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Research Article

PROXIMATE ANALYSIS, PHYTOCHEMICAL SCREENING AND HIGH RESOLUTION LIQUID CHROMATOGRAPHY MASS SPECTROPHOTOMETRY ANALYSIS OF ESSENTIAL OILS OF HEDYCHIUM CORONARIUM J. KOENIG, A MEDICINAL AND AROMATIC PLANT

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ABSTRACT

Medicinal plants have been used in all cultures as a source of medicine since ages. When parts of plants such as rhizomes, leaves or barks and other natural materials are used as drugs to treat illnesses, they are called crude drugs and the study of crude drugs is called pharmacognosy. Proximate analysis in plants gives valuable information and help to assess the quality of a crude drug. Beneficial effects of crude drugs are believed to be attributed to plant phytochemicals. *Hedychium coronarium* J. Koenig, a medicinal and aromatic plant of high value is an endangered and red-listed plant. The rhizome of this plant is used for the treatment of various diseases. Crude extracts prepared from the rhizomes show antibacterial and antifungal properties. Essential oils obtained from *Hedychium coronarium* are found to be rich in terpenes and used for bactericidal, fungicidal, medicinal and cosmetic applications. The present study assesses the quality of crude drugs prepared from this highly medicinal plant. Proximate analysis and phytochemical screening of rhizomes of *Hedychium coronarium,* which is frequently consumed as food and as medicine were carried out. Ash content was found to be low) when compared to the moisture content. The water extractive value was more than alcohol extractive. Preliminary tests carried out on phytochemicals revealed the presence of terpenoids and oils. High Resolution Liquid Chromatography Mass Spectrophotometry was carried to ascertain the different components of essential oils and revealed the presence of eucalyptol (1,8-cineole), caryophyllene oxide, camphor, linoleic acid, ricinin, phloroglucinol, 6-gingerol, carvone and arjungenin.

INTRODUCTION

जगत्येवमनौषधम्।

न किञ्चिद्विद्यते द्रव्यं वशान्नानार्थयोगयोः॥१०॥

(Sutra. Ch.9-verse 10, Ashthanga Hridaya).

The ancient physicians of India said and proved that "There is nothing in this Universe which is non-medicinal and which cannot be made use of for many purposes and by many modes. Knowledge and purpose of each substance is required to use it as medicine".

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This description rightly suggests that in principle all plants are potentially medicinal. There has been a renewed interest among scientists all over the world in plant-derived drugs for they offer practical and safe medical solutions for use by the masses. Increasing cost of personal health maintenance and growing awareness of the safety of natural products among industrialized countries/societies has led to the increase in overall demand for plant-derived drugs world-wide.

When certain parts of plants such as rhizomes, leaves or barks and other natural materials are used as drugs to treat illnesses they are called crude drugs. Crude drugs are in the form of decoction, juices, powder, paste, oils and whole plant extracts. Leaves and roots are the frequently used plant parts for treatment of diseases. External applications are used for rheumatism, poisonous bites and problems related

to hair and skin. Oral consumption is used for fever, cough, jaundice, indigestion, etc. It was discovered that the active principles in crude drugs are responsible for the medicinal properties and are present in minute amounts. They include alkaloids, flavonoids, glycosides, gums, oils, resins, saponins, tanins and waxes. These active principles are species, genus and family specific, are produced in small quantities and do not have any nutritive value to the plant. They are produced as by-products of primary metabolic pathways or in response to specific conditions or requirements. Hence are also known as secondary metabolites. These chemicals produce a definite physiological action on the human body [1]. Another broad category of terpenoids is essential oils, which are generally variable types of volatile terpenoids. Volatile terpenoids (isoprenes, monoterpenes, sesquiterpenes) are produced in all parts of the plants ie. roots, rhizomes, stem, leaves, fruits and flowers by the same biosynthetic pathway. A large number of terpenes are emitted by flowers [2]. Volatile terpenes have low molecular weight and high vapour pressure. These properties allow them to freely pass through the cellular membranes for releasing them into the adjacent environment[3]. Monoterpenes and sesquiterpenes are the most widely studied classes because of their extensive distribution in the plant kingdom and the role that they play in ecology. Of all terpenoids, monoterpenes constitute 53% followed by sesquiterpenes which is 28%, diterpenes are 1% and remaining are others (isoprenoids, hemi-, tri- and tetraterpenes)[4].

As the use of herbal medicines has increased all over the world, the global market for these products has also expanded rapidly. The lack of drug standardisation, information and quality control has led to a lacuna in using crude drugs [5]. There are more than one reason to this effect. Crude drugs may vary in composition and properties. Counterfeits and drugs of poor quality may degrade the clinical effects of ASU drugs i.e., Ayurveda, Siddha and Unani systems of drugs which may in turn affect their export value. The safety and quality of medicinal plant materials and finished products have become a major concern for health authorities, pharmaceutical industries and the public. Therefore, authentication is an important step for successful and reliable clinical applications and for further experimental studies on them. Several methods are used to authenticate herbal drugs such as taxonomic, macroscopic, microscopic, physicochemical, chemometric and spectral, chromatographic, chemical fingerprinting and using molecular markers [6].

Hedychium coronarium J. Koenig also known as Gulbakawali or Dolan Champa or butterfly ginger lily belongs to the family Zingiberaceae [7,8]. It is cultivated in many parts of the world and widely distributed in tropical and subtropical regions among China, India and South-East countries. In Central India, it is found in the Amarkantak region of Madhya Pradesh and Chattisgarh [9]. Even though it is widespread as an ornamental plant and used as raw material for the paper industry, the plant is under threat category. This plant has most recently been assessed for The IUCN Red List of Threatened Species in 2019. *Hedychium coronarium* is listed as Data Deficient [10].

The medicinal value of this plant in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita and Sushruta Samhita [11]. The different parts of the plant *Hedychium coronarium* find its use as ethnomedicine in various countries and in India by various tribes of Karnataka, Madhya Pradesh, Odisha, Arunachal Pradesh and Manipur [9]. The rhizome of the plant is used in the treatment of diabetes, cold, body aches, headache, lancinating pain, contusion, inflammation and rheumatic pain [12]. It has anti-cancerous, antioxidant, anti-hypertensive, diuretic, leishmanicidal, anti-malarial activities and used in irregular menstruation, piles bleeding and stone in urinary tract [9]. Recently, antifungal activity of *Hedychium coronarium* crude extracts has been reported. Cancer chemoprevention activity is also reported recently of labdane diterpenes from rhizomes of *Hedychium coronarium* which showed anti-inflammatory and cytotoxic activities [13]. Terpenoids from Hedychium oil showed antioxidant and antimicrobial properties. The rhizomes of this plant are also used in Chinese natural medicine. The flowers of *Hedychium coronarium* are widely cultivated for sweet perfume and also provide essential oil borneol, methyl salicylate, eugenol and methyl lanthranilate. Rhizome paste is applied on bruises and sprains. Literature mentions that *Hedychium coronarium* contains essential oils whose major contents are α-pinene, β-pinene, linalool, 1,8-cineole, α-terpineol, α-humulene and caryophyllene [14]. The biological activities of *Hedychium coronarium* are due to the presence of essential oils.

Considering the economic value, declining population, medicinal properties of every part of the plant especially the essential oils and the plant's endangered status, we have considered *Hedychium coronarium* J. Koenig as a model plant to study its phytochemicals extracted from the rhizomes and the analysis of the essential oils using high resolution liquid chromatography mass spectrophotometry. Prior to this, proximate analysis was thought to be appropriate to assess the quality of crude drug.

MATERIALS AND METHODS

Collection of Plant Material and Preparation of Sample

Fresh rhizomes of *Hedychium coronarium* were obtained from the greenhouse of Ramniranjan Jhunjhunwala College, Ghatkopar. They were washed in continuous, running water for 1 hour. They were again washed with Tween 20 and a soft brush was used to remove all sticking soil particles to obtain clean rhizomes. The cleaned rhizomes were deskinned and were cut into small pieces with a knife, placed in a petridish and kept in the oven for 24 hours at a temperature of 50°C. The dried rhizome pieces were now put in a mixer (high volts) and ground to a dry, fine powder. They were then sieved through a sieve (180µ), (Jayant Scientific Ind., Mumbai 400002) to obtain a fine powder of uniform size. The process was repeated with the remaining powder that was not ground finely. The fine powder was stored in a clean and dry glass bottle and was used for proximate analysis and for phytochemical screening. In addition some of the cleaned and deskinned rhizomes were grated to be used for extraction. The grated rhizomes were weighed and kept in a flask covered with aluminium foil. The parameters studied in proximate analysis were moisture content, water and alcohol extractives, total ash, water soluble ash and acid insoluble ash. The phytochemicals screened were terpenoids, alkaloids, flavonoids, glycosides, cardiac glycosides and volatile oils. Determination of all parameters were carried out in triplicates.

Preparation of plant Extracts for Phytochemical Screening

Preparation of Plant Extracts- Cold Extraction

A solution of 2 grams each of rhizome powder and freshly grated rhizomes in 100ml petroleum ether was prepared. The flask was covered tightly with a cotton plug and refrigerated for 24 hours. The extract was filtered and then used for preliminary phytochemical tests.

Preparation of plant extracts-reflux: (with rhizome powder)

A solution of 6 grams rhizome powder was prepared each in 60ml water, methanol, ethyl acetate and hexane. The solutions were refluxed at 69°C using a conical flask covered with a funnel for one hour in a water-bath. The respective extracts obtained were cooled and filtered twice- first with a regular filter paper and a second time using Whatman filter paper no. 1. The filtrate obtained was evaporated to dryness. The dried residue was weighed and reconstituted in the extraction solvents to obtain a 100mg/ml stock solution to be used for preliminary phytochemical tests.

Preparation of Plant Extracts- Reflux: (With freshly grated rhizomes)

A solution of 6 grams of freshly grated rhizome pieces was prepared each in 60ml water, methanol, ethyl acetate and hexane. The solutions were refluxed at 69°C using a conical flask covered with a funnel for one hour in a water-bath. The respective extracts obtained were cooled and filtered twice- first with a regular filter paper and a second time using Whatman filter paper no. 1. The filtrate obtained was evaporated to dryness. The dried residue was weighed and reconstituted in the extraction solvents to obtain a 100mg/ml stock solution to be used for preliminary phytochemical tests.

Preparation of plant extract for High Resolution Liquid Chromatography Mass spectrophotometry (HR- LCMS)

The ethyl acetate extract obtained from freshly grated rhizomes were cooled and filtered twice- first with a regular filter paper and a second time using Whatman filter paper no. 1. The filtrate obtained was evaporated to dryness. The dried residue was weighed and reconstituted in ethyl acetate to obtain a 100mg/ml stock solution. The analysis of the extracted essential oil was done by High Resolution Liquid Chromatography Mass Spectrophotometry. The instrument used was Q-Exactive Plus Biopharma, Thermo Scientific. The column details were Hypersil Gold 3 micron 100x2.1mm (Thermo Scientific). Solvent A was 0.1% formic acid in Milli-Q water and solvent B was methanol. The run time was 35 minute. The flow rate was 0.3ml/min. The Data Acquisition Software used was Thermo Scientific Xcalibur, Version 4.2.28.14. The Data Processing Software was Compound Discoverer 3.2 SP1.

Qualitative Phytochemical Analysis

The different extracts obtained through different extraction procedures using more than one type of sample source were subjected to phytochemical screening using standard methods described by Harborne, 1998.[15]

Test for Terpenoids

Vanillin- sulphuric acid reagent

To 1ml of extract, 1ml of vanillin-sulphuric acid reagent was added and heated. The tube was scored for expected colouration of purple-blue corresponding to presence of terpenoids.

Anisaldehyde reagent

To 1ml of extract, 1ml of anisaldehyde reagent was added and heated. The tube was scored for expected colouration of pink-purple corresponding to presence of terpenoids.

Test for alkaloids

Dragendorff reagent

To 1ml of extract, 1ml of Dragendorff reagent was added. The tube was scored for expected colouration of red-brown corresponding to presence of alkaloids.

Wagner's reagent

To 1ml of extract, 1ml of Wagner's reagent was added. The tube was scored for expected colouration of orange-red corresponding to presence of alkaloids.

Test for flavonoids

Pew's test

A pinch of zinc dust was added to 1ml of plant extract, followed by addition of concentrated hydrochloric acid. The tube was scored for expected colouration of cherry-red corresponding to presence of flavonoids.

Sodium hydroxide test

To 1ml of plant extract, 1ml of 10% aqueous sodium hydroxide and 1ml of 5% aqueous hydrochloric acid were added. The tube was scored for expected colouration of canary yellow corresponding to presence of flavonoids.

Test for Glycosides

Kedde's test

1ml of plant extract was mixed with 1ml of 2% ethanolic 2,3-dinitrobenzoic acid, followed by addition of 2-3 drops of 20% aqueous sodium hydroxide. The tube was scored for expected colouration of pink-purple or blue-violet corresponding to presence of glycosides.

Keller-Kiliani test

1ml of plant extract was added to a solution of glacial acetic acid to which 2-3 drops of 5% aqueous ferric chloride have been added. Concentrated sulphuric acid was added along the sides of the test tube without mixing the solutions. The tube was scored for expected brown-red coloured ring at the junction of the liquids and blue-green colour of the upper phase corresponding to the presence of glycosides with 2-deoxysugar moieties.

Test for cardiac glycosides

Baljet's reagent

To 1ml of plant extract, 1ml of Baljet's reagent was added. The tube was scored for expected colouration of orange-red colour corresponding to presence of cardiac glycosides.

Test for Volatile Oils

Characteristic odour

1ml of plant extract was tested for characteristic odour.

Filter paper test

2 drops of plant extract were added to a filter paper of 12.5cm diameter (Filtroll filter paper) and kept a room temperature. The filter paper was checked for a greasy spot and evaporation.

RESULTS AND DISCUSSION

Proximate analysis results of *Hedychium coronarium* for the parameters of moisture content, total ash, water soluble ash, acid insoluble ash and extractive values (water and alcohol) are presented in Table 1. These values of individual parameters have been compared with the standard values of species of Hedychium as given in Ayurvedic pharmacopoeia of India, Volume I.

Table 1: Proximate analysis values in percentage

S.No	Parameters	Observed values in percentage	Standard Values of *Hedychium spicatum* (closely related species) (The Ayurvedic Pharmacopoeia of India, Vol. I)
1	Moisture	15.80 ±0.163	-
2	Water extractive	15.080 ± 0.865	Not less than 8 percent
3	Alcohol extractive	4.0426 ±0.424	Not less than 4 percent
4	Total ash	3.616 ±0.840	Not more than 8 percent
5	Water soluble ash	1.676 ± 0.966	-
6	Acid insoluble ash	0.690±0.0282	Not more than 2 percent

*Observations are mean of three readings

Phytochemical screening of the samples in different forms subjected to different methods of extraction are summarized in table 2 and photo plate 1 & 2. The report showed the presence of terpenoids in all non-polar solvents like methanol, ethyl acetate, petroleum ether and hexane. Presence of volatile oils were ascertained with characteristic odour and filter paper test.

Table 2: Qualitative Phytochemical Analysis

Tests	Water extract		Methanol extract		Ethyl acetate extract		Petroleum Ether Extract (cold extract)		Hexane extract	
	rhizome powder	freshly grated	rhizome powder	freshly grated	rhizome powder	freshly grated	rhizome powder	freshly grated	rhizome powder	freshly grated
Terpenoids										
Vanillin-sulphuric acid reagent	-	-	+	+	+	+	+	+	+	+
Anisaldehyde-sulphuric acid reagent	-	-	+	+	+	+	+	+	+	+
Alkaloids										
Dragendroff's reagent test	-	-	-	-	-	-	-	-	-	-
Wagner's reagent test	-	-	-	-	-	-	-	-	-	-
Flavonoids										
Pew's test	-	-	-	-	-	-	-	-	-	-
Sodium Hydroxide test	-	-	-	-	-	-	-	-	-	-
Glycosides										
Kedde's test	-	-	-	-	-	-	-	-	-	-
Keller-Kelliani test	-	-	-	-	-	-	-	-	-	-
Cardiac Glycosides	-	-	-	-	-	-	-	-	-	-
Volatile oils										
Characteristic odour	-	-	+	+	+	+	+	+	+	+
Filter paper test	-	-	+	+	+	+	+	+	+	+

Note: '+'= detected, '-'= not detected

Photo plate 1: Test for terpenoids

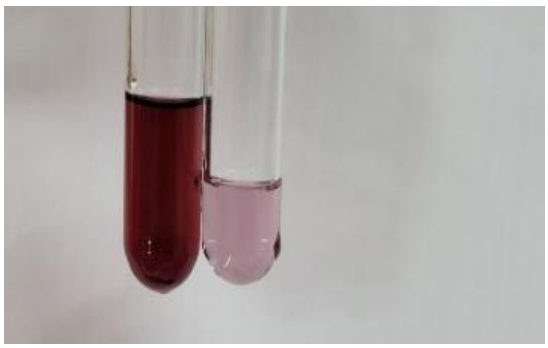
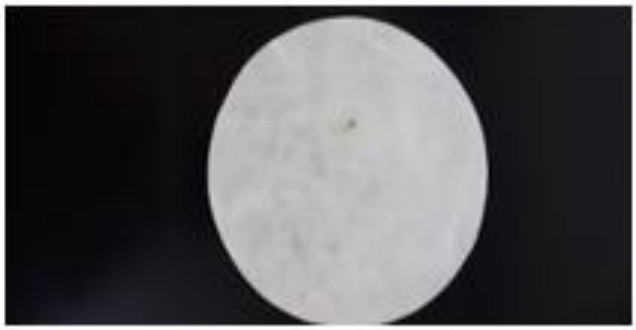


Photo plate 2 : Test for volatile oils

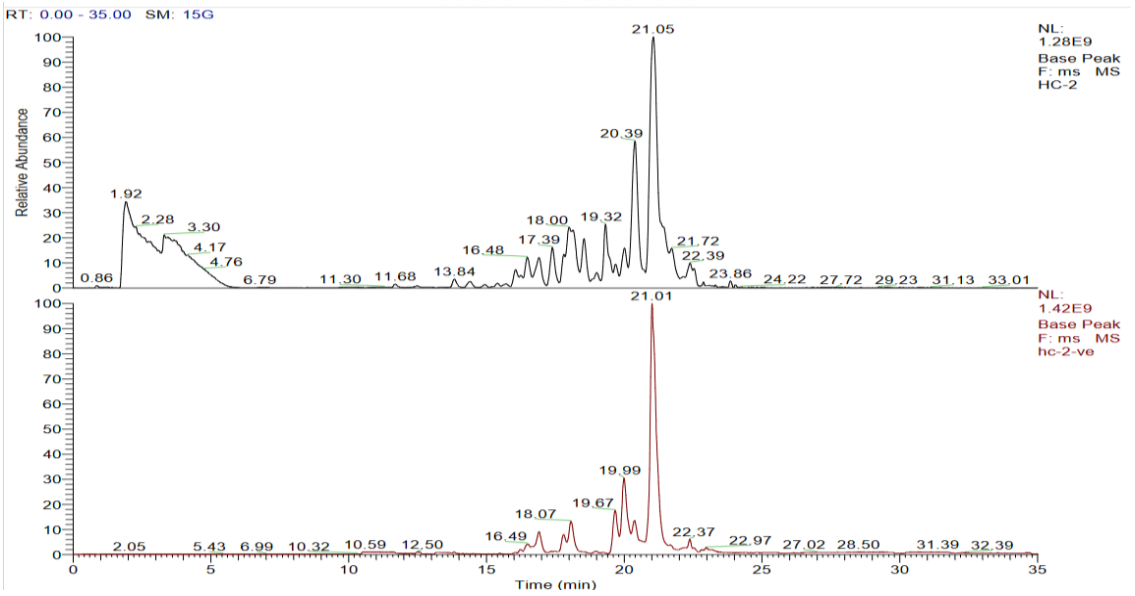


High Resolution Liquid Chromatography Mass Spectrophotometry analysis shows the presence of important metabolites as components of essential oils like eucalyptol (1,8-cineole), caryophyllene oxide, camphor, linoleic acid, ricinin, phloroglucinol, 6-gingerol, carvone (Table 3).

Table 3: List of Important Components in Essential oils of *Hedychium coronarium* by HR- LCMS

Name	Formula	Molecular Weight	Compound Class
Arjungenin	$C_{30}H_{48}O_6$	504.3451	Insect feeding deterrant
Caryophyllene oxide	$C_{15}H_{24}O$	220.1827	Endogenous metabolites; natural products/ medicines, excipients additives/ colourants
Camphor	$C_{10}H_{16}O$	152.1201	Endogenous metabolites, natural products/ medicines extractables, leachables, personal care products, cosmetics, Textile chemicals
Carvone	$C_{10}H_{14}O$	150.1045	Endogenous Metabolites; excipients/additives/ colorants
Eucalyptol (1,8-cineole)	$C_{10}H_{18}O$	154.1358	Metabolites; natural products/medicines/ colourants/ additives
6-Gingerol	$C_{17}H_{26}O_4$	294.1831	Endogenous metabolites, natural products/ Medicines
4-hydroxy coumarin	$C_9H_6O_3$	162.0317	Endogenous metabolites
Linoleic acid	$C_{18}H_{32}O_2$	280.2402	Endogenous metabolites
Methyl cinnamate	$C_{10}H_{10}O_2$	162.0681	Endogenous metabolites, personal care products/ Cosmetics
Palmitic acid	$C_{16}H_{32}O_2$	256.2402	Endogenous metabolites
Phloroglucinol	$C_6H_6O_3$	126.0317	Therapeutics/prescription drugs, endogenous metabolites, textile Chemicals, auxiliary/ dyes/ extractables, leachables
Ricinin	$C_8H_8N_2O_2$	164.0586	Natural toxins, endogenous metabolites
Vanillin	$C_8H_8O_3$	152.0473	Endogenous metabolites, personal care products/ cosmetics Industrial chemicals

Chromatogram



It has been reported that the moisture content in different members of Zingiberaceae remain close to 75% whereas in *Costus igneus,* member of the same family has only 7.4% moisture.[16,17]. The moisture content in *Hedychium spicatum* is 7.34%[18]. The moisture content in *Hedychium coronarium* is 15.80% in the present study. The moisture content is within the acceptable limits of about 6%-15% for most plant drugs[5]. High level of moisture in crude drugs is indicative of proneness to microbial attack. On the

other hand, low moisture content while eliminating the problem of infection reduces errors in the estimation of actual weight of drug material. It reduces the activities of hydrolytic enzymes which may destroy the active components [5]. The ash content of a crude drug represents the residue remaining after incineration that burns out all organic matter. It is thus a representative of total inorganic salts that occur in drugs naturally and adhere to it as carbonates, phosphates, silicates of sodium, potassium, calcium and magnesium. Total ash thus consists of carbonates, oxides, phosphates, silicates and silica. Crude drugs are sometimes adulterated with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drugs with different inorganic contents [19]. Acid insoluble ash which is a part of total ash insoluble in dilute hydrochloric acid is also recommended for certain drugs. Adhering dirt and sand maybe determined by acid insoluble ash content. Water soluble ash gives the crude estimate of the water-soluble extractable matter present in the ash [5]. Sometimes exhausted powder is added as an adulterant for crude drugs. Therefore, water soluble ash value is quite a reliable parameter to judge such types of adulteration. In the present study as against 0.690% acid insoluble ash, water soluble ash is nearly three times. This value is 1.66%. Water soluble ash in *Hedychium coronarium* is significantly high representing purity of the crude drug. The total ash in ginger rhizome which is a member of the same family is 6% and water-soluble ash content is 1.7% which is closer to the value of *Hedychium coronarium* rhizome[19]. The approximate measures of chemical constituents of a crude drug are obtained from extractives. These extractives are prepared using polar and non-polar solvents. It helps in selecting a suitable solvent. The water extractive and alcohol extractive values for *Hedychium coronarium* are 15.080% and 4.042% respectively whereas the water extractive and alcohol extractive values of ginger is 10% and 4.5% respectively[19]. In the lack of previous studies, these parameters provide referential information for proximate evaluation of *Hedychium coronarium* rhizomes.

The results of phytochemical screening tests of the samples indicate their preference towards non-polar solvents for extraction along with volatile oils. In all non-polar solvents, characteristic odour due to volatile oil was confirmed with filter paper test. A greasy spot was observed on the filter paper indicating the presence of fixed oil contents (Photo plate 2).

Extraction with ethyl acetate subjected to high resolution liquid chromatography mass spectrophotometry gave better resolution of bioactive compounds. The profile showed matching results with those obtained by earlier researchers.[20,21]. However, commonly reported bioactive molecules present in the essential oils like α-pinene, β-pinene and linalool could not be detected in our investigation. Rhizomes, being perennating organs, their tissue differentiation and corresponding metabolite make-up varied greatly at different point of time. The environmental conditions, the soil type also control the total reserves of rhizomes and the proliferation rate which is directly proportional to these conditions.

CONCLUSION

Proximate analysis is done to assess the quality of crude drugs. The results of proximate analysis of the rhizome powder of *Hedychium coronarium* for the parameters of moisture content, extractives (water & alcohol), total ash, water soluble ash and acid insoluble ash in percentage was found to be 15.80±0.163, 15.080 ±0.865, 4.0426±0.424, 3.616±0.840, 1.676±0.966, 0.690±0.0282 respectively. The phytochemical screening showed the presence of terpenoids and a characteristic odour was detected in all non-polar solvents. A greasy spot on the filter paper indicated the presence of fixed oil contents. The contents of the ethyl acetate extract were analysed by high resolution liquid chromatography mass spectrophotometry. The important bioactive molecules detected were arjungenin, caryophyllene oxide, gingerol, camphor, eucalyptol, linoleic acid, phloroglucinol, ricinin and vanillin.

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