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Evaluation of the antioxidant properties of the toothache plant Acmella oleracea cultivated in Mizoram, India

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ABSTRACT

Supplementary antioxidants are essential because of the innate production of reactive oxygen and nitrogen species through normal cellular metabolism. Phenolic compounds and flavonoids are known to be the principal antioxidant constituents of dietary and medicinal plants. As a widely used culinary and medicinal herb, Acmella oleracea was assessed for its antioxidant properties. Total flavonoid content was estimated based on the reaction between the plant extract and aluminium chloride. Total phenolic content was quantified using Folin-Ciocalteu reagent; and total antioxidant activity was determined by phosphomolybdate reaction. The total flavonoid evaluation showed that 1 g of the plant extract contains 28.7 mg of quercetin equivalent dry weight of the sample. In terms of phenolic content, 1 g of plant extract was estimated to contain 1.38 mg of gallic acid equivalent dry weight of the plant extract. The total antioxidant activity indicated that 1 g of plant extract contains 12.5 mg of ascorbic acid equivalent dry weight of the plant extract. These chemical estimations show that A. oleracea is a good source of antioxidants, and justify its medicinal and dietary uses.

Key Words: *Acmella oleracea*, total flavonoid, total phenol, total antioxidant.

INTRODUCTION

Acmella oleracea (L.) R.K. Jansen is popular medicinal as well as culinary plant in different parts of the world. It is a small perennial flowering herb classified under the family Asteraceae. It is generally believed to have originated from Peru, but now it has been introduced throughout tropical and subtropical regions including Africa, America, Borneo, India, Sri Lanka, and Southeast Asia [1,2]. It is noted for its distinct pungent smell and burning sensation that it produces upon eating. For its unique flavour, native Americans had been using it as a spice for various culinary preparation. Cooked as a whole plant, it is served as a common vegetable n India, Brazil, and Southeast Asia [3]. In different medicinal systems, it is used in a variety of health issues for its anaesthetic, anticonvulsant, antidiarrhoeal, antifungal, antiprotozoal, antiseptic, analgesic, antiulcer, antipyretic, antidiuretic, antiinflammatory, diuretic, aphrodisiac, and insecticidal activities [4,5]. One of its well-known application in dental

and oral health care. Its antiseptic and analgesic properties have been used for the treatment of sore throat, oral ulcer, gingivitis, and general toothache. It is for this reason that it has earned an English vernacular name the toothache plant [6].

A. oleracea is also used in the treatment of several debilitating diseases such as anaemia, haemorrhage, cancer, dysentery, gastrointestinal ulcer, rheumatism, scurvy, stammering, and xerostomia and snake bite [7-9]. It is also known for its effectiveness for infectious diseases such as malaria and helminthiasis [10]. It is also widely used in the treatment of disorders related to blood, basically for its cytotoxic, antioxidant, and vasorelaxant activities [8]. It has a potent antipyretic activity against Brewer's yeast-induced pyrexia, and this property is credited to its use in the treatment of high fever [6]. It is also known for its insecticidal activity suggesting its possible use in the control of agricultural pests and disease vectors. It reportedly kills important insects such as the common pest *Tuta absoluta* [11] and important vectors of infectious diseases including *Aedes aegyptii* [12].

In Indian traditional medicine, *A. oleracea* is known as an effective aphrodisiac, and is being used in the treatment of impotency [2]. These medicinal values have been appreciated from its variety of pharmacological properties such as anaesthetic, antiinflammatory, analgesic, antipyretic, antiobesity and diuretic activities [13-15]. It indicates significant antiinflammatory activity in experimental rats [14]. It has been also experimentally demonstrated to cause considerable increase the number of macrophages, specialised white blood cells vital for the most important immunological responses. This fact alone is a good rationale for its use in the treatment of rheumatism, a serious immune disorder [16]. Therefore, it is important to study the pharmacognostic values such as total flavonoid, total phenol, and total antioxidant contents of the plant.

MATERIALS AND METHODS

Collection of plant material

A. oleracea was harvested 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 prepared and 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 macerated to fine powder using motorised blender. The plant powder was subjected to continuous hot extraction in a Soxhlet apparatus using methanol as a solvent. Extraction was run for 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.

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 hour, absorbance was taken at 510 nm in a UV-Vis spectrophotometer (Evolution^{TM} 220). A standard curve was prepared with quercetin at different concentrations (10, 20, 40, 60, 80, and 100 µg/ml). From the calibration curve of the reference standard, the total flavonoid content was determined and expressed as milligrams of quercetin equivalent (QE/g) of dried extract.

Total phenolic content

The total phenolic content of the plant was determined by using the method of Mc Donald *et al.* [17] with modifications. 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 (which was diluted tenfold from the original stock). After 3 minutes, 4 ml of sodium carbonate solution (0.7 M) was added, and the mixture was allowed to stand for 1 hour 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 hour 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 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.

RESULTS

Total flavonoid content

The total flavonoid of *A. oleracea* extract was given by reactions with sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), and sodium hydroxide (NaOH). The total flavonoid concentration was expressed as weight in mg of quercetin equivalent per gram of dry weight. It was found that 1 g of the plant extract contains 28.7 mg of quercetin equivalent dry weight of the sample (Figure 1).

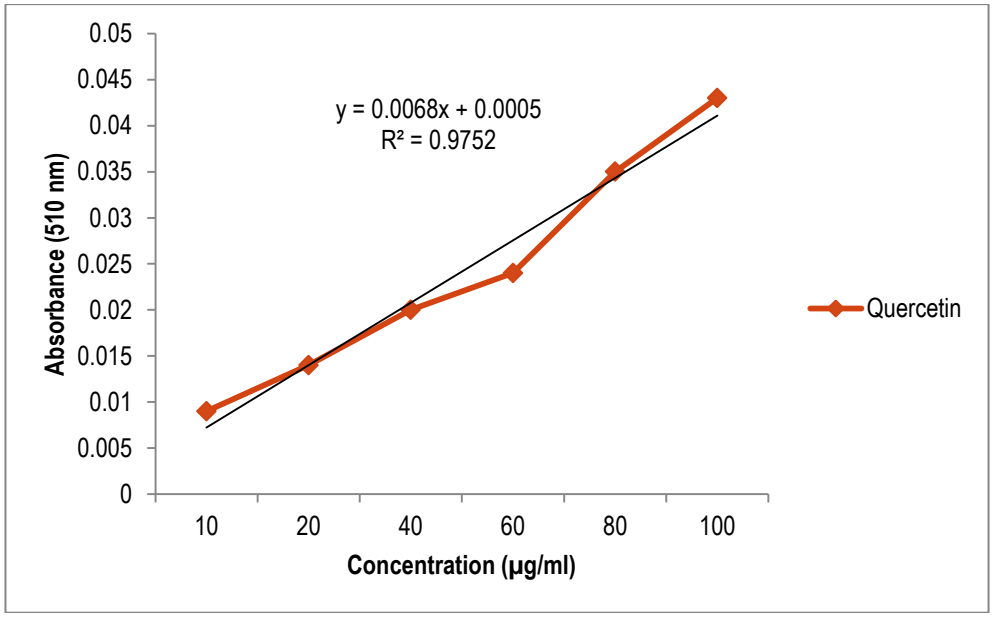

Figure 1: Total flavonoid content of *Acmella oleracea* estimated as quercetin equivalent.

Total phenolic content

Total phenolic concentration equivalent of gallic acid was estimated based on its reaction with Folin-Ciocalteu reagent. It was found that 1 g of plant extract contains 1.38 mg of gallic acid equivalent dry weight of the plant extract **(Figure 2)**.

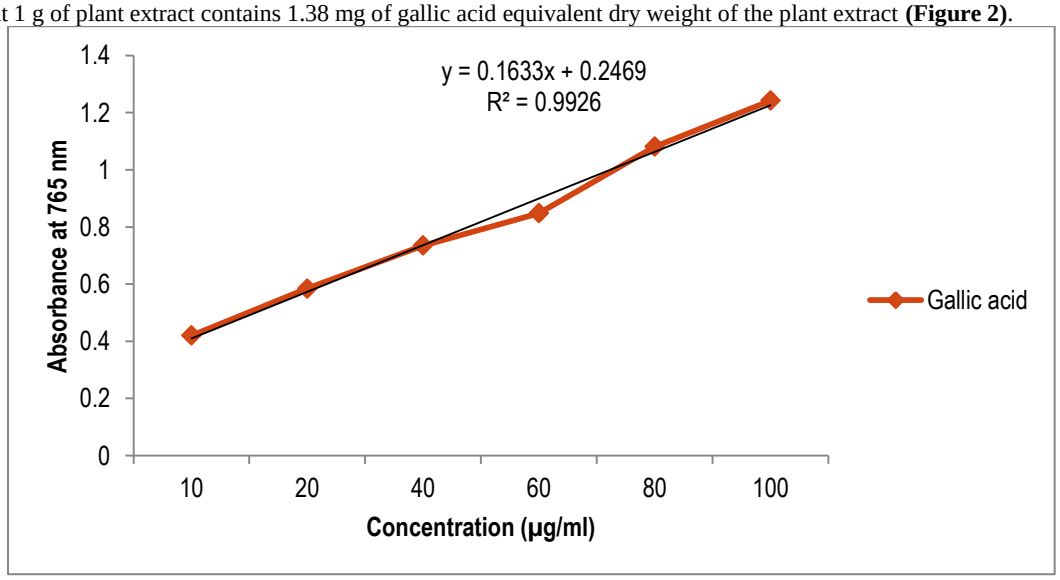

Figure 2: Total phenolic content of *Acmella oleracea* estimated as gallic acid equivalent.

Total antioxidant activity

The total antioxidant activity was estimated by reaction with sulphuric acid, sodium phosphate and ammonium molybdate. The reaction product shows that 1 g of plant extract contains 12.5 mg of ascorbic acid equivalent dry weight of the plant extract **(Figure 3).**

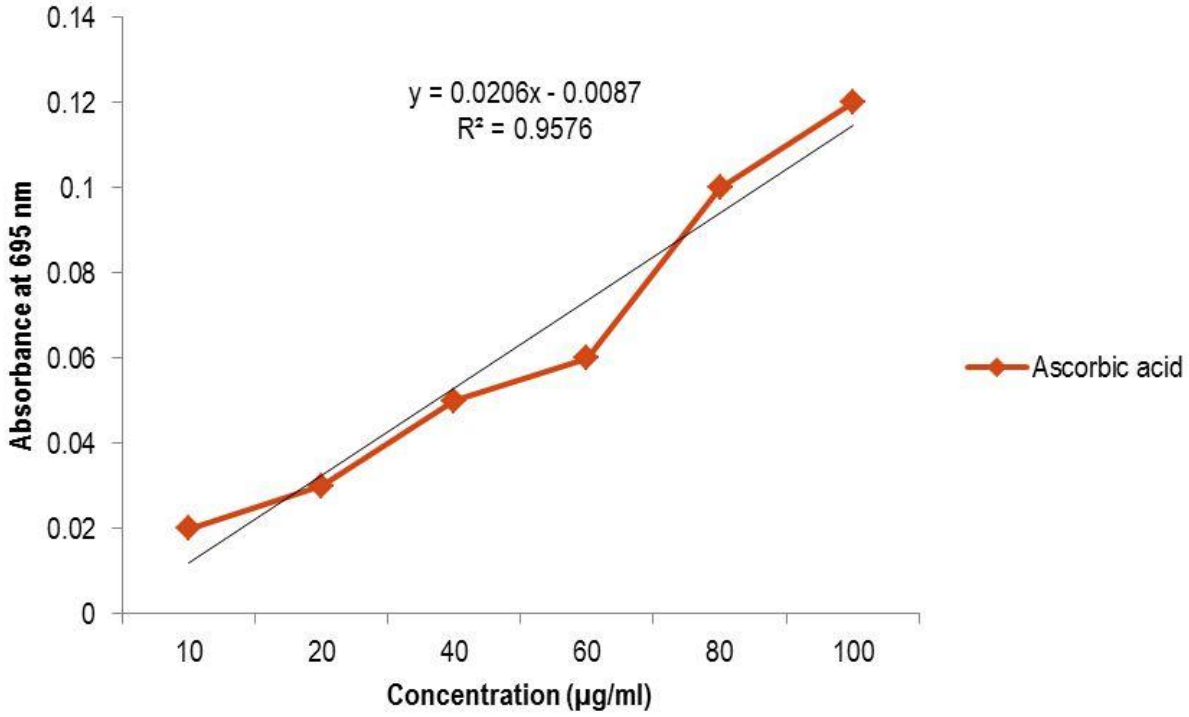

Figure 3: Total antioxidant activity of *Acmella oleracea* estimated against ascorbic acid.

DISCUSSION

Plants are the major source of exogenous antioxidants [18]. Reactive species, either oxygen derived or nitrogen derived, are produced in the body as a result of normal cellular metabolism reactions in the form of free radicals or non-radicals. The long-term effects of these species attributed to the pathogenesis of a number of diseases. This is due to their ability to cause 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 [19]. The antioxidant potential revealed by the total phenolic content, flavonoid content, and antioxidant reaction indicates that *A. oleracea* is a valuable medicinal and dietary plant.

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 [20]. 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. Medicinal plants in particular are among the best sources of antioxidant compounds [21,22].

Phenolic derivatives and flavonoids in plants are essential for general growth, reproduction, and defence against parasites and pests. Flavonoids themselves are a group of hydroxylated phenolic compounds having a benzo-γ-pyrone structure and are ubiquitously occurring in plants [23]. Specifically, these compounds act as natural antioxidants, antibiotics and natural pesticides in many plants. In fact, the best known biological properties of these phenolic compounds are their high antioxidant activities. As a dietary component, they are shown to have health-promoting properties due to their high antioxidant capacity both in vivo and in vitro models. Their antioxidant activity is known to be exerted by three mechanisms, (1) by suppressing of reactive species formation either by inhibition of enzymes or by chelating trace elements involved in the formation of free radical; (2) by directly scavenging the reactive species; and (3) by upregulating cellular defence molecules such as cytokines [24]. Further, their health benefits extend beyond just antioxidation, but are known to play pivotal role in the modulation of the immune system at various molecular levels [25]. Hence, it is important to make further investigations on the pharmacological properties of *A. oleracea.*

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