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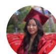
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RESEARCH ARTICLE

Some Phytochemical, Antimicrobial and Anticancer Tests for an Aqueous Extract of *Acmella oleracea*

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ABSTRACT:
Acmella oleracea (family Asteraceae) is known for its diverse applications such as food supplement, vegetable, antioxidant, antineoplastic, antimicrobial, ornamental plant and pig fodder. We prepared an aqueous extract from which the presence of alkaloids, tannins and saponins were detected. These chemical groups have been known for their wide-ranging therapeutic properties. The antibacterial activity was tested on Gram-negative bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*, and a Gram-positive species *Bacillus subtilis.* The plant extract showed no activity compared to that of the standard antibiotic tetracycline. Cytotoxic activity was also studied on cancer cell lines. We found an inverse relationship between treatment concentration and percent inhibition for HeLa (ATCC® CCL-2™) and V79 (ATCC® CCL-93™), however, the plant extract showed negligible effect on Dalton's lymphoma ascites even at the highest concentration tested. The lower degree of inhibitions observed at higher concentrations for HeLa and V79 suggests that the plant extract might confer proliferative effect rather than antimitogenic effects on the cancer cells. The results indicate that *A. oleracea* has unique medicinal properties, which in turn provide the rationale for further investigations.

KEYWORDS: *Acmella oleracea*, medicinal plant, antibacterial, cytotoxicity, cancer cells.

INTRODUCTION:
Arguably, plants and their products are the most important sources of useful substances in human health care system. The biological and chemical investigations of medicinal plants have fundamentally shaped the structure of modern pharmaceutical and medical sciences.[1] These plants offer a variety of benefits ranging from disease managements, nutritional diet, to cosmetics. This is because plants are unique organisms by producinga large number of primary and secondary metabolites which are innately useful for their own defense mechanisms and normal development.

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These chemical by-products arise from normal cellular activities and are successfully exploited as antioxidant, antimicrobial, and antineoplastic agents. Their contributions to human health and longevity are enormous.[2] Therefore, systematic study of established medicinal plants is of paramount importance to develop new or improved remedies for the complex illnesses and infections that affect both humans and veterinary subjects.

Acmella oleracea R. K. Jansen is a medicinal plant known for its diverse beneficial applications. It constitutes a part of common vegetable and food supplement.[3] The health benefits are obvious from its important bio-compounds that include many essential fatty acids such as octanoic acid, octadecanoic acid, hexadecanoic acid, as well as other phytochemicals like phenols, carotenoids, spermidine, and vitamins.[4,5] In addition, it is also known to be a rich source of linoleic acid (cis-9, 12-octadecadienoic acid), an essential omega

6-fatty acid, which is an essential nutritional supplement and is crucial for the synthesis of prostaglandins and cell membranes. Used as fodder, it is known to bea good nutrition for pigs.[6] It is also reportedly used in the treatment of scurvy and nephrolithiasis. Under experimental condition, its protective activity against acute lesions induced by ethanolin the gastrointestinal tract was demonstrated.[3,7]

To the Mizo people, *A. oleracea* is among the most common vegetables in traditional cuisine. The aerial portion is eaten raw or cooked. It is also acclaimed as a good medicine for fever, bacterial diseases and intestinal worm infection. In the light of its normal use as a vegetable, it can be easily assumed that it is a relatively safe medicinal plant and is thus a good candidate for lead compounds of pharmaceutical importance. We have studied some of its pharmacognostic properties using the methanol extracts.[8-10] In this study, the phytochemical screening and basic biological tests were performed for the aqueous extract to understand the pharmacological potentials on cancer and bacterial cells *in vitro*.

MATERIALS AND METHODS:

Chemicals:

MTT[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], sodium dodecyl sulphate, RPMI-1640 media and trypan blue were purchased from Sigma Aldrich Chemical Co., Kolkata, India. Hydrochloric acid and isobutanol were obtained from SD Fine Chemicals, Mumbai, India. Those otherwise mentioned were procured from HiMedia Laboratories Pvt. Ltd. Mumbai, India.

Cancer 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. Dalton's lymphoma ascites (DLA) was obtained from serial transplantation in Swiss albino mice.

Collection of plant material:

A. oleracea was collected from the farming area of Ngopa, Champhai district, Mizoram, India. Collection and preparation of the specimen were reported earlier.[9,10] It was authenticated at the Botanical Survey of India, Shillong, India, and a voucher specimen (No. PUC-A-17-1) is maintained at Pachhunga University College.

Preparation of extract:

The aerial parts with flowers were dried in shade for four weeks. 200g of the dried plants were macerated with double-distilled water (DDW) in an aspirator bottle. Extraction was done continuously for seven days with constant stirring at room temperature (20-23°C). The extract was filtered in Whatman Paper No 1 and then concentrated in rotary vacuum evaporator (Buchi Rotavapor® R-215) to obtain semi-solid mass. The crude extract was then stored in refrigerator at 4°C for further analysis.

Phytochemical detection:

Phytochemical screening was done according to standard protocols.[8,9,11] Phytochemical group test was performed for alkaloids (using Mayer's test, Dragendorff's test, Wagner's test, and Hager's test), carbohydrates (using Molisch's test, Fehling's test, Barfoed's test, and Benedict's test), phytosterols (using Liebermann-Burchard's test, and Salkowski reaction), glycosides (using Legal's test, Keller-Killiani's test, and Borntrager's test), tannins (using $FeCl_3$ test, $K_2Cr_3O_7$ test, and lead acetate test), saponins (using foam test), reducing sugars (using Fehling's test and Benedict's test), flavonoid (using Shinoda test and zinc hydrochloride reduction test), and proteins and amino acids (using biuret test and ninhydrin test).

Antimicrobial activity:

Antimicrobial activity was assessed by disc diffusion technique in which the agar plates were prepared by pour plate method.[12] Three species of Gram-negative bacteria such as *Klebsiella pneumoniae* (ATCC®10031™), *Pseudomonas aeruginosa* (ATCC® 10145™) and *Escherichia coli* (ATCC® 10536™), and one Gram-positive species *Bacillus subtilis* (ATCC® 11774™) were used. Different concentrations of the plant extract, namely 10, 20, 40, 60, 80, and 100 µg/ml were prepared in DDW and dimethyl sulfoxide (DMSO). Each sample was impregnated on 5 mm Whatman No 3 paper discs. 10 µg of tetracycline impregnated in Whatman paper disc was used as a standard reference, whereas dimethyl sulfoxide (DMSO) was used as control. The culture plates were then incubated at 37±1°C for 20 hours. The inhibition zones were observed and recorded, and then compared with that of the standard antibiotic.

MTT assay:

The inhibitory properties of the different extracts were tested by MTT assay using three cell cancer lines, *viz.* HeLa, V79 and Dalton's lymphoma ascites. Approximately 5000 cells from each cell line were inoculated into 96-well microtiter plates. Each cell line was treated separately with different concentrations of the plant extracts such as 10, 20, 40, 60, 80 and 100µg/ml. A separate group without plant extract was maintained as a negative control. Cytotoxicity assay was performed according to the method of Mosmann (1983).[13] The cell lines were incubated at 37°C for 48 h in a CO_2 incubator with an atmosphere of 5% CO_2 and 95% humidity. After the incubation, 20 µl of MTT was added to each well and were further incubated for 2-4 h. After the formation of formazan crystal, 100 µl of MTT lysis buffer was added to each well to dissolve the

formazan crystals. The cultures were then incubated overnight and the optical density was read at 570 nm in a microplate spectrophotometer (Spectra Max M2). Three replicates were processed for each sample. From the standard reading, the inhibition percentage for each treatment was calculated using the following formula:
Inhibition %: 100–(Treatment/Control) x100.

Statistical analyses:
All the statistical data acquisition and analyses were done using Origin Pro-8 (Origin Lab Corporation, Northampton, USA) and Microsoft excel 2016. The values of inhibitory concentration (IC_{50}) were calculated by linear regression analysis using standard graph obtained from the absorbance readings. Student's *t*-test was used to determine the differences in inhibition percentages between the cell lines. Statistical significance was taken at $P<0.5$. Pearson correlation was used to determine the effect of different concentrations on the inhibition percentage.

RESULTS:

From 200 g of *A. oleracea,* an extractive weight of 33.837 g obtained, which was 16.916% of the original sample. Various standard tests performed on the aqueous extract showed the presence of important bioactive compounds. The occurrence of alkaloids was confirmed by Mayer's test, Dragendorff's test, Wagner's test and Hager's test. Tannin was found to be present in one of the tests, i.e. lead acetate test, but not in $FeCl_3$ and $K_2Cr_3O_7$ tests. Saponins were detected in foam test. Carbohydrates, phytosterols, glycosides, reducing sugars, flavonoids, proteins and amino acids were not detected in any of the tests (Table 1).

Table 1: Phytochemical analysis of *A. oleracea* aqueous extract by various standard tests.

Sl. No.	Phytochemicals	Test	%
1.	Alkaloids	Mayer's test	+
		Dragendorff's test	+
		Wagner's test	+
		Hager's test	+
2.	Carbohydrates	Molisch's test	-
		Fehling's test	-
		Barfoed's test	-
		Benedict's test	-
3.	Phytosterols	Liebermann-Burchard's test	-
		Salkowski reaction	-
4.	Glycosides	Legal's test	-
		Keller-Killiani's test	-
		Borntrager's test	-
5.	Tannin	$FeCl_3$ test	-
		$K_2Cr_3O_7$ test	-
		Lead acetate test	+
6.	Saponins	Foam test	+
7.	Reducing sugars	Fehling's test	-
		Benedict's test	-
8.	Flavonoid	Shinoda test	-
		$ZnCl_2$ reduction test	-
9.	Proteins and amino acids	Biuret test	-
		Ninhydrin test	-

The plant extract at different concentrations, i.e. 10, 20, 50, 100 and 200 µg/ml showed lack of antibacterial activity against all the bacteria tested. In contrast, the standard antibiotic tetracycline showed distinct inhibition zones. For the drug, the inhibition zones were measured as 1.5 cm for *K. pneumoniae*, 1.6 cm for *P. aeruginosa*, 1.5 cm for *E. coli* and 1.4 cm for *B. subtilis*.

The plant extract exhibited cell-specific cytotoxic activity as shown in Figure 1 & 2 and Table 2. The activity was lowest on DLA with an IC_{50}value of -82.768 and highest for V79 with an IC_{50}value of -5.205.For HeLa, the IC_{50}value was -7.016. Comparison of the IC_{50} between the three cells showed significant difference between HeLa with V79 and V79 with DLA. However, no statistical difference significant was observed between HeLa and DLA (Table 3). For all the cell lines studied, strong and significant negative correlation was found between the treatment concentrations and IC_{50} (Table 4).

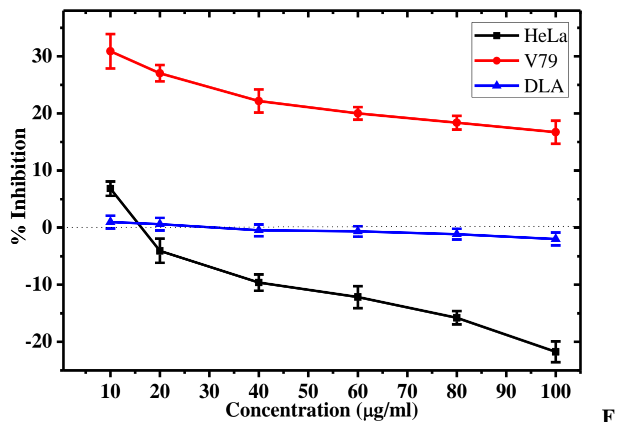

Figure 1: Inhibition percentage plotted against different aqueous extract concentrations of *A. oleracea* on HeLa, V79 and DLA cell lines.

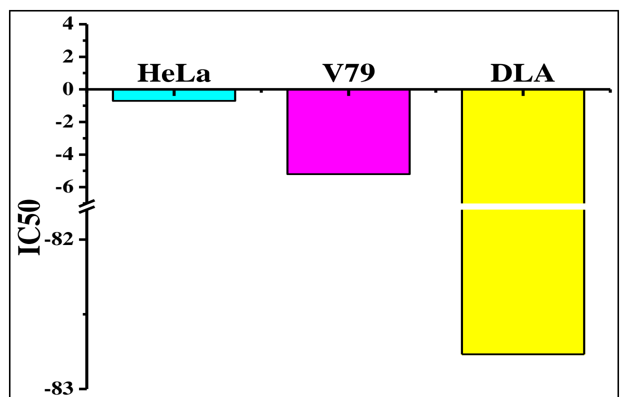

Figure 2: IC_{50} of different cell lines treated with aqueous extract of *A. oleracea*.

Table 2: MTT assay showing inhibition percentage and IC_{50} of HeLa, V79 and DLA cells treated with *A. oleracea* aqueous extract.

Conc. (µl/ml)	Inhibition percentage (mean± SEM)		
	HeLa	V79	DLA
10	6.839±1.272	30.886±3.021	0.976±1.117
20	-4.055±2.119	27.044±1.412	0.607±1.104
40	-9.624±1.414	22.183±2.028	-0.469±1.012
60	-12.164±1.916	20.007±1.109	-0.649±0.927
80	-15.779±1.171	18.375±1.184	-1.145±0.929
100	-21.739±1.818	16.709±2.029	-1.989±1.111
IC_{50}	-7.016	-5.205	-82.768

Table 3: Student's *t*-test between inhibition percentage of different cell lines.

Cell lines	*p*value
HeLa & V79	0.000*
HeLa & DLA	0.059
V79 & DLA	0.000*

*Significantly different at <0.05 level of significance.

Table 4: Pearson correlation showing the effect of the different aqueous extract concentrations on inhibition percentage.

Cells lines	*r*value
HeLa	-0.944*
V79	-0.957*
DLA	-0.913*

*Significantly different at <0.05 level of significance (2-tailed).

DISCUSSION:

Plants have been principal sources of chemical compounds used in health care systems including pharmaceutical drugs, nutritional products and cosmetics. Since the general methods of preparations are high temperature based aqueous extraction, the chemicals derived from such are assumed to be polar compounds.[14] Thus, analyses of the aqueous extract of potential medicinal plants and other consumable plants are important to know the therapeutic activities of the consumed nutrients and compounds or phytochemicals. These phytochemicals play the primary role in all the biochemical activities of the plants. In the present study, alkaloids are found to be present which suggests that the consumption of *A. oleracea* may have several health benefits. Alkaloids are the major sources of antimicrobial drugs including antibiotics and antimalarials.

Our study also showed that tannins and saponins are important constituents of the plant extract. Studies have shown that saponins possess fat-reducing (hypolipidemic) and anticancer activities. Saponins are also used in the synthesis of commercial sex hormones. Tannins are known to have antiseptic activity, thus, acting as a wound healing agent.[2] Therefore, the aqueous extract of *A. oleracea* may contribute significantly in the treatment of excessive and unwanted cholesterol and eventually obesity and hypertension. It may also be an effective source for the treatment of cancers and hormonal imbalance- related complications as well as for the treatment of wounds.

Other studies have shown that alcoholic extracts of the callus and stem of *A. oleracea* inhibited the growth of both Gram negative and Gram-positive bacteria such as *E. coli*, *Enterococcus faecalis, K. pneumoniae*, *Salmonella typhi*, *Staphylococcus aureus*, *S. epidermidis*, and *Streptococcus pyogenes*.[15] Another study also reported chloroform extract and methanol extract of *A. oleracea* to have inhibitory action against oral bacteria such as *Streptococcus mutans* and *Lactobacillus* sp.[16] However, our study showed that different concentrations of the aqueous extract of AO did not show any inhibitory activity toward the bacteria of our interest like *K. pneumoniae*, *P. aeruginosa*, *E. coli* and *B. subtilis*. This may be due geographical variation of the plant as it is known that *A. oleracea* cultivated in Mizoram has unique characteristics.

Related plants belonging to the family Asteraceae have been reported to have cytotoxic properties on different cell lines.[17,18] From our data (Figure 1), treatment of DLA with different concentrations of the plant extract showed no evident inhibition of the cell growth. However, for HeLa and V79 cells, there is an inverse relationship between treatment concentration and percent inhibition, suggesting possible proliferative potential of the extract on these two cell lines. The differences in the degrees of inhibition on different cell lines can be attributed to cell-specific activities. This further implies that the plant may have interesting compounds that is effective on a specific cancer type. A more meticulous analysis on different cells is required to further understand the pharmacological and medicinal potentials of this plant.

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CONFLICT OF INTEREST: None declared.

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