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A PRELIMINARY STUDY ON THE PHYTOCHEMICAL ANALYSIS AND GC-MS STUDIES OF PLECTRANTHUS AMBOINICUS

Biochemistry

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ABSTRACT

The present investigation was carried out to determine the qualitative analysis of phytochemical activities and possible chemical constituents of Plectranthus amboinicus (family:lamiaceae) leaves by GC-MS studies. Plectranthus Amboinicus (PA),commonly known as country borage,is a folkloric medicinal plant.The leaves are also used to treat malarial fever,congestive heart failure,hepatic and renal problems.Various flavonoids like quercetin,apigenin,luteonin, salvegenin,genkwanin and volatile oil are identified in the leaves of Plectranthus amboinicus.The ethanol extract of Plectranthus amboinicus contains 12 compounds were identified by GC-MS analysis.Most of the phytochemical compounds have pharmacological properties such as antioxidant,hypocholesterolemic,anticancer,diuretic,hepatoprotective and anti-inflammatory properties.

KEYWORDS

Plectranthus amboinicus, GC-MS, quercetin, apigenin, luteonin, salvegenin, genkwanin

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. Plant is man's friend in survival,giving him food and fuel and medicine from the days beyond drawn of civilization (Bose.T.K and Choudhary K.,1991).Plant continues to be a major source of medicine,as they have throughout human history (Prince.L and Prabakaran.P.,2011).These are non-nutritive chemicals that have protective or disease preventive property.

Fig: Plectranthus Amboinicus

Classification
Family : Lamiaceae
Genus : Plectranthus
Species : amboinicus
Botanical Name : Plectranthus amboinicus
Tamil Name : Karpuravalli

Plectranthus amboinicus (PA), commonly known as country borage, is a folkloric medicinal plant. The fresh juice of leaves of this plant is widely used for various illnesses including kidney stones (Warrier et al. 1995). It is scientifically evaluated for antimicrobial (Timer et al. 1992; Mele et al. 2006), antioxidant (Padma et al. 1998), antiseptic, anti-viral, anti-ochratoxigenic (Pushpa et al. 2009), and anti-malarial activities (Kaoua et al. 2008). Further, it is used in the treatment of asthma gastrointestinal disorders(Hernandez Canoa and Volpato. 2004). The leaves are also used to treat malarial fever,congestive heart failure, and hepatic and renal problems (de Albuquerque et al. 2007). Various flavonoids like quercetin, apigenin, luteonin, salveginin, genkwanin, and volatile oil are identified in the leaves of PA (Brieskorn and Riedel 1997).

Phytochemicals And Flavanoids

A number of other dietary antioxidant substances exist beyond the traditional vitamins discussed above. Many plant-derived substances,collectively termed "phytonutrients," or "phytochemicals," are becoming increasingly known for their antioxidant activity. Phenolic compounds such as flavonoids are ubiquitous within the plant kingdom approximately 3,000 flavonoid substances have been described.In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans, flavonoids appear to function as "biological response modifiers" (Cody, V et al., 1986).

Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity.The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages (Middleton. E ,1984).

Indigenous people all over the country have an instinctive knowledge of the therapeutic uses of the plants of their environment. Today, as conventional medicine pursues a more integrated approach to managing disease, natural products and select herbs that influence gastric functions are being revisited and evaluated for their overall health promoting factors. Some notable herbs and nutrients under investigation are garlic, turmeric, and cayenne and milk thistle.

MATERIALS AND METHODS

Collection And Identification Of Plant Material

The leaves of Plectranthus amboinicus (Family: Lamiaceae) were collected from the local region and the District of Tiruvannamalai, Tamil Nadu, India. The plant material were cleaned with distilled water, shade dried at room temperature and taxonomically identified. Voucher specimen has been retained in the Dept of Biotechnology, AEC, Tiruvannamalai. Tamilnadu, India. The shade dried plant material were powdered by using electric blender.

Preparation Of Plant Extract

The powdered plant material were extracted separately to exhaustion in a soxhlet apparatus using chloroform, ethyl acetate and ethanol solvent (Merk Chemicals, India) systems. All the extracts were filtered through a cotton plug followed by Whatman filter paper (No.1). All the solvents were completely removed by rotary vacuum evaporator. The extracts were freeze dried and stored in vacuum dedicator. The extract was collected and evaporated to dryness by using vacuum distillation unit.The final residue thus obtained was then subjected to GC-MS analysis.

Phytochemical Analysis Of Ethanolic Extract Of Plectranthus Amboinicus Leaves (PA)

The ethanol extract of Plectranthus amboinicus (PA) was a complex mixture of many constituents and 12 compounds were identified in this plant by GC-MS. Phytoconstituents such as Glycerin, Cyclobutane

carboxylic acid, 2-butyl ester, Dodecanoic acid, Tetradecanoic acid, n-Decanoic acid, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 3,5-di-tert-Butyl-4-hydroxyanisole, 9,12-Octadecadienoic acid (Z,Z)-, Octadecanoic acid, 1,2-Benzenedicarboxylic acid, diisooctyl ester and Squalene were identified in the ethanol extract of Plectranthus amboinicus by relating to the corresponding peak area through coupled GC-MS.In this plant also, most of phytochemical compounds have pharmacological properties such as antioxidant, hypocholesterolemic, anticancer, diuretic, hepatoprotective, and anti- inflammatory properties.

GC-MS Analysis

GC-MS Analysis of these extracts was carried out by following the method of (Hema et.al.,2010).GC-MS analysis were performed using a Perkin-Elmer GC clauses 500 system and Gas chromatograph interfaced to a mass spectrometer(GC-MS) equipped with a Elite-1,fused silica capillary column(30m x 0.25 mm ID x1xdf,composed of 100%

Identification Of Phytocomponents In Plectranthus Amboinicus (PA) Extracts (GC-MS study)

The identification of the chemical composition of ethanol extract of Plectranthus amboinicus was performed using a GC-MS spectrograph (Agilent 6890/Hewlett-Packard 5975) fitted with electron impact (EI) mode. The ethanol extract (2.0µl) of Plectranthus amboinicus was injected with a Hamilton syringe to the GC-MS manually for total ion chromato- 125 graphic analysis in split mode. In quantitative analysis, selected ion monitoring (SIM) mode was employed during the GC-MS analysis. SIM plot of the ion current resulting from very small mass range with only compounds of the selected mass were detected and plotted.

RESULTS AND DISCUSSION

Phytochemical Studies - GC-MS

Medicinal plants have been used by human being since early ages in traditional medicine due to their therapeutic potential and the search on medicinal plants has led to the discovery of novel drug candidates used against diverse diseases. Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health (Sermakkani et al.,2012). Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with variety of structural arrangements and properties.

The study of the bioactive constituents of higher plants represents a promising approach to the discovery of new drugs.

The investigation of plants used as remedies in traditional folk medicine can be a useful tool to identify several biologically active molecules from the 250,000 higher plants species (Mbwamboo et al., 1996).

In recent year's Gas chromatography – Mass Spectrum (GC-MS) studies have been increasingly applied for the analysis of medicinal plants to identify the structures of different phytoconstituents in plant extracts and biological samples with great success (Prasain et al., 2004). This technique has proved to be a valuable method for the analysis of essential oil, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds (Anjali et al., 2009).

From GC-MS analysis it was found that Plectranthus amboinicus extract showed a positive response for the presence of flavonoids, glycosides, saponins, steroids and tannins.This identification was done by comparison of their mass spectra on both columns with phytochemical and ethnobotanical databases libraries or with mass spectra from the literature (Adams, 2001; Jennings and Shibamoto, 1980) and home-made library. Most of the compounds 2-Methylthiolane, S,S-dioxide, Dodecanoic acid, Tetradecanoic acid, n-Hexadecanoic acid, 3,5-di-tert-Butyl-4-hydroxyanisole and Squalene belong to the group of antioxidant agents (Yoshida and Nihi, 2003). Based on data available in literature all these compounds could effectively contribute to the biological activities of Plectranthus amboinicus.

Since medicinal plants are endowed with complex chemical substances such as alkaloids, glycosides, flavonoids, saponins tannins etc., efforts are being made continuously to isolate and discover more potent plant principles for the treatment of various human ailments (Prajapathi et al., 2003). Different phytochemicals have been found to possess a wide range of activities; which may help in protection against chronic diseases. It is hypothesized that the presence flavonoids (Mota, et al., 2009), tannins (Jesus, 2012) , alkaloids (Falcao, et al.,2008) and hexadecanoic acid (Shabi et al.,2010) present in plant extracts are capable of protecting duodenal and peptic ulcer and Gastrointestinal damage induced by Gastro-Intestinal Toxin in both in vivo and in vitro studies. It is believed that the active principles extracted from medicinal plants produce better, specific and safe drugs (Hasrat et al., 2004) and may reduce the risk of toxicity and maintain their therapeutic efficacy when put for use in clinical application (Nan et al., 2003). The results of the preliminary phytochemical screening of Plectranthus amboinicus leaves extract reveal that plants have quite a number of phytochemical constituents which may be responsible for many pharmacological actions.

Table. Activity Of The Phytocomponents Identified In The Plectranthus Amboinicus Leaves [GC MS study]

No	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Com-poud Nature	Activity
1	3.71	Glycerin	C3H8O3	92	4.50	Alcohol	Preservative Antimicrobial
2	10.84	Cyclobutanecarboxylic acid, 2-butyl ester	C9H16O2	156	0.32	Ester compound	No activity reported
3	10.95	Dodecanoic acid	C12H24O2	200	1.51	Lauric acid	Antioxidant, Antibacterial, COX-1 & COX-2 inhibitor, Antiviral, Hypocholesterolemic, Candidicide.
3	10.95	Dodecanoic acid	C12H24O2	200	1.51	Lauric acid	Antioxidant, Antibacterial, COX-1 & COX-2 inhibitor, Antiviral, Hypocholesterolemic, Candidicide.
4	13.38	Tetradecanoic acid	C14H28O2	228	1.27	Myristic acid	Antioxidant, Cancer preventive, Nematicide, Lubricant Hypocholesterolemic
5	14.72	n-Decanoic acid	C10H20O2	172	0.20	Fatty acid	Insecticide
6	16.24	n-Hexadecanoic acid	C16H32O2	256	12.27	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
6	16.24	n-Hexadecanoic acid	C16H32O2	256	12.27	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
7	16.46	Hexadecanoic acid, ethyl ester	C18H36O2	284	0.52	Fatty acid ester	-do-
8	17.61	3,5-di-tert-Butyl-4-hydroxyanisole	C15H24O2	236	0.60	Anisole compound	Antioxidant
9	18.90	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	17.52	Linoleic acid	Anti inflammatoy, Hypocholesterolemic Cancer preventive, Hepatoprotectiv, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge

10	19.17	Octadecanoic acid	C18H36O2	284	2.19	Stearic acid	No activity reported
11	24.70	1,2Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	57.11	Plasticizer compound	Antimicrobial Antifouling
12	28.83	Squalene	C30H50	410	1.99	Triterpene	Antibacterial, Antioxidant, Antitumor,Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase inhibitor,Pesticide

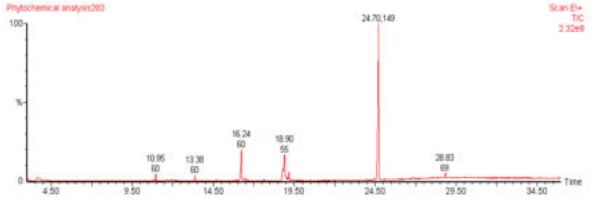

Figure: The Chromatogram Showing Different Compounds As Peaks Detected By Gas Chromatography–mass Spectrophotometry In Ethanolic Extract Of Plectranthus Amboinicus Leaves (PA)

CONCLUSION

Different phytochemical studies on Plectranthus amboinicus leaves revealed the presence of chemical compounds such as alkaloids,flavonoids, tannins and phenolic compounds. Plants are a rich source of secondary metabolites with interesting biological activities Plectranthus amboinicus leaves is widely available in south india has been used to treat various diseases.The present study explained the various pharmacological potentials due to the active compounds of the plant.

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