

Essential Oil Content and Antioxidant Activity of Methanolic Rhizome Extract Of Two Zingiberace Plants In The Hill Of Manipur

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ABSTRACT: In the present work, essential oil content, phytochemical constituents of rhizomes of two wild aromatic plants of Zingiberaceae family were tested in vitro. Protocols such as water distillation, 1,1-diphenyl-2-picryl hydrazyl radical(DPPH), Folin-Ciocalteu method, Aluminum Chloride have been used for evaluation. Rhizome extract were prepared with methanol by Soxhelt apparatus and essential oil extract with distilled water by Clevenger apparatus. The study reveals that the highest oil content was observed in *Alpinia officinarum*(0.3%). The total phenol content of *Alpinia galangal* showed the higher value (63.870 ± 0.353). Total flavonoid content of methanolic extract of *Alpinia officinarum* ,*Alpinia galangal* in terms of Quercetin equivalent (QE) was 33.66 ± 1.87 , 29.537 ± 2.151 of the extract respectively. In DPPH assay of *Alpinia galanga* (625.123 ± 0.155) showed the higher free radical scavenging activity compared with *Alpinia officinarum* (195.30 ± 0.52).

KEY WORDS: Antioxidant, Eessential Oil, DPPH, Quercetin, Clevenger Apparatus.

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I INTRODUCTION

In the ancient and modern cultures, medicinal plants play an important role in health care and essential oils in the plants have been of great interest due to a rich source of antioxidant capacities. Antioxidants are substances with free-radical chain reaction breaking properties. They are inhibitors of lipid peroxidation and are important not only for food protection, but also protects the cell against the oxidative damage [1]. Medicinal plants possess antioxidant activity because they contain antioxidative phytochemicals such as phenolic compounds, which may reduce the risk of cancer, cardiovascular disease and many other diseases [2] as well as reduce the antioxidative damage of cellular components. Phenolic compounds play a key role as antioxidants and may help to protect the cells against the oxidative damage caused by free-radicals [3]. Free radicals play a positive role in phagocytosis, energy production and regulation of cell growth etc. However, free radicals may also be damaging. Many biological molecules, such as lipids, proteins and deoxyribonucleic acids react with free radicals which is produced in the body and resulting in the imbalance between oxidants and antioxidants. Even though our body is protected by natural antioxidants defense but there is always a demand for antioxidants from natural sources [4]. Medicinal plants possess rich antioxidant potential which are the good supplements for the diseases associated with the stress [5]. Essential oil have been used for many medical products. They are plant secondary metabolites that are also used as natural sources of food flavor and preservatives. Essential oils are volatile, natural and complex compounds characterized by a strong odor, and are produced by aromatic plants as secondary metabolites [6]. Essential oils are frequently referred to as the “life force” of plants. These essential oils are extracted from flowers, leaves, stems, roots, seeds, barks and fruit rinds. These oils have potent antimicrobial factors, having wide range of therapeutic constituents. These oils are often used for their flavor and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines and cosmetics. An essential oil consist of chemical compounds which have hydrogen, carbon and oxygen as their building blocks. Essential oils containing aldehydes are helpful in treating inflammation, Candida and viral infections. Oils are commercially important, especially as ingredients in cosmetic and sanitary products and food preservatives and additives and as natural remedies. Therefore, both industrial and academic research fields are increasingly focusing on aromatic plants with biological properties that can be used to improve skin health.

The plant *Alpinia galanga* is a perennial herb and commonly known as great galangal. It contains important phytoconstituents such as alkaloids, saponins, terpenoids, phenolics and flavonoids [7]. It has a potential of anti-tumor, antioxidant, antifungal, antibacterial gastro protective, hypoglycaemic, hypolipidemic, anti-inflammatory substance [8]. *Alpinia galanga*'s oil has been reported to possess bioactivities against the cigarette beetle *Lasioderma serricorne*(F). *Alpinia galanga*'s essential oil contained main components viz.,

eucalyptol, IR- α -pinene, α -terpineol etc. These compounds have great potential to be developed into natural insecticides or repellents in controlling insects in stored grains and traditional medicinal material [9].

Alpinia officinarum is a rhizome belonging to the family, Zingiberaceae, cultivated in South East Asia. This rhizome has strong aromatic odour and dark reddish brown in colour. This rhizome is used to be against rheumatism, whooping cough in children, bad breath, dysentery, stomach ulcer and skin diseases [10]. Essential oil of *Alpinia officinarum* has been confirmed to possess bioactivities against *Coptotermes gestroi*, *Coptotermes curvignathus* and other pests [11]. The main chemical component of *Alpinia officinarum* oil were benzylacetone, 1,7-diphenyl-5-hydroxy-3-heptanone, guaiacylacetone and benzenepropanal [12].

However, currently, there is little information about the essential oil, phytochemical properties in two species from different sources in Manipur. Therefore, the present objectives of this study are to evaluate leaf extracts of each plants for their chemical profiles such as total phenol, flavonoid and antioxidant using methanol for extraction which is used in folk medicine in Manipur, India as well as to determine the volatile oil percentages.

II MATERIALS AND METHODS

2.1. Plant Material Collection

The present study included two wild plants which were *Alpinia galangal*, *Alpinia officinarum*. Fresh rhizomes collected from different places of Sadar Hills, Senapati District of Manipur. The collected plant species were used for essential oil extraction and quantitative phytochemical analysis.

2.2. Volatile oil extraction method

The fresh rhizome (250g) of two samples were subjected to hydro-distillation for 4-5 h using Clevenger apparatus until oil distillation is ceased. The oil was dried over anhydrous Na_2SO_4 and preserved with a sealed vial in the freezer at 4°C until further analysis. The volatile oil percentages were determined by using the following equation [13].

Volatile oil % = oil volume in the graduated tube / fresh weight of sample x 100.

2.3. Preparation of Plant Extract

The rhizomes of collected two wild medicinal plants were washed under running tap water to remove dust particles and rinsed again with distilled water. The rhizome samples were shaded; air dried for one week and evaporated water molecules under hot air oven at a temperature of 35°C- 40°C for 3 days. The dried rhizomes were ground into powder form by using grinder and stored in polythene bags for chemical analysis.

2.4. Solvent Extraction

Crude rhizome was prepared by Soxhlet extraction method. For methanolic extraction, each 20gm of powdered plant material was uniformly packed into thimble and extracted with 250ml of methanol separately at 65°C. The process of extraction continues till the solvent in siphon tube of an extractor become colourless. The aliquots were cooled down and taken in colour amber bottles and kept in refrigerator for further analysis.

2.4.1. Determination of Total Phenolic Content

The phenolic contents in the methanolic extract was estimated by Folin-Ciocalteu method with little modification as previously described [14,15]. 2.5 ml of 10% Folin-Ciocalteu reagent and 2ml of NaCO_3 (2% W/V) were added to 0.5ml of the sample (3 replicates) of each plant extract solution (1mg/ml). The mixture was allowed to stand at 20°C for 30 minutes and the absorbance of the developed colour was measured at 760 nm using UV-vis Spectrophotometer. Gallic acid was used as standard (1mg/ml) to calculate total phenolic content. All the tests were performed in triplicates. Total phenolic content in the plant extract was expressed as Gallic acid equivalent (mg of Gallic acid equivalent/g of dry weight sample) and was calculated by the formula [16].

$$T = CXV/M$$

Where, T = Total content of phenolic compound, mg/g of plant extract, in GAE;

C = Concentration of Gallic acid established from the Calibration curve, microgram/ml;

V = Volume of extract; M = Weight of methanolic plant extract, g.

2.4.2. Determination of Total Flavonoid Content

Aluminum Chloride spectrophotometric method was used to measure the flavonoid content of all plant extracts with slight modification [17,18,19]. 0.5ml of sample (3 replicates) of each plant extract solution (1mg/ml) was taken and it was added with 0.1ml of aluminum chloride (10%), 0.1ml of potassium acetate (1M) and 2.8ml of distilled water to make up volume to 3.5ml. The reaction mixture was kept at room temperature for 30min. The absorbance was measured at 415nm using UV-vis Spectrophotometer against the

suitable blank. Quercetin was used as standard (0.1mg/ml) to calculate total flavonoid content. The calibration curve was prepared using different concentrations of quercetin expressed in mg/gm dry weight.

2.4.3. Scavenging activity against 1,1-diphenyl-2-picryl hydrazyl radical(DPPH)

Methanol solution of DPPH was used as a reagent for the spectrophotometric assay with slightly modifications [20]. DPPH solution (0.004% w/v) was prepared in methanol. Standard ascorbic acid (0.5mg/ml) were prepared using methanol. Various concentrations(10-50µg/ml) of the rhizome extract and ascorbic acid were taken in test tubes and 1ml of freshly prepared DPPH solution were added, the test tubes were covered with aluminum foil to protect from light. The final volume in each test tube are made to 2ml with methanol. Absorbance was read against a blank at 517nm after incubation of the reaction mixtures for 30 minutes in dark room temperatures. Control sample was prepared containing the same volume of methanol and DPPH without any extract and reference ascorbic acid. Methanol was served as blank. The DPPH solution in methanol was prepared daily before the experiments. DPPH is a purple coloured stable free radical. When reduced it becomes the yellow coloured Diphenyl picryl hydrazine. All experiments were performed thrice and the results were averaged [21]. Percent inhibition was calculated using the following expression.

$$\% \text{ Inhibition} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Where A_{blank} and A_{sample} stand for absorption of the blank sample and absorption of tested extract solution respectively.

2.8. Statistical analysis

All experiments were performed in triplicate (n=3) and results were given as mean± standard deviation.

III. RESULTS AND DISCUSSION

3.1. Essential Oil Content

The essential oil content, organoleptic and texture are recorded as Table 2. Total oil content(%) of *Alpinia officinarum* and *Alpinia galangal* were 0.3%, 0.2% of the fresh matter.

3.2. Total Phenol Content

The total phenol contents of two crude extracts determined by folin-ciocalteu method were reported as gallic acid equivalent. The total phenol, flavonoid and antioxidant activity are shown in Table 2. The standard graph for gallic acid is represented in graph($y=0.007x-0.009, r^2=0.993$), where, y is absorbance at 760nm and x is concentration. The total phenolic content of the methanolic extract of *Alpinia galangal*, *Alpinia officinarum* were 63.870 ± 0.353 , 43.6 ± 0.395 , mg/g of gallic acid equivalent per gram of extract respectively.

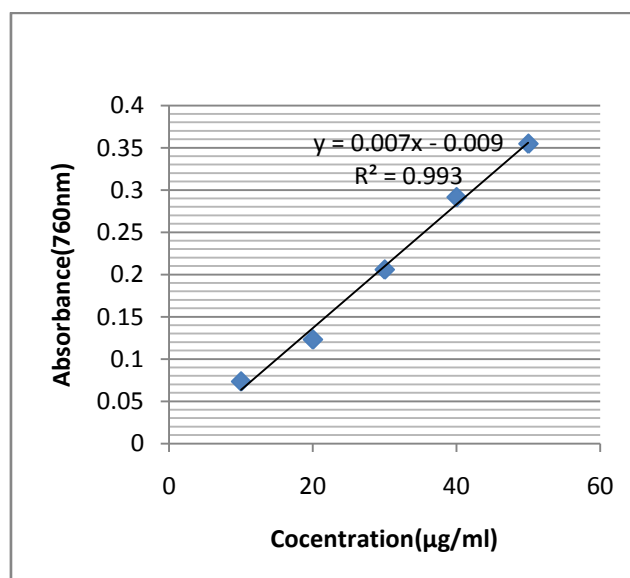


Figure 1: Gallic acid Regression curve

3.3. Total Flavonoid Content

The content of total flavonoid was measured by aluminium chloride method in term of quercetin equivalent($y=0.007x-0.035, r^2=0.997$) is shown in fig.2. The total flavonoid content of *Alpinia galang* and *Alpinia officinarum* were 29.537 ± 2.151 , 33.66 ± 1.87 mg/g of quercetin equivalent/gram of extract. *Alpinia officinarum* was found highest flavonoid content than *Alpinia galanga*.

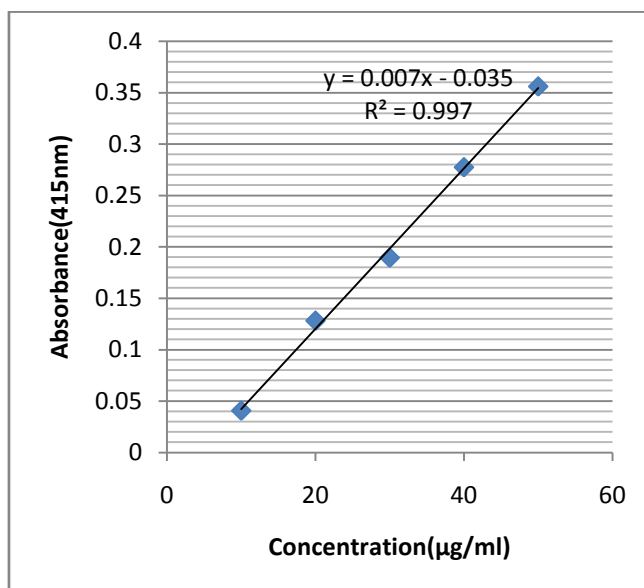


Figure2 : Graph represents regression curve of quercetin

3.4. DPPH Assay

The radical scavenging activity of rhizome extract of *A.galanga* and *A.officinarum* were assessed by DPPH radical scavenging assay. The crudes exhibited dose dependent scavenging of free radicals. The rhizome extract of *A.galanga* ($635.123 \pm 0.155 \mu\text{g/ml}$) scavenged free radicals more effectively when compared to *A.officinarum* ($195.30 \pm 0.52 \mu\text{g/ml}$). Essential oils served as an important bio-resource of antioxidants for using in the food industries. Flavonoids have been found to be antimicrobial substance against wide array of microorganisms in vitro [22]. Phenolic compounds posses biological properties such as anti-aging, anti-carcinogen, anti-inflammation, cardiovascular protection etc.[23]. Thus, from the present study volatile oil ,medicinal properties of the two wild aromatic plants can be identified based on the chemical compounds present on them.

Table 1: Total oil content % , Organoleptic study and texture of two wild aromatic plants.

Sample	Oil content (%)	Odour	Colour	Texture
<i>Alpinia galanga</i>	0.2	Sweet	Reddish	Fibrous rhizome
<i>Alpinia officinarum</i>	0.3	Sweet pungent	Whitish	Fibrous rhizome

Table2: Total phenol, flavonoid contents and scavenging % inhibition in the methanolic extracts

Sample	mg GA/g of extract X*	mg Q/ g of extract X*	% inhibition of extract X*
<i>Alpinia galanga</i>	63.870 ± 0.353	29.537 ± 2.151	625.123 ± 0.155
<i>Alpinia officinarum</i>	43.6 ± 0.395	33.66 ± 1.87	195.30 ± 0.52

GA= gallic acid ,Q= quercetin ,TA= tannic acid, X* = mean±standard deviation.

IV. CONCLUSION

From the present investigation, it could be concluded that the solvent play a vital role in the extraction of the constituents. *A.galanga* may be considered as good source of natural antioxidant to be used in medicinal and food products to promote human health and prevent diseases. This investigation suggests that exploring the source of natural antioxidant activity, phenolic and flavonoid contents in *A.galanga*, *A. officinarum* with help to re-introduce their use as food supplements and encourage their cultivation, conservation by home garden or by government authority before these plants are almost going to extinct due to deforestation and urbanization.

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