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Research Article

Phytochemical screening, antibacterial and allelopathic effects of few invasive plants of Kerala

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Abstract

Invasive species are often regarded as a threat to native flora. Many of them curtail the normal physiological functioning abilities of the native plants by growing over them, or by producing certain metabolites which control their growth potentials and seed germination abilities. The present study aims to find out the different bioactive compounds like alkaloids and terpenoids responsible for the vast spread of *Eupatorium odoratum*, *Vernonia cinerea*, *Mikania micrantha*, *Tridax procumbens*, *Pilea microphylla* and *Cuscuta reflexa* which are some of the major invasive plants of Kerala. Apart from these negative roles attributed to invasive plants, whether they possessed any beneficial roles was the prime concern of this study. Our study brings to light the allelopathic effects of invasive plants upon legume seeds. Different phytochemicals which are known to produce such effect were present in all these plants. Greatest allelopathic effects were exhibited by *C. reflexa* and *E. odoratum*. Against *Escherichia coli* bacteria, *E. odoratum* and *M. micrantha* showed highest zone of inhibition (20 mm, 15 mm) while against *Proteus vulgaris* bacteria, *C. reflexa, M. micrantha* and *T. procumbens* produced inhibition zones of 21 mm, 15 mm and 12 mm. Against *Pseudomonas aeruginosa* bacteria, *C. reflexa, M. micrantha* and *E. odoratum* produced inhibition zones of 16 mm, 13 mm and 12 mm. Alcoholic extract of *V. cinerea* showed comparatively high inhibition against *Staphylococcus aureus* bacteria (10 mm). *V. cinerea* showed inhibitiory effects against *E. coli*, *S. aureus* and *P. vulgaris* (11 mm, 10 mm and 9 mm). Similarly, *P. microphylla* showed inhibition only against *P. vulgaris* and *P. aeruginosa* (10 mm and 8 mm).

Keywords

Invasive plants; allelopathy; phytochemicals; antibacterial; inhibition zone

Citation

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Introduction

Biodiversity refers to the variety of living organisms. It is the distribution, number, variety and variability of living organisms over time. Habitat loss, introduced species, population growth, pollution and over consumption are regarded as the key reasons for loss of biodiversity. After habitat loss, invasive species are considered to be

the second largest threat to biodiversity. An invasive species is a plant, or an animal species that is not found in its native place and has a tendency to spread to a new area or location and affects the native plants/animals living in that environment. Common features of invasive exotics include their ability to reproduce both asexually and sexually, fast growth, rapid rate of reproduction, high dispersal ability, tolerance against a wide range of environmental conditions and the ability to adapt or modify themselves in order to suit to the newly inhabited area. Invasive species can compete with natives for food, water, mineral nutrients and space. They often reduce light availability, moisture content, nutrient sources, and space available to native species. Some invasive plants produce secondary metabolites that inhibit the growth and development of native plants.

An introduced species turned to an invasive species by outcompeting the native species for resources such as nutrients, light, physical space, water or food thereby establishing a colony of their own by gradually replacing the existing species. Invasive species sometimes coexist with native species by adapting to the environment much rapidly and increasing in number and thereby modify the environment for their suitability. Invasive plants are more likely to have potent secondary metabolites which have been reported to have multiple activities like anti-herbivory, antifungal, antimicrobial and allelopathic effects which may provide the plants with several advantages in their new environments (1). The presence of different chemical compounds in invasive plants gave super powers to them (2).

This study of few selected invasive plants like *Eupatorium odoratum, Mikania micrantha, Vernonia cinerea, Pilea microphylla, Tridax procumbens* and *Cuscuta reflexa* aims to find out the comparative phytochemical analysis, allelopathic effects and antimicrobial abilities, if any, for these groups of plants.

Materials and methods

Preparation of extract

Healthy plant specimens of *Eupatorium odoratum, Mikania micrantha, Tridax procumbens, Vernonia cinerea, Pilea microphylla* and *Cuscuta reflexa* were collected from Maharaja's College campus. The collected plant materials were washed under running tap water, shade dried, powdered and then stored in airtight containers. For phytochemical analysis, 10 g powder from each of the whole plant samples were weighed accurately and separately extracted in 50 ml alcohol, acetone, chloroform and centrifuged. The supernatant was collected and kept in refrigerator till further use.

Phytochemical analysis

Alcohol, acetone and chloroform extracts of *E. odoratum, M. micrantha, T. procumbens, V. cinerea, P. microphylla* and *C. reflexa* were used for phytochemical studies as per standard procedures (3).

Antimicrobial Assay

Preparation of culture medium: 28 g of nutrient agar was weighed and transferred into beaker containing 1 L distilled water. Gently heated the contents to dissolve the medium and covered the mouth of the beaker with aluminium foil. Petriplates and nutrient agar containing beaker was placed in autoclave and sterilized. Further operations were done in laminar air flow chamber. The sterilized agar medium was poured into petridishes and allowed to solidify at room temperature and kept in an incubator in inverted position for 24 h.

Four strains of bacteria available at Maharaja's College Botany lab were used for the study. The bacterial strains selected for study were *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Staphylococcus aureus. S. aureus* is gram positive and the rest were gram negative.

The experiment was done in a laminar air flow chamber. The bacterial culture in nutrient broth was swabbed using buds over the solidified agar medium. A cork borer was used to prepare well in the medium. The medium was kept in the incubator for 2 to 3 h. Extracts of the plants (1 g in 20 ml acetone, alcohol and chloroform) of 5% concentration were filled in the wells prepared. Ampicillin (0.2 g in 100 ml) was kept as the positive control and the solvent in which the plant extracts were prepared (alcohol, acetone and chloroform) were taken as the negative control respectively. The petriplates were then inverted and kept in the incubator at 37ºC for 24 h for the optimum growth of the organisms. After the stipulated period of time, petriplates were taken out, zone of inhibition was recorded using scale.

Allellopathic study

For the allelopathic studies, fresh leaves of *E. odoratum, M. micrantha, V. cinerea, T. procumbens, P. microphylla* and *C. reflexa* were collected and washed well under running tap water. All the extracts were prepared using distilled water in 1% concentration. The prepared extracts were filtered and kept in marked bottles for further use. Petridishes were washed properly in tap water and kept in hot air oven for complete drying. Petridishes were labeled and to each of them, fresh absorbent cotton was kept and extract was poured into each of them. Twenty five seeds each in two petridishes (duplicate) of the selected legume variety was placed as control (pure distilled water without any of the plant extracts), and also in different solvent extracts of the selected plants under study. Rate of germination

were recorded at every 24, 48 and 72 hours after treatment and photographs were taken using camera. The seeds were considered as germinated when the radicle emerged and the germination percentage was calculated using the formula:

$$\text{Germination Percentage} = \frac{\text{Number of seeds germinated x 100}}{\text{Total number of seeds sown.}}$$

Results

Phytochemical Analysis

Preliminary phytochemical analysis of the whole plant extract of *E. odoratum* revealed all the major phytoconstituents like alkaloids, terpenoids, protein, phytosterols, saponins, flavonoids, phenol and tannins (Supplementary Table 1). Both alcoholic and acetonic extracts of *E. odoratum* showed more or less similar results and chloroform was not at all effective in extraction of phytocompounds. In the case of *V. cinerea*, phytoconstituents were comparatively more in alcoholic extracts (Supplementary Table 2). Only alkaloids, terpenoids and tannins were consistently present in different solvent extracts of *V. cinerea*. Alcoholic extract of *M. micrantha* revealed comparatively higher number of phytoconstituents than both acetonic and chloroform extracts (Supplementary Table 3) and the phytochemicals were saponins, flavonoids, phenols and tannins. In case of *T. procumbens*, alkaloids, terpenoids and phytosterols were present in all the three solvents selected for extraction. Saponins were present only in alcohol extract of *T. procumbens* (Supplementary Table 4). Phytochemical studies of *P. microphylla* revealed the presence of terpenoids and phytosterols in all the three solvents selected for study, while saponins, flavonoids and protein were present only in alcoholic extract (Supplementary Table 5). *C. reflexa* revealed the presence of phytosterols in all the three solvents and flavonoids and tannins were present in both alcoholic and acetonic extracts (Supplementary Table 6). Terpenoids, proteins, saponins and phenols were present in alcoholic extract only.

Antibacterial studies

Antibacterial studies of whole plant extracts yielded interesting results. Acetonic plant extracts against *E. coli, E. odoratum* showed the highest zone of inhibition (20 mm) followed by *T. procumbens* (12 mm), *V. cinerea* (11 mm), *P. microphylla* and *M. micrantha* (10 mm) (Supplementary Fig. 2-B). The positive control (antibiotic) obtained 22 mm inhibition zone and negative control (acetone) obtained 12 mm. *C. reflexa* extract showed no inhibition at all against *E. coli*. Acetonic extracts against *P. vulgaris* showed maximum inhibition by *M. micrantha* (15 mm) followed by *T. procumbens (*12 mm) (Supplementary Fig. 2-A), *P. microphylla* (11 mm), *V. cinerea* (9 mm) and *C. reflexa* (7 mm). The positive control obtained 15 mm inhibition zone and negative control obtained 10 mm. Acetonic extracts against *P. aeruginosa* showed maximum inhibition by *M. micrantha* (13 mm) followed by *P. microphylla* (8 mm) (Supplementary Fig. 2-C), *T. procumbens* (7 mm) and *V. cinerea* (6 mm). The positive control obtained 32 mm inhibition zone and negative control obtained 10 mm. *C. reflexa* showed no rate of inhibition at all. Acetonic extracts against *S. aureus* was in the following order *V. cinerea* (10 mm) > *C. reflexa* (7 mm) and *P. microphylla* (6 mm). The positive control obtained 20 mm inhibition zone and negative control obtained 6 mm.

Alcoholic plant extracts against the different bacteria showed maximum inhibition by *E. odoratum*. No plant extracts in alcohol was effective against *E. coli*. Inhibition zone was obtained for *E. odoratum* plant extracts only against *P. vulgaris* and *S. aureus* (7 mm and 5 mm) (Supplementary Fig. 2-E). The positive control obtained 16 mm inhibition zone and 25 mm respectively against both *P. vulgaris* and *S. aureus* bacteria and negative control obtained no zones of inhibition in both cases. Only *T. procumbens* alcoholic extract was effective against *P. aeruginosa* bacteria (9 mm), here the positive control obtained 24 mm and negative control obtained 7 mm (Supplementary Fig. 2-F).

Chloroform extracts of *M. micrantha, T. procumbens* and *V. cinerea* were effective against *E. coli* (15 mm, 5 mm and 3 mm) (Supplementary Fig. 2-H), where the positive control was (35 mm) and negative control (chloroform) was 0 mm. Chloroform extracts of *C. reflexa, M. micrantha, P. microphylla, E. odoratum, T. procumbens* and *V. cinerea* were effective against *P. vulgaris* (21 mm, 17 mm, 12 mm, 11 mm, 5 cm and 5 mm respectively) (Supplementary Fig. 2-I, J & K), here the positive control was 19 mm and negative control was 9 mm. Chloroform extracts of *C. reflexa, E. odoratum*, *V. cinerea* and *T. procumbens* were effective against *P. aeruginosa* (16 mm, 12 mm, 6 mm, and 5 mm) respectively (Supplementary Fig. 2-G & K). Here the positive control was 26 mm and negative control was 9 mm.

Allellopathic studies

Seed germination studies of selected legume revealed interesting features (Table 1). All the seeds of the selected legume germinated under control, *i.e.* in pure distilled water after 24 h of treatment. The seeds treated with fresh plant extracts of *C. reflexa* and *E. odoratum* did not germinate at all even after 72 h. Seed germination in *M. micrantha* showed 24% (12 seeds germinated out of 50 seeds selected for study) followed by 32% in *P. microphylla* (16 seeds germinated out of 50 seeds selected for study) 56% in *T. procumbens* (28 seeds germinated out of 50 seeds selected for

Table 1. Seed germination studies of selected invasive plants

Name of the plant	Number of seeds germinated out of 50 seeds	Percentage of seed germination	Percentage of seed germination (Control)
Eupatorium odoratum	Nil	0%	100%
Cuscuta reflexa	Nil	0%	100%
Mikania micrantha	12	24%	100%
Pilea microphylla	16	32%	100%
Tridax procumbens	28	56%	100%
Vernonia cinerea	32	64%	100%

study) and 64% in *V. cinerea* (32 seeds germinated out of 50 seeds selected for study). The allelopathic effect is considered to be highest for *C. reflexa* and *E. odoratum* followed by *M. micrantha* and *P. microphylla*. Allelopathic effect may be in reduced form for both *V. cinerea* and *T. procumbens* as above 50% seeds germinated in both of them.

Discussion

In the present study, the plants selected belonged to Asteraceae, Convoluvulaceae and Urtricaceae families. Asteraceae have the peculiarity of numerous seeds and pappus hairs that help them for long dispersal through wind and thus contributing to their invasive nature. From the study, *M. micrantha, E. odoratum* and *C. reflexa* grow over many native plants thereby reducing the availability of sunlight, curtailing the photosynthetic abilities and suspend the growth of host plants.

Phytochemical analysis of the different plant extracts revealed almost all the major phytoconstituents including alkaloids, phytosterols, diterpenes, flavonoids, saponins, tannins, phenols, etc. The present study on *E. odoratum* revealed the presence of alkaloids, flavonoids, phytosterols and diterpenes. Studies by Nayak *et al.* (4), revealed the presence of alkaloids in the phytochemical analysis of *E. odoratum*. Germination studies revealed the effects of certain phytochemicals which checked the normal process of germination. Seed germination could not even take place in the extracts of *E. odoratum*. According to Hoque *et al*. (5), different concentrations of *E. odoratum* leaf extracts caused significant inhibitory effects on germination, root and shoot elongation and development of lateral roots of receptor crops. Further it has been proposed that inhibitory effects were proportional to the concentration of the extracts and higher concentration has the stronger inhibitory effect (6-8). Acetonee extracts of *E. odoratum* were active against *E.coli*. From Supplementary Fig. 2-B it can be understood that against *E. coli, E. odoratum* showed the maximum zone of inhibition (20 mm). It was almost equal to positive control (antibiotic control) (22 mm) and higher than negative control (12 mm). Alcoholic extracts of *E. odoratum* showed inhibition against *S. aureus* (5 mm) (Supplementary Fig. 2-E) and *P. vulgaris* bacteria (7 mm) (Supplementary Fig. 2-E). Chloroform extracts of *E. odoratum* showed inhibition against *P. vulgaris* (11 mm) (Supplementary Fig. 2-K) and *P. aeruginosa* (12 mm) (Supplementary Fig. 2-K). Studies by Jai Sunder *et al.* (9), revealed that ethanol and methanol extracts of *E. odoratum* leaves showed maximum antibacterial activity against *Salmonella pullorum, S. aureus, E. coli, Enterobacter aerogenes* and *Pseudomonas aeruginosa*. Studies of Singh *et al.* (10), proved the antimicrobial activity of aqueous and methanolic leaf extract of *E. odoratum* against bacteria of clinical and non-clinical origin.

Analysis of *V. cinerea* whole plant extract revealed the presence of alkaloids, tannins, diterpenes, phytosterols and flavonoids. Haque *et al.* (11), conducted phytochemical screening of *V. cinerea* and showed the presence of steroids, triterpenoids and esters in the methanolic extract of stem bark and the leaves of the plant. Our study was in confirmation with the above said results. Allelopathic studies revealed 64% seed germination in *V. cinerea*. Studies of Purohit and Rosalin (12) revealed that the quantitative estimation of alkaloid contents of *V. cinerea* is 17.5% and they attribute allellopathic effects of the same may be due to this high concentration of alkaloids. Our studies also confirmed the presence of alkaloids in all the solvents selected for the study. Against *P. vulgaris and E. coli*, acetone extracts of *V. cinerea* showed inhibition zone of 9 mm and 11 mm respectively which was higher than negative control (6 mm) (Supplementary Fig. 2-D). Similarly, chloroform extracts of *V. cinerea* showed 10 mm zone of inhibition against *E. coli* and 5 mm against *P. vulgaris*. As per the studies of Gupta *et al.* (13), the benzene extract of *V. cinerea* showed a broad spectrum of antibacterial activity.

The phytochemical analysis of *M. micrantha* showed the presence of tannins, phenol, saponins and flavonoids in alcohol and tannins and diterpenes in acetone and carbohydrates and diterpenes in chloroform extracts. Phytochemical

studies of the crude extracts of *M. micrantha* by Matawali *et al*. (14), had detected the presence of tannins, polyphenols, alkaloids, saponins and triterpenoids. Studies of *M. scandens* by Banerjee *et al.* (15), revealed the presence of alkaloids, flavonoids, tannins and steroids. In *Mikania micrantha* seed germination percentage was 24%. Studies of Wong (16), pointed out that *M. micrantha* extracts can significantly reduce the dry weight and nitrogen content of tomato seedlings and legume cover crops. Acetone extracts of *M. micrantha* were active against *P. vulgaris, P. aeruginosa* and *E. coli* which obtained 15 mm, 13 mm and 10 mm zone of inhibition (Supplementary Fig. 2-A). Alcoholic extracts could not bring about inhibition against microbes under study. Chloroform extracts of *M. micrantha*, showed zone of inhibition of 15 mm against *E. coli* (Supplementary Fig. 2-H). Against *Proteus* bacteria, *M. micrantha* obtained 15 mm zone of inhibition as can be understood from the Supplementary Fig. 2-I & J). Some of the earlier studies reported that *M. micrantha* contained sesquiterpene lactones, diterpenes, flavonoids and phenolic compounds that mostly responsible for allelopathic response, antibacterial and anticancer activities (17-20).

The preliminary analysis of *T. procumbens* revealed the presence of alkaloids, diterpenes and phytosterols in all the solvents (alcohol, acetone, & chloroform) selected for the study and the alcoholic extract revealed the presence of saponins also. The chloroform extract of leaves of *T. procumbens* by Sawant and Godghate (21) revealed the presence of steroids, saponins, alkaloids, coumarins, aminoacids, diterpenes, phenols and flavonoids. Our studies are in confirmation with the presence of above said components except coumarins, phenols and flavonoids. Allelopathic studies brings out 56% seed germination in *Tridax*, (28 seeds germinated out of 50 seeds selected for study). According to Manonmani *et al*. (22), aqueous leaf extracts of different concentrations of *T. procumbens* was found to have inhibitory effect on germination, root shoot elongation and fresh and dry weight of receptor plants and they concluded that the inhibitory effect was much more pronounced at higher concentrations. Acetone extracts of *T. procumbens* were active against *E. coli, P. vulgaris* and *P. aeruginosa.* From Supplementary Fig. 2-A & B, it can be understood that against *E. coli and P. vulgaris, T. procumbens* showed 12 mm zone of inhibition and against *P. aeruginosa*, *T. procumbens* produced 7 mm zone of inhibition. Alcoholic extracts of *Tridax* was found to be active only against *P. aeruginosa* bacteria (9 mm) (Supplementary Fig. 2-F). Chloroform extracts of *T. procumbens* showed zone of inhibition of 5 mm against *E. coli* (Supplementary Fig. 2-H). As per the studies of Bharathi *et al*. (23), the ethyl acetate extract of *T. procumbens* showed significant zone of inhibition against *S. aureus, S. typhi* and *B. cereus* than other two bacterial strains *Klebsiella pneumonia* and *E. coli* which showed lesser inhibition zones. Our studies showed comparatively higher inhibition against *E. coli* in acetone extracts.

Phytochemical analysis of *P. microphylla* whole plant showed the presence of phytosterols and diterpenes in all the solvents selected for study. Alcoholic extract brings out saponins, flavonoids, protein and tannin in addition to the above said phytoconstituents. Phytochemical studies by Chahardehi *et al*. (24), showed high concentrations of phenol and flavonoids in them. Allelopathic studies revealed 32% seed germination in *Pilea,* (16 seeds germinated out of 50 seeds selected for study). From the literature available, much conclusive support has not been obtained regarding the allelopathic properties of *P. microphylla*. Antibacterial activity was comparatively less in *Pilea*, both acetonic and chloroform extracts showed active inhibition only against *P. vulgaris*. Against *P. aeruginosa*, *P. microphylla* acetone extracts obtained maximum inhibition zone of 8 mm (Supplementary Fig. 2-C). Chloroform extracts of *P. microphylla* exhibited 10 mm inhibition against *P. vulgaris* where the positive control was 19 mm and negative control was 9 mm (Supplementary Fig. 2-I). According to Chahardehi *et al*. (24), crude extracts of *P. microphylla* inhibited growth of bacteria like *B. cereus*, *B. subtilis, S. aureus* and *E. coli*.

Phytochemical analysis of *C. reflexa* could bring out the presence of phytosterols in all the three solvents selected for study but flavonoids and tannins were present in acetone and alcohole extracts only. Protein, phenol, diterpenes and saponins were present only in alcoholic extract. As per the studies of Tapsya *et al.* (25), preliminary phytochemical screening revealed alkaloids, glycosides and flavonoids as the major groups of phytochemicals present in the extracts. The seeds treated with fresh plant extracts of *C. reflexa* did not germinate at all even after 72 h. According to Yu *et al.* (26), there are allelochemicals in *C. australis* and many *Cuscuta* species which inhibited the growth and germination of seeds including weeds that can influence plant population density. Only chloroform extracts showed inhibitory activity against bacteria under study while both acetonic and alcoholic extracts were not effective. Chloroform extracts of *C. reflexa* brings about 21 mm inhibition zone against *P. vulgaris,* where the positive control was 19 mm and negative control was 9 mm (Supplementary Fig. 2-K). Here *C. reflexa* got values higher than the positive control which can be considered as a good result. Against *P. aeruginosa*, C. *reflexa* got 16 mm against positive control of 26 mm and negative control of 9 mm (Supplementary Fig. 2-K). Studies using ethanolic whole plant extracts of *C. reflexa* exhibited highest bactericidal properties against *E. coli*, followed by *B. cereus* and *S. aureus* but was not effective against *S. typhi* regardless of extract concentration (27). Studies revealed that

terpenoids and phytosterols help plants against biotic or abiotic stresses and they are treated as signal molecules to attract the insects of pollination (28). From the present study, it can be learned that these compounds which are found in all the invasive plants selected, may help the plants to survive and propagate better under stressed conditions.

In conclusion, it can be inferred that, against *E. coli*, *E. odoratum* acetone (20 mm) and *M. micrantha* chloroform (15 mm) extracts are the best ones for inhibition where as in case of *P. vulgaris*, *C. reflexa* chloroform extract (21 mm) produced high effects followed by *M. micrantha* chloroform (15 mm). Against *P. aeruginosa*, *C. reflexa* chloroform (16 mm) and *M. micrantha* acetone (13 mm) performed well. In the case of *S. aureus*, only acetone extracts of *V. cinerea*, *C. reflexa*, *P. microphylla* performed better (10 mm, 7 mm and 6 mm). Alcoholic extract of *E. odoratum* extract could bring out 5 mm zone of inhibition against *S. aureus* bacteria.

Conclusion

The selected invasive plants possessed several secondary metabolites which may be responsible for their invasive nature. Another major fact was that many of these compounds possessed by them proved to be efficient bactericidal agents (alkaloids, flavonoids, phenols, phytosterols, diterpenes, tannins and saponins) which can be used for synthesizing valuable drugs against both gram positive and gram negative organisms. *Eupatorium odoratum*, *Mikania micrantha* and *Cuscuta reflexa* showed comparatively high antibacterial results against *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. Many of these secondary metabolites like alkaloids, saponins flavonoids etc which possessed strong antimicrobial, anti-infla mmatory, antitumor, antispasmodic and other such medicinal properties which can be utilized by thorough scientific investigation and isolation of individual compounds in them. This may help in the formulation of many wonder drugs for combating several harmful/ resistant microbes, disorders, etc. for the future generations to come.

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Conflict of interest

The authors have no conflict of interest.

Authors' contribution

GCU, AA and SG collected the specimens for study, conducted experiments and documented the data. JMJ designed and supported the experiments, supervised throughout the process and written up the whole manuscript.

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