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Phytochemical analysis of *in vivo* and *in vitro* plants of *Hedychium coronarium* (J.) Koenig: a preliminary report

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ABSTRACT

Hedychium coronarium (J.) Koenig is a medicinal and aromatic herb. That plant is widely used in different traditional system of medicines for the treatment of various diseases. In this study, a preliminary comparative phytochemical analysis was done between the leaf, rhizome and root extract of normal field grown (*in vivo*) plant and tissue culture regenerated (*in vitro*) plants. The results revealed the presence of saponins, flavonoids, tannins and phenolics as well as absence of steroids and terpenoids in various extracts of different plant parts of both *in vivo* and *in vitro* plants. These findings could provide the basis for the production of new drugs for treating various ailments.

Hedychium coronarium (J.) Koenig (Gurudalanga) is a perennial, erect and rhizomatous herb of the family Zingiberaceae. It is commonly known as butterfly ginger or butterfly lily or white garland lily. This plant has curative properties and is used in traditional systems of medicine like Ayurveda, Homeopathy, Siddha and Unani. Its rhizome is used as a folkloric medicine for treatment of fever, headache, inflammation, insomnia, diabetes, tonsillitis, infected nostrils and sharp pain due to rheumatism (Bhandary *et al*., 1995; Jain *et al*., 1995; Bisht *et al*., 2012; Verma and Bansal, 2012; Kiem *et al*., 2012; Chen *et al*., 2013; Mohanty *et al*., 2013). Its root is also medicinally used for the treatment of urinary stone (Bahuguna and Kumar, 2014). The essential oil, derived from various parts like leaves, flowers and rhizome of the plant, possesses antimicrobial, anti-inflammatory and analgesic activities (Bisht *et al*., 2012). Besides, the juice of the mature seeds is used for the treatment of hair and skin (Parida *et al*., 2013).

Studies revealed that the secondary metabolites including saponins, tannins, flavonoids, alkaloids, terpenoids, etc. have immense curative properties and are responsible for the medicinal use of the plants (Savithramma et al., 2011). Like other medicinal plants, the therapeutic properties of *H. coronarium* are also possibly due to presence of different secondary metabolites. So, phytochemical analysis of plant parts of this valuable medicinal plant is necessary to evaluate the presence of phytoconstituents. Till date only a handful of reports are available on compound isolation and structural elucidation as well as phytochemical analysis by using different solvent extracts (methanol, aqueous, petroleum ether, ethanol and chloroform) of different parts (rhizomes and roots) of *H. coronarium* (Sah *et al*., 2012; Chen *et al.,* 2013; Singh *et al*., 2013; Singh and Bag, 2013; Bahuguna and Kumar, 2014). Chen *et al*., (2013) isolated and determined the structure of four new labdane-type diterpenoids from the rhizome of *H. coronarium.* Rest of

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the reports on phytochemical analysis are of preliminary in nature (Sah *et al*., 2012; Singh *et al*., 2013; Singh and Bag, 2013; Bahuguna and Kumar, 2014). To the best of our knowledge, there is no literature on phytochemical screening of leaves of *H. coronarium*. Most importantly there is not a single report on phytochemical analysis of *in vitro* regenerated plants of *H. coronarium*. Therefore, the objective of this work was preliminary phytochemical screening of original field grown mother plant (*in vivo*) and *in vitro* regenerated soil established (acclimatized) (*in vitro*) plants, which can be the basis for discovery of new drugs.

In this study the plant materials -leaf, root and rhizome- were collected from the *in vivo* grown mother plant and *in vitro* regenerated soil established plants of *H. coronarium* from the garden of Department of Botany, Ravenshaw University, Cuttack, Odisha, India. The collected parts of both the sources were washed thoroughly in tap water and then rinsed in distilled water. All the plant materials were cut into small pieces (0.5-1.0 cm) followed by drying under shade and final drying in an oven (Bio Techno Lab, India) at a temperature of 40 ^{0}C for 3-4 days. They were then coarsely powdered and stored in air tight container at room temperature for further experiments. Five solvents, based on their polarity, namely aqueous, n-hexane, methanol, acetone and chloroform were selected for extraction purpose. Five grams of powered tissue of both *in vivo* and *in vitro* plant source were each individually dispensed in 60 ml screw capped tubes (Borosil, India) containing 30 ml each of aqueous (distilled water), n-hexane, methanol, acetone and chloroform. All these were kept at room temperature (25-28 ^{0}C) for 24 h. Then the extracts were filtered through Whatman No. 1 filter paper. The collected filtrates were used for the preliminary phytochemical analysis by methods of Harborne (1973).

Test for tannins: To test the presence of tannins, 1 ml of extract was taken in a test tube and dissolved in 50 ml of distilled water followed by heating for 10 min. After cooling few drops of 1% lead acetate [$Pb(C_2H_3O_2)_2$] was added, change of colour of the sample from yellow to green and formation of dark green precipitate indicated the presence of tannins. *Test for saponins*: To confirm the presence of saponins, 1 ml of extract was diluted with 20 ml distilled water and was agitated for 5-10 min. The formation of approximately 1 cm layer of foam at the top indicated the presence of saponins. *Test for flavonoids*: Few drops of 10% sodium hydroxide (NaOH) were added to 1 ml of the extract. An intense yellow colour of plant extract became colourless on addition of few drops of dilute hydrochloric acid (HCl) which indicated the presence of flavonoids. *Test for phenolics*: Three-four drops of 10% ferric chloride ($FeCl_3$) was added to 1 ml of the extract and 2 ml of distilled water. Formation of bluish black colour showed the presence of phenolic compounds. *Test for steroids*: To check the presence of steroids, 1 ml of extract was dissolved in 10 ml chloroform ($CHCl_3$). Then equal volume of concentrated sulphuric acid (H_2SO_4) was added carefully through the side walls of test tube. Development of two differently coloured layers (red and green) indicated the presence of steroids. *Test for terpenoids*: Formation of a reddish brown interface indicated the presence of terpenoids, when 5 ml of extract was mixed with 2 ml of chloroform ($CHCl_3$) followed by addition of 3 ml of concentrated sulphuric acid (H_2SO_4).

Adequate work is yet to be done on *H. coronarium* to know the presence of secondary metabolites in different plant parts in addition to the active principles behind the therapeutic properties of the plant. Our preliminary phytochemical screening revealed the presence of saponins in all the extracts of both *in vivo* and *in vitro* leaf samples whereas, flavonoids were found in all extracts except n-hexane of both samples. Of the five extracts tested, it was found that tannins and phenolic compounds were present only in three extracts i.e. aqueous, methanol and acetone extract of both leaf samples (Table 1).

The phytochemical screening of *in vivo* and *in vitro* root extracts of *H. coronarium* revealed the presence of saponins in all the extracts of both samples. Tannins and flavonoids were present in both *in vivo* and *in vitro* samples in aqueous, methanol and acetone extract. Besides, phenolic compounds were observed in methanol extract of both the samples. Interestingly, acetone extract analysis for phenolic compounds revealed their presence only in *in vivo* sample (Table 1).

All extracts of both sources of rhizome samples showed the presence of saponins (Table 1). However, flavonoids were present in all the extract of both samples except n-hexane extract. Phenolic compounds were observed in both samples of aqueous, acetone and chloroform extracts, whereas methanolic extracts of only *in vitro* sample showed the presence of phenols. Aqueous, acetone and methanol extract analysis of both the samples indicated the presence of tannins. However, chloroform extract analysis for tannins revealed their presence only in *in vivo* sample.

Further in this study, the phytochemical screening of all the extracts of root, rhizome as well as leaf as the sources for steroids and terpenoids were negative. This result is not in agreement with the report of Singh and Bag (2013) and Singh *et al*. (2013), where aqueous and methanolic extracts of rhizome showed the presence of steroids and terpenoids. The presence of saponins in all the extracts of rhizome, root

Table 1

Preliminary phytochemical screening of *in vivo* and *in vitro* leaf, root and rhizome of *H. coronarium*

	Tannins		Saponins		Flavonoids		Phenolic compounds		Steroids		Terpenoids	
	In vivo	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*
						Leaf						
Aqueous	+	+	+	+	+	+	+	+	-	-	-	-
n-hexane	-	-	+	+	-	-	-	-	-	-	-	-
Methanol	+	+	+	+	+	+	+	+	-	-	-	-
Acetone	+	+	+	+	+	+	+	+	-	-	-	-
Chloroform	-	-	+	+	+	+	-	-	-	-	-	-
						Root						
Aqueous	+	+	+	+	+	+	-	-	-	-	-	-
n-hexane	-	-	+	+	-	-	-	-	-	-	-	-
Methanol	+	+	+	+	+	+	+	+	-	-	-	-
Acetone	+	+	+	+	+	+	+	-	-	-	-	-
Chloroform	-	-	+	+	-	-	-	-	-	-	-	-
						Rhizome						
Aqueous	+	+	+	+	+	+	+	+	-	-	-	-
n-hexane	-	-	+	+	-	-	-	-	-	-	-	-
Methanol	+	+	+	+	+	+	-	+	-	-	-	-
Acetone	+	+	+	+	+	+	+	+	-	-	-	-
Chloroform	+	-	+	+	+	+	+	+	-	-	-	-

(+ = Presence; - = Absence)

and leaf of this study corroborates earlier reports (Sah *et al*., 2012; Singh and Bag, 2013; Singh *et al*., 2013) on the presence of saponins in rhizome and roots.

Before the pharmaceutical use of *in vitro* regenerated plants, there is always need to check and compare the phytochemicals of *in vitro* regenerated plants with that of a field grown control mother plant for qualitative and quantitative confirmation and promising biological activity. The phytochemical analysis of leaf, rhizome and root of both *in vivo* and *in vitro* plants of *H. coronarium* in this study revealed the presence of saponins, flavonoids, tannins, and phenolic compounds. Saponins and flavonoids are known for their anti-inflammatory and antimicrobial effects (George *et al*., 2002; Cushnie and Lamb, 2005; Antonisamy *et al*., 2012). Tannins have also vital role in inflammation (Antonisamy *et al*., 2012). Phenolic compounds and flavonoids have been reported to contribute for the antioxidant activities of medicinal plants (Verma *et al*., 2012). Thus, it may be assumed that the medicinal properties of *H. coronarium* is attributed to saponins, tannins, flavonoids and other phytochemicals present therein. In conclusion, the finding suggested that the *in vitro* regenerated plants have potential to be used as raw material for pharma industries. However, further work is necessary to isolate, characterize and compare the active constituents present in *in vivo* and *in vitro* regenerated *H. coronarium*.

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