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Pharmacognostic Study of the Medicinal Plant *Acmella oleracea*: Analysis of its Chemical Components and Properties

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INTRODUCTION

Medicinal plants have played crucial roles in various traditional systems around the world, as well as in the discovery and development of pharmaceutical drugs (Hammond *et al*., 1999; Taylor, 2013). In spite of the boom in pharmaceutical industry during the last century, the need for novel drugs is more pressing than ever due to either, or both, the adverse effects of the drugs or extensive evolution of drug resistance in the target pathogens (Butler and Newman, 2008; Cragg and Newman, 2013). A vast collection of research has indicated that many medicinal plants, which are relatively unexplored, offer promising sources for novel drugs (Saklani and Kutty, 2008; Itokawa *et al*., 2008; Li and Vederas, 2009; Brusotti *et al*., 2014).

Acmella oleracea (L.) R.K. Jansen (synonyms *Spilanthes acmella* Murr. or *Spilanthes oleracea* L.) is a small and perennial flowering herb of the family Asteraceae. It is native to Brazil and Peru, and is cultivated throughout the year as ornamental, culinary and medicinal plant. It is now widely distributed throughout tropical and subtropical regions, such as Africa, America, Borneo, India, Sri Lanka and Asia (Tiwari *et al*., 2011; Prachayasittikul *et al*., 2013). Its flowers and leaves have a characteristic pungent odour and burning taste, and when touched and rubbed it produces tingling sensation and numbness on the skin. Because of this unique flavour, it is widely used as vegetable and culinary additive. In various traditional medicines, it is one of the most extensively used medicinal plants because of its acclaimed anaesthetic, anticonvulsant, antiseptic, antifungal, antiprotozoal, antidiarrhoeal, analgesic, antiulcer, antipyretic, antidiuretic, antiinflammatory, diuretic, aphrodisiac, and insecticidal properties (Dubey *et al*., 2013; Paulraj *et al*., 2013). Its

antiseptic and analgesic properties have been exploited in dental health care in the treatment of various oral problems including gingivitis, oral ulcer, sore throat, and general toothache. As such it has been given a nickname toothache plant. These properties have been attributed to the analgesic and disinfectant activity of the plant (Chakraborty *et al.,* 2010).

A. oleracea has also been used for the treatments of terminal afflictions including anaemia, cancer, dysentery, gastrointestinal ulcer, rheumatism, and snake bite (Prachayasittikul *et al*., 2009; Dubey *et al*., 2013). It is also a source of antimalarial therapy in the traditional medicines in India and Africa (Spelman *et al.,* 2011). It is being used in Indian medicine as a powerful aphrodisiac for cases of impotency, and has been claimed to improve sexual function in men (Prachayasittikul *et al*., 2013). It has been experimentally shown that it indeed has several pharmacological properties, such as anaesthetic, antiinflammatory, analgesic, antipyretic, antiobesity and diuretic activities (Ratnasooriya *et al*., 2004; Wu *et al.,* 2008; Chakraborty *et al.,* 2010). Using experimental rats, the antiinflammatory activity has been demonstrated (Chakraborty *et al*., 2004). Its beneficial roles in the functions of blood has been vindicated by its cytotoxic, antioxidant, and vasorelaxant activities (Prachayasittikul *et al*., 2009). Its use for the treatment of rheumatism has been substantiated by the fact that it can induce macrophage concentration in the blood, thereby enhancing the immunomodulatory activity of the immune cells (Savadi *et al.,* 2010). The aerial aqueous extract was demonstrated to show antipyretic activity against Brewer's yeast-induced pyrexia, thereby supporting its use as a therapy for high fever (Chakraborty *et al.,* 2010). The insecticidal activity has been demonstrated 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). Thus, it is essential that more of its chemistry is understood to make its way into proper pharmaceutical medication.

Materials and Methods

Collection of plant material

The fully matured flowering plants (Fig. 1) were collected from Ngopa, Champhai District, Mizoram, India (located at 23.8861° latitude north and 93.2119° longitude east), in 2015. A voucher specimen is maintained at the herbarium section of the Department of



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

Botany, Pachhunga University College, Aizawl, Mizoram. The aerial parts of the plant were dried in the shade.

Preparation of plant extracts

A known quantity of the dried plants was ground to powder using electric grinder. A pre-weighed sample was loaded in a 5l-capacity thimble of Soxhlet apparatus, and extraction was done successively by hot continuous extraction with solvents such as hexane and methanol. Hexane extraction was run for 48 h, while methanol extraction required longer period, i.e. 72 h. The extracts were concentrated by recovering the solvents using vacuum rotary evaporator (Buchi Rotavapor® R-215). The crude extracts were obtained as semi-solid mass, and were stored at 4°C until further analysis.

Phytochemical detection

The different extracts were subjected to preliminary phytochemical screening to identify the presence of phytoconstituents according to the standard procedures such as Meyer's test, Dragendroff's test, Wagner's test, Hager's test, Molisch's test, Fehling's test, Barfoed's test, Benedict's test, Liebermann-Burchard's test, Salkwoski reaction, Legal's test, Keller Killiani's test, $FeCl_3$ test, $K_2Cr_3O_7$ test, lead acetate test, foam test, Fehling's test, Benedict's test, Shinoda test, Zn hydrochloride reduction test, Biuret test, and ninhydrin test.

Antimicrobial activity

The antimicrobial activity was evaluated by disc diffusion method using Gram-negative bacteria *Escherichia coli*, and Gram-positive species *Bacillus subtilis*. Bacteria were cultured in Mueller-Hinton agar in culture dishes under sterile condition. The plant extracts were impregnated on absorbent disks (Whatman Antibiotic Assay Discs). Control disc will contain only the solvent. The antibiotic tetracycline was used as a positive reference drug. Each experiment was done in triplicate. After incubation at 37°C for 18-20 h, the size of inhibition zone was observed.

Antioxidant activity

The antioxidant activity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) stable free radical scavenging activity following the method of Blois, 1958. 1 ml of 0.1 mM solution of DPPH in methanol and 3 ml of plant extracts were incubated at 37°C for 30 minutes. Butylated hydroxytoluene (BHT) was used as reference standard. Absorbance was measured at 517 nm against control using 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 as follows:

I = (Abs control – Abs sample) / Abs control X 100

Result and Discussion

The extractive weight and extractive value of *A. oleracea* were calculated from the yield of extraction as shown in Table 1. The extractive value is higher in methanol with 17.94% yield and hexane extract have an extractive value of 4.07%.

The phytochemical screening of the hexane and methanol extracts shows the presence of phytochemicals such as phytosterols, tannins, reducing sugars and carbohydrates (Table 2). Tannins, reducing sugars and carbohydrates were detected only in the methanol extract. Phytosterols were detected in all the tests for both the hexane and methanol extracts. However, the detection protocols used did not show the presence of important phytochemicals such as alkaloids, flavonoids, and saponins.

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

Table 2: Detection of chemical composition of *A. oleracea* using different biochemical tests.

Sl. No.	Phytochemicals	Name of test	Hexane extract	Methanol extract
1.	Alkaloid	Mayer's test	-	-
		Dragendorff's test	-	-
2.	Carbohydrate	Molisch's test	-	-
		Fehling's test	-	+
		Barfoed's test	-	+
		Benedict's test	-	+
3.	Phytosterol	Liebermann-Burchard's test	+	+
		Salkwoski reaction	+	+
4.	Glycoside	Legal's test	-	-
		Keller-Killiani's test	-	-
5.	Tannin	$FeCl_3$ test	-	+
		$K_2Cr_3O_7$ test	-	+
		Lead acetate test	-	+
6.	Saponin	Foam test	-	-
7.	Reducing sugar	Fehling's test	-	+
		Benedict's test	-	+
8.	Flavonoid	Shinoda test	-	-
		Zn hydrochloride reduction test	-	-

The antioxidant activity of the methanol extract was determined by DPPH free radical scavenging assay (Fig. 2). Varying concentrations of the plant extract were used, namely 10, 20, 40, 60, 80, and 100 µg/ml. Similar concentrations were used for BHT. Both tests indicated concentration-dependent activity against DPPH. BHT however exhibited higher activity at all concentrations tested. From the standard graph, IC_{50} of standard BHT and the plant extract was calculated. The IC_{50} of extract was 13.773 mg/ml while that of the standard was 28.098 mg/ml.

Antimicrobial activity was tested against *Escherichia coli* and *Bacillus subtilis* using tetracycline as a standard antibiotic. The crude extracts at 10 mg/ml and 20 mg/ml concentrations were found to cause no inhibition while the standard drug showed a distinct zone of inhibition (Fig. 3). Hence, the hexane and methanol extracts do not exhibit antibacterial activity.

A. oleracea has been a fascinating plant because of its wide range of application in traditional medicine complimented by its use as foodstuff. Its continued use as a therapy for anaemia, cancer, dysentery, malaria, rheumatism, snake bite, toothache, and so on (Prachayasittikul *et al.*, 2009; Chakraborty *et al.*, 2010; Spelman *et al.*, 2011; Dubey *et al.*, 2013), make it more interesting to investigate on its biochemical properties. A number of

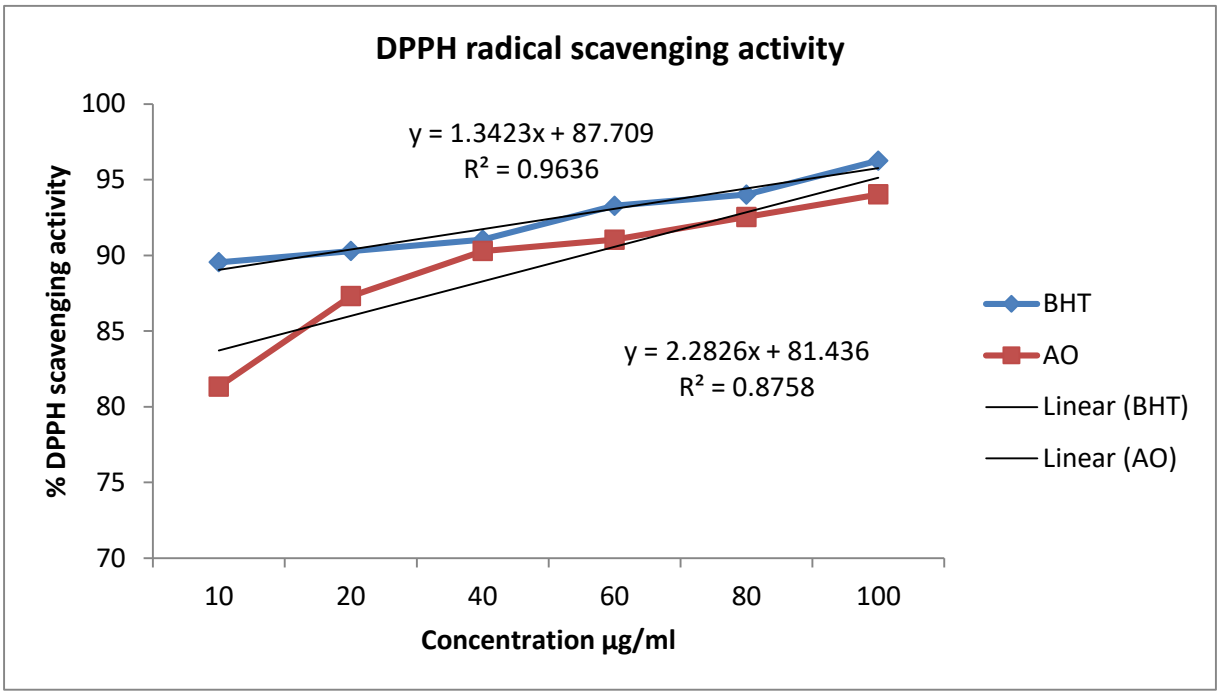


Fig. 2: Free radical scavenging activity of *A. oleracea* on DPPH. (AO = *A. oleracea*; BHT = butylated hydroxytoluene.)

bioactive compounds have been reported in *A. oleracea*, including alkylamides, phenolic compounds, coumarin, sterols, and triterpenoids (Prachayasittikul *et al.,* 2009). The pungent, tingling, numbing, and mouth-watering effects are due to spilanthol or affinin, an olefinic alkylamide with an isobutyl side chain, which is also the principal bioactive compound in *Heliopsis longipes* (Molinatorres *et al.,* 1996), and other species of *Acmella* (Chung *et al.*, 2008). The leaves of *A. oleracea* contain various compounds such as 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, *a*- and *β*-amyrins), essential oils (e.g. limonene and *β*-caryophyllene), sesquiterpenes, *a*- and *β*-bisabolenes and cadinenes, flavonoid glucoside and a mixture of long chain hydrocarbons (Sahu *et al*., 2011; Tiwari *et al*., 2011).

The present study also confirmed the presence of phytosterols, tannins, reducing sugars, and carbohydrates. Phytosterols such as β-sitosterol, stigmasterol and campesterol, are well established compounds clinically beneficial for cardiovascular diseases, colon and breast cancer (Choudhary and Tran, 2011). A number of tannins have been documented to be effective antitumour, antimutagenic, anticancer, antibacterial, and antiviral agents (Okuda, 2005). Tannin-rich plants are most often used for their wound-healing and anti-inflammatory properties (de Sousa Araújo *et al*., 2008). A complex

carbohydrate, rhamnogalacturonan, isolated from leaves, reportedly showed healing effect on acute ulcers induced by ethanol in rats (Maria-Ferreira *et al.*, 2014).

The methanol extract showed similar antioxidant activity as that of butylated hydroxytoluene (BHT), but with less intensity. But the potency for scavenging DPPH was greater for the plant extract having IC_{50} of 13.773 mg/ml, while that of the standard BHT was 28.098 mg/ml. Free radicals such as reactive oxygen and nitrogen species, which are generated *de novo* and cause damage to DNA, lipids, proteins, and other biomolecules. Oxidative stress is intimately associated with several health issues such as cardiovascular, neurodegenerative, cancer and even aging (López-Alarcón and Denicola, 2013). Exogenous antioxidants are necessary to effectively delay cellular oxidation by converting the harmful free radicals to benign molecules or by scavenging them (Young and Woodside, 2001). The antioxidant action is not limited to scavenging free radicals but includes upregulation of antioxidant and detoxifying enzymes, modulation of redox cell signaling and gene expression. Antioxidant defenses inside the body, such as superoxide dismutases, hydrogen peroxide-removing enzymes, metal binding proteins, are inadequate to prevent the harmful oxidation completely, hence dietary antioxidants are the major guards for oxidation processes (Halliwell, 1996). Medicinal plants in general are among the best sources of antioxidant compounds (Rice-Evans *et al.*, 1996; Scartezzini and Speroni, 2000; Carocho and Ferreira, 2013).

Fig. 3: IC_{50} values of plant extract and standard solution.

Plant extract and standard	**IC_{50} value (mg/ml)**
Butylated hydoxy toluene	28.092
Acmella oleracea	13.773

The chloroform and ethyl acetate extracts of *A. oleracea* have also been shown to exhibit antioxidant activity (Wongsawatkul *et al*., 2008). The antiinflammatory action was shown to be exerted through inhibition of NF-κB pathway, which in turn caused reduction in mRNA level and protein expression of COX-2 and iNOS. This finally is attributed to mechanism by which the plant induces free radical scavenging activity. Wu *et al.* (2008) found that the acetate extract has the highest antioxidant activity using different radical scavenging assays.

Antimicrobial test was performed against *Escherichia coli* and *Bacillus subtilis*. Pathogenic *E. coli* is the causative bacterium of debilitating, sometimes lethal, diseases of the gastrointestinal, urinary, or central nervous system (Kaper *et al*., 2004). On the other hand, *Bacillus subtilis* is rather harmless even in the gastrointestinal tract. *A. oleracea* extract did not show any antibacterial activity on these two commonly tested bacteria. However, Arora *et al*. (2011) has observed that the ethyl acetate and methanol extracts exhibited the strong antimicrobial activity against *Klebsiella pneumoniae*. The study showed that ethyl

acetate extract was two-fold more potent than the standard antibiotic doxycycline. In other studies, the chloroform extract significantly reduced the propagation of *Streptococcus pyogenes* (Prachayasittikul *et al.,* 2009), and *Salmonella typhi* (de Alcantara *et al.,* 2015). But, similar to the present study, Prachayasittikul *et al.* (2009) found no activity for the methanol and ethyl acetate extracts. The general 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).

Summary

Acmella oleracea (L.) R.K. Jansen is a perennial flowering herb belonging to the family Asteraceae. It is among the most widely used medicinal and culinary plants in various traditional systems. It grows and propagates easily in the wild, and is also cultivated extensively. For its medicinal properties it has been extensively applied as antiseptic, antifungal, antidiarrhoeal, analgesic, anaesthetic, antimalarial, antiulcer, antipyretic, diuretic, and antiinflammatory agent. However, there is insufficient information on its chemical properties. Using the whole plant, crude extracts were prepared using Soxhlet extractor and concentrated in a rotary vacuum evaporator. Principal extracting solvents such as hexane and methanol were used. Biochemical detections showed the presence of important bioactive groups, including carbohydrates, phytosterols, and tannins. Antioxidant activity is an important pharmacological factor that can reduce the adverse biological effects of free radicals. The antioxidant activity of the plant extract was examined by studying its ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH). Increasing concentration of the extract, *viz*. 10, 20, 40, 60, 80, and 100 µg/ml showed increased activity, similar to those of the standard butylated hydroxytoluene (BHT). Its probable antibacterial activity was tested against a Gram-negative bacteria *Escherichia coli*, and a Gram-positive species *Bacillus subtilis,* which are commonly found in the gastrointestinal tract of humans. However, the plant extract failed to show significant activity in comparison to the antibiotic tetracycline. But there are reports that the chloroform extract of the same plant induced significant inhibitory action on different bacteria. The fact suggests either that the antimicrobial principle is retained only in a specific extract, or that there are different antimicrobial compounds that are selectively effective against different species. It can be concluded that *A. acmella oleracea* indeed contains important properties for therapeutic applications, and the specific pharmacological properties are yet to be explored.

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