International Journal of Chemistry and Pharmaceutical Sciences

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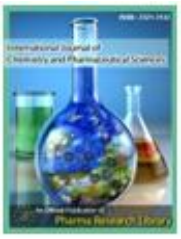

Research Article Open Access

Comparitive Evaluation of Antibacterial Activity of *Acmella calva, Crotalaria ovalifolia* Wall. –Potential Medicinal Herbs

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A B S T R A C T

The present study deals with the evaluation of antibacterial activity of two medicinally important plants *Acmella calva* (DC.) R. K. Jansen and *Crotalaria ovalifolia* Wall. belonging to the family Asteraceae. Evaluation of the antibacterial activity was carried out using two solvents viz., ethanol and water which were tested against two human pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*). In *Acmella calva*, among the two solvent extracts the ethanol extract showed significant activity against both Gram-negative and Gram-positive bacteria. Among the tested extracts (root, aerial parts and flower) the ethanol extract of flower showed maximum antimicrobial activity against both the tested microorganisms than the aqueous extract. In *Crotalaria ovalifolia*, ethanol extract was more effective as antibacterial agent than the corresponding aqueous extract for the two pathogens. Among the two plants, the flower (ethanol) extract of *Crotalaria ovalifolia* showed maximum inhibition and the root extract of both the plants showed minimum inhibition.

Keywords: Antibacterial, *Acmella calva, Crotalaria ovalifolia, Escherichia coli* and *Staphylococcus aureus.*

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Article History: Received 25 November 2016, **Accepted** 29 December 2016, **Available Online** 27 January 2017

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Manuscript ID: IJCPS3288

PAPER-QR CODE

Citation: C. Yogeshwari. Comparitive Evaluation of Antibacterial Activity of *Acmella Calva (DC.) R. K. Jansen and Crotalaria Ovalifolia* Wall.–Potential Medicinal Herbs. *Int. J. Chem, Pharm, Sci.*, 2017, 5(1): 18-23.

1. Introduction

The use of herbal medicine for the treatment of diseases and infections is as old as mankind. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. In India, the knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha [1]. As many diseases are caused due to the microbial infections, the evaluation of antimicrobial activity seems to be an essential part of medicinal phytochemistry. Now-a-days, an increasing number of microbial agents are becoming more resistant to commercial antimicrobial compounds [2]. The necessity to develop new drugs requires varied strategies, among them, the bio prospection of secondary metabolites produced by medicinal plants plays an important role [3]. *Acmella calva* (DC.) R. K. Jansen belonging to the family Asteraceae is an erect annual herb attaining a height of 50-60 cm and it has yellow cone like flowers. It is commonly known as Akarkara or toothache plant. It has been used as folk medicine since ancient times to cure severe toothache, affections of throat and gums, stomatitis, paralysis of tongue and psoriasis. *Crotalaria ovalifolia* Wall. also belongs to the family Asteraceae. It is a prostrate herb, stem wiry, leaves simple, ovate, orbicular, sparsely pubescent, up to 1.2 inch long, 0.7 inch broad, herbaceous, with pubescence hair all over the body. Flowers arranged in racemes, few flowered, flowers 0.6 inch long, pod 1 inch long and stalked, oblong, inflated. However, very little information is available on such activity of medicinal plants and out of the 4,00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities [4]. Hence, in the present study phytochemical screening and antimicrobial activity of 2 important medicinal plants *Acmella calva* and *Crotalaria ovalifolia* was carried out. This study will be very much helpful for the development of any plant-based drugs in the future.

2. Materials and Methods

Collection and identification of the plant material

Healthy whole plants of *Acmella calva* and *Crotalaria ovalifolia* were collected near Kandhara falls of Wayanad, Kerala and Thindal, Erode, Tamilnadu respectively. The collected study plants were identified with the help of the existing floras [5].

Extraction of plant materials: The collected plant parts were cleaned, shade dried and powdered by a mechanical grinder. Fifteen grams of pulverized plant materials were soaked in 100 ml of solvents (aqueous, ethanol) and incubated for 24 hrs. They were filtered using standard Whatmann filter paper No.1 and the filtrate was allowed to evaporate at low temperature (10°C). The extracts were stored in refrigerator and used for further analysis.

Antibacterial Activity of *Acmella calva* and *Crotalaria ovalifolia*

Micro-Organisms: The bacterial organisms used in the present study were *Escherichia coli* and *Staphylococcus aureus*. The cultures were collected from Kovai Medical Centre and Hospital (KMCH), Erode, Tamil Nadu, India and were used as antimicrobial test organisms. *Escherichia coli* is a motile gram negative bacterium. It is the most common cause of diarrhoea, septic emiameningitis and urinary tract infections. It is also responsible for 50% of traveller's diarrhoea. Some patients have fecaleukocytes, haemolytic-urinic syndrome or thrombotic thrombocytopenia purpurea. *Staphylococcus aureus* is a non-motile gram positive bacterium. It is the most common cause of skin infections and is responsible for various diseases including mild skin infections [impetigo, pimples, boils, etc.], invasive diseases [wound infections, chest pain, osteomyelitis, bacteremia with metastatic complications] and toxin mediated diseases [food poisoning, toxin shock syndrome or TSS, scaled skin syndrome, etc.].

Preparation of Inoculum

The gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*) were precultured in nutrient broth overnight in a rotary shaker at 37°C. They were centrifuged at 10,000 rpm for 5 minutes and the pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A610 mm).

Composition of Bacterial Medium

Peptone	: 5g/l
Sodium Chloride	: 5g/l
Yeast	: 3g/l
Beef extract	: 3g/l

Nutrient Agar medium

Peptone	: 0.5g/l
Beef extract	: 0.3g/l
Agar	: 1.5g/l
NaCl	: 0.5g/l
Distilled water	: 1000ml
pH	: 6.8

Antibacterial activity: The autoclaved media (Nutrient agar media) was poured into the petridishes. The test strain (0.2ml) was inoculated into the media to inoculum size (108 cells/ml). The plant extract was tested against *Escherichia coli* and *Staphylococcus aureus* for antibacterial activity by using the Agar well diffusion assay.

Agar Well Diffusion Method

Procedure: The antimicrobial activity was tested using the whole plant extract of *Acmella calva* and *Crotalaria ovalifolia.* The inoculum of micro-organisms was prepared from bacterial culture. About 15-20ml of Nutrient agar medium was poured into the sterilized glass petridishes and was allowed to solidify. One drop of bacterial strain was spread over the medium by a rod. Wells of 5 mm in diameter and about 2 cm apart were punctured in the culture media by using sterile corkborer. About 1 ml of the plant extract was added to the wells. Inoculated plates were incubated at 37° C for 24 hrs. Antibacterial activities were evaluated by measuring the diameter of inhibition zones.

3. Results and Discussion

Results: Antimicrobial activity was performed with the aim of identifying the active compounds. In the present investigation, the antimicrobial activity of aqueous and ethanol extracts of *Acmella calva* and *Crotalaria ovalifolia* against the test microorganisms *Escherichia coli* and

Staphylococcus aureus were qualitatively assessed by the zone of inhibition and minimum inhibitory concentration. The root extracts of *Acmella calva* exhibited strong antimicrobial effects against the tested microorganisms with the inhibition zones ranged between 0.5-1.8 cm^2 (Table–1). It was found that 100 µg/ml ethanolic root extract exhibited significant activity against both the test organisms. 75 µg/ml root extract have moderate activity, whereas 25µg/ml showed minimum inhibitory activity. Effect of aqueous and ethanol extracts of aerial parts of *Acmella calva* was presented in Table-2. The gram positive bacterium was more susceptible than the gram negative bacteria. Ethanol extract showed higher activity than aqueous extract.

The different solvent extracts (aqueous and ethanol) of *Acmella calva* flower head showed effective antimicrobial activity against the test pathogens which is presented in Table-3. Both the bacteria showed maximum inhibition for flower extracts (aqueous and ethanol). Gram-negative bacteria showed least susceptible activity. Among the two solvent extracts, the ethanol extract showed significant activity against both Gram-negative and Gram-positive bacteria. Among the tested extracts (root, aerial parts and flower) the ethanol extract of flower showed maximum antimicrobial activity against both the tested microorganisms than the aqueous extract. The data pertaining to the antimicrobial potential of aqueous and ethanol extracts of root of *Crotalaria ovalifolia* was presented in Table-4. The results revealed variability in the inhibitory concentration of each extract against pathogenic bacteria. The diameters of growth inhibition zone were in the range of 0.6cm^2 to 1.3cm^2. The highest inhibition zone was observed in ethanol extract against *Staphylococcus aureus*. On the other hand, aqueous extract showed low activity against tested microorganisms. Among the bacteria, *Staphylococcus aureus* was more susceptible than *Escherichia coli.*

The antimicrobial activity of aqueous and ethanol extracts of aerial parts of *Crotalaria ovalifolia* was investigated (Table-5). Both the plant extracts (aqueous and ethanol) used against the pathogenic organisms have showed varied degree of antimicrobial activity against the pathogens and they showed maximum zone of inhibition (2.4cm^2). The collective analysis of antimicrobial activity of ethanol extract indicated that *Escherichia coli* was more susceptible than *Staphylococcus aureus* (2.2cm^2). In the case of aqueous extract, the pathogen *Staphylococcus aureus* showed maximum inhibition (1.6cm^2) followed by *Escherichia coli.*

The antimicrobial activity of the flower extract of *Crotalaria ovalifolia* was furnished in Table-6. The aqueous and ethanolic extracts exhibited different degrees of antibacterial activity. Aqueous extract of *Crotalaria ovalifolia* flower showed minimum activity against *Escherichia coli* and maximum activity against *Staphylococcus aureus*. Ethanol extract was more effective as antibacterial agent than the corresponding aqueous extract for the two pathogens.

In the present study, two plant species (*Acmella calva* and *Crotalaria ovalifolia*) were selected and two solvents (aqueous and ethanol) were tested for antibacterial activity against two human pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*). Among the two plants, the flower (ethanol) extract of *Crotalaria ovalifolia* showed maximum inhibition and the root extract of both the plants showed minimum inhibition. In general the pathogen *Staphylococcus aureus* was more susceptible than *Escherichia coli.*

Discussion

The antibacterial activity of *Acmella calva* extracts against *Escherichia coli* and *Staphylococcus aureus* showed that the flower extracts were more effective than the extracts of aerial parts and roots. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent. Aqueous and ethanol extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity.

The aqueous and ethanol extracts were more active against the Gram positive bacterial strain *(Staphylococcus aureus*) than the Gram negative bacterial strain (*Escherichia coli*). This was in agreement with the previous work [6]. The present work was in line with the previous works[7,8] on *Acmella calva* inflorescence and on flower head of *Spilanthes acmella* respectively. In the present investigation, the solvent extracts of flower head of *Acmella calva* showed maximum antibacterial activity than any other part. The more activity of flower bud of *Acmella calva* might be due to the presence of phytochemical composition of the species. Further the presence of some antimicrobial secondary metabolites such as alkaloids and phenols may also be explained as a factor for suppressing the colonial growth of tested microorganisms.

The 100 µg/ml ethanolic fractions of *Crotalaria ovalifolia* demonstrated reasonable activity against *Escherichia coli* when compared to *Staphylococcus aureus,* where the activity was less and the pathogen was less susceptible to the extracts. This is because the Gram-positive ones possess outer membrane surrounding the cell wall and these differential activities also may be due to nature and concentration of antimicrobial compounds and their mode of action. A previous study [9] on *Tarchonanthus camphoratus* demonstrated the same results.

In general, the antimicrobial activity of flower of *Crotalaria ovalifolia* was generally higher than the other parts which is supported by the previously reported results [10]. In the present investigation, higher concentrations of aqueous and ethanol extracts exhibited antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, whereas lower concentration of ethanol and aqueous extracts fail to inhibit the growth of *S. aureus* and *E. coli*. This is in accordance with Thenmozhi *et al*. [11] who reported that the lower concentration of methanol extract of *Emilia sonchifolia* possess less antibacterial activity against all the tested microorganisms.

Table 1A: Effect of Aqueous and Ethanolic Root Extracts of *Acmella Calva*

Name of the Bacteria	Aqueous								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.6	1.2	0.5	0.6	1.2	0.5	0.6	1.2
Staphylococcus aureus	0.5	0.6	1.2	0.5	0.7	1.4	0.5	0.8	1.6

Table 1B: Effect of Aqueous and Ethanolic Root Extracts of *Acmella Calva*

Name of the Bacteria	Ethanol								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.7	1.4	0.5	0.7	1.4	0.5	0.8	1.6
Staphylococcus aureus	0.5	0.8	1.6	0.5	0.8	1.6	0.5	0.9	1.8

A_1- Area of well in cm^2
A_2- Area of zone of inhibition in cm^2 (including area of well)
RMI- A2 / A_1

Table 2A: Effect of Aqueous and Ethanolic Aerial Parts Extracts of *Acmella Calva*

Name of the Bacteria	Aqueous								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.6	1.2	0.5	0.8	1.6	0.5	0.9	1.8
Staphylococcus aureus	0.5	0.8	1.6	0.5	0.8	1.6	0.5	1.0	2.0

Table 2B: Effect of Aqueous and Ethanolic Aerial Parts Extracts of *Acmella Calva*

Name of the Bacteria	Ethanol								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.8	1.6	0.5	1.0	2.0	0.5	0.9	1.8
Staphylococcus aureus	0.5	0.8	1.6	0.5	1.2	2.4	0.5	1.2	2.4

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

Table 3A: Effect of Aqueous and Ethanolic Flower Extracts of *Acmella Calva*

Name of the Bacteria	Aqueous								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.5	1.0	0.5	0.6	1.2	0.5	0.8	1.6
Staphylococcus aureus	0.5	0.5	1.0	0.5	0.7	1.4	0.5	0.9	1.8

Table 3B: Effect of Aqueous and Ethanolic Flower Extracts of *Acmella Calva*

Name of the Bacteria	Ethanol								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.6	1.2	0.5	0.7	1.4	0.5	1.0	2.0
Staphylococcus aureus	0.5	0.7	1.4	0.5	0.7	1.4	0.5	1.2	2.4

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

Table 4A: Effect of Aqueous and Ethanolic Root Extracts *Crotalaria Ovalifolia*

Name of the Bacteria	Aqueous								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.5	1.0	0.5	0.6	1.2	0.5	1.0	2
Staphylococcus aureus	0.5	0.5	1.0	0.5	0.7	1.4	0.5	1.2	2.4

Table 4B: Effect of Aqueous and Ethanolic Root Extracts *Crotalaria Ovalifolia*

Name of the Bacteria	Ethanol								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.5	1.0	0.5	0.5	1.0	0.5	0.8	1.6
Staphylococcus aureus	0.5	0.5	1.0	0.5	0.6	1.2	0.5	0.8	1.6

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

Table 5A: Effect of Aqueous and Ethanolic Aerial Parts Extracts of *Crotalaria Ovalifolia*

Name of the Bacteria	Aqueous								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.6	1.2	0.5	0.5	1.0	0.5	0.6	1.2
Staphylococcus aureus	0.5	0.6	1.2	0.5	0.7	1.4	0.5	0.8	1.6

Table 5B: Effect of Aqueous and Ethanolic Aerial Parts Extracts of *Crotalaria Ovalifolia*

Name of the Bacteria	Ethanol								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.6	1.2	0.5	0.7	1.4	0.5	1.2	2.4
Staphylococcus aureus	0.5	0.7	1.4	0.5	0.8	1.6	0.5	1.1	2.2

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

Table 6A: Effect of Aqueous and Ethanolic Flower Extracts of *Crotalaria Ovalifolia*

Name of the Bacteria	Aqueous								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.8	1.6	0.5	0.8	1.6	0.5	1.1	2.2
Staphylococcus aureus	0.5	0.8	1.6	0.5	0.9	1.8	0.5	1.2	2.4

Table 6B: Effect of Aqueous and Ethanolic Flower Extracts of *Crotalaria Ovalifolia*

Name of the Bacteria	Ethanol								
	25µg/ml			75µg/ml			100µg/ml		
	A_1	A_2	RMI	A_1	A_2	RMI	A_1	A_2	RMI
Escherichia coli	0.5	0.8	1.6	0.5	0.6	1.2	0.5	1.3	2.6
Staphylococcus aureus	0.5	0.8	1.6	0.5	0.7	1.4	0.5	1.1	2.2

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

4. Conclusion

The study has confirmed the antibacterial potentials of the two plant species *Acmella calva* and *Crotalaria ovalifolia*. Therefore, we have to explore the potential of these plants which possess high therapeutic value and many other uses.

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