PHYTOCHEMICAL, BIOLOGICAL SCREENING OF CAPSELLA BURSA AND HEDYCHIUM CORONARIUM AND FORMULATIONS OF OINTMENT OF HEDYCHIUM CORONARIUM EXTRACT

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Abstract:
A thorough phytochemical investigation of Hedychiumcoronarium J. Koenig, a plant with important therapeutic qualities, is presented in this work. The study looks into the existence of distinct secondary metabolites in various plant parts, both naturally occurring and in vitro, using methanolic extraction. These substances are evaluated both qualitatively and quantitatively using thin-layer chromatography (TLC). The results show that alkaloids, phenolic chemicals, glycosides, saponins, carbohydrates, flavonoids, reducing sugar, steroids, and terpenoids are present. Understanding the potential of plants in both conventional and modern medicine is aided by this research.
Keywords: medicinal plants, Heydchiumcoronarium, secondary metabolites, thin layer chromatography, phytochemical screening.
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1. Introduction

Nature has always been a first - rate drugstore, with its enormous range of plants that are known to have effective therapeutic qualities. Herbs are nature's gift to mankind and herbal renaissance is blooming across the world. Herbal medicines are assumed to be of great importance in the primary healthcare of individuals and local communities in many developing countries [1] . Medicinal herbs contain substances known to modern and ancient civilizations for their healing properties [2], [3], [4], [5], [6] and traditional use of these plants for their primary health care needs that mainly involves use of plant extracts and their active components[7] . Many of these natural products have been used as sources of a large number of pharmaceuticals, agrochemicals, flavours, fragrance ingredients, food additives, and pesticides [8], [9] . Products of primary

metabolism such as amino acids, carbohydrates and proteins are essential for continuance of life processes, whereas others like alkaloids, phenolics, steroids, terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological important [10].

According to World Health Organization (WHO) in 2008 more than 80 % of the world population relies on traditional medicines for

their primary health care needs[11] . Traditional & folklore medicine plays an important role in health services around the globe. Hedychiumcoronarium J. Koenig belonging to family Zingiberaceae, has many common names including butterfly ginger, butterfly lily, cinnamon jasmine, garland flower, and ginger lily, and is critically endangered in central India [12] , widely cultivated in Southeast Asian countries, South China, Taiwan, Japan, and Brazil.

This plant has tremendous medicinal properties. All plant parts are used in traditional as well as modern medicine. In Thailand boiled leaves of H. coronarium are applied to relieve stiff and sore joints [13] . In Peninsular Malaysia, boiled leaves are eaten for indigestion [14] . The base of the stem is used in swellings. The stem contains 43 - 48% cellulose and is useful in making paper. As an ornamental, it is cultivated for its sweet - scented flowers and attractive green foliage. Hedychium flowers are widely cultivated for their perfume, essence and ethnomedicine [15], [16] . The scent ranges from the rich gardenia like fragrance of H. coronarium to scents reminiscent of citrus, clove and coconut [17] . In Cuba, where it is the national flower it is known as the ‘‘flor de mariposa’’ (literally, ‘‘butterfly flower’’).

2. Materials and Methods

Collection of Plant material

The fresh parts of Hedychiumcoronarium were collected from the garden of approximately 1 year old plant and in vitro grown plants (approx.3 - 4 months old) rhizome, leaf and stem were performed. Both nature grown and hardened in vitro regenerated plants were collected and washed thoroughly under running tap water and then rinsed in distilled water, they were allowed to dry for some time. The leaves, rhizome and stem were separated and shade dried without any contamination for about 3 to 4 weeks. The dried plant sample was powdered in a blender and stored in airtight container.

Preperation of Extracts:

Ethanolic extract: Approximately 2g crude powder was extracted by cold percolation in methanol for 2 days. It was filtered and the filtrate was concentrated and used for further analysis.

Qualitative analysis by Thin Layer Chromatography Extract was to begin with, checked by Thin Layer Chromatography (TLC) on analytical plates over silica gel. TLC was carried out to isolate the principle components that were present in most effective extracts of plant. The different solvent systems of different polarities were prepared and TLC studies were carried out to select the solvent system capable of showing better resolution.

Method The glass plate coated with silica gel F254 was activated at 100ºC for 1 hr. in oven. The sample (5µl, 10µl, 15µl) of methanolic fraction of each sample was loaded with the help of a micropipette about 2 cm above the edge of the plate, as a small spot of solution and developed in a TLC chamber using suitable mobile phase. After the spot dried, the plate was propped vertically in a chromatography chamber pre - saturated with the solvent. The spot on the plate was positioned above the level of the solvent. The mobile phase used was Toluene: Ethyl acetate (93: 7), Hexane: Benzene: Methanol (1: 1: 1). The movement of the analyze was expressed by its retention factor (Rf). Values were calculated for different sample. The developed chromatograms were dried at room temperature and visualized under UV light and their Rf values were calculated using the formula.

$$R_f = \frac{\text{Distance run by sample}}{\text{Distance run by solvent}}$$

(R_f- Retention factor)

Phytochemical Analysis

The Phytochemical which are present in the extracts of H. coronarium were determined and quantified by standard procedures. The Phytochemical tests were carried out as described [18], [19], [20] . Qualitative Phytochemical screening of the aqueous and methanolic fraction of the nature grown plants (approx.1 year old) and in vitro grown plants (approx.3 - 4 months old) rhizome, leaf and stem were performed.

● **Test for tannins:** About 0.5g of powder sample of nature grown and in vitro grown plant parts was boiled in 20 ml of distilled water in a test tube and then filtered. The normal filtration method is used for the process, which includes a conical flask and filter paper.0.1 % $FeCl_3$ is added to the prepared samples and observed for brownish green or a blue black colouration, which shows the presence of tannins.

● **Test for Saponins:** About 2g of powdered sample was boiled with 20 ml of distilled water in a water bath and then filtered.10 ml of the filtered sample is mixed with 5 ml of distilled water in a test tube and shake it vigorously to obtain a stable persistent froth. The frothing is mixed with few drops of olive oil to observe the formation of emulsion, which indicates the presence of saponins.

● **Sodium bicarbonate test:** About 0.5ml of methanolic extract and few drops of sodium bicarbonate solution was added to it and shaken well. Appearance of a honey comb like frothing shows the presence of saponin.

● **Test for flavonoids:** Aqueous extract of plat sample few drops of 1% NH_3 solution is added in a test tube. Yellow colour is observed to confirm the presence of flavonoid.

● **Test for Terpenoids:** 0.5 ml of aqueous extract of the prepared sample was mixed with 2ml of $CHCl_3$ in a test tube.3 ml of concentrated H_2SO_4 is added carefully to the mixture to form a layer. A reddish brown ring like structure is formed at interface confirms the presence of terpenoids in the sample.

● **Test for Alkaloids:**

Wagner's test: 0.5ml of methanolic extract, add 2 - 3 drops of Wagner's reagent (freshly prepared solution containing 2g iodine and 6g of potassium iodide in 100ml water) appearance of brown or reddish precipitate shows positive test for alkaloid.

Mayer's test: 0.5 g of crude powder was defatted with 5% ethyl ether for 15 min, and the defatted sample was extracted for 20min with 5ml of aq. HCl on a boiling water bath. Then the mixture was centrifuged for 10min at 3000rpm.1ml of the filtrate was treated with few drops of Mayer's reagent (freshly prepared solution containing 1.36g mercuric chloride and 5g of potassium iodide, dissolved in 100 ml of water). Cream colour precipitate shows the presence of alkaloid.

● **Test for Cardiac glycosides:** 5ml of methanolic extract is added with 2ml of glacial acetic acid and 2 drops of $FeCl_3$ solution in it. Conc. HCl is added from the walls of the tube. Development of a brown colour at the junction indicates the presence of de - oxy sugars, characteristic of cardenolides.

3. Result and Discussion

Phytochemical analysis

The result confirms the presence of constituents which are known tit medicinal as well as physiological activities [21] . The preliminary Phytochemical analysis of the methanol extracts of H. coronarium (Table 1) revealed the presence of medicinally active constituents like flavonoids (Fig.5),
triterpenoids (Fig.6), saponin (Fig.7), alkaloid (Fig.8) and steroids in all the parts indicated the presence of several group of compounds in both nature grown as well as in vitro derived plant parts (Figs.1 - 4). From this study, findings showed that the leaves have more phytochemicals than the rhizome and stem. Presence of Alkaloid, Anthraquinones, Phenols, Flavonoid, Carbohydrate, Triterpenoids, Reducing sugar, Cardiac glycosides, Saponin, Steroid in nature grown and in vitro grown leaf extracts confirmed the presence of rich bioactive principles.

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Figure 1: Different parts of Hedychiumcoronarium J. Koenig, nature grown plant (Fig 1), in vitro hardened plant (Fig.2), nature grown rhizome (Fig.3), in vitro grown rhizome (Fig.4).

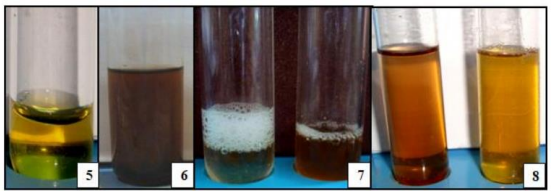

Figure 2: Test of flavonoid (Yellow colour Fig.5), test of terpenoid (Reddish brown coloration Fig.6), test of saponin (Frothing Fig.7), cardiac glycoside test (Brown ring Fig.8).

Table 1: Preliminary Phytochemical analysis of the methanol extracts of H. coronarium

S. No.	Secondary metabolite	Rhizome		Leaf		Stem	
		Nature grown	*In vitro*	Nature grown	*In vitro*	Nature grown	*In vitro*
1.	Alkaloid	+ve	+ve	+ve	+ve	+ve	+ve
2.	Anthraquinones	- ve	- ve	- ve	- ve	- ve	- ve
3.	Phenols	- ve	- ve	+ve	+ve	- ve	- ve
4.	Flavonoid	+ve	+ve	+ve	+ve	+ve	+ve
5.	Carbohydrate	+ve	+ve	+ve	+ve	+ve	+ve
6.	Triterpenoids	+ve	+ve	+ve	+ve	+ve	+ve
7.	Reducing sugar	+ve	+ve	+ve	+ve	+ve	+ve
8.	Cardiac glycosides	- ve	- ve	+ve	+ve	- ve	- ve
9.	Saponin	+ve	+ve	+ve	+ve	- ve	- ve
10.	Steroid	+ve	+ve	+ve	+ve	+ve	+ve

TLC (Thin Layer Chromatography)

Thin - layer chromatography continues to be an important method for qualitative analysis of plant products because of its inherent advantages—many samples can be analyzed simultaneously and quickly and multiple separation techniques and detection procedures can be applied. Extraction is an important step involved in the isolation of bioactive compounds from the plants with medicinal value.

The aim of every extraction process is rapid and effective isolation of compounds by using minimum amount of solvent. Traditional methods, for example percolation, exhaustive soxhlet extraction or direct extraction with boiling solvent under reflux are most often used [26] . Methanol is more frequently used than ethanol and acetone because of its higher extraction efficiency [27] . Extraction is removal of desired substances from undesired ones. Successful extraction involves selection of right solvent which can extract out maximum quantity of targeted chemicals, while minimizing the interference of unwanted components.

The present study has concentrated on the assessment of relative amount of the secondary metabolite in nature grown and in in vitro regenerated plant parts (stem, leaf, and rhizome). The Rf value of Rhizome and Root was 0.40 cm (Fig.10) which shows the presence of cineol (terpene). In nature grown leaves and in vitro regenerated leaves Rf value was 0.43 cm (Fig.9) which shows the presence of citral (terpene) in the TLC plate. The Rf value of the spots shows the presence of terpenoids in the present sample. All the samples also showed various additional spots between Rf value of 0.23 to 0.43.

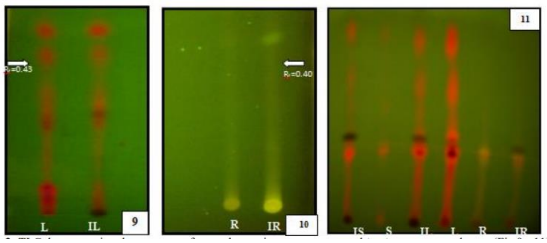

Figure 3: TLC demonstrating the presence of several spots in nature grown and in vitro regenerated parts (Fig.9 - 11). (IS - In vitro regenerated stem, S - Nature grown stem, IL - In vitro regenerated leaf, L - Nature grown leaf, R - Nature grown rhizome, IR - In vitro regenerated rhizome).

Maximum spots were observed in the in vitro regenerated leaf sample. TLC analysis of the in vitro regenerated stem, leaf and rhizome showed the present of terpenoid, saponins and flavonoids. The Rf value obtained in natural and in vitro regenerated leaf shows 0.30, 0.43, 0.46, 0.6 and in vitro root showed 0.40 respectively, suggesting the presence of many secondary metabolites in the sample. Results obtained from TLC of all extracts shows very good results that directing towards the presence of number of phytochemicals. Obtained phytochemicals gives different Rf values in different solvent system. Obtained photochemical shows variation in their Rf values provides a very important information in understanding of their polarity and also helps in the choosing of suitable solvent system for separation of pure

compounds buy column chromatography [28] .

4. Conclusion

This study reveals a wide variety of secondary metabolites and offers insightful information about the phytochemical composition of Hedychiumcoronarium J. Koenig. The research highlights the potential of plants as a source of bioactive chemicals for therapeutic purposes through rigorous extraction and TLC analysis. These results open up new avenues for research on medicinal plants and will be useful in the development of therapeutic medicines utilizing Hedychiumcoronarium.

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