Pharmacognostic Study on *Acmella oleraceae* Murr. (Asteraceae), a Therapeutically Important Medicinal Plant Grown in Sri Lanka

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ABSTRACT

Acmella oleraceae **Murr. (Asteraceae) is a therapeutically important annual or short-lived perennial herb which has been widely used in Ayurveda and folk systems of medicine in Sri Lanka. Present study compared the quantitative physico-chemical parameters, qualitative phytochemical contents, total antioxidant capacity (TAC) using Ferric Reducing Antioxidant Power (FRAP) assay and cytotoxicity by means of brine shrimp toxicity assay of leaves, stems and flower extracts of *A. oleraceae*. The highest values for all the physico-chemical parameters, TAC and total phenolics are found in leaves of *A. oleraceae*. However, the presence of higher brine shrimp toxicity in flower extracts scientifically validated the use of flower in pharmaceutical and medicinal purposes in Ayurveda and traditional systems of medicine in Sri Lanka. Order of cytotoxicity potency was flower>leaf>stem. Quantitative (physico-chemical), qualitative [phytochemical and Thin Layer Chromatography (TLC) fingerprints], antioxidant and brine shrimp toxicity information generated through the present study could be effectively used for the quality control and standardization process of different parts of *A. oleracea* in order to validate/upgrade the Sri Lankan pharmacopoeia.**

KEYWORDS: *Acmella oleraceae*, Antioxidant capacity, Cytotoxicity, Phenolics, Phytochemicals

INTRODUCTION

Acmella oleraceae Murr. (Asteraceae) is a therapeutically important annual or short-lived perennial herb, 20–60 cm tall, with a prostrate or ascending branched cylindrical stem. Leaves are simple ovate opposite leaves without stipules. The flowers are yellow, non-fragrant with five petals on long glabrous peduncles (Jayaweera, 1981). *A. oleraceae* has been widely used in Ayurveda and folk systems of medicine as an anti-inflammatory, antiseptic and anesthetic drug since historic times (Dias *et al.*, 2011). In traditional medicine, flowers have been chewed to relieve toothache and infection of throat and to paralyze the tongue (Jirovetz *et al.*, 2006). Moreover, the chloroform extract of *Acmella* species have been successfully used to inhibit tobacco-induced mutagenesis (Sukumaran and Kuttan, 1995). Chemically, the plant contain secondary metabolite alkylamide, spilanthol (Figure 1), which is responsible for an array of bioactivities such as antioxidant activity, anti-inflammatory activity (Boonen et *al.*, 2010; Dias *et al.*, 2011), oral health care (Hebbar *et al.*, 2004), diuratic activity (Ratnasooriya *et al.*, 2004) and treatment for tooth ache (Ong and Nordiana, 1999). Flowers have been reported as the highest anti-inflammatory activity.

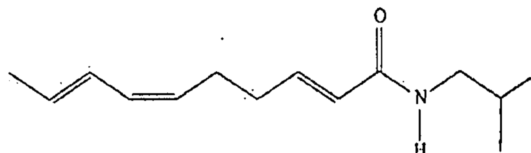

Figure 1. Structure of spilanthol

Medicinal value of the plant lies on the chemical active substances present in the plant and physiological action it creates in the human body. Thus, it is important to ensure the quality and the standards of herbal material. Although this plant has been used in many traditional and Ayurveda treatments, information on quality control and standardization is yet to be investigated. Therefore, the present study was undertaken to establish basic standards for quality control and standardization of *A. oleraceae* through established protocols.

MATERIALS AND METHODS

Location

Experiment was carried out during January to April 2013 at the laboratory of Herbal Technology Section of Industrial Technology Institute (ITI), Colombo 07, Sri Lanka and the laboratory of the Department of Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila. (NWP)

Physico-chemical Analysis

Physico-chemical analysis of moisture content, total ash, water soluble ash, acid insoluble ash, total extractable matter in hot and cold systems were determined as methods described in WHO guidelines (Anon, 1998).

Sample Preparation for Chemical Analysis

Leaf, Stem and flower samples were cut into pieces and air dried for three days at room temperature (28±2 °C). Samples were coarsely powdered by using an electrical grinder. Powdered plant material (15 g) was extracted with 50 mL of methanol by using Soxhlet apparatus and concentrated under reduced pressure at 45 °C using rotovapour.

Phytochemical Screening

The phytochemical screening tests for alkaloids, flavonoids. saponins, steroid glycosides and tannins were performed according to the method described by Farnsworth (1966).

Thin Layer Chromatography

The Thin Layer Chromatography (TLC) was performed according to the method described in WHO guidelines with some modifications. About 8 µL of the extract was spotted on TLC plates (Pre-coated silica gel 60 A 20 X 20 cm; 0.2 mm thickness) and developed in chloroform: dichloromethane: cyclohexane: methanol (5:4:1:0.4) mobile phase. They were observed under UV 366 nm and after spraying with Vanillin-Sulfuric acid. Then R_f values and colour of the spots were recorded.

Determination of Total Phenolic Content

The total phenolic content was determined using a modified Folin-Ciocaltue method (Abeysinghe *et al.*, 2007). Absorbance was measured at 760 nm using spectrophotometer (Shimadzu, UV Mini 1240, Japan). Gallic acid was used as the standard solution.

Determination of Total Antioxidant Capacity

Total antioxidant capacity was determined using Ferric Reducing Antioxidant Power (FRAP) assay as described by Benzie and Stain (1996). Methanolic extract (100 µL) of the extract was mixed with 900 µL of freshly prepared FRAP reagent of pH 3.6 containing 2.5 mL of 10 mmol/L, 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution in 40 mmol/L, HCl plus 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 300 mol/L acetate buffer. Absorbance of the reaction was measured at 593 nm using the spectrophotometer (Shimadzu, UV Mini 1240, Japan) after incubating for 4 min. The trolox was used as the standard solution.

Brine Shrimp Toxicity Assay

Brine shrimp assay was carried out as described by Michael, 1956 with slight modifications. *Artemia salina* eggs were incubated in 500 mL of brine water under illumination at 28 °C for 24 hr and larvae were transferred to 12 well plates containing 1 mL of aerated artificial brine water. The extracts of 3 different concentrations (5 ppm, 25 ppm and 50 ppm) were added into the wells and left for 24 hr at 28 °C. Artificial brine water was used as the control. The numbers of death larvae were counted under light microscope.

Statistical Analysis

Statistical comparison of mean values were performed by general linear model (GLM) of ANOVA followed by Turkey multiple range test and LC_{50} values of Brine Shrimp Toxicity Assay were performed by Probit analysis using Minitab 15 version and presented as means ± SEM. with 95% confidential level.

RESULTS AND DISCUSSION

Present study compared the physico-chemical parameters, qualitative phytochemical contents, total antioxidant capacity and cytotoxicity of leaves, stems and flower extracts of *A. oleraceae*

Table 1. Physico-chemical parameters of different parts of *Acmella oleraceae*

Part of the plant	Moisture content	Ash			Extractable matter	
		Total Ash (%)	Water Soluble Ash (%)	Acid Insoluble Ash (%)	Hot Extraction method (%)	Cold Maceration method (%)
Leaf	10.48±0.12^a	18.31±0.36^a	12.66±0.23^a	0.93±0.16^a	18.04±1.78^a	17.38±0.27^a
Stem	10.41±0.02^a	18.28±0.26^a	10.64±0.60^b	0.78±0.08^a	16.58±0.10^a	16.54±0.21^a
Flower	8.48±0.36^b	11.22±0.14^b	6.89±0.12^c	0.72±0.09^a	10.17±0.30^b	9.05±0.51^b

Means followed by same letter in each column are not significantly different at 0.05 level

Physico-chemical parameters such as moisture content, total ash content, water soluble ash content, acid insoluble ash content and extractable matter content play an important role in quality control and standardization by means of stability, purity and phytochemical composition of an herbal drug (Bharat, 2010). Moreover, maintaining of moisture content below 10% is desired for herbal preparations. As shown in Table 1, present study demonstrates moisture content of all samples is $\leq$ 10 %. Further, moisture content of flower was significantly different ($p < 0.05$) from leaf and stem samples. Since the ash represent the physiological (derived from plant itself such as calcium oxalate and silicate) and non-physiological (accumulated from external environment such as sand, soil, adulterants etc.) impurities, determination of these parameters are vital important in order to maintain the purity of the herbal medicine (Rao and Xiang, 2009; and Bharat, 2010; Kunle *et al.*, 2012). Present study reveals the total ash and water soluble ash contents were significantly ($p<0.05$) lower in flowers compared to the leaves and stem (Table 1).

Extractable matter percentage is one of the parameters used for the characterization of botanical drugs (Bharat, 2010). Comparatively higher extractable matter contents were observed in the extracts of all plant parts (leaf, stem and flower) obtained through hot extraction method over the cold extraction method. Presence of higher extractable matter in hot extraction may be due to the boiling of materials in higher temperature during the extraction process.

Secondary metabolites are responsible for the therapeutic properties of a plant and it may vary from part to part in the same plant. Preliminary investigation of phytochemicals is important for the quantitative estimation and for the locating of pharmacologically active chemical compounds (Sharanabasappa *et al.*, 2007). Current study exhibited the presence of alkaloids, flavanoids, saponins, steroid glycosides and tannins in different parts (leaf, stem and flower) of *A. oleraceae* (Table 2).

Table 2. Qualitative phytochemical parameters of *Acmella oleraceae*

Phytochemicals	Part of the plant		
	Leaf	Stem	Flower
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Steroid Glycosides	+	+	+
Tannins	+	+	+

+: Presence; - : Absence

Thin Layer chromatography (TLC) is the widely used analytical method in herbal drug standardization process due to its simplicity and cost effectiveness. As shown in Figure 2, TLC finger print profiles observed under UV 366 nm exhibited the highest number of spots in leaf extracts (12 spots) followed by stem (10 spots) and flowers (8 spots). A prominent, bright light green colour spot (R_f – 0.78) was characteristic for flower sample while it was not observed in leaf and stem samples. After spraying the colour reagent (vanillin sulfuric acid) 12 spots for leaf and 8 spots for stem and flower were observed. A blue color spot (R_f – 0.67) was observed only in leaf sample. However, some common spots for all samples were observed in TLC chromatogram.

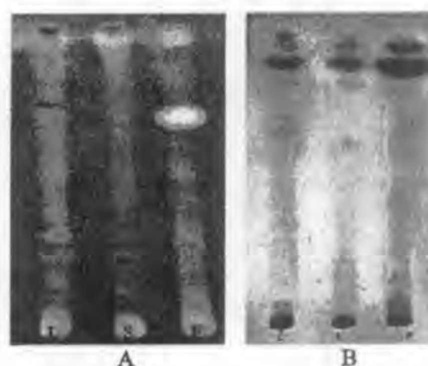

Figure 2. Thin Layer Chromatogram of different parts of *Acmella oleraceae*
A-under UV 366 nm; B- after spraying Vanillin sulphuric acid; L-Leaf; S- stem; F- flower

Antioxidants and phenolics are secondary metabolites which are mainly responsible for the defense mechanisms of a plant (Pourmorad *et al.*, 2006). The phenolic contents were significantly varied in leaves, stems and flowers of the plant (Table 3). The results demonstrated that phenolic content was increased as leaf > flower > stem with the values of 7.59 ± 1.26, 5.34 ± 0.75 and 1.65 ± 0.35 mg gallic acid equivalent (GAE) /g dry matter, respectively. Moreover, the Total Antioxidant Capacity (TAC) of tested extracts was also exhibited in same order as observed in phenolic contents. The TAC was also significantly different among the three samples leaf, stem and flower. The values of TAC were 5.29 ± 0.85, 3. 42 ± 0.59 and 1.42 ± 0.40 mg trolox equivalent (TE)/ g dry matter for leaf, flower and stem samples respectively. This study revealed the significantly high antioxidant capacity and total phenolic contents in leaves and flowers validating the use of both leaves and flowers in pharmaceutical and medical purposes.

Table 3. Contents of total phenolics and total antioxidant capacity (TAC) and cytotoxicity of *Acmella oleraceae*

Part of the plant	Total phenolics (mg GAE per g of dry matter)	TAC (mg TE per g of dry matter)	Brine Shrimp Toxicity Assay	
			LC_{50} (ppm) (after 24 hr)	95% Fiducial CI
Leaf	7.59 ± 1.26 [a]	5.29 ± 0.85 [a]	15.89 ± 3.46 [a]	8.30 – 22.92
Stem	1.65 ± 0.35 [b]	1. 42 ± 0.40 [b]	23.87 ± 4.28 [b]	15.39 – 34.07
Flower	5.34 ± 0.75 [c]	3. 42 ± 0.59 [c]	9.75 ± 1.79 [a]	6.16 – 13.66

Means followed by same letter in each column are not significantly different at 0.05 level; GAE=gallic acid equivalent; TE= trolox equivalent; LC= lethal concentration.

Since brine shrimp toxicity assay has a good correlation with cytotoxic activities, it is commonly used as a preliminary tool for screening cytotoxicity in plant crude extracts (Vivas *et al.*, 2005). As shown in Table 3 all the parts of *A. oleraceae* exhibited toxicity. Order of potency was flower > leaf > stem. Flowers showed the lowest LC_{50} value (9.75 ± 1.79) indicating the highest toxicity. Our results are in accordance with Sharmin *et al.* (2012), who proved the presence of cytotoxicity in leaf stem and flowers of *A. oleraceae.*

CONCLUSIONS

The present study compared physico-chemical parameters, phytochemical analysis, antioxidant activity and cytotoxicity of leaf, stem and flower extracts of *A. oleraceae* for the first time in Sri Lanka.

Presence of higher brine shrimp toxicity in flower extracts scientifically validates the use of flower in pharmaceutical and medicinal purposes in Ayurveda and traditional systems of medicine in Sri Lanka

Quantitative (physico-chemical), qualitative (Phytochemical and TLC fingerprints) antioxidant and brine shrimp toxicity information generated through the present study could be effectively used for the quality control and standardization process of different parts of *A. oleracea* in order to validate/upgrade the Sri Lankan pharmacopeia.

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