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PHYTOCHEMICAL ANALYSES, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *ACMELLA OLERACEA*, A VARIETY GROWN IN MIZORAM

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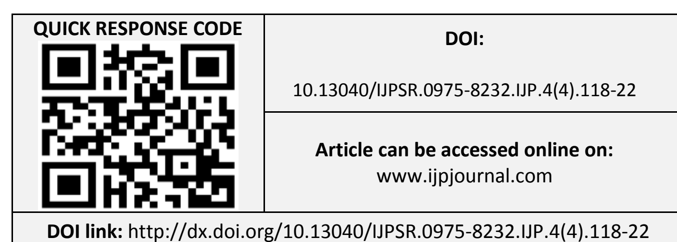
ABSTRACT: *Acmella oleracea* (L.) R.K. Jansen (family Asteraceae) is an interesting plant because of its wide range of medicinal and culinary uses. It has been traditionally used in the treatments of anaemia, body ache, cancer, gastric ulcer and infections, gingivitis, gout, inflammations, laceration, malaria, stammering, and worm infection (helminthiasis). Different extracts of the plants were prepared, and the methanol extract showed highest number of bioactive compounds, including carbohydrates, phytosterols, and tannins. The antioxidant activity was estimated from the rate of scavenging of the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). The plant extract showed similar concentration-dependent scavenging activity as that of butylated hydroxytoluene (BHT). The total antioxidant activity was estimated against ascorbic acid, and it showed higher value than BHT. The methanol extract was treated with two Gram-negative bacteria *Pseudomonas euroginosa* and *Escherichia coli*, and two Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis.* However, no antibacterial activity was observed. Nonetheless, the study provides important information on the basic pharmacological properties of the plant.

INTRODUCTION: *Acmella oleracea* (L.) R.K. Jansen is a perennial flowering herb belonging to the family Asteraceae. Native to Peru, it has been introduced throughout tropical and subtropical regions including Africa, America, Borneo, India, Sri Lanka, and Southeast Asia. [1, 2] It has distinct pungent smell and burning taste upon chewing. Native Americans use it as a spice for its unique aroma. In India, Brazil, and Southeast Asia, it is served as a common vegetable.[3]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In different traditional medicine, it is known to have anaesthetic, anticonvulsant, antiseptic, antifungal, anti-protozoal, anti-diarrhoeal, analgesic, antiulcer, anti-pyretic, anti-tumour, anti-inflammatory, diuretic, aphrodisiac, and insecticidal activities. [4, 5] For its antiseptic and analgesic properties, its extract is used in oral health care for the treatment of sore throat, oral ulcer, gingivitis, and general toothache. It is because of this application that it is given an English vernacular name the toothache plant.[6]

A. oleracea is also used for clinical diseases such as anaemia, haemorrhage, cancer, dysentery, gastrointestinal ulcer, rheumatism, scurvy, stammering, and xerostomia and snake bite.[7-9] It is also used in infectious diseases such as malaria and

helminthiasis.[10] Its cytotoxic, antioxidant, and vasorelaxant activities have been attributed to its use in the treatment of blood disorders.[8] Its antipyretic activity against Brewer's yeast-induced pyrexia is credited to its use in high fever.[6] It also has insecticidal activity against the pest *Tuta absoluta*,[11] and vectors of infectious diseases including *Aedes aegyptii*.[12]

In Indian medicine, it is used as an effective aphrodisiac, and is being prescribed for cases of impotency. [2] All these medicinal applications have been supported from its pharmacological properties such as anaesthetic, anti-inflammatory, analgesic, antipyretic, anti-obesity and diuretic activities. [13-15] The anti-inflammatory activity has been demonstrated in experimental rats. [14] It is also experimentally shown to increase the number of macrophages, specialised white blood cells vital for various immunological responses. This supports the the use of the plant in the treatment of rheumatism. [16] This study therefore aims at chemical analysis and tests for some biological activities of the plant.

MATERIALS AND METHODS:

Collection of plant material: *A. oleracea* was collected from the plantation field in the village of Ngopa, Champhai District, Mizoram, India (located between 23.8861° latitude north and 93.2119° longitude east) in 2015. A voucher specimen was identified and is maintained at the herbarium section of the Department of Botany, Pachhunga University College, Aizawl, Mizoram. The aerial parts, *i.e.* leaves and flowers, of the plant were dried in a thermostat oven at 45 °C.

Preparation of plant extracts: The dried plants were pulverised to powder using mortar and pestle. The plant powder was subjected to continuous hot extraction in a Soxhlet apparatus using different solvents of increasing polarity, namely hexane, chloroform, and methanol. Hexane extraction was run for 48 h, while chloroform and methanol extraction required 72 h. The extracts were concentrated in a vacuum rotary evaporator (Buchi Rotavapor® R-215). The plant extracts were produced in the form of semi-solid mass, and were refrigerated at 4 °C until further use.

Phytochemical detection: The various chemical components were screened using standard protocols. Alkaloids were tested by Meyer's test, and Dragendroff's test; carbohydrates by Wagner's test, Hager's test, Molisch's test, Fehling's test, Barfoed's test, and Benedict's test; phytosterols by Liebermann-Burchard's test, and Salkwoski reaction; glycosides by Legal's test, and Keller Killiani's test; tannins by $FeCl_3$ test, $K_2Cr_3O_7$ test, and lead acetate test; saponin by foam test; reducing sugars by Fehling's test, and Benedict's test; flavonoids by Shinoda test, and zinc hydrochloride reduction test; and amino acids by Biuret test, and ninhydrin test.

Antimicrobial activity: The antimicrobial activity was determined using a disk diffusion method. Two Gram-negative bacteria *Pseudomonas euroginosa* and *Escherichia coli*, and two Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, were used. The bacteria were grown in culture dishes containing Mueller-Hinton agar. The plant extracts were impregnated on absorbent disks (Whatman Antibiotic Assay Discs). Control experiment was maintained containing the bacteria culture only. For standard reference, tetracycline was used. The experiment was prepared in triplicate. The culture dishes were maintained at a 37±1 °C. After 20 h, the size of bacterial growth and the corresponding inhibition zones were noted.

Antioxidant activity: Estimation of the antioxidant activity followed the method of Blois. [17] Briefly, a stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was used as a substrate. Antioxidant activity was shown by the scavenging of DPPH. 1 ml of 0.1 mM solution of DPPH in methanol and 3 ml of the methanol extract were incubated at 37±1 °C for 30 minutes. For the standard reference, butylated hydroxytoluene (BHT) was used. Absorbance was measured at 517 nm against control in a UV-Visible spectrophotometer (Evolution™, Thermo Scientific). The percentage of inhibition was calculated by comparing the absorbance values of the test samples with those of the controls.

The inhibition percentage (I) was calculated using the formula:

$$I = (\text{Abs control} - \text{Abs sample}) / \text{Abs control} \times 100$$

Total antioxidant activity: The total antioxidant activity was determined by phosphomolybdate estimation using ascorbic acid as a standard. 0.1 ml of sample solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After incubation at 95 °C, absorbance was measured at 695 nm.

RESULT: Biochemical detections using various methods show the presence of important phytochemicals in *A. oleracea* such as phytosterols, tannins, reducing sugars and carbohydrates (**Table 1**). The methanol extract indicated maximum variety of compound including phytosterols, tannins, reducing sugars and carbohydrates in all the tests, except in Molisch's test for carbohydrate. The hexane showed the presence of phytosterols in both Liebermann-Burchard's and Salkwoski tests. However, other important phytochemicals such as alkaloids, flavonoids, and saponins could not be detected in any of the tests used.

TABLE 1: CHEMICAL COMPOSITION OF *A. OLERACEA* USING DIFFERENT BIOCHEMICAL TESTS

Sl. No.	Phytochemicals	Name of test	Hexane	Chloroform	Methanol
1.	Alkaloids	Meyer's test	-	-	-
		Dragendroff'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	+	-	+
		Salkwoski reaction	+	-	+
4.	Glycosides	Legal's test	-	-	-
		Keller Killiani'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	-	-	-
		Zinc hydrochloride reduction test	-	-	-
9.	Proteins and amino acids	Biuret test	-	-	-
		Ninhydrin test	-	-	-

The antioxidant activity was estimated by the method of DPPH free radical scavenging assay using the methanol extract (**Fig. 1**). Increasing concentrations of the plant extract were prepared from 10, 20, 40, 60, 80 to 100 µg/ml. Exactly similar concentrations were used for the reference compound BHT. Both the extract and BHT showed concentration-dependent activity against DPPH, *i.e.* increased scavenging activity with increased concentration. BHT appeared to be more potent than the plant extract at all concentrations tested. IC_{50} of standard BHT and the plant extract was calculated from the standard graph. The plant extract showed IC_{50} of 13.773 mg/ml while for BHT it was 28.098 mg/ml. The total antioxidant activity was estimated as ascorbic acid equivalent *i.e.* 85 mg/ml.

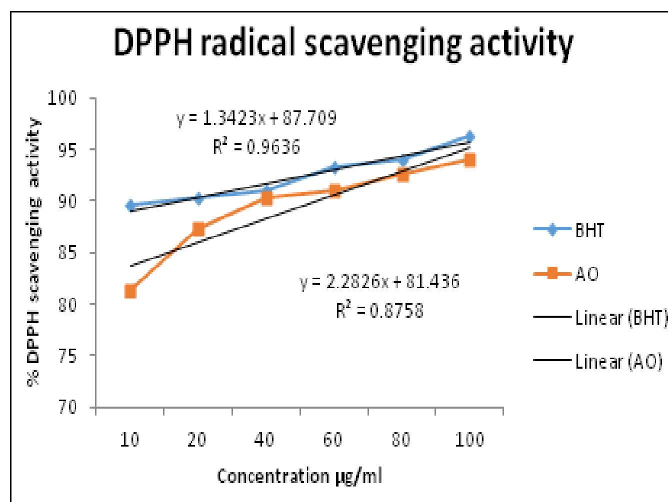

FIG. 1: ANTIOXIDANT ACTIVITY OF *A. OLERACEA* USING SCAVENGING ACTIVITY OF 2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH)

Antimicrobial activity was assessed upon two commonly available bacteria, *viz. Pseudomonas euroginosa* and *Escherichia coli* (both Gram-negative), and *Staphylococcus aureus* and *Bacillus subtilis* (both Gram-positive). The antibiotic tetracycline was used as a positive treatment. Using the equivalent concentrations (10 and 20 mg/ml) as that of the drug, the methanol extract of the plant did not produce any significant zones of inhibition, all the bacteria grew normally; while the standard drug showed a distinct zone of inhibition.

DISCUSSION: In the present study, the presence of important bioactive compounds in *A. oleraceae* was confirmed. Phytosterols, tannins, reducing sugars, and carbohydrates were detected. The leaves of *A. oleracea* have been reported to contain alkamides, amides, carbohydrates, tannins, steroids, carotenoids, essential oils, sesquiterpenes and amino acids, [8, 18] phytosterols (e.g. β-sitosterol, stigmasterol, α- and β-amyrins), essential oils (e.g. limonene and β-caryophyllene), sesquiterpenes, α- and β-bisabolenes and cadinenes, flavonoid glucoside and a mixture of long chain hydrocarbons.[1, 19] The most well-known phytosterols such as β-sitosterol, stigmasterol and campesterol, are well established to be pharmacologically beneficial for their therapeutic actions in cardiovascular diseases, colon and breast cancer. [20]

The methanol extract exhibited concentration-dependent antioxidant activity like that of butylated hydroxytoluene (BHT). Although its overall activity was lower than that of BHT, it showed higher total activity with an IC_{50} of 13.773 mg/ml, compared to BHT having an IC_{50} of 28.098 mg/ml. Free radicals are responsible for physiological oxidative stress, which is the cause of health problems including several cardiovascular, neurodegenerative, cancer and even aging. [21] Antioxidants are required to downplay the oxidation process by converting the harmful free radicals to harmless molecules or by destroying them. [22] Hence dietary antioxidants are the principle sources of defense for cellular oxidation. [23, 24]

Antimicrobial activity was not observed against four bacteria, *P. euroginosa* and *E. coli*, *S. aureus* and *B. subtilis.* However, is has been reported that closely related species showed antibacterial activity against *Klebsiella pneumoniae*, [25] *Streptococcus pyogenes*, [8] *Salmonella typhi*. [26] But, similar to the present study, Prachayasittikul *et al.* [8] found no antibacterial activity for *Spilanthes acmella*. These data coupled with the present study suggest that the antibacterial property of *A. oleracea* may be highly species specific.

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