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Anticariogenic Activity of *Acmella calva* (DC.) R.K. Jansen against Selected Bacterial Pathogens

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Powdered whole plant, leaves and inflorescence materials of *Acmella calva* (DC.) R.K.Jansen extracted with different solvents viz., aqueous, chloroform, acetone, ethanol and petroleum ether. The extracts were tested against three dental bacterial pathogens such as *Streptococcus mutans, Streptococcus salivarius, Porphyromonas gingivalis* and one lactic acid bacteria like *Lactobacillus brevis* for its antibacterial activity. The results obtained showed that among the five solvent extracts tested, the chloroform extract of whole plant, leaves and inflorescence was found to be the most active against all the tested bacteria. The results suggested that *A. calva* can be used a potential medicinal plant for further investigations in medical microbiology against infective pathogens.

Key words: *Acmella calva,* anticariogenic activity, tooth caries pathogens.

Medicinal values of plants and herbs are immense and they are recommended for various ailments as they are cheaper, more effective and have fewer side effects. Of the 2,50,000 higher plant species on earth, more than 80,000 species are reported to have general medicinal properties and around 5000 species have specific therapeutic value (Sharma *et al*., 1991). *Acmella calva* belongs to the family Asteraceae, it is an important medicinal plant with rich source of therapeutic constituents and is being administered as a traditional folk medicine for years to cure toothaches, stammering, rheumatism, fever and stomatitis (Bunyapraphatsara and Chokechareunporn, 1999).

The whole plant and its different parts are of great medicinal significance for its therapeutic, medicinal, aromatic or savory qualities. They also play vital role as an antimicrobial agent. In addition, its extract is an active component added to body and beauty care cosmetics as a fast acting muscle relaxant to accelerate repair of functional wrinkles (Belfer, 2007). The plant extract is also widely used for stimulating, reorganizing and strengthening the collagen network in anti-age applications, e.g. in antiwrinkle cream formulations (Schubnel, 2007; Demarne and Passaro, 2005). A number of phytochemical constituents have been isolated from the *A. calva*., like un-saturated alkamide, spilanthol - (2 E ,6 Z, 8 E) -deca-2,6,8-trienoic acid N -isobutyl amide, with the highest concentration (1.25%) found in the flowers (Ramsewak *et al*., 1999). The *Acmella calva* is also one of the active ingredients in compositions for acute or long-term treatment of microbial infections, particularly, oral pathogenic

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microorganisms, dental caries, periodontosis, gum disease, gum bleeding and plaque reduction (Adler, 2006). In this context, the present study explores the anticariogenic activity of various extracts of *A. calva* against a set of tooth caries causing pathogens.

MATERIAL AND METHODS

Plant sample collection

Acmella calva (DC.) R.K. Jansen plants were collected from Herbal Garden of Department of Botany, Holy Cross College, Tiruchirappalli, Tamil Nadu and was identified by Dr.S.John Britto SJ and Voucher specimen deposited in the Rapinat Herbarium and Center for Molecular Systematics, St.Joseph's College (Campus), Tiruchirappalli, Tamil Nadu, under the voucher code number: PA-001.

Preparation of crude extracts

Whole plants, leaves and inflorescence were harvested; samples were cleaned and shade dried. The materials were powered by a mechanical grinder and were extracted following the method of Bakus and Green (1981). Fifteen grams of ground plant materials were soaked in 100 ml of solvents (aqueous, chloroform, acetone, ethanol and petroleum ether) and filtered using standard Whatman No.1 filter paper. Filtrate was transferred into vials and allowed to evaporate at low pressure using Buchi Rotavapor at 10°C. The extracts were then stored in refrigerator for further analysis.

Partial purification of crude extract

Partial purification of crude extracts was carried out using DEAE Cellulose anion exchange chromatography by the method of Stempion (1970).

Protein estimation

Estimation of protein in the partially purified crude extract was carried out by the method of Lowry *et al*. (1951). Bovine Serum Albumin (BSA) solution was used as standard and diluted at concentrations ranging from 20-100 mg/l. To 0.5 ml of the protein solution, 0.7 ml of Lowry solution was added, shaken well and incubated for 20 minutes at room temperature in dark. Then 0.5 ml of diluted Folin reagent was added, mixed well and incubated for 30 minutes at room temperature in dark condition. A blank was also prepared and after 30 minutes the optical density of the solution was read at 750 nm in Spectronic 20D. A standard graph was obtained by plotting absorbance against concentration to estimate the amount of protein present in the sample.

Anticariogenic activity

The anticariogenic activity of the plant extracts was investigated using the Nutrient agar plate method. Tooth caries causing bacterial strains of microorganisms viz: *Streptococcus mutans, Streptococcus salivarius,* oral pathogen like *Porphyromonas gingivalis* and lactic acid bacteria like *Lactobacillus brevis* were obtained from Easma Institute of Technology, Karur, Tamil Nadu, India and diluted bacterial culture (100 µl) was spread over sterile nutrient agar plates using sterile glass L - rod. The Whatman No.1 filter paper discs with a diameter of 6 mm were dipped into 10 µl of each extract and were placed in the centre of inoculated petri dishes. The plates were incubated for 24 hours at 37ºC and the zone of inhibition was measured in mm (millimeter). Extracts that possessed high efficacy to inhibit bacterial cell growth were analyzed and recorded.

RESULTS

The total volume of crude extracts obtained from the whole plant, leaves and

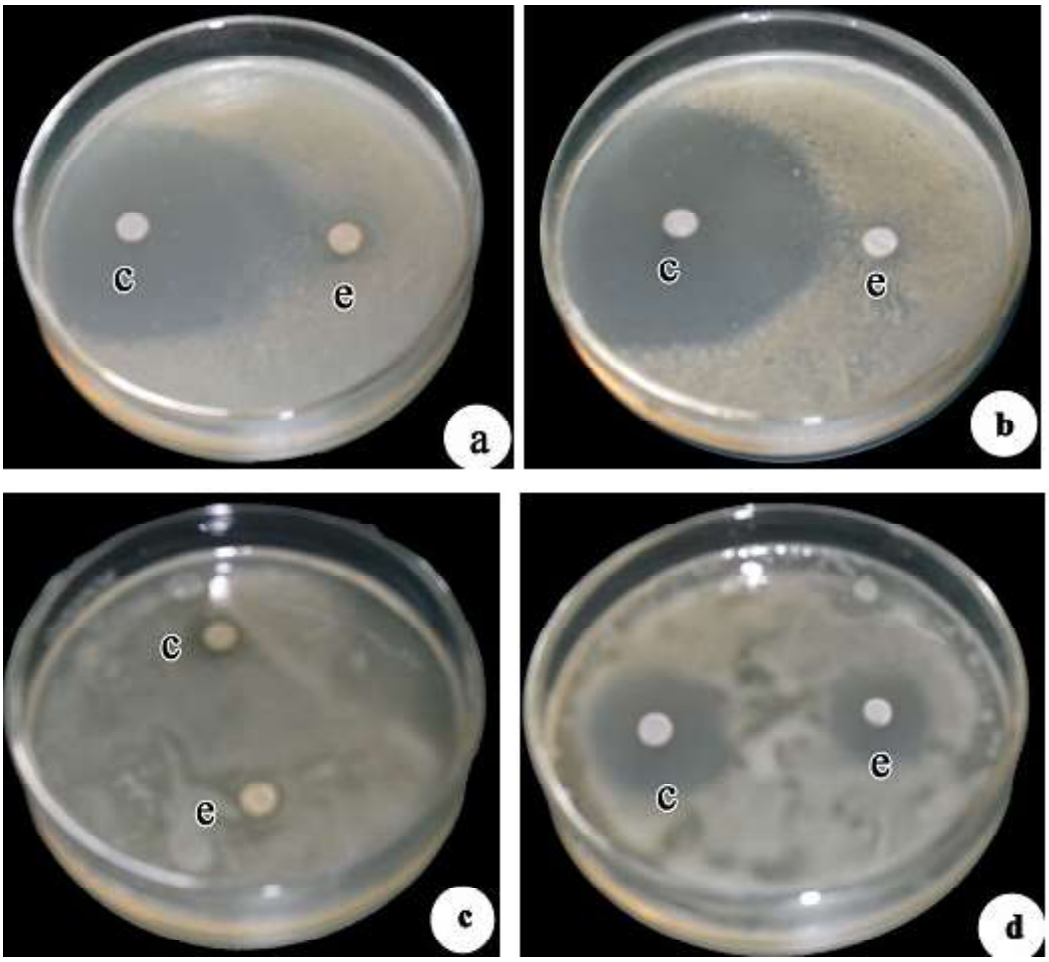
Table 1. Different crude extracts of *A. calva*

Plant Parts	Solvents	Volume of crude extract (ml/15 gm)
Whole plant	Aqueous	32
	Chloroform	26
	Acetone	25
	Ethanol	30
	Petroleum ether	28
Leaves	Aqueous	30
	Chloroform	25
	Acetone	33
	Ethanol	30
	Petroleum ether	25
Inflorescence	Aqueous	33
	Chloroform	30
	Acetone	27
	Ethanol	30
	Petroleum ether	29

inflorescence in different solvents are listed in Table 1 which shows that maximum volume of crude extract (33ml) was obtained for the acetone and aqueous extract of leaves and inflorescence respectively.

The protein content of the partially purified crude extracts (whole plant, leaves and inflorescence) was analyzed and recorded (Table 2). The obtained results indicated that the highest amount of protein (2.8 mg/ml) was found in the aqueous and acetone extracts of the inflorescence of the plant.

The results of the anticariogenic activity of the *A. calva* are tabulated in Table 3. The chloroform extract of whole plant showed the highest inhibition zone of 36 mm diameter against *S. mutans* (Fig. 1a) and it was found to be a potential inhibitor of *S. mutans*. It was followed by the chloroform extract of leaves and inflorescence which showed activity against *S. salivarius, P. gingivalis* and *L. brevis* (Fig. 1b, c and d). Acetone, ethanol and petroleum ether extracts *A. calva* showed moderate inhibition on these microbes. The chloroform extract of the



a). Chloroform and ethanol extracts of whole plant on *S. mutans*
b). Chloroform and ethanol extracts of inflorescence on *S. salivarius*
c). Chloroform and ethanol extracts of leaves on *P. gingivalis*
d). Chloroform and ethanol extracts of leaves on *L. brevis*

Fig. 1. Anticariogenic activity of different solvent extracts of whole plant, leaves and inflorescence of *A. calva*

Table 2. Quantitative estimation of protein from different partially purified crude extracts of whole plant, leaves and inflorescence extracts of *A. calva*

Plant parts	Extracts	Concentration of protein in mg/ml
Whole plant	Aqueous	2.7
	Chloroform	2.4
	Acetone	1.9
	Ethanol	2.6
	Petroleum ether	1.5
Leaves	Aqueous	1.8
	Chloroform	1.2
	Acetone	0.9
	Ethanol	1.9
	Petroleum ether	0.9
Inflorescence	Aqueous	2.8
	Chloroform	1.6
	Acetone	2.8
	Ethanol	2.5
	Petroleum ether	2.3

plant parts showed more inhibitory effect against bacterial pathogens than the other extracts. However, the aqueous extract of the three parts did not show any zone of inhibition on tested bacteria.

DISCUSSION

Oral diseases continue to be a major health problem worldwide (Peterson *et al*., 2005). The link between oral diseases and the activities of microbial species that form part of the microbiota of the oral cavity is well established (Jenkinson and Lamont, 2005). The development of dental caries involves acidogenic and aciduric Gram-positive bacteria (*Streptococci mutans, Lactobacilli* and *Actinomycetes*) (Loesche, 2007). Bacterial resistance to most of the antibiotics commonly used to treat oral infections (penicillins and cephalosporins, erythromycin, tetracycline and derivatives and metronidazole) have been widely detected (Bidault *et al*., 2007) and has prompted the need for alternative prevention and treatment options and products for oral diseases that are safe, effective and economical. There have been numerous reports of the use of traditional plants and natural products for the treatment of oral diseases. In the present study one such medicinal plant *A. calva* was analyzed for its anticariogenic activity. The results showed that

Table 3. Anticariogenic activity of *A. calva* using different extracts (aqueous, chloroform, acetone, ethanol and petroleum ether)

Plant parts	Extracts	Zone of inhibition (mm)			
		Streptococcus mutans	*Streptococcus salivarious*	*Porphyromonas gingivalis*	*Lactobacillus brevis*
Whole plant	Aqueous	-	-	-	-
	Chloroform	36	28	26	19
	Acetone	-	-	8	8
	Ethanol	8	-	19	19
	Petroleum ether	-	12	8	8
Leaves	Aqueous	-	-	-	-
	Chloroform	17	27	29	29
	Acetone	-	-	-	-
	Ethanol	-	-	7	7
	Petroleum ether	-	-	-	-
Inflorescence	Aqueous	-	-	-	-
	Chloroform	18	31	28	24
	Acetone	-	-	8	8
	Ethanol	-	-	8	8
	Petroleum ether	8	-	7	7

of all the extracts used (aqueous, chloroform, acetone, ethanol and petroleum ether), the chloroform extract of the plant parts showed more inhibitory effect against bacterial pathogens than the other extracts. This tends to show that the active ingredients of the plant parts are better extracted with chloroform than with other solvents. The absence or reduced amount of anticariogenic activity in the aqueous, acetone and petroleum ether extracts indicates the insolubility of the active ingredients in these solvent. The present study confirms the fact that the Asteraceae family to which *A. calva* belongs to have a very effective anticariogenic activity against bacterial pathogens and the results obtained are in compliance with the investigations carried out by several other researchers (Tichy and Novak, 1998; Jae-Kwan Hwang *et al*., 2004; Yatsuda *et al*., 2005; Jose and Beegum, 2007 and Cha *et al*., 2005, 2007).

CONCLUSION

The present study provides considerable evidence that *A. calva* has the potential to be developed and used as an effective therapy for oral diseases. Based on these encouraging results, further studies and trials can be conducted to test the safety and efficacy of this plant against oral diseases causing pathogens.

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