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Research Article

ANTIMICROBIAL ACTIVITY OF *IN VITRO* RAISED *ACMELLA CALVA* (DC.) R.K.JANSEN

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ABSTRACT

In vitro raised *Acmella calva* (DC.) R.K. Jansen was extracted using various solvent systems and screened for their phytochemical and antimicrobial activity using disc diffusion method. *Acmella calva* was raised through *in vitro* culture of nodal segments. Murashige and Skoog (MS) medium supplemented with 2.22µM BA was found to be most effective in inducing plantlet growth. The methanol and water extract exhibited good antibacterial and antifungal activity. These finding is important for pharmacology and potential to develop natural active constituents from these improved elite lines would be useful and profitable.

Keywords: Antimicrobial activity, *Acmella calva* (DC.) R.K. Jansen, Minimum Inhibitory Concentration (MIC), Phytochemical, Murashige and Skoog (MS) media.

INTRODUCTION

Acmella calva (DC.) R.K.Jansen (Synonym: *Spilanthes acmella* (L.) Murr) is an important medicinal and ornamental plant belonging to Asteraceae family, commonly known as "Akarkara" or "toothache plant"[1]. It grown in the tropics and subtropical parts of the world and can be found in damp pastures, at swamp margins, on rocks near the sea and as a weed of roadsides[2]. It is mainly found in the shola under stories of the high hills of Nilgiris, Western Ghats, India[3]. It is an erect annual herb attaining a height of 50 - 60 cm and it has yellow cone like flowers[4]. The genus consists around 60 species[5] spread throughout Mexico and Central America, Cuba, Curacao, India and Tanzania[6]. In India, the plants have been growing in the northern and southern hills and plateaus. There are around five species of Spilanthes are growing in India[7]. The extract of this genus has been used as folk medicine since ancient times to cure severe toothache, affections of throat and gums, stomatitis, paralysis of tongue, and psoriasis[8]. Flowers and leaves of the plant have a pungent taste and have been used as a spice for appetizers and as folk medicine for stammering, toothache, stomatitis, and throat complaints[9, 10]. It is a rich source of therapeutic elements and has been well documented for its anti-inflammatory, pesticidal, larvicidal, antibacterial, and antifungal properties[10-13]. The flowers of *Acmella calva* used as a remedy for stammering children in western parts of India[14]. The plant is highly toxic to adult houseflies, *Anopheline* and *Culicine* larvae. The hexane extract of dried flower buds of *Acmella calva* contains bioactive N-isobutylamides effective against the housefly, *Aedes aegypti* larvae[10]. The plant has marked larvicidal potential against the *Culex quinquefasciatus*[15]. The roots, flower heads and the whole aerial part yield a compound known as spilanthol, is a powerful stimulant, sialogogue and local anesthetic[3]. Recently, scopoletin has also been detected in Spilanthes flower buds[13] treating cardiovascular disease, tumors and thyroid disorders[16]. The present study is to report the development of an efficient *in vitro* regeneration protocol for the growth of *Acmella calva* using nodal explant and evaluates the antimicrobial activity and phytochemical screening of *in vitro* derived plants.

MATERIALS AND METHODS

Plant source and media preparation

Acmella calva (DC.) R.K. Jansen was collected from the foot hills of Vellingiri, Tamil Nadu (India) in the month of August, 2010 and was identified by the Taxonomy section of Botany Department, Karpagam University, Coimbatore, India. Voucher sample was prepared and deposited in the Herbarium for reference. The nodal explants were surface sterilized using 70% ethanol and 0.1% mercuric chloride (Hi media, India) and inoculated on MS basal medium at pH 5.6, supplemented with 0.088M sucrose, 0.8% agar and BA 2.22 µM (Data not shown) for multiple shoots induction. The *in vitro* nodal explants obtained from the multiple shoots were used for further experiments. The cultures were transferred to fresh medium at 4-week intervals.

Extract of plant material

Table 1: Percentage yield and color of the extracts from *Acmella calva* in various solvents.

Extracts	Percentage yield (w/w)	Color
Petroleum ether	1.07%	Green
Benzene	0.26%	Green
Acetone	0.94%	Green
Methanol	0.60%	Light Green
Distilled Water	1.13%	Pale yellow

In vitro grown plants were washed with distilled water and dried on room temperature under shade. Dried plants were ground into fine power. A total 100 g powered material successively extracted with petroleum ether, benzene, acetone, methanol and water by soxhlation. For each gram of dry material 2 mL of solvent were used[17]. After extraction, each extract was passed through Whatman filter paper No . 1. The extracts were evaporated to complete dryness by vacuum distillation and stored in refrigerator for further use.

Qualitative phytochemical analysis

The color and percentage yield was noted. Qualitative phytochemical analysis was carried out according to the methods described by pavala rani[18], Sudhanshu[19], Raaman[20].

Microorganisms used

The test organisms used included *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Enterococcus faecalis* ATCC 29212 (*E. faecalis*), *Escherichia coli* ATCC 25922 (*E. coli*), *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Candida albicans* (*C. albicans*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Staphylococcus epidermidis* (*S. epidermidis*). All the strains were collected from American Type Culture Collection, Manassas, USA and Kovai Medical Centre and Hospital (KMCH), Coimbatore, India. The organisms were subculture on Nutrient agar (NA) slants and incubated at 37 ºC for 24 h. The organisms were then stored at 4 ºC until needed.

Media preparation and antimicrobial activity

The antimicrobial assay was performed by agar disc diffusion method. The molten potato dextrose agar (PDA) was distributed to sterilized petri dishes. 100 µl of the inoculums were (10^8

cfu/mL) spreaded onto the petri plate containing PDA. The sterile filter paper disc (0.6 cm in diameter), individually impregnated with 25 µl of the test extract at concentration of 1 mg/mL in Dimethyl sulfoxide (DMSO), allowed to dry and was introduced on the upper layer of the seeded PDA plate. All the plates were incubated at 37 ºC for 24 h. For each bacterial strain, positive and negative controls were maintained. For positive control tetracycline disc (30 µg/disc) and for negative control disc containing DMSO was used instead of the extract. The result was obtained by measuring the zone of inhibition in millimeters. The experiment was done three times and the mean values are presented.

Minimum Inhibitory Concentration

Minimum Inhibitory Concentration (MIC) was checked according to Bauer[21] with modifications, briefly 9 mL of nutrient broth was taken in test tubes for each microorganism. Various concentrations of plant extracts ranging 0.125, 0.250, 0.375, 0.500, 0.625, 0.750, 0.875, 1.0, 1.125 and 1.250 mg/mL (w/v) concentration were incorporated into the broth and the tubes were then inoculated with 0.1 mL of inoculums of respective microorganism (10^5 CFU/mL) and kept at 37 ºC for 24 h. The test tube containing the lowest concentration of extract showing reduction in turbidity when compared with control was regarded as MIC of that extract.

Table 2: Preliminary phytochemical analysis of *in vitro* derived plantlet of *Acmella calva*

Phytochemical constituents	Petroleum ether	Benzene	Acetone	Methanol	Distilled water
Carbohydrates					
i) Molish test	-	-	+	+	+
ii) Fehling test	-	-	+	+	-
iii) Benedict test	-	-	-	+	+
Phenolic compound					
i) Ferric chloride	+	+	+	+	-
ii Lead acetate	-	-	+	+	+
Flavonoids	+	+	+	+	+
Cardiac glycoside	-	-	-	-	-
Alkaloid test					
i)Dragon droff test	-	-	+	+	+
ii)Mayer's test	-	-	+	+	+
Phlobatannins	-	-	-	-	+
Terpinoids	+	+	+	+	-
Glycoside					
i) Borntrager test	-	-	+	+	+
Tannins	-	-	+	+	+
Saponins	-	+	+	+	+
Phlobatannins test	-	-	-	-	+
Phytosterol test	+	+	+	-	-
Gum and Mucilage	-	-	-	-	+

Here, +: present, -: not present

Table 3: Antimicrobial activity as zone of inhibition (mm) of *in vitro* derived plantlet of *Acmella calva.*

Microorganism	Petroleum ether	Benzene	Acetone	Methanol	Water	Positive control (Tetracycline)	Negative control (DMSO)
S. aureus	-	-	-	11.0±1.35	8.0±0.35	28.0±0.24	-
P. aeruginosa	-	-	-	.10.0±0.0	-	20.0±0.50	-
E. faecalis	-	-	11.0±0.0	8.0±0.0	12.0±0.70	18.0±0.19	-
K. pneumonia	-	-	8.0±0.0	-	10.0±0.0	18.0±0.24	-
S. epidermidis	-	-	-	10.5±0.35	-	20.0±0.14	-
C. albicans	-	-	8.5±0.70	7.0±0.14	8.0±0.35	18.0±0.35	-

RESULTS

In vitro propagated plantlets of *Acmella calva* on the MS media (Fig 1) supplemented with 0.088M sucrose, 0.8% agar and BA (2.22 µM) were used for preliminary phytochemical analysis and antimicrobial activity.

Preliminary phytochemical analysis of *in vitro* derived plantlet of *Acmella calva*

The maximum percentage yield was observed in water extract and minimum in benzene extraction. Color changes recorded was green to pale yellow in petroleum ether to distilled water (Table 1). The phytochemical screening of *in vitro* derived plantlets of *Acmella calva* (Table 2) revealed the presence of carbohydrates, phenol, alkaloids, terpenoids, glycosides, Tannins and saponins. The Preliminary Phytochemical analysis revealed that the water extract shows the maximum phytochemical constituents followed by acetone and methanol.

Antimicrobial screening of *in vitro* derived plantlet of *Acmella calva*

The antimicrobial activities of various solvent extracts of *in vitro* derived *Acmella calva* at the concentration of 1 mg/mL showed significant variations against six microorganisms tested as shown in Table 3. Among the five extracts, methanol extract shows wide range of antimicrobial potential against four bacteria and a fungi, followed by water extract and then by acetone. No activity was observed on petroleum ether and benzene extracts. The largest zone of inhibition was found on water extract against *Enterococcus faecalis* (12 mm). Methanol extract of *in vitro* derived *Acmella calva* was effective against *Staphylococcus aureus* (11 mm) and *Staphylococcus epidermidis* (10.5 mm) as well as the acetone extract of *Acmella calva* shows effective against *Enterococcus faecalis* (11.0 mm).

Table 4: Minimal inhibitory concentration of *in vitro* raised plant extract against microorganisms

Microorganisms	MIC of methanol extract (mg/ml)
S. aureus	1.0
P. aeruginosa	0.5
E. faecalis	1.25
S. epidermidis	0.75
C. albicans	1.0

The methanol, acetone and water extracts of *in vitro* derived *Acmella calva* showed the most potent inhibition over *Enterococcus faecalis* and *Candida albicans*. Whereas minimum activity was observed against *Candida albicans* (7 mm) when methanol extract used. The

diameter of zone of inhibition for each extracts against the microbes were compared with standard positive control (Tetracycline 30 mcg/disc), No zone of inhibition was found on negative control.

Fig. 1: *In vitro* derived multiple shoots of *Acmella calva*.

The present study was the first to report the antimicrobial activity of *in vitro* derived plantlets of A*cmella calva*. It also possesses remarkable activity against many pathogens as well as leads to development of new ideas in the field of antimicrobial activity on *in vitro* generated plantlets.

Minimum Inhibitory Concentration of *in vitro* derived plantlet of *Acmella calva*

Minimum Inhibitory Concentration (MIC) of active methanol extract is shown in Table 4. *In vitro* derived *Acmella calva* showed the strongest antimicrobial activity with MIC values of 0.5 mg/mL, against *Pseudomonas aeruginosa* followed by *Staphylococcus epidermidis* (MIC of 0.75 mg/mL). The *in vitro* derived *Acmella calva* showed the strongest activity over *Pseudomonas aeruginosa* and moderate activity against *Staphylococcus epidermidis, Klebsiella pneumonia, Candida albicans*, *Staphylococcus aureus* and *Enterococcus faecalis*.

Due to the presence of phytochemical properties there is also difference in the antimicrobial activity among species. The potential of antimicrobial compounds from plants lead to the development of phytomedicine against pathogens. Plant-based antimicrobial products have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials[22].

DISCUSSION

The phytochemical result was similar to the results of Shanthi and Amutha[23] in which the aqueous extract of *in vivo Acmella calva* showed positive result for reducing sugar, anthraquinone, flavanoids, saponins, tannins, alkaloids and glycosides as well as saponins were present in all the extracts of whole plant. The chemical constituents present in the *in vitro* derived plant extract shows that the plant was pharmacological important. Flavonoids, terpenes, tannins and steroids are known to have antimicrobial and bactericidal properties against several pathogens[24, 25]. Yadav[26] reported that the alcohol extract of *in vivo Spillanthus acmella* showed the presence of alkaloids, carbohydrates, tannins, steroids, carotenoids, sesquiterpenes and amino acids.

Antimicrobial results were obtained by Panthi and Chaudhary[27] on *Acmella calva* inflorescence and by Sabitha[28] on petroleum ether extract of flower head of *Spilanthes acmella* against fungi *Aspergillus niger* (1.1 cm) *and Aspergillus parasiticus* (0.9 cm). The result of Ali[29], in which the methanol extract of *Spilanthes* leaves shows activity against *Staphylococcus aureus* (15 mm), *Pseudomonas aeruginosa* (8 mm) and *Klebsiella species* (7 mm). The antimicrobial activity of A*cmella calva* extract may be due to the presence of spilanthol, alkamides, non-volatile sesquiterpenoids and saponins[9, 30, 31].

Minimal inhibitory concentration was similar to Holetz[32], stated that the ethanol extract of S*pillanthus acmella* is greater than 1mg/mL for *P. aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. Available literature determine that the strong activity is due to the MIC values in the range between 0.05-0.50 mg/mL, then the moderate activity was in the range between 0.6-1.50 mg/mL and weak activity was above 1.50 mg/mL[33].

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REFERENCES

1. Yadav K, Singh N. *In vitro* flowering of shoots regenerated from cultured nodal explants of *Spilanthes acmella* Murr. - an ornamental cum medicinal herb. Analele Universităţii din Oradea - Fascicula Biologie. 2011; 18:66-70.
2. Singh M, Chaturvedi R. Improved clonal propagation of *Spilanthes acmella* Murr for production of scopoletin. Plant Cell Tiss Organ Cult. 2010; 103:243–253.
3. Senthilkumar P, Paulsamy S, Vijayakumar KK, Kalimuthu K. *In vitro* Regeneration of the Medicinal Herb of Nilgiri shola, *Acmella calva* L. from Leaf Derived Callus. Plant Tissue Cult Biotechnol. 2007; 17(2):109-114.
4. Purabi D, Kalita MC. *In vitro* clonal propagation and organogenesis in *Spilanthes acmella* (L.) Murray a herbal pesticidal plant of North-East India. J Plant Biochem Biotechnol. 2005; 14:69-71.
5. Willis JC. *A dictionary of the flowering plants and ferns*. Cambridge: Cambridge University Press. 1977.
6. Jansen RK. Systematics of Acmella (Asteraceae: Heliantheae). Syst Bot Mongr. 1985; 8:115.
7. Pandey V, Agrawal V, Raghavendra K, Dash AP. Strong larvicidal activity of three species of *Spilanthes* (Akarkara) against malaria (*Anopheles stephensi* Liston, *Anopheles culicifacies*, species C) and filaria vector (*Culex quinquefasciatus* Say). Parasitol Res. 2007; 102:171-174.
8. Anonymous. The Wealth of India: a dictionary of Indian raw materials and industrial products, Vol 10. New Delhi: CSIR; 1989.
9. Nakatani N, Nagashiwa M. Pungent alkamides from *Spilanthes acmella* L. var. oleraceae. Clark. Biosci Biotechnol Biochem. 1992; 56(5):759-762.
10. Ramsewak RS, Erickson AJ, Nair MG. Bioactive N-isobutylamides from the flower buds of *Spilanthes acmella*. Phytochem. 1999; 51:729–732.
11. Saritha KV, Prakash E, Ramamurthy N, Naidu CV. Micropropagation of *Spilanthes acmella* Murr. Biol Plant. 2002; 45:581–584.
12. Wu LC, Fan NC, Lin MH, Chu IR, Huang SJ, Hu CY, Han SY. Anti-inflammatory effect of spilanthol from *Spilanthes acmella* on murine macrophage by down-regulating LPS-induced inflammatory mediators. J Agric Food Chem. 2008; 56:2341–2349.
13. Prachayasittikul S, Suphapong S, Worachartcheewan A, Lawung R, Ruchirawat S, Prachayasittikul V. Bioactive metabolites from *Spilanthes acmella* Murr. Mol. 2009; 14:850–867.
14. Ansari AH, Mukharya DK, Saxena VK. Analgesic study of n-isobutyl-4, 5- decadienamide isolated from the flowers of *Spilanthes acmella* (Murr). Indian J Pharm Sci. 1988; 50:106.
15. Pitasawat B, Choochote W, Kanjanapothi D, Panthong A, Jitpakdi A, Chaithong U. Screening for larvicidal activity of ten carminative plants. Southeast Asian J Trop Med Public Health. 1998; 29(3):660-662.
16. Taguchi G, Yoshizawa K, Kodaira R, Hayashida N, Okazaki M. Plant hormone regulation on scopoletin metabolism from culture medium into tobacco cells. Plant Sci. 2001; 5:905–911.
17. Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK, Van Staden J. Preliminary screening of some

traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. J Ethnopharmacol. 1999; 68:267–274.
18. Pavala rani N, Moorthi C, Senthamarai R, Kathiresan K. A study to explore the pharmacognostic and phytochemical screening of *Artemisia nilagirica* leaves found in nilgiris district of Tamil nadu. Int J Pharm Phar Sci. 2012; 4(4):441-447.
19. Sudhanshu, Mittal S, Rao N, Menghani E. Phytochemical and antimicrobial activity of *Withania coagulans* (stocks) dunal (fruit). Int J Pharm Phar Sci. 2012; 4(4):387-389.
20. Raaman, N. Phytochemical techniques. New Delhi: New India publishing agency.
21. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Patholv 1966; 45:493-496.
22. Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick J, editor. Perspectives on New Crops and New Uses. Alexandria: ASHS Press; 1999, p.457-462.
23. Shanthi P, Amudha P. Evaluation of the phytochemical constituents of A*cmella calva* (DC.) R.K. Jansen. Int J Pharma Biosci. 2010; 1(4):308-314.
24. Usman H, Abdulrahaman FI, Ladan AA. Phytochemical and antimicrobial Evaluation of *Tribulus terrestrisi* L (Zygophylaceae) Growing in Nigeria. Res J Biol Sci. 2007; 2(3):244-247.
25. Hassan MA, Oyewale AO, Amupitan JO, Abdullahi MS, Okonkwo EM. Prelimirary phytochemical and antimicrobial investigation of crude extract of root bark of *Deterium Microcarpum*. J Chem Sci Niger. 2004; 29:36-49.
26. Rajesh Y, Nita Y, Murli DK, Rudraprabhu S. Preliminary studies on diuretic effect of *Spilanthes acmella* leaves extracts in rats. Int J Pharmacy Pharmaceut Sci. 2011; 3(3):245-247.
27. Panthi MP, Chaudhary RP. Antibacterial activity of some selected folklore medicinal plants from west Nepal. Sci World. 2006; 4(4):16-21.
28. Sabitha AR, Suryanarayana UM. Antifungal potential of flower head extract of *Spilanthes acmella* Linn. Afr J Biomed Res. 2006; 9:67-69.
29. Sekendar MA, Saiful MI, Masudur MR, Rabiul Islam, Ekramul I, Rafikul MI. Antibacterial and cytotoxic activity of methanol extract of *Spilanthes calva* (dc) leaves. Int j pharmaceut sci. 2011; 2(7):1707-1711.
30. Krishnaswami NR, Prasanna S, Seahadri TR, Vedantham TNC. α and ß- Amyrin esters and sitosterol glucoside from *Spilanthes acmella*. Phytochem. 1975; 14:1666-1667.
31. Mukharya DKK, Ansari AH. Olean-12-en-3-O-ß-D-Galactopyranosyl (1-4)-O-alpha-l- rhamnopyranoside: a new triteropenoidal saponin from the roots of *Spilanthes acmella* (Murr). Indian J Chem. 1986; 26:81-87.
32. Fabíola BH, Greisiele L P, Neviton RS, Diógenes AGC, Clso VN, Benedito PDF. Screening of Some Plants Used in the Brazilian Folk Medicine for the Treatment of Infectious Diseases. Mem Inst Oswaldo Cruz. 2002; 97(7):1027-1031.
33. Diaz MAN, Rossi CC, Mendonca VR, Silva DM, Ribon ADOB, Aguilar AP, GD Muñoz. Screening of medicinal plants for antibacterial activities on *Staphylococcus aureus* strains isolated from bovine mastitis. Braz J Pharmacog. 2009; 20(5):724-728.