Original Research

Science Vision 16 (4)
October-December
ISSN (print) 0975-6175
ISSN (online) 2229-6026

Phytochemical investigations on the medicinal plant *Acmella oleracea* cultivated in Mizoram, India

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Received 21 October 2016 | Revised 26 November 2016 | Accepted 2 December 2016

Abstract

Species of the genus *Acmella* are perennial plants and are well known as traditional medicines and as vegetables in tropical regions. Of these, *A. oleracea* (L.) R.K. Jansen is the most widely used, and is cultivated as an ornamental plant and as a crop. In traditional medicine, it is used for the treatment of haemorrhage, helminthiasis, gingivitis, laceration, malaria, oral and gastric ulcer and infections, dysentery, urinary calculi, and inflammations. Biochemical tests of the methanol indicated presence of important bioactive compounds, such as phytosterols, and tannins. Assessment of the free radical scavenging activity showed that it has concentration-dependent antioxidant activity similar to that of the standard butylated hydroxytoluene (BHT). The half maximal inhibitory concentration (IC_{50}) of the plant extract was 13.773 mg/ml, while it was 28.098 mg/ml for BHT. The total antioxidant activity was 85 mg/ml calculated against the standard ascorbic acid. The total phenol content was estimated using Folin-Ciocalteu-gallic acid reaction, and was determined to be 1.38 GAE mg/g. Using the aluminum chloride-quercetin assay, the total flavonoid content was calculated to be 28.7 QE mg/g.

Key words: *A. oleracea*, antioxidant activity, total phenol, total flavonoid.

Introduction

A widely acclaimed ornamental, culinary and medicinal plant, *Acmella oleracea* (L.) R.K. Jansen is a small and perennial flowering herb, and is the most well-known among the family Asteraceae. As far as its botanical origin is concerned, it is presumed to be native to Peru, and from then is exported for cultivation to other parts of the world. It is now widely distributed throughout tropical and subtropical regions including Africa, America, Borneo, India, Sri Lanka, and Southeast.[1,2] It is rather easily cultivated because it grows throughout the year, and easily grow in the wild. It is mostly known for its unique taste for which it is used in a variety

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of foodstuff. Its leaves and flowers contain a distinct odour and mild burning taste in the mouth when eaten. This flavour renders it as a special ingredient in culinary preparations or even as a vegetable. For its strong pungent flavour it is used in the America as a spice. It is a common vegetable in many parts of India, Brazil, and Southeast Asia.[3]

Its broad range of applications in traditional medicines includes uses as anaesthetic, anticonvulsant, antiseptic, antifungal, antiprotozoal, antidiarrhoeal, analgesic, antiulcer, antipyretic, antidiuretic, antiinflammatory, diuretic, aphrodisiac, and insecticidal agent.[4,5] When it is vigorously rubbed on the skin it also produces a tingling sensation and numbness, giving rise soothing effect. For its antimicrobial and pain-relieving properties, the plant extract has been an important commercial product in oral health care for the treatment of various mouth ailments such as gingivitis, oral ulcer, sore throat, and general toothache.[6] It is for this reason that it has been attributed a common name the toothache plant.

In more serious medical conditions *A. oleracea* is also an important source of treatment. It has been used as the medicine for many infectious diseases and life-threatening conditions such as different blood diseases, anaemia, haemorhhage, cancer, dysentery, gastrointestinal ulcer, rheumatism, and snake bite.[4,7] In the most malaria-endemic areas such as in India and Africa, it has been regarded as one of the most useful antimalarial medication.[8] It is also used in the treatment debilitating clinical conditions including gout, helmithiasis (intestinal infection), hepatosis, prostate cancer, scurvy, stammering, and xerostomia (dry mouth).[9] Its use in the treatment of blood related disorders has been attributed to its known cytotoxic, antioxidant, and vasorelaxant activities.[7] Further, its antipyretic activity against Brewer's yeast-induced pyrexia likely supports its use in the treatment of high fevers.[6]

It is known to be a strong natural insect killer. For example, its insecticidal activity has been shown against the pest *Tuta absoluta*,[10] and vectors of infectious diseases including *Aedes aegyptii*.[11] In Indian traditional medicine, it is widely employed as one of the most effective aphrodisiacs (libido enhancers), and is commonly prescribed for cases of impotency.[2] All these medical benefits have been warranted through its various important pharmacological properties such as anaesthetic, antiinflammatory, analgesic, antipyretic, antiobesity and diuretic activities.[6,12,13] Its antiinflammatory activity has been validated in experimental rats.[14] It is also shown to increase the production of specialised white blood cells called macrophages, which are one of the basic cells in preventing infection by pathogens. This will explain its antiinflammatory actions and its use in the treatment of rheumatism.[15] However, many details of its chemical properties are yet to be defined to make it as an empirically-proven therapeutic agent.

Materials and Methods

Collection of plant material

A. oleracea was harvested during October-November 2015 from Ngopa, a village in Champhai District, Mizoram, India (located between 23.8861° latitude north and 93.2119° longitude east). Only the fully mature and flowering plants were selected (Fig. 1). A voucher specimen was

Fig. 1: *A. oleracea* in plantation.

prepared and is maintained at the herbarium section of the Department of Botany, Pachhunga University College, Aizawl, Mizoram. The roots and the main stems were discarded, and the aerial parts of the plant were dried under a regulated temperature at 45°C in a thermostat incubator.

Preparation of plant extract

The dried plants were weighed in a balance and recorded, and then they were ground to fine powder using mortar and pestle. A pre-weighed powder was loaded into thimble of Soxhlet extractor having a 5l-capacity collection flask. The plant powder was subjected to continuous hot extraction using methanol. Total refluxing took 72 h. The plant extract was concentrated in a vacuum rotary evaporator (Buchi Rotavapor® R -215). The final extract was in the form of semi-solid mass, which was 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.

Antioxidant activity

The antioxidant activity was estimated by the method of Blois (1958).[16] In brief, 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 plant extracts were incubated at 37±1°C for 30 minutes. For the standard reference, butylated hydroxytoluene (BHT) was prepared in similar mixture as that of the extracts. Absorbance was measured at 517 nm against control using a 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, 28mM sodium phosphate and 4 mM ammonium molybdate). After incubation at 95°C, absorbance was measured at 695 nm using UV-vis spectrophotometer (Evolution™, Thermo Scientific).

Total phenol content

The total phenol content of the plant was estimated by using the modified method of Folin -Ciocalteu assay.[17,18] 1 ml of methanolic solution of gallic acid (with each concentration of 10, 20, 40, 60, 80, and 100 μg/ml) was mixed with 5 ml Folin–Ciocalteu reagent (initially diluted tenfold). 4 ml of sodium carbonate solution (0.7 M) was added after 3 minutes, and the mixture was left to stand for 1 hr at room temperature. Absorbance was measured at 765 nm using UV-Vis spectrophotometer. 1 ml extract (50 μg/ml) was also mixed with the reagents above and after 1 hr the absorbance was measured. A standard calibration curve was prepared from the absorbance readings at the different concentrations of gallic acid. The amount of total phenolic compounds was calculated from the calibration curve. The result was expressed as milligrams of gallic acid equivalent (GAE) per gram of the dried extract.

Total flavonoid content

The total flavonoid content of the plant was determined by the aluminum chloride ($AlCl_3$) method. 1 ml of the plant extract (50 µg/ml) was mixed with 2 ml of distilled water. 3 ml of 5% sodium nitrite ($NaNO_2$) and 0.3 ml of 10% $AlCl_3$ were added after 5 minutes. 2 ml of NaOH (1 M) was added after 6 minutes, and the volume was made up to 10 ml with distilled water. Absorbance reading was taken at 510 nm after 1 hr. 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 determined from the calibration curve of quercetin. The final value was expressed as milligrams of quercetin equivalent (QE) per gram of the dried extract.

Result

Biochemical detections using various methods show the presence of important phytochemicals such as phytosterols and tannins (Table 1). However, other important phytochemicals such as alkaloids, flavonoids, and saponins could not be detected in any of the tests used.

The antioxidant activity was estimated by the method of DPPH free radical scavenging assay using the methanol extract (Fig. 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 BHT it was 28.098 mg/ml. The total antioxidant activity was estimated as ascorbic acid equivalent i.e. 85 mg/ml.

Using Folin-Ciocalteu-gallic acid reaction, the total phenol content of methanol extract of *A. oleracea* was determined to be 1.38 GAE mg/g. From the aluminum chloride-quercetin assay, the total flavonoid content was calculated to be 28.7 QE mg/g.

Discussion

Species of *Acmella*, especially *A. oleracea*, have been studied and documented for their beneficial roles in health various as acclaimed in traditional medicines. Their antiseptic, antifungal, antidiarrhoeal, analgesic, anaesthetic, antimalarial, antiulcer, antipyretic, antiinflammatory, diuretic, and vasorelaxant properties have been use for the treatments of debilitating diseases including anaemia, cancer, dysentery, malaria, rheu-

Table 1: Some chemical constituents of *A. oleracea*.

Sl. No.	Phytochemicals	Name of test	Methanol
1.	Alkaloids	Meyer's test	-
		Dragendroff's test	-
		Wagner's test	-
		Hager'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	-

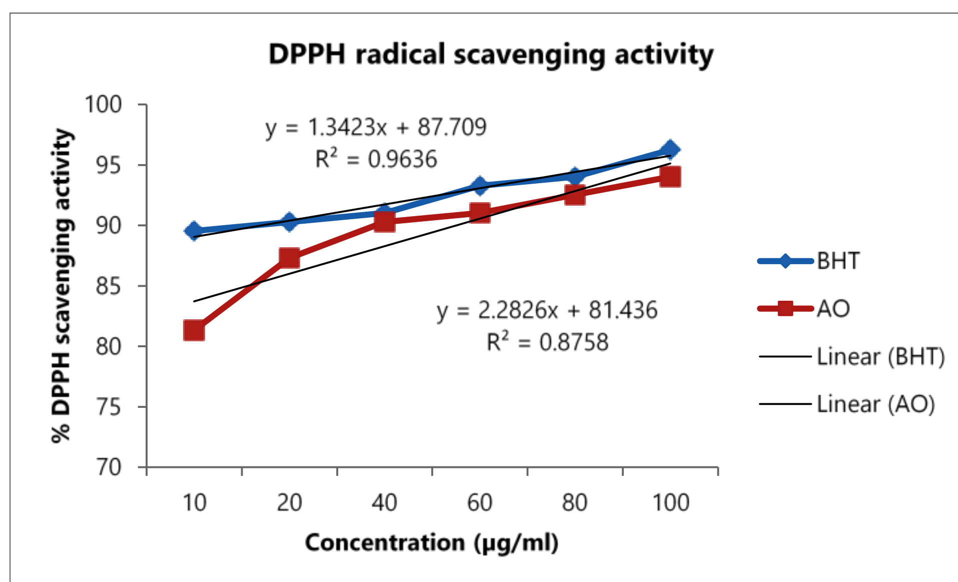

Fig. 2: Free radical scavenging activity of *A. oleracea* on DPPH. (AO = A. oleracea; BHT = butylated hydroxytoluene.)

matism, snake bite, toothache, and ulcer.[4,6-8] The unique odour and taste are due to an olefinic alkylamide spilanthol, or sometimes affinin, in the species of *Acmella*.[19] The leaves of *Acmella* are contain important compounds including alkamides, amides, carbohydrates, tannins, steroids, carotenoids, essential oils, sesquiterpenes and amino acids,[7,20] 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.[1,21]

In the present study, the presence of phytosterols and tannins was confirmed. Important phytosterols such as β-sitosterol, stigmasterol and campesterol are known for their therapeutic actions in cardiovascular diseases, colon and breast cancer.[22] Tannins from different plants are shown to have antitumour, antimutagenic, anticancer, antibacterial, and antiviral activities.[23] Tannin-rich plants are popuarly used for their healing activity in wounds and inflammations.[24] Maria-Ferreira *et al.* recently reported that rhamnogalacturonan, a complex pectic carbohydrate present in *A. oleracea*, could heal acute ulcers in rats.[25]

In the present study, the methanol extract exhibited concentration-dependent antioxidant activity similar to that of butylated hydroxytoluene (BHT). But its overall activity was lower than that of BHT. Free radicals such as reactive oxygen and nitrogen species, which are the products of normal physiological metabolism, are responsible for progressive damage to DNA, lipids, proteins, and other vital biomolecules. The effect called oxidative stress is in turn the pathogenesis of clinical health problems including many cardiovascular, neurodegenerative, cancer and even aging.[26] Exogenous antioxidants from foodstuffs are required to decrease or inhibit the oxidation process by making the free radicals to harmless byproducts or by destroying them.[27] Hence dietary antioxidants constitute the principle source of defense for cellular oxidation.[28,29]

Acknowledgement

This is a part of Major Research Project un-

der the University Grants Commission's [MRP-BIOC-2013-36855, sanction F. No. 43-47/2014 (SR) of 22/8/2015] to KLC. PBL is a UGC Project Fellow. The authors are deeply indebted to Dr. H. Lalhlenmawia, Head, Department of Pharmacy, RIPANS, for providing all the facilities and technical supports.

REFERENCES

1. Tiwari, K.L., Jadhav, S.K., & Joshi, V. (2011). An updated review on medicinal herb genus *Spilanthes*. *Zhong Xi Yi Jie He Xue Bao* (*Journal of Chinese Integrated Medicine*), 9, 1170–1178.
2. Prachayasittikul, V., Prachayasittikul, S., Ruchirawat, S., & Prachayasittikul, V. (2013). High therapeutic potential of *Spilanthes acmella*: a review. *EXCLI Journal*, 12, 291–312.
3. Lim, T.K. (2014). *Edible Medicinal and Non-Medicinal Plants: Volume 7, Flowers*. Dordrecht, Netherlands: Springer Science+Business Media. pp. 163–174.
4. Dubey, S., Maity, S., Singh, M., Saraf, S.A., & Saha, S. (2013). Phytochemistry, pharmacology and toxicology of *Spilanthes acmella*: a review. *Advances in Pharmacological Sciences*, 2013, 423750.
5. Paulraj, J., Govindarajan, R., & Palpu, P. (2013). The genus *Spilanthes* ethnopharmacology, phytochemistry, and pharmacological properties: a review. *Advances in Pharmacological Sciences*, 2013, 510298.
6. Chakraborty, A., Devi, B.R., Sanjebam, R., Khumbong, S., & Thokchom, I.S. (2010). Preliminary studies on local anesthetic and antipyretic activities of *Spilanthes acmella* Murr. in experimental animal models. *Indian Journal of Pharmacology*, 42, 277–279.
7. Prachayasittikul, S., Suphapong, S., Worachartcheewan, A., Lawung, R., Ruchirawat, S., & Prachayasittikul, V. (2009). Bioactive metabolites from *Spilanthes acmella* Murr. *Molecules*, 14, 850–867.
8. Spelman, K., Depoix, D., McCray, M., Mouray, E., & Grellier, P. (2011). The traditional medicine *Spilanthes acmella*, and the alkylamides spilanthol and undeca-2e-ene-8, 10-diynoic acid isobutylamide, demonstrate in vitro and in vivo antimalarial activity. *Phytotherapy Research*, 25, 1098–1101.
9. Duke, J.A. (2008). *Duke's Handbook of Medicinal Plants of Latin America.* Boca Raton, Florida USA: CRC Press. pp. 13–14.
10. Moreno, S.C., Carvalho, G.A., Picanço, M.C., Morais, E.G., & Pereira, R.M. (2012). Bioactivity of compounds from *Acmella oleracea* against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and selectivity to two non-target species. *Pest Management Science*, 68, 386–393.
11. Simas, N.K., Dellamora, E.D.C.L., Schripsema, J., Lage, C.L.S., de Oliveira Filho, A.M., Wessjohann, L., Porzel, A., & Kuster, R.M. (2013). Acetylenic 2-phenylethylamides and new isobutylamides from *Acmella oleracea* (L.) RK Jansen, a Brazilian spice with larvicidal activity on *Aedes aegypti*. *Phytochemistry Letters*, 6, 67–72.
12. Ratnasooriya, W.D., Pieris, K.P., Samaratunga, U., & Jayakody, J.R. (2004). Diuretic activity of *Spilanthes acmella* flowers in rats. *Journal of Ethnopharmacology*, 91, 317–320.
13. Wu, L.C., Fan, N.C., Lin, M.H., Chu, I.R., Huang, S.J., Hu, C.Y., & Han, S.Y. (2008). Anti-inflammatory effect of spilanthol from *Spilanthes acmella* on murine macrophage by down-regulating LPS-induced inflammatory mediators. *Journal of Agricultural and Food Chemistry*, 56, 2341–2349.
14. Chakraborty, A.R.K.B., Devi, R.K., Rita, S., Sharatchandra, K.H., & Singh, T.I. (2004). Preliminary studies on antiinflammatory and analgesic activities of *Spilanthes acmella* in experimental animal models. *Indian Journal of Pharmacology*, 36, 148–150.
15. Savadi, R.V., Yadav, R., & Yadav, N. (2010). Study on immunomodulatory activity of ethanolic extract of *Spilanthes acmella* Murr. leaves. *Indian Journal of Natural Products and Resources*, 1, 204–207.
16. Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1199–1200.
17. Singleton, V.L., & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.
18. Singleton, V.L., Orthofer, R., & Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152–178.
19. Chung, K.-F., Kono, Y., Wang, C.-M., & Peng, C.I. (2008). Notes on *Acmella* (Asteraceae: Heliantheae) in Taiwan. *Botanical Studies*, 49, 73–82.
20. Nagashima, M., & Nakatani, N. (1992). LC-LS analysis and structure determination of pungent alkamides from *Spilanthes acmella* L. flowers. *Lebensmittel-Wissenschaft+ Technologie*, 25, 417–421.
21. Sahu, J., Jain, K., Jain, B., & Sahu, R. K. (2011). A review on phytopharmacology and micropropagation of *Spilanthes acmella*. *Pharmacologyonline Newslett*, 2, 1105–1110.
22. Choudhary, S., & Tran, L. (2011). Phytosterols: perspectives in human nutrition and clinical therapy. *Current Medicinal Chemistry*, 18, 4557–4567.
23. Okuda, T. (2005). Systematics and health effects of chemically distinct tannins in medicinal plants. *Phytochemistry*, 66, 2012–2031.
24. de Sousa Araújo, T.A., Alencar, N.L., de Amorim, E.L.C.,

& de Albuquerque, U.P. (2008). A new approach to study medicinal plants with tannins and flavonoids contents from the local knowledge. *Journal of Ethnopharmacology*, 120, 72–80.

25. Maria-Ferreira, D., Da Silva, L.M., Mendes, D.A.G.B., de Almeida Cabrini, D., Nascimento, A.M., Iacomini, M., Cipriani, T.R., Santos, A.R.S., de Paula Werner, M.F., & Baggio, C.H. (2014). Rhamnogalacturonan from *Acmella oleracea* (L.) RK Jansen: gastroprotective and ulcer healing properties in rats. *PloS One*, 9, e84762.
26. López-Alarcón, C., & Denicola, A. (2013). Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. *Analytica Chimica Acta*, 763, 1–10.
27. Young, I.S., & Woodside, J.V. (2001). Antioxidants in health and disease. *Journal of Clinical Pathology*, 54, 176–186.
28. Halliwell, B. (1996). Antioxidants in human health and disease. *Annual Review of Nutrition*, 16, 33–50.
29. Carocho, M., & Ferreira, I.C. (2013). A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, 51, 15–25.