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RESEARCH ARTICLE

Antibacterial property of plant extracts against few bacteria

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SUMMARY

An investigation was carried out to study the effect of aqueous and ethanolic root extracts of *Anacyclus pyrethrum* and *Clitoria ternatea* on *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescence* and *Staphylococcus faecalis.* The ethanolic root extract of study plants exhibited Higher Relative Magnitude of inhibition against *Klebsiella pneumoniae*.In general all the tested bacteria were inhibited by both the root extracts. Regarding the concentration 100 µl/ml was more inhibitory than any other concentration.

Key Words **:** Plant extract, Relative magnitude of inhibition, Antibacterial activity

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In recent times, a renewed interest in the screening of medicinal plants for antimicrobial activity has been noticed because India is a hot spot of plant diversity and its vast plant resources are still fully untapped for their antipathogen activity. India is a leading third world country practicing the ancient herbal medicine authoritatively traditional wisdom in health is always realized with medicinal plants in our country. Now a days increasing recognition on raising importance of herbal remedies in western countries is observed in modern medicine too. Increase in demand for neutraceuticals is felt seriously in developed countries. India is exporting the medicinal and aromatic plants worth about Rs. 2000 million annually.

In this regard India has a unique position in the world, where a number of recognized indigenous systems of medicine *viz.*, Ayurveda, Siddha, Unani, Homeopathy, Yoga and Naturopathy are being utilized for the health care of people.

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The demand for plant based medicines, health products, pharmaceuticals, food supplement, cosmetics etc are increasing in both developing and developed countries, due to the growing recognition that the natural products are non-toxic, have less side effects and easily available at affordable prices (Ncube *et al*., 2008).

According to world health organization, medicinal plants are the best source to obtain a variety of newer herbal drugs. About 80 per cent of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficacy. The world is now looking towards India for new drugs to manage various challenging diseases because of its rich biodiversity of medicinal plants. This worldwide interest in medicinal plants and the development of microbial resistance to the available antibiotics has led the authors to investigate the medicinal value of few medicinal plants.

MATERIALS AND METHODS

Plant materials were collected from Revenue Village of Thindal, Erode District, and confirmed with authentic herbarium specimens available in the Botany Department, Vellalar College for Women, Thindal, Erode.

Fresh leaves were collected and shade dried under room

temperature. The dried were grained into a course powder and macerated by using mortar and pestle. A soxhlet apparatus was used for extracting compounds from the powder. 20 gram dried powder was picked with thimble and then subjected to extraction with the water and ethanol separately. The extracts were concentrated by evaporation under room temperature and later used for antibacterial activity.

Qualitative priliminary study :

All the extracts were subjected to preliminary phytochemical tests followed by the methods of Herborne, (1973); Sadasivam and Manickam (1996); Trease and Evans, (1989). Tests for alkaloids, anthroquinone, flavonoids, cardiac glycosides, coumarins, phenols, saponins, steroids, tannins and triterpenoids.

Quantitative analysis :

Various methods used for estimations are,

- Assay of amino acids (Moore and Stein, 1948)
- Assay of μ - amylase (Nanmori, 1988)
- Assay of b- amylase (Nanmori, 1988)
- Assay of carbohydrate by Anthrone method (Hedge and Hofretier, 1962)
- Assay of DNA by diphenylamine method (Sadasivam *et al*., 1975)
- Assay of glutamine (Colowick *et al*., 1951)
- Assay of protein by Lowry's method (Lowry *et al*., 1951)
- Assay of proline (Bates *et al*., 1973)
- Assay of phenolic compounds (Bray and Thorne, 1954)
- Assay of RNA by orcinol method (Sadasivam *et al*., 1975)

Bacterial cultures used in this study were obtained from MTCC, Chandigarh. All the cultures (*Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescence* and *Staphylococcus faecalis)* were grown in Muller- Hilton agar medium. The inoculums were used for antibacterial assay. The media and the test bacterial cultures were poured into dishes. The test strain (0.2 ml) was inoculated into the media to inoculum size (108 cells/ml) when the temperature reached 40-42^{0}C. Care was taken to ensure proper homogenization. The extracts were tested for antibacterial activity in the agar well diffusion assay (Perez *et al*., 1990) against selected bacterial.

RESULTS AND DISCUSSION

The qualitative analysis of the extracts from the root sample of *Anacyclus pyrethrum* showed the presence of phytochemical constituents such as alkaloids, flavonoids, phenols, saponins, tannins and triterrpenoids. At the same time the phytochemical constituents like antheroquinone, cardiac glycosides, coumarins and steroids were absent. Root sample of Clitoria *ternatea* showed the presence of alkaloids, antheroquinone, flavonoids, coumarins, phenols and tannins. The quantitative analysis of root samples of the present study has been illustrated in Table 2.

The data presented in Table 3 and 4 show the antibacterial activity of root extracts of *Anacyclus pyrethrum* against *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescence* and *Staphylococcus faecalis.* Antibacterial potential varied from species to species of test organisms. The ethanol extract was found to be more effective against *Escherichia coli* and *Pseudomonas fluorescence* than *Bacillus cereus, Klebsiella*

Table 1 : Qualitative analysis of root extract

Name of the plants	Name of the constituents									
	Alkaloids	Anthroquinone	Flavonoids	Cardiac Glycosides	Coumarins	Phenols	Saponins	Steroids	Tannins	Triterpenoids
Anacyclus Pyrethrum	+	-	+	+	+	+	+	-	+	+
Clitoria ternatea	+	+	+	-	+	+	-	-	+	-

(+) - Presence of phytochemical constituent, (-) - Absence of phytochemical constituent

Table 2 : Quantitative analysis of root extract

Name of the plants	Name of the constituents (*mg/g*)									
	Amino acid	α- Amylase	β-Amylase	Carbohydrate	DNA	Glutamine	Protein	Proline	Phenols	RNA
Anacyclus Pyrethrum	23.5	36	15	23	34.5	34.5	28.5	31	36	6
Clitoria ternatea	22.1	23	18	23	31.2	30	28	27	30.2	25.3

Table 3 : Effect of ethanol extract of *Anacyclus pyrethrum* on bacteria (RMI- cm^2)

Name of the bacterium	Concentration of root extract (µl/ml)														
	20			40			60			80			100		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Bacillus cereus	0.5	0.2	0.4	0.5	0.3	0.6	0.5	0.3	0.6	0.5	0.4	0.8	0.5	0.6	1.2
Escherichia coli	0.5	1.5	3..0	0.5	1.5	3.0	0.5	0.5.	1.0	0.5	1.3	2.6	0.5	1.6	3.2
Klebsiella pneumoniae	0.5	0.3	0.6	0.5	0.4	0.8	0.5	o.4	0.8	0.5	0.3	0.6	0.5	0.6	1.2
Pseudomonas fluorescence	0.5	1.0	2.0	0.5	1.2	2.4	0.5	1.1	2.2	0.5	1.3	2.6	0.5	1.7	3.4
Staphylococcus faecalis	0.5	0.2	0.4	0.5	0.1	0.2	0.5	0	0	0.5	0	0	0.5	0.1	0.2

Table 4 : Effect of aqueous extract of *Anacyclus pyrethrum* on bacteria (RMI-cm^2)

Name of the bacterium	Concentration of root extract (µl/ml)														
	20			40			60			80			100		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Bacillus cereus	0.5	0.2	0.4	0.5	0.2	0.4	0.5	0.1	0.2	0.5	0.3	0.6	0.5	0.4	0.8
Escherichia coli	0.5	0.3	0.6	0.5	0.3	0.6	0.5	0.2	0.4	0.5	0.4	0.8	0.5	0.5	1.0
Klebsiella pneumoniae	0.5	0	0	0.5	0	0	0.5	0.1	0.2	0.5	0.2	0.4	0.5	0.2	0.4
Pseudomonas fluorescence	0.5	0.5	1.0	0.5	0.5	1.0	0.5	0.3	0.6	0.5	0.6	1.2	0.5	1.5	3.0
Staphylococcus faecalis	0.5	0.2	0.4	0.5	0.2	0.4	0.5	0.5	1.0	0.5	0.4	0.8	0.5	1.0	2.0

A_1 = Area of well in cm^2
A_2 = Area of zone of inhibition in cm^2 (including area of well)
RMI = A_2 / A_1

pneumoniae and *Staphylococcus faecalis*. The Aqueous extract was more inhibitory on *Escherichia coli, Pseudomonas fluorescence* and *Staphylococcus faecalis* and less inhibitory on *Klebsiella pneumoniae* and *Bacillus cereus.*

Table 5 and 6 show the antibacterial activity of root extracts of *Clitoria ternatea* against some bacteria. The results clearly show that plant extracts were specific in action against the growth of bacteria. Ethanol extract was more effective followed by aqueous extract. Ethanol extract showed much inhibition against *Klebsiella pneumoniae* and *Pseudomonas fluorescence*. Regarding the dilution 100 µl/ml root extract was more effective than any other concentration. The minimum

Table 5 : Effect of ethanol extract of *Clitoria ternatea* on bacteria (RMI-cm^2)

Name of the bacterium	Concentration of root extract (µl/ml)														
	20			40			60			80			100		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Bacillus cereus	0.5	0.3	0.6	0.5	0.3	0.6	0.5	0.5	1.0	0.5	0.6	1.2	0.5	0.8	1.6
Escherichia coli	0.5	0.3	0.6	0.5	0.4	0.8	0.5	0.6	1.2	0.5	0.9	1.8	0.5	1.1	2.2
Klebsiella pneumoniae	0.5	0.5	1.0	0.5	0.7	1.4	0.5	0.9	1.8	0.5	1.4	2.8	0.5	1.6	3.2
Pseudomonas fluorescence	0.5	0.4	0.8	0.5	1.1	2.2	0.5	1.0	2.0	0.5	1.4	2.8	0.5	1.4	2.8
Staphylococcus faecalis	0.5	0.6	1.2	0.5	0.6	1.2	0.5	0.9	1.8	0.5	1.1	2.2	0.5	1.2	2.2

Table 6 : Effect of aqueous extract of *Clitoria ternatea* on bacteria (RMI cm^2)

Name of the bacterium	Concentration of root extract (µl/ml)														
	20			40			60			80			100		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Bacillus cereus	0.5	0.4	0.8	0.5	0.4	0.8	0.5	0.5	1.0	0.5	0.7	1.4	0.5	0.7	1.4
Escherichia coli	0.5	0.3	0.6	0.5	0.5	1.0	0.5	0.6	1.2	0.5	0.6	1.2	0.5	1.2	2.4
Klebsiella pneumoniae	0.5	0.5	1.0	0.5	0.6	1.2	0.5	0.7	1.4	0.5	1.0	2.0	0.5	1.5	3.0
Pseudomonas fluorescence	0.5	0.4	0.8	0.5	0.5	1.0	0.5	0.8	1.6	0.5	1.0	2.0	0.5	1.3	2.6
Staphylococcus faecalis	0.5	0.5	1.0	0.5	0.5	1.0	0.5	0.8	1.6	0.5	1.0	2.0	0.5	1.0	2.0

A_1 = Area of well in cm^2
A_2 = Area of zone of inhibition in cm^2 (including area of well)
RMI = A_2 / A_1

inhibitory concentration of both the extract against all the test organism was found to be 20 µl/ml. *Bacillus cereus* was sensitive to ethanolic extracts of *Anacyclus pyrethrum* and *Clitoria ternatea.* In the present investigation ethanolic extracts of the study plants exhibited higher relative magnitude of inhibition (RMI) against *Klebsiella pneumoniae.* This credit to ethanol extraction was supposed to because ethanol in an organic solvent and will dissolve organic compounds better than aqueous extract and also liberate the active component required for antibacterial activity. Rajendhran *et al.* (1998) reported that the volatile components of acetone extracts of *Ocimum sanctum* were more effective against all the tested microorganisms *viz., Staphylococcus aureus, Escherichia coli and klebsiella* species. Dubey (2004) studied the effect of *Cassia elata* leaf extract against *Staphylococcus aureus* and *Escherichia coli.* Antibacterial property of ethanol and methanol leaf extracts of *Spathodea campanulata* was already proved against standard strains of *Klebsiella pneumonia* (Parekh and Chanda, 2007). In the present study the plant root extracts showed significant antibacterial action against *Staphylococcus faecalis.* It is in line with work of Dhanabalan *et al.* (2008) in *Tridax procumbens*. According to them the methanolic and aqueous extract of *Tridax procumbens* showed inhibitory activity against *Staphylococcus aureus.* It has also been supported by Bae *et al*. (1998).

Conclusion :

From the present study it can be concluded that plants showing high antimicrobial activity should be analyzed owing to the presence of secondary metabolites. All the selected plant extracts are having antimicrobial activity and these plants can be used as sources for new drugs. In conclusion solvent extract method was proved to be more efficient when compared to aqueous extracts in all the plants studied.

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