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Research Article

Antioxidant and cytotoxic properties of *Acmella oleracea*

P.B. Lalthanpuii[1,3], B. Lalruatfela[1], K. Vanlaldinpuia[2], H.T. Lalremsanga[3] and K. Lalchhandama[1]*
[1]Department of Zoology, [2]Department of Chemistry, Pachhunga University College, Aizawl-796001, Mizoram, India
[3]Department of Zoology, Mizoram University, Tanhril-796004, Mizoram, India

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ABSTRACT

Acmella oleracea has been widely used in various traditional medicines for the treatment of different diseases ranging from infection, helminthiasis, peptic ulcer to dental abscess. In this experiment, we analysed the antioxidant property of the methanol extract of *A. oleracea* by hydroxyl radical-scavenging assay and hydrogen peroxide-scavenging assay. In both the scavenging assays, the plant extract showed free radical scavenging activities in a concentration-dependent manner. The cytotoxic property was also determined by MTT assay, which indicated a potential cytotoxic effect on cancer cell lines such as HeLa (ATCC® CCL-2™) and V79 (ATCC® CCL-93™) cells. Higher cytotoxicity was noticed on V79 with an IC_{50} of 54.341 µg/ml. The plant extract was only moderately toxic on HeLa. The results indicated that the plant contains important bioactive compounds having pharmacological properties.

Keywords: *Acmella oleracea*, antioxidant, cytotoxicity, HeLa cell, free radical scavenging, V79 cell

INTRODUCTION

Acmella oleracea (R.K. Jansen) is an annual herb belonging to the family Asteraceae (Abeysiri *et al.,* 2013). It is generally believed to be originated from Peru and is one of the most important native plants of the Amazon (da Silva Borges *et al*., 2016; da Silva *et al*., 2016). The plant is mostly cultivated in fresh cultivation areas and grows best in clayey soil which is rich in organic matters. It is often cultivated in low open spaces and may ascend up to an altitude of about 1200 m (Lim, 2014). It is usually sown in April and can attain maturity within 6-8 months. The leaves and stem are frequently consumed either cooked or used as a seasoning to enhance the flavor of other food items. The aerial part of the plant is hot in taste and produces numbness or tingling sensation in the mouth upon mastication. This sensation is thought to be enhanced when the plant, along with the mature yellow flower, is consumed (Paulraj *et al.*, 2013).

Traditionally, *A. oleracea* is used for the treatment of flatulence, constipation, peptic ulcer, liver abscess, toothache and intestinal helminth infection (Neamsuvan and Ruangrit, 2017). It is also used for the treatment of dysentery, snakebite, articular rheumatism and tuberculosis (Tiwari *et al*., 2011). Beside these medicinal uses, the aqueous extract of the plant is used as a stupefying agent for fishes in small rivers and streams of Mizoram, India. Extracts of *A. oleracea* had been found to exert certain activities such as antimicrobial, antioxidant, diuretic, larvicidal, analgesic and mosquitocidal activities (Dubey *et al.*, 2014). It was found to contain many important bioactive compounds such as spilanthol, α- and β-amyrinester, stigmasterol, miricilic alcohol glycosides, sitosterol, saponins and triterpenes which are responsible for antioxidant activities and other therapeutic uses (Lemos *et al.*, 1991). Among the chemical components, spilanthol is considered to be the major active compound which can impart various important biological activities. Spilanthol is an

*Corresponding author e-mail: chhandama@gmail.com

important secondary metabolite which is claimed to be responsible for antioxidant, antimicrobial, cytotoxicity and anti-inflammatory bio-activities (Franca *et al.,* 2016).

In view of these diverse traditional usage and pharmacological potential, *A. oleracea* is considered to be a good alternative to various commercial drugs. In this experiment, we aimed to determine the antioxidant and cytotoxic properties of *A. oleracea*.

MATERIALS AND METHODS

Cell lines

Human cervical carcinoma cells HeLa (ATCC® CCL-2™) and Chinese hamster carcinoma cell V79 (ATCC® CCL-93™) were procured from National Centre for Cell Science, Pune, India.

Sample collection and authentication

The aerial parts of mature *A. oleracea* were collected from cultivation area of Ngopa, Champhai District, Mizoram (23.8861°N, 93.2119°E). Herbarium specimen was identified at the Botanical Survey of India (BSI), Shillong, Meghalaya, and a voucher specimen is maintained in the Department of Botany, Pachhunga University, College for further references (Accession no. PUC-A-17-1).

Preparation of extracts

Room-dried samples were grounded into powder using mortar and pestle. A known quantity of the sample was packed for extraction in a Soxhlet apparatus. Hot continuous extraction was performed for 72 hours using methanol as the solvent. The extract was concentrated using a rotary evaporator and the crude extract was obtained as a semi solid mass which was stored in a refrigerator maintained at 4°C for further analysis. The extract was evaporated and stored in a refrigerator at 4°C for further analysis.

Hydroxyl radical-scavenging assay

Hydroxyl radical (HO•)-scavenging assay was done according to the protocol of Halliwell and Gutteridge (1989). Different concentrations (viz. 10, 20, 40, 60. 80 and 100 µg/ml) were prepared each of the plant extract and ascorbic acid by dissolving in distilled water. To each concentration, 0.1 ml of 1 mM EDTA, 0.01 ml of 10 mM $FeCl_3$, 0.1 ml of 10 mM H_2O_2 and 0.36 ml of 10 mM deoxyribose was added. Further, 0.33 ml of phosphate buffer (pH 7.4) and 0.1 ml of 0.1 mM ascorbic acid was added and then incubated for an hour at 37°C. One ml of incubated solutions was mixed with 1 ml of trichloroacetic acid (TCA) and 1 ml of 0.5% thiobarbituric acid (TBA). The solutions were then heated at 80°C to develop pink chromagen for 10-20 minutes. After cooling at room temperature, the absorbance was taken at 532 nm using UV-Vis spectrophotometer (EVOLUTION 201). The results were calculated as percent inhibition of the deoxyribose attack using the following formula:

$$\% \text{ of HO•} = [AC - AS/AC] \times 100$$

Where, AC is absorbace of control and AS is absorbance of the test sample or standard. For each sample, the result was presented in IC_{50} (Sample concentration that produced 50% inhibition of hydroxyl radical).

Hydrogen peroxide radical-scavenging assay

The ability of *A. oleracea* methanol extract to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.* (1989). Briefly, 40 mM of hydrogen peroxide was prepared in phosphate buffer (pH 7.4) and different concentrations of extract and standard ascorbic acid was added separately to the hydrogen peroxide solution (0.6 mL, 40 mM). After 10-minute interval, the absorbance was taken at 230 nm against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide was calculated using the following formula:

$$\% \text{ of } H_2O_2 = [(AC - AS)/AC] \times 100$$

Where AC is the absorbance of control and AS is the absorbance of the sample or standard.

MTT assay

The inhibition percentage of the methanol extract was analyzed using HeLa and V79. For this, approximately 5000 cells from each cell lines were seeded into several wells of a 96 well microplates. The cell lines were treated with 10, 20, 40, 60, 80, and 100 µl/ml of the methanol extract. A separate group where no extract was added served as blank. MTT assay was performed according to standard protocol (Mosmann, 1983). The cell lines were immediately treated as the case may be. The cultures were then incubated at 37°C for 48 h in a CO_2 incubator in an atmosphere of 5% CO_2 and 95% humidity. After 48 h incubation, 20 µl of MTT was added to each well and the cells were incubated for another 2-4 h. After the formation of formazan crystal, 100 µl of MTT lysis buffer was added to each well to dissolve the crystals. The cultures were further incubated overnight and the optical

density was taken at 570 nm in a microplate spectrophotometer (SpectraMax M2). Usually three wells were used for each concentration in each group. The inhibition percentage of the cells was calculated by the following formula:

Inhibition % = 100-(Treatment/Control) X 100.

Statistical analyses

All statistical analyses were performed using OriginPro-8 (OriginLab Corporation, Northampton, USA) and Microsoft Excel 2013. The values of IC_{50} were calculated from linear regression analysis. To determine difference in inhibition percentages between the cell lines, student's *t*-test was employed.

RESULTS

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of *A. oleracea* is shown in Figure 1. The methanol extract and standard both showed concentration-dependent increase in scavenging activities. The activity of the extract was found to be lower than the standard at all concentrations tested. Concentrations of 10 and 100 µg/ml showed the lowest and highest scavenging activity respectively for both the methanol extract and standard tests. The lowest scavenging activity for methanol extract was 13.34% while it was 20.32% for the standard. The highest scavenging activities of the methanol extract and standard were 58.70% and 70.58% respectively. The IC_{50} of the extract was found to be 5.193 while that of the standard was 4.111.

Table 1: Cytotoxicity of the methanol extract of *A. oleracea* on cell lines

Cell line	Concentration (µg/ml)	Absorbance mean±SEM	% inhibition	IC_{50}
Control	0	0.682±0.022	0.000	
HeLa	10	0.653±0.024	4.250	116.865
	20	0.784±0.009	-14.899	
	40	0.809±0.010	-18.613	
	60	0.869±0.022	-27.308	
	80	0.891±0.020	-30.581	
	100	0.964±0.045	-41.280	
V79	10	0.700±0.014	28.574*	54.341
	20	0.749±0.009	23.610*	
	40	0.847±0.013	13.581*	
	60	0.930±0.018	5.150*	
	80	0.952±0.035	2.941*	
	100	1.040±0.025	-6.068	

*Significant difference ($p<0.05$) against control

H_2O_2 Scavenging activity

The H_2O_2 scavenging activity of methanol extract of *A. oleracea* is shown in Figure 2. A concentration-dependent increase in scavenging activity was observed. For the methanol extract, the highest scavenging activity was demonstrated by 100 µg/ml with 56.04% scavenging activity while the lowest scavenging activity was shown by 10 µg/ml at 67.67%. For the standard samples, the highest and lowest scavenging activities were 79.96% and 32.54% presented by concentrations of 100 and 10 µg/ml respectively (Figure 2). The IC_{50} of the methanol extract was 5.2776 while that of the standard was 3.235.

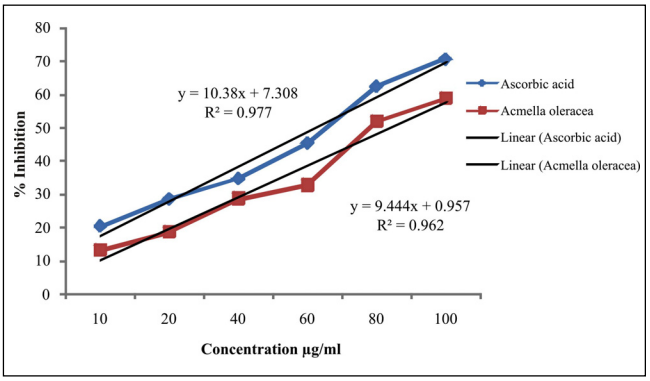

Figure 1: Scavenging activity of methanol extract of *A. oleracea* as determined by hydroxyl radical scavenging assay

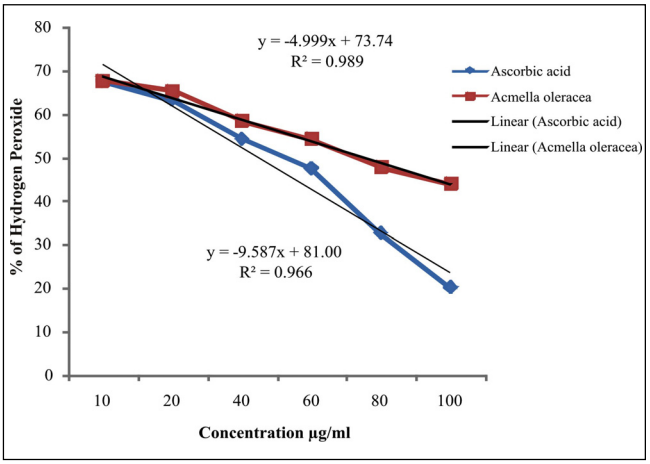

Figure 2: Scavenging activity of methanol extract of *A. oleracea* as determined by hydrogen peroxide scavenging assay

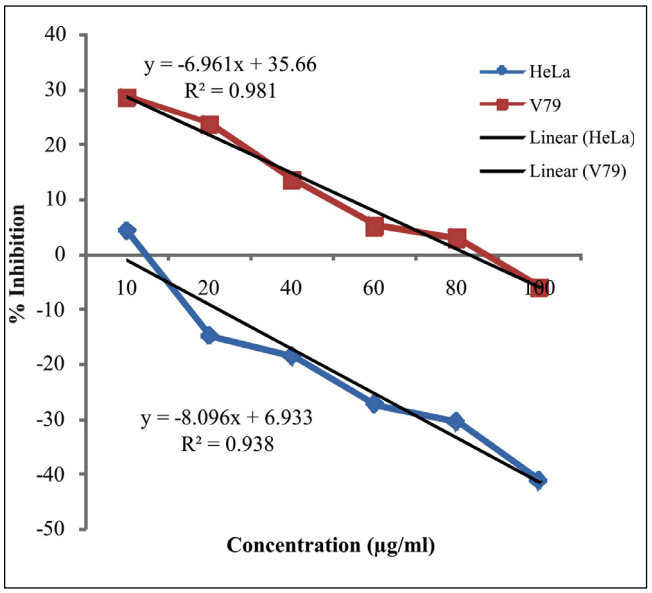

Figure 3: Graph showing the inhibition percentage of HeLa and V79 cell lines treated with methanol extract of *A. oleracea*

MTT assay

Our result showed that of methanol extract had higher cytotoxicity upon V79 cells with the IC_{50} value of 54.341 µg/ml. There was significant inhibition at lower concentrations ($p<0.05$). The IC_{50} on HeLa was 116.865 µg/ml, indicating only moderate cytotoxicity. However, the plant extract indicated proliferative effect instead of inhibition.

DISCUSSION

Different extracts of *A. oleracea* have been reported to hold numerous important functions. Hexane extract of the flower was earlier accounted to act as a larvicide against three important mosquito species such as *Anopheles stephensi*, *A. culicifacies,* and *Culex quinquefasciatus* (Pandey *et al.*, 2007). Besides this, the plant was also reported to show antimicrobial activity against bacteria such as *Klebsiella pneumonia*, *Basicillus subtilis* and *Escherichia coli* as well as an antifungal activity against *Aspergillus Niger* (Bedi *et al.,* 2017). Mouse treated with methanol extract also presented an anti-inflammatory function which can suppress neutrophilic inflammation in the lungs of the treated animal (Kim *et al.,* 2018). Due to these many beneficial properties, *A. oleracea* has been the emerging target plant for pharmaceutical research. In the present study, the antioxidant activities and cytotoxic property of methanol extract of *A. oleracea* have been demonstrated.

Antioxidants and antioxidant enzymes are group of bioactive compounds that are responsible for maintaining oxidative equilibrium of a biological system and also play essential roles in the elimination of free radicals which might be the product of cellular oxidative stress. Free radicals often steal electrons from biomolecules resulting in structural and functional alterations. Therefore, the potency of plants to eliminate free radicals, i.e. their antioxidant potential, plays crucial role in determining their therapeutic tendency (Jagetia *et al.,* 2012; Gaschler and Stockwell, 2017). Chemicals like hydrogen peroxide has been known to have the capability to cross plasma membranes and oxidize cellular compounds such as nucleic acids, lipids, proteins resulting in the inactivation of certain genes (Wongsawatkul *et al.,* 2008).

Earlier studies had shown that *A. oleracea* possessed antioxidant property in a concentration dependent manner where concentration was directly proportional to activity. This was determined by DPPH radical scavenging assay, analyses of total antioxidant content, total phenolic and flavonoid contents, nitric oxide scavenging assay and study of the reducing power (Lalthanpuii *et al.,* 2017a,b,c,d). In this study, we have also found that the antioxidant property showed a concentration dependent increase in scavenging activity by hydroxyl radical scavenging assay. Our result of the hydrogen peroxide scavenging assay showed that the scavenging activity was low when the concentration of the extract was low and as a result the remaining H_2O_2 remained high, thus result in a higher absorbance. Accordingly, at higher concentrations, the residual hydrogen peroxide was less which led to a lower absorbance. We have earlier reported the presence of phytosterols and tannins as the bioactive components (Lalthanpuii *et al.*, 2017a,d). Recently, a series of chromatographic preparations (column, thin layer chromatography, and HPLC) of the methanol and hexane extracts indicates alkylamides as the major bioactive compounds (unpublished data). These may be attributed to the pharmacological activities of the plant observed in this study.

The toxicity of two extracts of *A. oleracea* on the microbe *Artemiasarina* had been demonstrated (Ahmed *et al.*, 2013), however, the cytotoxic property of *A. oleracea* has not been studied so far. Other species of plants from the same family as the current plant of our interest, i.e. Asteraceae were reported to exert cytotoxicity on different cell lines. Panc-1 cells treated with 100 µg/ml of methanol extract of *Eclipta prostrate* showed cell survival value of 0.50 (George *et al.*,

2010). Similarly, methanol extract of *Bidens pilosa* also showed significant cytotoxic effect against HeLa and KB cells (Lemos *et al.*, 1991). From our experiment (Figure 3), different concentrations of the methanol extract showed a concentration-dependent cytotoxic effects. However, the plant extract appeared to have no significant effect on HeLa. It may be assumed that the treatment resulted in the promotion of cell proliferation, thus, resulting in the negative inhibition percentage as shown in our result. As the effects of the plant extract differ depending on the type of cells treated, the effects of the methanol extract of *A. oleracea* on different cells may differ significantly, therefore, needs more in-depth studies of the biological activities of this plant.

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