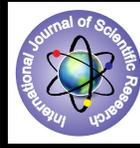


Phytochemical Analysis of Six Aromatic Plants



Botany

KEYWORDS : Alpinia galanga, Elsholtzia balanda, phytochemicals and flavonoids

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ABSTRACT

North-eastern India has been known for its rich biological diversity. For this study, six aromatic plants such as *Alpinia galanga*, *Hedychium marginatum*, *Zanthoxylum limonella*, *Elsholtzia balanda*, *Amomum aromaticum* and *Eryngium foetidum* were selected. The aim of the present study was to investigate the presence of phytochemicals and to determine the total phenolic and flavonoid contents of the selected aromatic plants. All the six aromatic plants showed variations in the presence of phytochemical constituents. *Alpinia galanga* (75.01mg/gm) showed the highest value of total phenolic contents among the six aromatic plants. In case of total flavonoid content, *Elsholtzia balanda* (36.05 mg/gm) showed the highest value among the selected plants.

Introduction

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousand of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs, antihepatotoxic compounds (Yadav and Agarwala 2011).

The aromatic plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the aromatic plants, leaves, vegetables, and roots that have defense mechanism and protect from various diseases (Nosro et al. 2000). Aromatic plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesised by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan 1999).

Materials and method

Collection and preparation of plant extract: -The collected aromatic plant species were washed in tap water and then rinsed in distilled water. They were cut into small pieces, shaded, air dried for 7 days and finally dried in an oven at a temperature of 35 – 40°C for 2 days. The dried plants were pulverized by using grinder to obtain a powdered form. Rhizomes, roots and leaves were powdered separately. Crude extracts of each plant were prepared by Soxhlet extraction method. For the methanolic extraction, each 20g of dried and powdered plant material was uniformly

packed into a thimble and extracted with 250 ml of methanol at 65°C. The process of extraction continues for 24 hours or until the solvent in siphon tube of an extractor become colourless. The extract was taken in the amber bottles and kept in refrigerator for future used.

Phytochemical testing were performed for the identification of various active chemical constituents like alkaloids, proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroid and terpenoids. For each phytochemical testing, the test was conducted differently by using several testing methods in order to confirm its positive result more accurately. Wagner's test, Mayer's test and Dragendorff's test were performed for the identification of alkaloids, Millon's test, Ninhydrin test and Xanthoproteic test for proteins; Fehling's test, Benedict's test, Molisch's test and Iodine test for carbohydrates; Shinoda test and Alkaline reagent test for flavonoids; Liebermann's test, Keller-kilani test and Ferric chloride test for glycosides; Salkowski's test for steroids and other remaining tests are performed

Quantitative phytochemical analysis

Determination of total phenolic content

Total phenol content was estimated using Folin-Ciocalteu reagent (McDonald et al 2001). To 1 ml of each extract (100µg/ml) in methanol, 5 ml of Folin-Ciocalteu reagent (diluted ten-fold) and 4 ml (75 g/litre) of Na₂CO₃ were added. The mixture was allowed to stand at 20° C for 30 minutes and absorbance of the developed colour was recorded at 765 nm using UV-VIS spectrophotometer. 1 ml aliquots of 20, 40, 60, 80, 100 µg/ml methanolic gallic acid solutions were used as standard for calibration curve. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass) which is a common referrence compound.

Total flavonoids determination

Aluminium chloride colorimetric method was used for flavonoids determination (Chang et al 2002). Each plant extract (0.5 ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min-

utes. The absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 $\mu\text{g/ml}$ in methanol.

Table 1: Phytochemical constituents of six aromatic plants

Plant: Botanical name (Local name)	Alkaloids	Proteins	Carbohydrates	Phenols-Tannins	Flavonoids	Saponins	Glycosides	Steroid	Terpenoids
<i>Alpinia galanga</i> (Kanghoo)	+	+	+	+	+	+	+	+	+
<i>Amomum aromaticum</i> (Namra)	+	+	+	+	+	+	+	+	+
<i>Elsholtzia balanda</i> (Lomba)	+	+	+	+	+	+	+	+	+
<i>Eryngium foetidum</i> (Shamaroi)	+	+	+	+	+	+	+	+	+
<i>Hedychium marginatum</i> (Takhelei angangba)	+	+	-	+	+	+	+	+	+
<i>Zanthoxylum limonella</i> (Ngang)	+	+	-	+	+	-	+	+	+

(+) = presence of phytochemicals and (-) = absence of phytochemicals

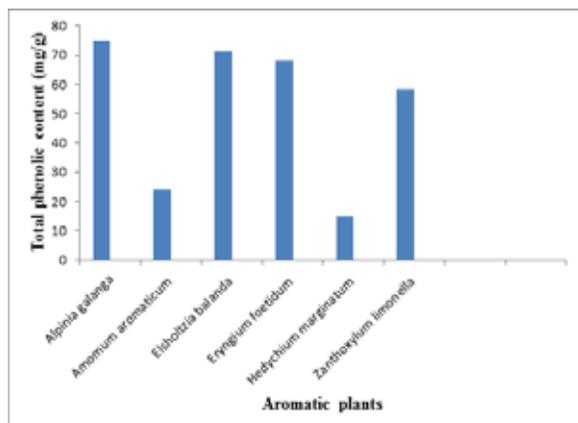


Figure 1: Total phenolic content in six aromatic plants

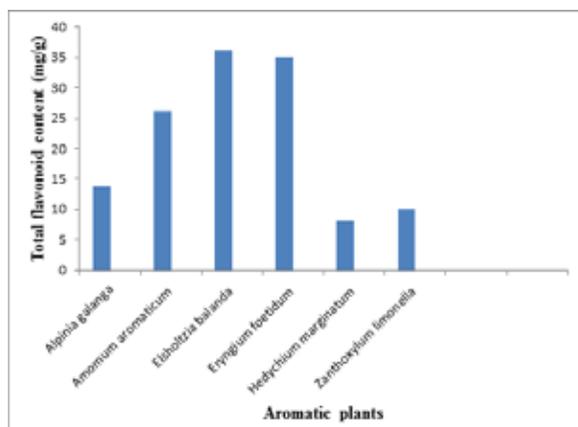


Figure 2: Total flavonoid content in six aromatic plants

Result and Discussion

Alpinia galanga, *Amomum aromaticum*, *Elsholtzia balanda* and *Eryngium foetidum* showed the presence of all phytochemical constituents tested (Table 1). *Hedychium marginatum* showed the presence of almost all phytochemical constituents except carbohydrates whereas carbohydrates and saponins were observed to be absent in *Zanthoxylum limonella*.

Total phenolic content was found to be highest in *Alpinia galanga* followed by *Elsholtzia balanda* (Figure 1) whereas flavonoid content was found to be highest in *Elsholtzia balanda* followed by *Eryngium foetidum* (Figure 2).

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh et al 2007). They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities (Han et al 2007). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc (Ali et al 2008). Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesised by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall. They are also effective antioxidant and show strong anticancer activities (Okwu 2004).

The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, haemolytic activity, cholesterol binding properties and bitterness. Steroids have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties in their cytotoxicity (Yadav and Agarwala 2011). Several workers have reported the analgesic, antispasmodic and antibacterial properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports (Nyarko and Addy 1990). The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Acknowledgement

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