Whole plant	Scabies, myalgia, aphrodisiac
<i>Bridelia montana</i>	Bark, Root Leaf	Stomach pains, sore eyes and headaches.
<i>Cassia occidentalis</i>	Whole plant	Boils, Spasm. Hysteria, Whooping cough
<i>Catharanthus roseus</i>	Leaves and roots	Anti-mitotic and Anti-microtubule agents
<i>Centella asiatica</i>	Whole Plant	Diuretic, treatment of leprosy, use as brain tonic and stimulates hair growth.
<i>Cleome viscosa</i>	Leaves and seeds	Anthelmintic, carminative, diaphoretic and rubefacient.
<i>Coleus forskohlii</i>	Roots	Treat heart and lung diseases, intestinal spasms, insomnia and convulsions. Antispasmodic.
<i>Coriandrum sativum</i>	Fruits	Colic, Laxative, Blood purifier, Indigestion, sore throat
<i>Derris scandens</i>	Stem	Arthritis, Anti-inflammatory
<i>Eichhornia crassipes</i>	Whole plant	Biomass, soil reclamation
<i>Embllica officinalis</i>	Fruit	Aperient, Carminative, Diuretic, Aphrodisiac, Laxative, Astringent and Refrigerant.
<i>Gmelina arborea</i>	leaves and roots	Gonorrhoea, catarrh of bladder, cough, cleaning the ulcers, insanity, epilepsy, fevers, indigestion, nerve tonic.
<i>Gynandropsis gynandra</i>	Leaf	Anti-irritant
<i>Hildegardia populifolia</i>	Stem bark	Dog bite, Malaria.
<i>Hiptage benghalensis</i>	Bark and seeds	Dysentery, piles, leprosy, colic, dyspepsia, chronic chest complaints, , spleen diseases, jaundice, bilious, calculi
<i>Holarrhena antidysenterica</i>	Leaves and bark	Insecticidal, cough, inflammation, skin diseases and leprosy
<i>Hyptis suaveolens</i>	Leaves	Antispasmodic, antirheumatic and antisporific
<i>Kyllinga nemoralis</i>	Whole Plant	Promotes action of liver, and relief prunitus
<i>Lantana camara</i>	Whole Plant	Antidote to snake venom, Malaria, wounds

Table 1. Cont.

		cuts ulcers, Eczema, Tumours
<i>Melia azedarach</i>	Leaves,	Vermifuge, Insecticide, Astringent, Tonic and Antispetic. It possesses anti diabetic, anti bacterial and anti viral
<i>Mimosa pudica</i>	Whole Plant	Menorrhagia, piles, Skin wounds Diarrhoea, Hydrocele, Whooping caught, Filiriasis
<i>Moringa heterophylla</i>	Roots, Seeds,	Antibiotic Anti-inflammatory and Diabetes
<i>Muntinga calabria</i>	Leaves	Antiseptic
<i>Murraya Koenigii</i>	Leaves	Skin diseases, Heminthiasis, Hyperdipsia, Pruritus, etc.
<i>Ocimum sanctum</i>	Leaves, Seeds	Malaria, bronchitis, colds, fevers, absorption, arthritis.
<i>Peltophorum pterocarpum</i>	Whole plant	Reclamation
<i>Phyllanthus niruri</i>	Leaves or herb	Jaundice, Diabetes
<i>Plumeria rubra</i>	Leaves	Ulcers, leprosy, inflammations, rubefacient.
<i>Pongamia pinnata</i>	Bark, seeds	Anti malaria , skin disease, rheumatic and leprous sores
<i>Ricinus communis</i>	Leaves	Jaundice, sores,
<i>Salvadora persica</i>	Twigs, roots	Antimicrobial and dental diseases
<i>Scoparia dulcis</i>	Leaves, bark, roots	Used for upper respiratory problems, congestion, menstrual disorders, fever, wounds and hemorrhoids
<i>Sesbania grandiflora</i>	Flowers	Treat gonorrhoea and for curing infection of the cornea.
<i>Strychnos nuxvomica</i>	Seeds	Cholera, chronic wounds, Ulcers, paralysis, Diabetes
<i>Suaeda maritima</i>	Whole plant	Bioremediation
<i>Tephrosia pumila</i>	Root	Rheumatism, fevers, pulmonary problems, bladder disorders, Coughing, hair loss, and reproductive disorders
<i>Tephrosia tinctoria</i>	Root	Antisymphilitic
<i>Tephrosia villosa</i>	Root, Leaves, Bark	Anthelmintic, alexiteric, leprosy, ulcers, antipyretic, cures diseases of liver, spleen, heart, blood, asthma etc.
<i>Terminalia chebula</i>	Fruit	Antimicrobial, digestive problems, mouthwash/gargle, astringent, and douche for vaginitis.
<i>Tinospora cordifolia</i>	Stem	Analgesic and anti-inflammatory.
<i>Tridax procumbens</i>	Whole plant	Antimicrobial, Anti-oxidant and Anti-inflammatory
<i>Vitex pentaphyllal</i>	Aerial parts	Foetid discharges, Febrifuge Rheumatism affections, catarrhal
<i>Withania somnifera</i>	Leaves	Sore eyes, Febrifuge, ulcers Cure sterility of women sedative

Table 2: Minimum inhibitory Concentration (MIC) analysis of fifty plants methanolic extracts on *A. tumefaciens*



against *A. tumefaciens* and *Terminalia chebula* also given very nice activity with 25mg/ml conc. Most of the tested plants have reported moderate activity with 55-100mg/ml conc. Among them *A. farnaciana*, *C. forskohlii*, *G. arborea*, *H. antidysenterica*, *H. populipolia*, *H. sueolences*, *L. camara*, *M. azedarach* and *P. pinnata* were shown good moderate activity with 75mg/ml concentration and finally least bioactivity was found with only one plant *H. bengalenses* at 300mg/ml conc. Therefore, this

result may suggest that methanolic extracts of screened plants would be helpful in treating crown gall disease in plants. In particular, the authors may recommend that the methanolic extract of *S. dulcis* can be used as potent biocide to treat the crown gall disease caused by *A. tumefaciens*. The observed findings suggested the further work on all the selected plants to evaluate their potential for use as antibacterials to treat human and plant related diseases.

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