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Chapter 49

Chemical Constituents and Some Biological Properties of the Traditional Herbal Medicine *Acmella oleracea* (Asteraceae)

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ABSTRACT: *Acmella oleracea* is one of the most widely used traditional medicinal and culinary plants. It is easily available both in the wild and in cultivation. It is variously used in the treatments of anaemia, body ache, cancer, gastric ulcer and infections, gingivitis, gout, inflammations, laceration, malaria, stammering, and intestinal worm infection. The whole plant extracts were prepared with hexane, methanol, and chloroform. The methanol extract contained the most number of bioactive compounds, including carbohydrates, phytosterols, and tannins. The chloroform extract had carbohydrates and phytosterols. The hexane extract contained only phytosterols. The plant extract showed concentration-dependent scavenging activity of free radical, and has an almost the same antioxidant potency as that of the standard butylated hydroxytoluene (BHT). Two anthropo-pathogenic bacterial species, namely a Gram-negative *Pseudomonas euroginosa* and a Gram-positive *Staphylococcus aureus*, were used for antibacterial activity. However, the plant extract failed to inhibit the bacteria in comparison to treatment with tetracycline. But there are reports of high activity against other species such *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia*, *Micrococcus luteus*, and different *Staphylococcus* species. The differential activity suggests that either the antibacterial principle is lost in the extraction or the antibacterial activity is species-specific. The study provided the evidence that *A. oleraceae* possesses chemical components that are potentially useful in therapeutic applications.

***Keywords**: Acmella oleracea, Antioxidant Activity, Medicinal Plant, Antimicrobial Activity.*

INTRODUCTION

Acmella oleracea (L.) R.K. Jansen is a rather small and perennial flowering herb belonging to the family Asteraceae. Considered to have originated from Peru, it is now introduced and now widely distributed throughout tropical and subtropical regions including Africa, America, Borneo, India, Sri Lanka, and Southeast Asia (Tiwari *et al.,* 2011; Prachayasittikul *et al.*, 2013). It is cultivated throughout the year as ornamental, culinary and medicinal plant. Its flowers and leaves produce a distinct pungent smell and irritating taste when chewed. It also produces a tingling sensation and numbness when rubbed against the skin. For this unique flavour, it is remains as an important vegetable and culinary additive. In the America, for its pungent flavour it is used as a spice. In India, Brazil, and Southeast Asia, it is a common vegetable (Lim, 2014). It is one of the most widely used medicinal plants in various ethnomedicinal systems because of its broad range of pharmacological properties including an aesthetic, anticonvulsant, antiseptic, antifungal, antiprotozoal, antidiarrhoeal, analgesic, antiulcer, antipyretic, antidiuretic, antiinflammatory, diuretic, aphrodisiac, and insecticidal activities (Dubey *et al.*, 2013; Paulraj *et al.*, 2013). As antiseptic and pain-relievingagent, it has been employed in oral health care for the treatment of various mouthailments such as gingivitis, oral ulcer, sore throat, and general toothache. For this reason, it has been earned a moniker toothache plant. These properties have been ascribed to the analgesic and antimicrobial activities of the plant (Chakraborty *et al.,* 2010).

A. oleracea is also applied in a curative of a number of infectious diseases and life-threatening afflictions such asblood diseases and bleeding, cancer, dysentery, gastrointestinal ulcer, rheumatism, and snake bite (Prachayasittikul *et al.*, 2009; Dubey *et al.*, 2013). In India and Africa, it is widely acclaimed as an antimalarial medication (Spelman *et al.,* 2011). It is used additionally in the treatment of gout, helminthiasis (intestinal infection), hepatosis, prostate cancer, scurvy, stammering, and xerostomia (dry mouth) (Duke, 2008). Its cytotoxic, antioxidant, and vasorelaxant activities have been confirmed indicating that there are good rationales for its use in the treatment of blood disorders (Prachayasittikul *et al.*, 2009). Its use in the treatment of high fever can be credited to the fact that it reported show antipyretic activity against Brewer's yeast-induced pyrexia (Chakraborty *et al.,*

2010). Its insecticidal activity has been investigated against different insects. It is active against the pest *Tuta absoluta* (Moreno *et al.,* 2012), and vectors of infectious diseases including *Aedes aegyptii* (Ramsewak *et al*., 1999; Simas *et al.*, 2013). In Indian medicine, it is regarded as one of the most effective aphrodisiac, and isbeing prescribed for cases of impotency, and to enhance libido in men (Prachayasittikul *et al.*, 2013). Much of these applications have been experimentally verified showing that the plant indeed has important pharmacological properties, includinganaesthetic, antiinflammatory, analgesic, antipyretic, antiobesity and diuretic activities (Ratnasooriya *et al.*, 2004; Wu *et al.,* 2008; Chakraborty *et al.,* 2010). The antiinflammatory activity has been clearly defined in experimental rats (Chakraborty *et al.*, 2004). It is also experimentally demonstrated toelevate the number of specialised white blood cells called macrophages, which are vital for various immunological responses. This lends credence to the use of the plant for the treatment of rheumatism (Savadi *et al.,* 2010). It is therefore imperative that the details of its chemical and biological properties are analysed so as to make it a scientifically-proven therapeutic agent.

MATERIALS AND METHODS

Collection of Plant Material

A. oleracea was collected in 2015 from Ngopa, Champhai District, Mizoram, India (located between 23.8861° latitude north and 93.2119° longitude east). The aerial parts of the plant were dried at 45–50°C in a hot air oven.

Preparation of Plant Extracts

The dried plants were pulverised to powder, and subjected to continuous hot extraction using hexane, chloroform, and methanol. The different extracts were concentrated by recycleing the solvents in a vacuum rotary evaporator (Buchi Rotavapor® R-215). The plant extracts were obtained assemi-solid mass, and were refrigerated at 4°C.

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 Zn hydrochloride reduction test; and amino acids by Biuret test, and ninhydrin test.

Antimicrobial Activity

The antimicrobial activity was determined using a disk diffusion method. Gram-negative bacteria *Escherichia coli*, and Gram-positive species *Bacillus subtilis* were grown in Mueller-Hinton agar. The plant extracts were impregnated on absorbent disks (Whatman papers). For standard and positive reference, tetracycline was impregnated to a separate disk. The experiment was prepared in triplicate. The culture dishes were maintained at a 37 ± 1°C. After 18–20 h, the size of bacterial growth and the corresponding inhibition zones were observed.

Antioxidant Activity

The antioxidant activity was estimated by the method of Blois (1958). 1 ml of 0.1 mM solution of DPPH (2, 2-diphenyl-1-picrylhydrazyl) mixed with methanol and 3 ml of plant extracts were incubated at 37 ± 1°C for 30 minutes. For the standard reference, butylated hydroxytoluene (BHT) was prepared similarly. Absorbance was measured at 517 nm. 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 phospho-molybdate 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.

Total Phenol Content

The total phenolic content was determined by Folin–Ciocalteu assay. Calibration curve was prepared by mixing 1 ml of methanolic solution of gallic acid (10, 20, 40, 60, 80, and 100 µg/ml) with 5 ml Folin–Ciocalteu reagent (diluted tenfold). After 3 minutes, 4 ml of sodium carbonate solution (0.7 M) was added, and the mixture was left to stand for 1 hr at room temperature. Absorbance was measured at 765 nm. 1 ml extract (50 µg/ml) was also mixed with the reagents above and after 1 hr the absorbance was measured to determine total plant phenolic content. From the calibration curve, the amount of phenolic compounds was determined and expressed as milligrams of gallic acid equivalent (GAE)/g of the dried extract.

Total Flavonoid Content

The total flavonoid content of the plant was determined by the aluminum chloride method. 1 ml of the extract (50 µg/ml) was mixed with 2 ml of distilled water. After 5 minutes,

3 ml of 5% sodium nitrite ($NaNO_2$) and 0.3 ml of 10% aluminum chloride ($AlCl_3$) were added. After 6 minutes, 2 ml of NaOH (1 M) was added, and the volume was made up to 10 ml with distilled water. After 1 hr, absorbance reading was taken at 510 nm. A standard curve was prepared with quercetin at different concentrations (5, 10, 20, 40, 60, 80, and 100 μg/ml). The total flavonoid content was expressed as milligrams of quercetin equivalent (QE/g) of dried extract.

RESULT

From the initial total weight (2.8 kg) of *A. oleracea*, the extraction values were calculated in terms of extractive weight and extractive value of as given in Table 1. The extractive value is higher in methanol with 17.94% yield and hexane extract have an extractive value of 4.07%.

Biochemical detections using various methods show the presence of important phytochemicals such as phytosterols, tannins, reducing sugars and carbohydrates (Table 2). The methanol extract indicated maximum variety of compound includ-ing phytosterols, tannins, reducing sugars and carbo-hydrates in all the tests, except in Molisch's test for carbo-hydrate. The hexane showed the presence of phytosterols in both Liebermann-Burchard's and Salkwoskitests. However, other important phytochemicals such as alkaloids, flavonoids, and saponins could not be detected in any of the tests used.

Table 1: Extraction Yield of *A. oleracea* Using Soxhlet Extractor and Rotary Vacuum Evaporator

Solvent	*Total Weight (g)*	*Extractive Weight (g)*	*Extractive Value (%)*
Hexane	2,800	113.904	4.07
Methanol	2,800	502.194	17.94
Chloroform	2,800	78.638	2.81

Table 2: Detection of 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	–	–	–
		Zn 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 (Figure 2). 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 BHTit was 28.098 mg/ml.The total antioxidant activity was estimated as ascorbic acid equivalent, *i.e.* 85 mg/ml.

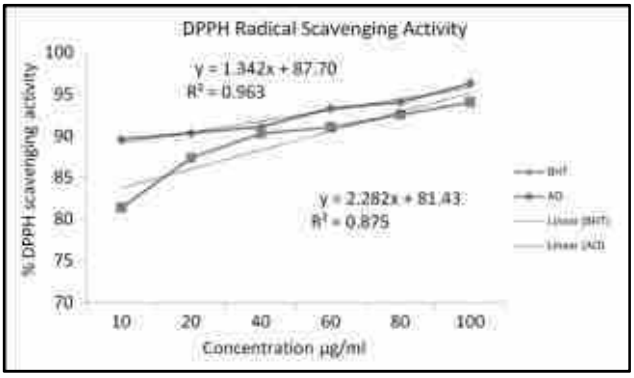

Fig. 1: Photograph of of *A. oleracea*

Compound	*IC_{50} Value (mg/ml)*
Butylated hydoxytoluene	28.092
Acmella oleracea extract	13.773

Fig. 2: Free Radical Scavenging Activity of *A. oleracea* on DPPH. (AO = *A. oleracea*; BHT = butylated hydroxytoluene)

Antimicrobial activity was assessed upon two commonly available bacteria, *viz.* a Gram-negative *Pseudomonas eurogenosa* and a Gram-positive *Staphylococcus aureus.* The antibiotic tetracycline was used as a standard positive reference. Using the equivalent concentrations (10 and 20 mg/ml) as that of the drug, the plant extracts did not produce any significant zones of inhibition, while the standard drug showed a distinct zone of inhibition.

DISCUSSION

A. oleracea has received quite a lot of pharmacological attention because of its wide range of therapeutic application in various traditional medicines. Its antiseptic, antifungal, antidiarrhoeal, analgesic, anaesthetic, antimalarial, antiulcer, antipyretic, antiinflammatory, diuretic, and vaso relaxant properties have been use for the treatments of debilitating diseases including anaemia, cancer, dysentery, malaria, rheumatism, snake bite, toothache, and ulcer (Prachayasittikul *et al.*, 2009; Chakraborty *et al.,* 2010; Spelman *et al.,* 2011; Dubey *et al.*, 2013. There are some reports on the bioactive compounds present in *A. oleracea* and those include alkylamides, phenolic compounds, coumarin, sterols, and triterpenoids (Prachayasittikul *et al.,* 2009). The unique odour and taste that produce irritating and numbing sensation, and sialogogue (increased salivation) effects, are found to be due to the presence of an olefinic alkylamide having an isobutyl side chain called spilanthol, or sometime affinin, which is also documented to be the major bioactive principle in *Heliopsis longipes* (Molinatorres *et al.,* 1996), and other species of *Acmella* (Chung *et al.*, 2008). The leaves of *A. oleracea* are snalysed to contain several important compounds including alkamides, amides, carbohydrates, tannins, steroids, carotenoids, essential oils, sesquiterpenes and amino acids (Nagashima and Nakatani, 1992; Ramsewak *et al.,* 1999; Prachayasittikul *et al.,* 2009), 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 (Sahu *et al*., 2011; Tiwari *et al*., 2011).

In the present analysis also, some vital organic compounds were confirmed such as phytosterols, tannins, reducing sugars, and carbohydrates. The most well-known phytosterols such as β-sitosterol, stigmasterol and campesterol, are well established to bepharmacologically beneficial for their therapeutic actions in cardiovascular diseases, colon and breast cancer (Choudhary and Tran, 2011). A variety of tannins from different plants have been known to be useful asantitumour, antimutagenic, anticancer, antibacterial, and antiviral agents (Okuda, 2005). Tannin-rich plants are most widely used for their curing wounds and inflammations (de Sousa Araújo *et al.*, 2008). In a recent report, Maria-Ferreira *et al.* (2014) found that rhamnogalacturonan, which is a complex carbohydrate isolated from the leaves of *A. oleracea*, healed acute ulcers in rats induced by ethanol.

In the present study, the methanol extract exhibitedconcentration-dependent antioxidant activity similar to that of butylated hydroxytoluene (BHT). But its overall activity was lower than that of BHT. Nonetheless the plant extract had higher potency with anIC_{50} of 13.773 mg/ml in terms for scavenging DPPH, than BHT having an IC_{50} of 28.098 mg/ml. Free radicals such as reactive oxygen and nitrogen species, produced in the body because of normal cellular metabolism, are known to be responsible for inherent damage to DNA, lipids, proteins, and other vital biomolecules. The effect called oxidative stress is deeply rooted with several health problems including a number of cardiovascular, neurodegenerative, cancer and even aging (López-Alarcón and Denicola, 2013). The body does not have sufficient antioxidant to prevent the harmful effects. Antioxidant defenses inside the body, such as superoxide dismutases, hydrogen peroxide-removing enzymes, metal binding proteins, are inadequate to prevent the harmful oxidation completely. Thus, antioxidants from external sources are required to downplay the oxidation process by

converting the harmful free radicals to harmless molecules or by destroying them (Young and Woodside, 2001). The antioxidant action is not limited to scavenging free radicals but extends to upregulation of antioxidant and detoxifying enzymes, modulation of redox cell signaling and gene expression. Hence dietary antioxidants are the principle sources of defense for cellular oxidation (Halliwell, 1996). Medicinal plants in particular are among the best sources of antioxidant compounds (Rice-Evans *et al.*, 1996; Scartezzini and Speroni, 2000; Carocho and Ferreira, 2013).

The chloroform and ethyl acetate extracts of *A. oleracea*-have also been shown to exhibit significant antioxidant activity (Wongsawatkul *et al*., 2008). There is a link between the antioxidant and antiinflammatory actions. The antiinflammatory action isproduced by inhibition of NF-κB pathway, which in turn causes reduction in mRNA level and protein expression of *COX-2* and *iNOS*. Increased translation from these genes ultimate free radical scavenging activity. Wu *et al.* (2008) indeed showed that the acetate extract has the highest antioxidant activity using different radical scavenging assays.

Antimicrobial activity was tested against two different bacteria, a Gram-negative *Pseudomonas euroginosa* and a Gram-positive *Staphylococcus aureus. P.euroginosa* is a major pathogen among patients with immunosuppression, cystic fibrosis, malignancy, and trauma (Cross *et al*., 1983). It is regarded as the most important gram-negative pathogen in medical centres (Gellatly and Hancock, 2013). Due to its high prevalence, it has evolved multi-drug resistance, posing further threats to hospital care systems (degli Atti *et al*., 2014). *S. aureus* commonly infects nose, respiratory tract, and skin, causing a range of disease, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis (Thomar *et al.*, 2016). Disk diffusion method showed that *A. oleracea* extract did not have antibacterial activity on these two bacteria. However, is has been reported that the ethyl acetate and methanol extracts of the same plant showed high inhibitory effect on the bacterium *Klebsiella pneumoniae* (Arora *et al*., 2011). The study further reported that the ethyl acetate extract was two-fold more potent than the pharmaceutical antibiotic doxycycline. In other studies, Prachayasittikul *et al.* (2009) found that the chloroform extract significantly reduced the propagation of *Streptococcus pyogenes*, and de Alcantara *et al.* (2015) also reported similar activity against *Salmonella typhi*. But, similar to the present study, Prachayasittikul *et al.* (2009) found no activity for either the methanol and ethyl acetate extracts. The broad-spectrum antimicrobial activity is also confirmed against the pathogenic fungi *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moniliformis, F. oxysporium* (Rani and Murty, 2006), *Rhizopus arrhigus*, *Rhizopus stolonifer* (Arora *et al*., 2011), and *Saccharomyces cerevisiae* (Prachayasittikul *et al.,* 2009).

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