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Subhash Sirangi
Plant systematics laboratory,
Department of Botany,
Kakatiya University, Warangal,
Telangana, India

Ajmeera Ragan
Plant systematics laboratory,
Department of Botany,
Kakatiya University, Warangal,
Telangana, India

Correspondence
Ajmeera Ragan
Plant systematics laboratory,
Department of Botany,
Kakatiya University, Warangal,
Telangana, India

Phytochemical analysis and Evaluation of Antimicrobial activity in Ethnomedicinal herb *Corynandra chelidonii* Var. *pallae* (Cleomaceae)

Subhash Sirangi and Ajmeera Ragan

Abstract
Phytochemistry is mainly concerned with enormous varieties of secondary plant metabolites which are biosynthesized by plants. Preliminary phytochemical investigations of leaves extract have been carried out by using different solvents (methanol, ethyl acetate and n-hexane) for the presence of various phytoconstituents. Phytochemical analysis of various solvent extracts of leaves had shown the presence of alkaloids, glycosides, Tanins, Phenols, Flavonoids, and Steroids. And also leaf extract evaluated for antibacterial activity against gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus)* and gram negative bacteria *(Escherichia coli*, *Enterobacter aerogenes)* two concentrations of extracts 600µg/ml and 900µg/ml were used. The extract of leaf inhibited the growth of all the test samples. The zone of inhibition increased with increase in concentration of the test solution. High inhibition zone was observed in methanol extracts with 900µg/ml concentration in *Enterobacter aerogenes (*18mm).

Keywords: Antibacterial activity; Inhibition zone; Leaf extract; *Corynandra.*

1. Introduction
A new variety of *Cleome chelidonii* was describedfrom the littoral waters of Pakhal reservoir (Pakhal Forest Reserve, Warangal district, northern Telangana). (Reddy & Raju, 2001) [25]. This herb is commonly called *Adavi avalu* and the seeds are used as condiment. These species is having ethnomedicinal, ecological and economic importance. *Cleome chelidonii* and *Cleome viscosa* contain glucocapparin and glucocleomin Callus and suspension cultures of *Cleome chelidonii* produced glucosinolates (Songsak & Lockwood, 2004) [23]. *Cleome chelidonii* is generally known to be used for the treatment of colic, dysentery, headache, otitis and rheumatism (Kirtikar & Basu, 1991) [11]. Recently, *Corynandra* Schard. ex Spreng., the earlier name for the segregated genus *Arivelia* Raf. of Cleome (Cleomaceae) was reinstated (Cohrane & Iltis, 2014) [6]. Accordingly, *Cleome feline* L.f., *Cleome flava* (Banks ex DC.) and *Cleome viscoasa* L. were transferred to *Corynandra*. Hence, *Cleome chelidonii* L.f. is to be called *Corynandra chelidonii* (L.f) Cocharane & Iltis. The local endemic taxon *Cleome chelidonii* var. *pallae* becomes *Corynandra chelidonii* var. *pallae* (reddy & raju) V.S. Raju, ined**.** (V.S. Raju, per. comm.) Which grows as perennial in the local water bodies with 3-6 foliate leaves, pink flowers and 2-3 inch pods. It is a rare endemic and its population is on decline and it is not seen at pakhal over the past three years.

Materials and Methods
Plant material
Corynandra chelidonii. var. *pallae* collected from pakhal Reservoir (pakhal Forest Reserve, Warangal district, northern Telangana. The identified and authenticated plants were established in research field in department of botany kakatiya University, Warangal. The plant material required for phytchemical studies.

Preparation of the Extracts
About 250gr of air-dried plant leaf material was extracted in soxhlet assembly with n-hexane, ethyl acetate and methanol. Each time before extracting with the next solvent, the powdered material was dried. Each extract was concentrated by using rotary vacuum evaporator. All the solvents used for this entire work were of analytical reagent grade (Merck, Mumbai).

Qualitative Chemical Tests
The n-hexane, ethyl acetate, methanol extracts of leaf powder were subjected to determine the presence of following bioactive compounds by using the standard qualitative procedures

(Sofowora 1993; Harborne 1998) [22, 10]. Phytochemical analysis are the presence of medicinally active constituents.

Test for alkaloids

A small portion of the extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was carefully tested with various alkaloidal reagents such as Mayer's reagent, Dragondroff's reagent and Wagner's reagent.

Test for glycosides

Cardiac glycoside

Keller-Killani test- To 2 ml of extract, glacial acetic acid, one drop 5 % ferric chloride and concentrated sulphuric acid were added. Appearance of reddish brown colour at the junction of the two liquid layers indicates the presence of cardiac glycosides.

Anthraquinone glycosides

Borntrager's Test – To 3 ml extract dilute sulphuric acid was added, boiled and filtered. To the cold filtrate equal volume benzene or chloroform was added. The organic layer was separated and ammonia was added. Ammonical layer turns pink or red.

Saponin glycosides

Foam test – The extract and powder were mixed vigorously with water.

Test for tannins and phenolic compounds

Small quantity of various extracts were taken separately in water tested for the presence of phenolic compounds and tannins with

(a) Dilute ferric chloride solution (5%) - violet colour

(b) 1% solution of gelatin with 10%NaCl - white precipitate

(c) 10% lead acetate solution - white precipitate

Test for flavanoids

(a) With aqueous solution of sodium hydroxide blue to violet colour (Anthrocyanins), yellow colour (Flavones), yellow to orange (Flavonones).

(b) Lead acetate test: When aqueous basic lead acetate was added to test sample produces reddish brown precipitate.

(c) Shinoda's test – the extracts were dissolved in alcohol, to that a piece of magnesium and followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.

Test for terpenoids

Noller's test: The substance was warmed with tin and thionyl chloride. Pink coloration indicates the presence of triterpenoids.

Test for steroids

Salkowski test: Few drops of concentrated sulphuric acid were added to the test samples in chloroform, a red colour appears at the lower layer indicates the presence of sterols.

Antimicrobial activity

Microorganisms used

The following gram positive and gram negative pathogenic cultures were used for testing the antibacterial activity of plant extracts.

Bacterial cultures/Test organisms

Gram negative -*Escherichia coli*, *Enterobacter aerogenes*

Gram positive- *Bacillus subtilis, Staphylococcus aureus,*

Streptomycin is used as a control.

Media used for the assay

NAM medium (Nutrient agar medium)

Peptone-0.5g, Beff extract-0.3g, NaCl- 0.5g, Agar-2gm, DH_2O-100mL

Preparation of sample/test solution for antibacterial activity

A concentration of 600µg/ml and 900µg/ml of each solvent extract of different plant parts was prepared in DMSO (which did not influence the microbial growth).

Preparation of the extracts

Later finely powdered using an electric blender and stored in air tight containers for future use. The shade dried powder (25gm) was used for the extraction with 150 ml 80% methanol for 24 hours by Soxhlet equipment and filtered through 0.45 µm membrane filter. This filtrate was evaporated under reduced pressure and dried in a rotator evaporator at 55 °C. Dried extracts were stored in screw cap bottles at -20 °C and used as stock. Further, the same was diluted by using distilled water to arrive at different concentrations (1:1, 1:2 and 1:3).

Preparation of inoculum

By the standard method of inoculation (Bauer *et al*., 1966) an inoculating loop was touched each of four or five well isolated colonies of the same morphological type and inoculum was inoculated into 5ml of nutrient broth. The broth cultures were allowed to incubate at 37 ºC for 24hrs until a slight visible turbidity appeared. The turbidity of actively growing broth cultures was then adjusted with broth to obtain a half of MC Farland standard (1×10^8 to 5×10^8 cfu/ml). This was used as a starting inoculum for the assay.

Antimicrobial assay by agar well diffusion method:

The antimicrobial assay was carried out by agar well diffusion or agar cup plate method (Desta 2005) [7].

Agar cup plate method

A standardized 1 to 2×10^4 cfu/ml 0.5 MC Farland standards was introduced onto the surface of sterile agar plate and evenly distributed the inoculum by using a sterile glass spreader. Simultaneously 8 mm wells were cut from the plate using a sterile cork borer.

50µl of extract at a concentration of 600 and 900µg/ml was introduced into each well. The agar plates were incubated aerobically at 37 ºC. After 24hrs, the inhibition zones were measured with a ruler and compared with the control well containing only DMSO and 10 mg/ml of Streptomycin as standard.

Results and Discussion

Phytochemical analysis conducted in the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowora, 1993 Harborne, 1998) [22, 10]. The leaf extracts of *Corynandra chelidonii* var. *pallae* revealed the presence of phytochemicals such as alkaloids, glycosides, tannins, flavonoids, sterols, phenols. Alkaloids have been associated with medicinal uses for centuries and one of their common

biological properties is their cytotoxicity (Nobori *et al.,* 1994) [14]. Several workers have reported the analgesic (Antherden, 1969) [2] antisplasmodic and antibacterial (Stray, 1998; Okwu, and Okwu, 2004) [4, 17] properties of alkaloids. Glycosides were present in leaf extracts of *Corynandra chelidonii* var. *pallae* Glycosides are known to lower the blood pressure according to many reports (Nyarko and Addy 1990) [15]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al.,* 2007) [21] and they are present in leaf extracts of *Corynandra chelidonii* var. *pallae.* They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al.,* 2007) [9]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown and Rice-Evans, 1998; Krings and Berger, 2001) [5, 12]. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. (Ali *et al.,* 2008) [1]. The extracts of *Corynandra chelidonii* var. *pallae* leaf extracts were also revealed to contain flavonoids. They are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of micro organisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996) [13]. They are effective antioxidants and show strong anticancer activities (Del-Rio *et al.,* 1997; Okwu, 2004) [8, 18]. Sterols have been reported to have antibacterial properties (Raquel, 2007) [19] and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001) [16]. Leaf extract evaluated for antibacterial activity against gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus)* and gram negative bacteria *(Escherichia coli*, *Enterobacter aerogenes)* two concentrations of extracts 600µg/ml and 900µg/ml were used. The extract of leaf inhibited the growth of all the test samples. The zone of inhibition increased with increase in concentration of the test solution. High inhibition zone was observed in methanol extracts with 900µg/ml concentration in *Enterobacter aerogenes (*18mm). Methanol extract has exhibited highest and significant antibacterial activity against all seven bacteria in *Cleome viscosa* (Saradha *et al* 2010*)* [20] the antimicrobial activity of the ethanolic extract and its various fractions of *Cleome rutidosperma* were studied (Bose *et al* 2007) [4].

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Table 1: Analysis of phytochemicals from leaf extracts of *C.chelidonii.*

S. No	Phytochemicals	Test	Methanol	Ethyl Acetate	n-Hexane
1	Alkaloids	Mayer's reagent	+	+	+
		Dragondroff's Reagent/ Hagers	+	+	+
		Wagner's reagent	+	+	+
2	Glycosides				
	a)Cardiac Glycosides	Keller-Killani test	+	+	-
	b)Anthraquinone Glycosides	Borntrager's Test	+	-	+
	c)Saponin Glycosides	Foam test	+	+	+
3	Steroids	Salkowaski test	+	+	+
4	Tanins&Phenols	Dilute ferric chloride solution (5%)	+	+	+
		1% solution of gelatin with 10%NaCl	+	+	-
		10% lead acetate solution	+	-	+
5	Flavonoids	sodium hydroxide	-	+	-
		Lead acetate test	+	+	+
		Shinoda's test	+	-	-
7	Terpenoids	Noller's test	-	-	-
8	Steroids	Salkowski test	+	+	+

Note: + ve indicates positive result, whereas – ve indicates negative results

Table 2: Antibacterial activity in DMSO extraction of *C. Chelidonii.*

Plant material	Conc. (µg/ml	*Stryphylococcus arens*	*Bacillus subtilis*	*Escherichia coli*	*Enterobacter aerogenes*
		Zone of inhibition (in mm)			
Methanol	600	13	11	12	15
	900	15	15	14	18
Ethanol	600	11	05	11	15
	900	13	07	13	17
n-Hexane	600	10	06	10	11
	900	14	08	14	15

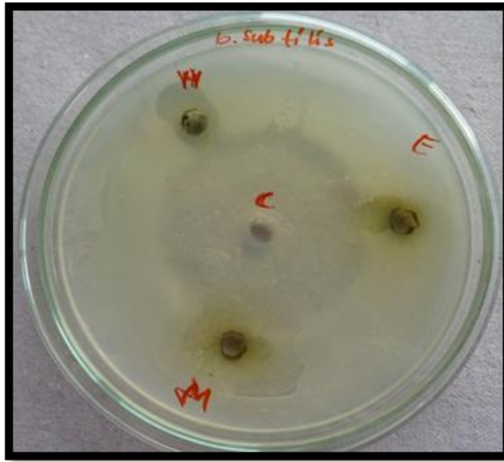

Plate 1: Zone of inhibition shown by *Bacillus subtilis*

Plate 2: Zone of inhibition shown *Staphylococcus aureus*

Plate 3: Zone of inhibition shown by *Escherichia coli*

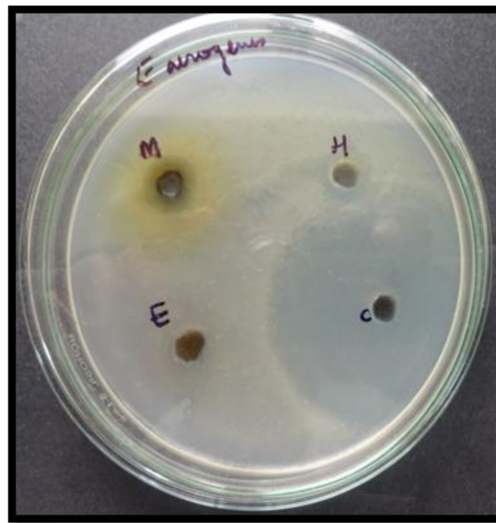

Plate 4: Zone of inhibition shown *Enterobacter aerogenes*

References

1. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahuand A. Bora U Indian medicinal herbs as source of antioxidants. Food Res Int. 2008; 41:1-15.
2. Antherden LM. Textbook of Pharmaceutical Chemistry, 8th edn, Oxford University Press, London, 1969.
3. Bauer AW. Kirby WM. Sherris JC. Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45:493-496.
4. Bose A, Mondal S, Gupta JK, Ghosh1 TSS, Debbhuti D. A study on antimicrobial activity of *Cleome rutidosperma* DC Int j of pharma sci. 2007; 7-1:132-134.
5. Brown JE, Rice-Evans CA. Luteolin rich artichoke extract protects low density lipoprotein from oxidation *in vitro*. Free Radical Res. 1998; 29:247-255.
6. Cochrane TS, Iltis HH. Studies in the Cleomaceae VII: Five new combinations in *Corynandra*, an earlier name for *Arivela*. Novon. 2014; 23(1):21-26.
7. Desta B. Antimicrobial activity of 63 medicinal plants. J. Ethnopharmacol. 2005; 100:168-175.
8. Del-Rio A, Obdululio BG, Casfillo J, Main FG, Ortuno A. Uses and properties of citrus flavonoids. J Agric Food Chem. 1997; 45:4505-4515.
9. Han X, Shen T, Lou H. Dietry polyphenols and their biological significance. Int J Mol Sci. 2007, 950-988.
10. Harborne JB. Phytochemical methods guide to modern Technique of Plant analysis. 3rd Edition, Chapman and Hall, London. 1998.
11. Kirtikar KR, Basu BD. Indian Medicinal Plants. Periodical Expert Book Agency. New Delhi, India, 1991; 3:2171-2172.
12. Krings U, Berger RG. Antioxidant activity of roasted foods. Food Chem. 2001; 72:223-229.
13. Marjorie C. Plant products as antimicrobial agents. Clincal Microbiol Rev. 1996; 12:64-582.
14. Nobori T, Miurak K, Wu DJ, Takabayashik LA, Carson DA. Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature. 1994; 46:753-756.
15. Nyarko AA, Addy ME. Effects of aqueous extract of Adenia cissampeloides on blood pressure and serum analyte of hypertensive patients. Phytotherapy Res. 1990; 4(1):25-28.
16. Okwu DE. Evaluation of chemical composition of medicinal plants belonging to Euphorbiaceae. Pak Vet J. 2001; 14:160-162.
17. Okwu DE. Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. J Sustain Agric Environ. 2004; 6(1):30-37.
18. Okwu DE, Okwu ME. Chemical composition of Spondias mombin linn. Plant parts. J Sustain Agric Environ. 2004; 6(2):140-147.
19. Raquel FE. Bacterial lipid composition and antimicrobial

efficacy of cationic steroid coppounds. Biochemica et Biophysica Acta. 2007, 2500-2509.
20. Saradha *et al. In vitro* antibacterial activity of *Cleome viscosa* Pharma science monitor. 2010; 2:1.
21. Singh R, Singh SK, Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis A. Cunn Food Chem Toxicol. 2007; 45:1216-1223.
22. Sofowora A. Medicinal plants and Traditional medicine in Africa. John Wily and Sons, New York. 1993; 2:6-56, 351998:12-16.
23. Songsak T, Lockwood GB. Production of two volatile glucosinolate hydrolysis compounds in *Nasturtium montanum* and *Cleome chelidonii* plant cell cultures. Fitoterapia. 2004; 75(3):296-301.
24. Stray F. The Natural Guide to Medicinal herbs And Plants. Tiger Books International, London. 1998, 12-16.
25. Reddy CS, Raju VS. A new variety of *Cleome chelidonii* L. f. (Cleomaceae). J Econ Taxon Bot. 2001; 25(1):217-218.