SUMMARY
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Plants have been used in human healthcare system from time immemorial. The World Health Organization (WHO) has listed 20,000 medicinal plants globally (Gupta and Chadha 1995) and India’s contribution is 15-20% (Singh 2000). According to the WHO estimate, about 80% of the population in the developing countries depends directly on plants for its medicines (Pareek 1996; Mukhopadhyay 1998). More than 2000 drugs used in India are of plant origin (Dikshit 1999). Plant resources are depleting globally at an alarming rate and a number of economically and medicinally important plant species are under threat of extinction. In the last few decades over-exploitation of forest resources has led to species loss. As a result, 20-25% of existing plant species in India has become endangered (Laloo et al. 2006).

India is known for its traditional health care system Ayurveda, in this system of medicine plant based drugs play an important role in the management of diseases and disorders. The country is also well known for its diversity of plant species being one of the important hot spots of mega biodiversity of the world (Anon 1998). More than 8000 species of plants are used to prepare 10,000 herbal drug formulations (Rao and Patil 2005). The active principle responsible for the management of microbial pathogens from these plants has not been identified and their mode of action also not clearly established. Thus there is paucity of information with reference to biological activities of these plants. Hence there is a need to isolate and characterize the active principles responsible for the biological activities in general and antimicrobial activities in particular.

Considering these an attempt has been made to screen a few plants of the local flora used in traditional medicine for their antimicrobial potential with the following objectives

➢ To isolate and characterize antimicrobial agents of plant origin.
➢ To test their efficacy on some human and plant pathogenic bacteria and fungi.
➢ To determine Minimal Inhibitory Concentration and to test their efficacy in vivo on seed system.

In the present investigation, based on the routine screening of plants of the local flora for antimicrobial activity, five plants viz., Prosopis juliflora Swartz. (Fabaceae), Acacia nilotica (L.) Willd. Ex. Delile ssp. indica (Benth) Brenan
Oxalis corniculata L. (Oxalidaceae), Samanea saman Prain. (Fabaceae) and Punica granatum L. (Punicaceae) were selected. These plants were tested for their antibacterial potential against wide range of human pathogenic bacteria and phytopathogenic bacteria and phytopathogenic fungi.

**Test organisms**

*Human pathogenic bacteria*

 Cultures of *Proteus mirabilis*, *Citrobacter* sp., *Klebsiella* sp., *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* A, *Salmonella paratyphi* B, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Sh. flexneri* and *Sh. sonnei* were obtained from the government medical college hospital and other hospitals in Mysore, Karnataka and their characteristic diagnostic features were studied to confirm their identification.

*Phytopathogenic bacteria*

 Authentic pure cultures of phytopathogenic *Xanthomonas axonopodis* pv. *malvacearum* (*X. a. pv. m.*) isolated from cotton (*Gossypium herbaceum* L.), *Xanthomonas axonopodis* pv. *phaseoli* (*X. a. pv. p.*) isolated from French bean (*Phaseolus vulgaris* L.) and *Xanthomonas campestris* pv. *vesicatoria* (*X. c. pv. v.*) isolated from tomato (*Lycopersicon esculentum* Mill.) were obtained from DANIDA lab, DOS in Applied Botany and Biotechnology, University of Mysore, Mysore, India and their characteristic diagnostic features were studied.

*Phytopathogenic fungi*

 Sorghum seeds were collected from the farmer’s field, regulated market and retail shops to isolate the important phytopathogenic fungi associated with the seeds. The collected seed samples were subjected to standard blotter method. Twenty-five seeds per plate were plated on three layer moistened sterile blotter discs in petriplates. These plates were incubated at 22±2°C under alternating cycles of 12/12h of near ultraviolet (NUV) light and darkness for seven days. On the seventh day of incubation samples were screened for seed mycoflora with the help of stereo binocular microscope and also with the help of a compound microscope. Associated fungi were identified based on the growth characteristics, colony and spore morphological characters using standard manuals. Six species of *Fusarium* viz., *F. equiseti*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, *F. semitectum* and *F. solani*, three species of *Drechslera* viz., *D. tetramera*, *D. hawaiensis* and *D. halodes* which were frequently associated in higher percentage with sorghum seeds.
were isolated and their pure cultures were maintained. *F. graminearum* and *F. lateritium* were isolated from the diseased tissue of mulberry suffering from shoot blight and bud blight. Three important isolates of phytopathogenic *Alternaria alternata* were selected for the study. They were isolated from the seeds of paddy, seeds of sunflower and leaf of tobacco. These strains are known to cause seed rot and seed discoloration of paddy, seed rot and seedling blight in sunflower and leaf spot in tobacco, which are important crops of this region. All the test fungi were studied for their colony morphology and other characteristic diagnostic features.

The thesis comprises of six chapters. The first chapter deals with the general introduction about the problems and prospects of antimicrobials of plant origin.

The second chapter deals with antibacterial and antifungal activity of aqueous extract of all the five test plants against all the test pathogenic microbes following the procedures of cup diffusion and poisoned food technique. The results revealed that aqueous extract of all the plants have significant inhibitory activity against *Xanthomonas* pathovars. Inhibitory activity against human pathogenic bacteria was also observed in all the aqueous extracts, but antibacterial activity varied with the fourteen pathogenic bacteria tested. Aqueous extracts of all the plants were tested in different concentrations viz., 10, 20, 30, 40 and 50μl. Maximum inhibitory activity was observed in 50μl concentration against all the test pathogenic bacteria.

Among the different plants tested for antifungal activity, *Prosopis juliflora* was active against all the test phytopathogenic fungi. Hence different concentration of aqueous extract viz., 4, 8, 12, 16, 20 and 24% were tested against all the test fungi. Maximum inhibition of the mycelial growth was observed at 24% concentration.

The interesting observation is that aqueous extracts of all the plants retained antibacterial activity even after sterilization suggesting that the bioactive principle is thermostable.

The third chapter deals with the solvent extraction of dried leaves of all the test plants. Organic solvents such as petroleum ether, benzene, chloroform, ethanol and methanol in increasing polarity basis were selected for the extraction. All the extracts were obtained by successive extraction in Soxhlet extractor and evaporated to dryness in rotary flash evaporator. The antibacterial and antifungal activity assay of the solvent extracts against all the test pathogenic bacteria and
fungi revealed that methanol extract of all the plants showed strong antibacterial activity against human pathogenic and phytopathogenic bacteria followed by ethanol and chloroform. Least or no activity was observed in petroleum ether and benzene extract.

Significant antifungal activity was observed in the methanol and the ethanol extracts of *Prosopis juliflora* against all the fourteen phytopathogenic fungi.

The fourth chapter deals with phytochemical analysis of all the test plants following standard procedures of Roberts *et al.* 1981, Becknett and Stenlake (1986), Harborne (1998) and Anon (1985). The preliminary phytochemical analysis involved the tests for presence or absence of important plant metabolites such as alkaloids, carbohydrates and glycosides, phytosterols, fixed oils and fats, phenolic compounds/tannins, saponins, flavonoids, proteins and aminoacids, gums and mucilages and volatile oils. The results revealed that *Prosopis juliflora* showed the presence of alkaloids, carbohydrates and glycosides, phytosterols, phenolic compounds/tannins, saponins, flavonoids, proteins and aminoacids and gums and mucilages in both methanol and ethanol extract. Chloroform extract revealed the presence of alkaloids and petroleum ether extract revealed the presence of phytosterols.

In case of *Acacia nilotica* methanol and ethanol extracts recorded the presence of carbohydrates and glycosides, phytosterols, phenolic compounds/tannins, saponins, flavonoids, proteins and aminoacids and gums and mucilages. Alkaloids, fixed oils and fats and volatile oils were absent. Phytosterols were present in petroleum ether extract and flavonoids were present in chloroform extract.

Methanol and ethanol extract of *Punica granatum* recorded the presence of alkaloids, carbohydrates and glycosides, phytosterols, phenolic compounds/tannins, flavonoids, proteins and aminoacids. Fixed oils and fats, saponins, gums and mucilages and volatile oils were absent. Chloroform extract recorded the presence of alkaloids and flavonoids and petroleum ether extract recorded the presence of alkaloids and phytosterols.

Presence of carbohydrates and glycosides, phytosterols, phenolic compounds/tannins, flavonoids, proteins and aminoacids and volatile oils were observed in methanol and ethanol extracts of *Oxalis corniculata* where as it was
found negative for alkaloids, fixed oils and fats and gums and mucilages. Chloroform extract recorded the presence of flavonoids and petroleum ether for phytosterols.

*Samanea saman*, phytochemical analysis revealed the presence of alkaloids, carbohydrates and glycosides, phytosterols, phenolic compounds/tannins, saponins and flavonoids, proteins and amino acids and mucilages. Fixed oils and fats and volatile oils were absent. Chloroform extract was found to contain alkaloids and flavonoids and petroleum ether extract was found to contain phytosterols.

Thin layer chromatographic studies were conducted to identify the best elutant for clear separation of the compounds present in different solvent extract viz., petroleum ether, benzene, chloroform, methanol and ethanol. It was observed that chloroform as an elutant was the best for separation. Colours of the different bands that are easily observable when exposed to iodine and under UV light at 254 nm and 365 nm were recorded and tabulated in the respective tables.

Chapter five deals with isolation and characterization of the active principle responsible for antibacterial and antifungal activity. *Prosopis juliflora* which recorded highly significant antibacterial activity against *Xanthomonas* pathovars, *Staph. aureus* and *Strep. faecalis* and antifungal activity against all the fourteen test phytopathogenic fungi was further selected for isolation and characterization of active principle responsible for the activity. Simultaneously an attempt was made to isolate the active fractions responsible for the antibacterial activity in other four test plants.

Methanol extract of powdered leaves of *Prosopis juliflora* was subjected to fractionation following the procedures of Harborne (1998). The result confirmed that the alkaloid fraction of the plant is responsible for the antifungal and antibacterial activity. Isolation of active fraction (alkaloid) from the methanol extract of *P. juliflora* was done following the procedures of Becknett and Stenlake (1986) and Harborne (1998). This fraction also recorded highly significant antibacterial and antifungal activity. The active fraction thus obtained by the above procedure was subjected to TLC separation on silica gel G (Merck) with methanol: acetic acid (100:1) as elutant, resulting in separation of three bands at Rf values 0.47, 0.56 and 0.70 (Harborne 1998). Each band was recovered in to methanol, purified by TLC and again subjected to antibacterial and antifungal activity assay.
The results confirmed that the band at Rf value 0.47 is responsible for the desired activity.

The melting point of the active principle was measured by the capillary method and it has not been corrected. The UV spectrum was recorded on a Jeol UV spectrophotometer. The IR spectrum was recorded on a Shimadzu FT-IR spectrophotometer. Mass spectra were obtained on a Fennigan 4021 mass spectrophotometer at an ionizing energy of 35 eV.

The isolated pure alkaloid was then subjected to following spectral analysis

- $^1$H 1-dimensional spectrum
- $^{13}$C 1-dimensional spectrum
- Conventional $^1$H-$^{13}$C correlation spectrum (HSQC)
- Variant of the $^1$H-$^{13}$C correlation spectrum that distinguishes C with an even number of attached 1Hs from C with an odd number of attached $^1$Hs.

The characteristic parameters observed for the active principle is; UV (EtOH) $\lambda_{max}$ 235 nm; IR $v_{max}$ 3300-3350 (sharp, NH), 1745 (CO), 1600 (C=C) cm$^{-1}$; MS (relative intensity): (m/z) 224.15 (M$^+$, 22), 200 (M$^+$, 3) 95 (100). The compound was obtained as yellow semisolid in 30 mg/100g of leaves, m.p. 59-61°C. Anal. Calc. for C$_{12}$H$_{20}$N$_2$O$_2$: C, 64.29; H, 9.01; N, 12.47; O, 14.23%. Found Anal. Cal. for C, 64.28%; H, 9.02%; N, 12.49%; O, 14.21%.

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Proposed structure of Julifloravizole based on $^1$H and $^{13}$C NMR spectroscopy
The spectral analysis clearly revealed the structure of the compound as 1H-Imidazole-4-carboxylic acid 2-ethyl-hexyl ester. Neither the Beilstein nor the sciFinder Scholar databases contained this compound, indicating that the current work is the first to describe it. Thus the compound is named as Julifloravizole and the above structure is suggested.

Stability studies of the isolated novel Julifloravizole indicated that the compound is thermostable, stable during six months of storage without decrease in antibacterial and antifungal activity against all the test pathogens.

Julifloravizole showed fungicidal and bactericidal activity, which was confirmed by further studies.

Minimal Inhibitory Concentration studies of Julifloravizole revealed that the low concentration of the active principle is enough to completely inhibit the mycelial growth of almost all the fungi tested except F. lateritium at 400 ppm concentration.

Comparative efficacy studies of Julifloravizole with synthetic fungicides such as Blitox, Captan, Dithane M-45 and Thiram at recommended dose of 2000 ppm revealed that low concentration of Julifloravizole is effective in complete inhibition of the mycelial growth of the test fungi.

The active principle recorded highly significant antibacterial activity against all the Xanthomonas pathovars tested. MIC of the active principle was recorded as 4μg/ml, 3μg/ml and 2μg/ml to X. a. pv. m, X. a. pv. p. and X. c. pv. v. respectively. Comparative efficacy of the alkaloid with synthetic antibiotics Bact-805 and K-cycline tested at the recommended dosage revealed that the antibacterial activity of the active principle is highly significant against X. c. pv. v. and X. a. pv. p. and was found equal to the antibiotics tested in case of X. a. pv. m.

The active principle recorded antibacterial activity only against Staph. aureus and Strep. faecalis. MIC of the active principle was found to be 1 μg/ml and 2μg/ml for Staph. aureus and Strep. faecalis respectively. Comparative efficacy of the alkaloid Julifloravizole with the synthetic antibiotics revealed that the antibacterial activity is equal to that of gentamycin and streptomycin in case of Staph. aureus. In case of Strep. faecalis, the alkaloid was highly significant when compared with streptomycin and gentamycin.

Antibacterial activity guided fractionation of solvent extracts of other plants such as Acacia nilotica, Punica granatum, Samanea saman and Oxalis corniculata.
revealed that acidic fraction of *Acaica nilotica*, neutral fraction of the *Punica granatum*, alkaloid fraction of *Samanea saman* and phenolic fraction of *Oxalis corniculata* recorded significant activity.

**Against phytopathogenic bacteria**

Acidic fraction of the *A. nilotica* showed MIC of 4, 3 and 2μg/ml for *X. a. pv. m. X. a. pv. p.* and *X. c. pv. v.* respectively.

MIC of phenolic fraction of *O. corniculata* against *X. a. pv. m. X. a. pv. p.* and *X. c. pv. v.* were found to be 5, 5 and 4μg/ml respectively.

MIC of the alkaloid fraction of *S. saman* were found to be 6, 6, 4μg/ml for *X. a. pv. m. X. a. pv. p.* and *X. c. pv. v.* respectively.

Neutral fraction of the *P. granatum* showed MIC for *X. a. pv. m. X. a. pv. p.* and *X. c. pv. v.* was 5, 4 and 3μg/ml respectively.

MIC of the isolated active fractions was also studied in the range of 0-20 μg/ml; the results revealed that MIC varied for each test human pathogenic bacteria among the different fractions of the test plants.

The sixth chapter deals with the biological activities of Julifloravizole such as antibacterial, antifungal and antioxidant property. The antibacterial activity was tested against *Staph. aureus* which was identified and isolated from the hospital environment by Andersen sampler (Andersen 1958). The results revealed that MIC of the Julifloravizole is 2μg/ml. At MIC it was observed that antibacterial activity of the alkaloid was highly significant compared with synthetic antibiotics viz., Roxithromycin, Pefloxacin, Oxacilin, Amoxycillin, Cephatoxime, Penicillin, Cefozolin, Amikacin and Methicillin in 10 mcg/disc.

The antifungal potential was tested *in vivo* on sorghum seeds. Different concentrations of Julifloravizole viz., 250, 500 and 750 ppm was treated to sorghum seeds for different periods of 1h, 2h, 3h and 4h. The results revealed the significant reduction in the percent incidence of *Fusarium* sp., *Alternaria* sp., *Curvularia* sp., *Drechslera* sp., and other fungi over control. Maximum reduction was observed at 750 ppm concentration for 4h. with increase in seed germination and seedling vigour.

Comparative efficacy of the Julifloravizole with synthetic fungicides such as captan, blitox, dithane M-45 and thiram was also conducted on the seed system. The results revealed that the low concentration of alkaloids is highly effective in controlling seed borne pathogens compared to synthetic fungicides.
Seed storage studies of the sorghum seeds treated with Julifloravizole and control seeds revealed that the active principle is highly effective even up to six months of storage. The seed mycoflora, seed germination, seedling vigour, total water soluble protein and carbohydrate content was studied during the storage for both treated and control seeds at one month interval for six months period. The results revealed no change in the nutritional parameters in the seeds treated with active principle, where as control seeds showed great fluctuation in the total water soluble and carbohydrate content. After five months of storage seedling vigour and germination decreased marginally.

All the results were subjected to statistical analysis using SPSS for windows software.

Significant radical scavenging activity was also observed in the Julifloravizole tested by DPPH analysis. Radical scavenging activity was 59% compared with control ethanol at 40μg/ml.

The present investigation is successful in isolating and characterizing a novel active principle responsible for different biological activities from a medicinal plant Prosopis juliflora. Julifloravizole is a compound of interest isolated from the plant origin which recorded highly significant antibacterial activity both against plant and human pathogenic bacteria and a wide variety of phytopathogenic fungi. Moderate radical scavenging activity has also been demonstrated. The MIC studies have indicated its potential even at low concentrations. The possible use of this compound in the management of Staphylococcal infections in human beings needs to be investigated based on further toxicological studies. The utility of the active principle in the management of seed borne fungi and the prevention of biodeteriration of grains during storage has been demonstrated.

The present investigations has also demonstrated the need for further investigations on the isolation and characteristaion of the active principles from the other four plants viz., Acacia nilotica, Oxalis corniculata, Samanea saman and Punica granatum which have significant antimicrobial activity.