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Chromatographic and spectroscopic fingerprint analysis of *Hedychium coronarium* rhizome extracts through HPLC, GC-MS and FTIR

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ABSTRACT

Hedychium coronarium J. koenig. is an important medicinal and aromatic plant of Zingiberaceae. Bioactive constituents present in the extract have created a lot of interest because of its tremendous biological activity. The biggest challenge lies in the authentic identification of the drug, hence it is of utmost importance to develop spectral and chromatographic fingerprint that represents pharmacologically active and chemically characteristic components of *H. coronarium*. The present study was initiated to carry out chemical fingerprinting using HPLC, FTIR and GC-MS. A total of twelve absorption peaks were present in the IR spectra which can be used to characterize the extract. This study also attempts to develop HPLC fingerprint of the rhizame extract. Observation on HPLC spectra shows the presence of twelve distinct peaks. The IR spectra show the presence of peak at frequencies between ranges from 467.65 to 3327.57 cm^{-1}. FTIR analysis confirmed the presence of alkane, alkene, alcohol, amines, acid and halogens in the extract. A total of 21 phytoconstituents was identified in the methanolic extract from the rhizome extract of the plant by GC-MS. These chemical fingerprinting would be of commercial significance for quality control and authentication of *H. coronarium* extracts.

1. Introduction

Herbal medicines and their preparations are being used extensively throughout the world for combating various diseases. Inferior quality of herbal drug is a hindrance for its globalization and modernization. Hence ensuring their quality is of utmost importance. Quality control of herbal drug is more challenging since it involves a high level of chemical complexity due to diverse bioactive components. Furthermore, loss of principal active components may result in loss of pharmacological action of the plant. Therefore, significant efforts have been made for qualitative and quantitative characterization of samples using fingerprint technologies. (Lin *et. al.*, 2001). WHO also stresses the importance of analytical techniques for standardization of herbal drugs.

Hedychium coronarium J. Koenig (family *Zingiberaceae*) commonly known as Butterfly ginger is a rhizomatous herb widely cultivated in tropical and subtropical regions (Ray *et. al*., 2016). Rhizome extracts of *H.* coronarium are used as drug in traditional herbal medicine for the treatment of tonsillitis, infected nostrils, tumor, and fever. It is also used as a febrifuge, tonic, excitant and anti-rheumatic in the Ayurvedic system of Indian medicine (Jain *et. al*., 1995). The essential oil extracted from leaves, flowers and rhizome of this plant have potent antimicrobial, antifungal, anti-inflammatory, antibacterial and analgesic effects. Coronarin D, a labdane diterpenoid present in *H. coronarium* possess diverse pharmacological activities supported by various investigations. Coronarin D has shown to inhibit NF-Kβ leading to induction of apoptosis (Kunnumakkara *et. al*, 2008) and inhibit the release of *β*-hexosaminidase from RBL-2H3 cells (Morikawa *et.al*, 2002).Pharmacological actions reported are antibacterial (Reuk-ngam *et. al.*, 2014) and antifungal activities (Kaomongkolgit *et. al.*, 2012). In the present study chemical fingerprint has been developed using HPLC, FTIR and GCMS to control the quality of herbal drug of *H. coronarium*.

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2. Materials and methods

2.1. Plant source and extract preparation

Rhizomes of *H. coronarium* was collected in October 2015, at the flowering stage in the Ushabali valley (latitude 19° 56' 31.3" N, longitude 83° 39' 22.3" E), Phulbani Territorial Forest Division, at an elevation of 745 m above the sea level. The plant was authenticated by Dr. P.C. Panda, Principal Scientist, (Taxonomy and Conservation Division, RPRC, Bhubaneswar) and voucher specimen of plant (9741) was housed in the herbarium of Regional Plant Resource Centre, Bhubaneswar. Rhizomes were shade dried, powdered and subjected to extraction in solvent methanol using soxhlet apparatus for 8 h. The extract was filtered and the solvent was removed in a rotary evaporator at 50°C. Extracts were stored at 4°C for future analysis.

2.2. Chromatographic conditions of HPLC analysis

HPLC system for chromatographic analysis consisted of a separation module (Waters 600E) equipped with Empower software (Waters) and comprising of quaternary pump, an in-line vacuum degasser and a photodiode array detector (Waters 2996). The chromatographic separation was carried out on a Cosmosil C_8 column (250 × 4.6 mm, 5 ìm i.d.) using an isocratic elution. The mobile phase consisted of a mixture of solvent acetonitrile (80%, A) and water (20%, B). The solvent flow rate was 1.0 ml/min. The injection volume was 10 µl and the column temperature was ambient. The photo diode array detector wavelength was set at 254 nm.

2.3. Fourier Transform Infrared Spectrophotometer analysis

Dried powder of methanolic extract was used for FTIR analysis. 10 mg of the extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare sample disc. The powdered sample was loaded in FTIR spectrophotometer (Jasco, FTIR-6000 series), with a scan range from 400 to 4000 cm^{-1}.

2.4. Gas Chromatography mass spectrometry analysis

GC-MS analysis of this extract was performed using THERMO TRACE 1300 gas chromatography equipped with THERMO TSQ 8000 mass detector. 0.1 µl of the extract was injected in split less mode using helium as the carrier gas at a constant flow rate of 1 ml/min. The column used was TG 5MS (30 m x 0.25 mm, 0.25 µm) silica capillary column. For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Ion source temperature is maintained at 230°C. The injector temperature is maintained at 250°C whereas transfer line temperature is maintained at 280°C. The mass spectrometer was scanned from a mass range of 50-600 amu. Compounds were identified using the NIST Mass Spectral Database.

3. Results and discussion

In this work, a method based on reverse phase HPLC separation combined with PDA detection has been developed for analysis in *H. coronarium*. An isocratic elution was chosen since it is simple, requires only one pump and minimizes the variation of baseline and ghost peaks. For RP-HPLC, various columns are available, but a Cosmosil C_8 column (250 × 4.6 mm, 5 ìm i.d.) was preferred because its peak shape and resolution were better. Among the different mobile phases employed, acetonitrile and water (80:20 v/v) was found to be suitable for analysis. Further, a flow rate of 1 ml/min and an injection volume of 10 ìl along with UV detection at 254 nm provided the optimal conditions for analysis of the compounds. The results from HPLC crude extract profile for rhizome extract of *H. coronarium* showed the presence of 12 peaks at the retention time between 0 to 12 minutes (Fig. 1).

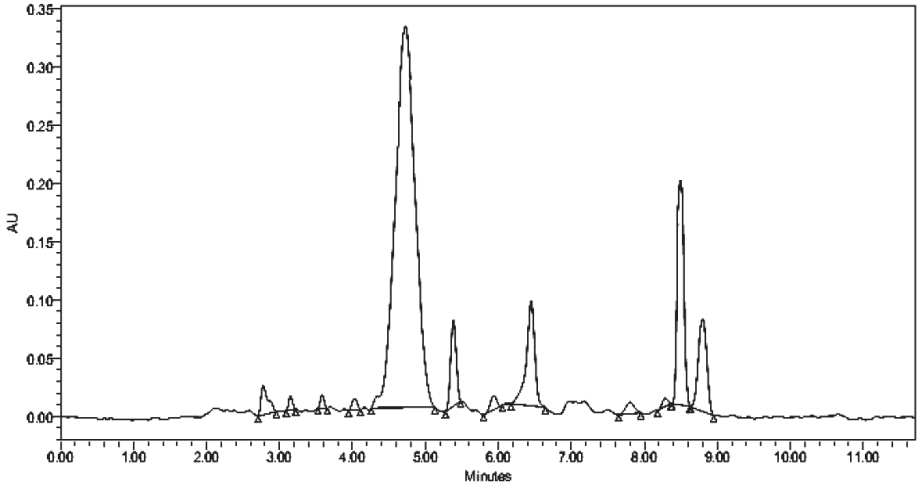

Fig. 1: HPLC Chromatogram of *H.coronarium* rhizome methanolic extract.

The peak spectrum of rhizome extract showed the highest peak spectrum at 4.703, 8.491 min. while, at 6.448, 8.797, 5.382 and 2.777 min showed a moderate peak spectrum (Table 1).

Table 1

HPLC results of rhizome extract of *H.coronarium*

Peak Name	RT	Area	Area %	Height
Peak 1	2.777	168911	1.70	25204
Peak 2	3.150	44555	0.45	12223
Peak 3	3.585	41272	0.41	11492
Peak 4	4.029	48879	0.49	9717
Peak 5	4.703	6206929	62.32	327504
Peak 6	5.382	394219	3.96	73869
Peak 7	5.930	73511	0.74	12230
Peak 8	6.448	723480	7.26	90439
Peak 9	7.802	82169	0.82	9808
Peak 10	8.282	37710	0.38	7243
Peak 11	8.491	1496133	15.02	297653
Peak 12	8.797	642461	6.45	79280

RT: Retention time in minutes (min).

Table 2

FTIR spectral peak values and functional groups of rhizome extracts of *H. coronarium*

S.No	Peak values	Functional groups
1	3327.57	Amine
2	2919.70	Alkane
3	1650.77	Alkene
4	1385.60	Alkane
5	1265.07	Carboxylic acid
6	1175.40	Amine
7	1057.76	Alcohol
8	827.31	Aromatic alkene
9	775.24	Aromatic alkene
10	698.11	Aromatic alkene
11	625.79	Aromatic alkene
12	467.65	Halogen

Peak values are expressed in wave number (cm^{-1})

Results of FTIR spectroscopic analysis in the methanolic extracts of *H. coronarium* have revealed the existence of various chemical constituents (Fig. 2). The absorption bands, the wave number (cm^{-1}) of prominent peaks obtained from absorption spectra are described in Table 2.

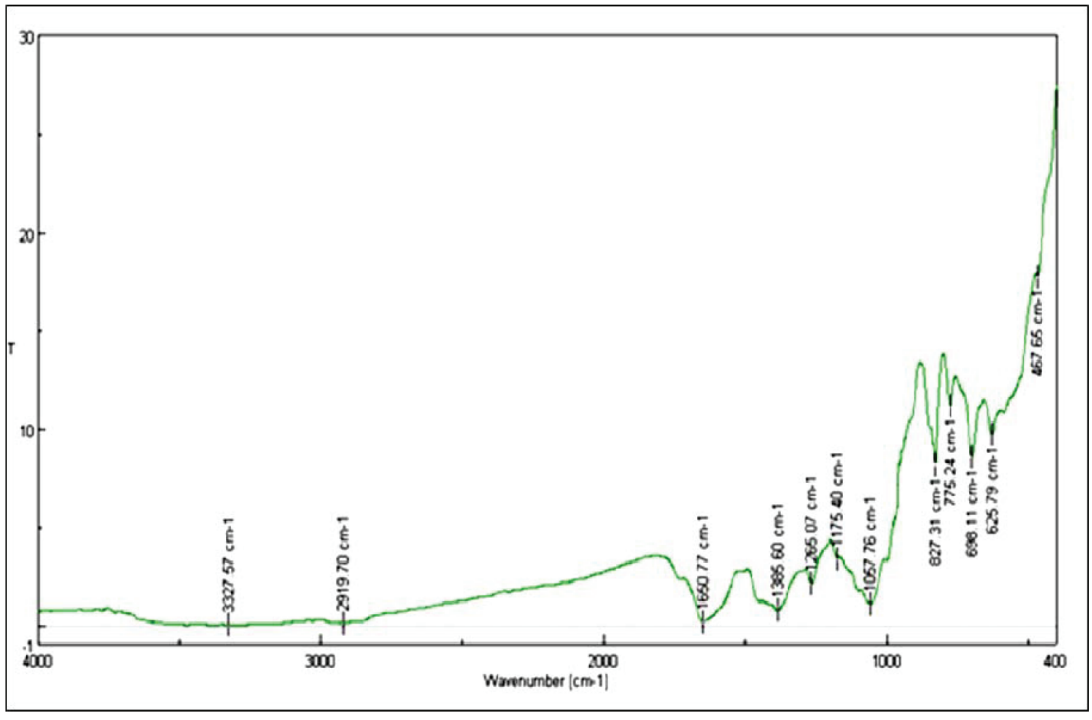

Fig. 2: FTIR Spectra of *H. coronarium* rhizome methanolic extract

The IR spectrum of methanolic extract reveals structural information about major and minor constituents. The functional group identification is attributed to the stretching and bending vibrations of the compound.

GC-MS chromatogram analysis of the methanolic extract of *H. coronarium* showed 21 peaks which indicating the presence of twenty one phytochemical constituents (Fig. 3).

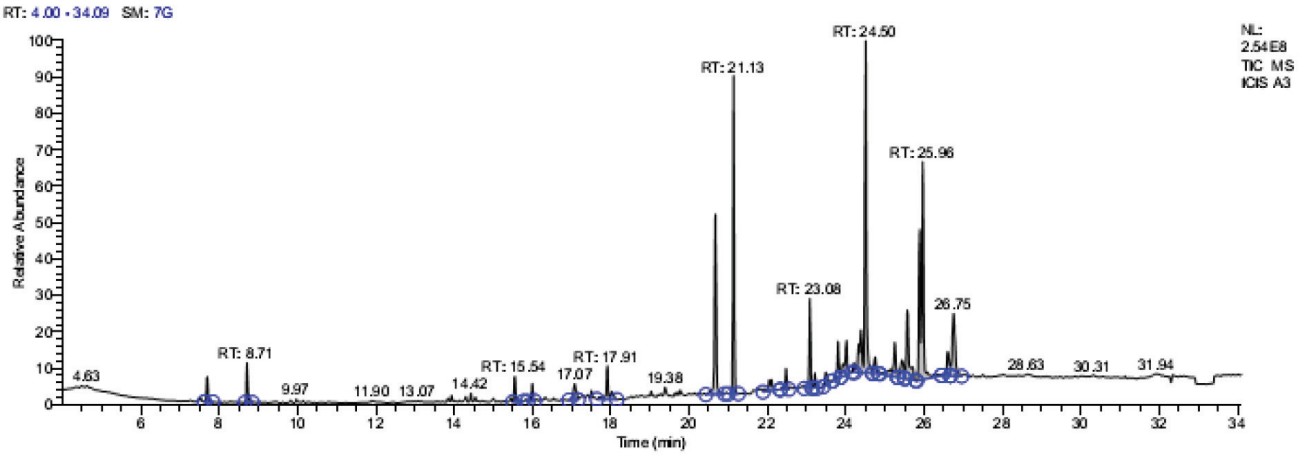

Fig. 3: GC-MS chromatogram of *H. coronarium* methanolic rhizome extract

On comparison of the mass spectra of the constituents with the NIST library, the phytocompounds were characterized and identified. Of the compounds identified, the most prevailing compounds were Spiro [furan-2(5H), 2'(1'H)-naphtho [2,1-b] furan]-5-one (19.89%), Retinoyl-â-glucuronide 6',3'-lactone (18.21%), 9-cis-Retinal (12.07%) and ã-Carboethoxy-ã-butyrolactone (9.56%).

4. Conclusion

The combinative approach of these chemical fingerprinting techniques would help in evaluating quality consistency of herbal drug of *H. coronarium*.

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References

Jain, S. K., Fernandes, V. F., Lata, S. and Ayub, A. (1995). Indo-Amazonian ethnobotanic connections-similar uses of some common plants. Ethnobotany 7:29-37.

Kunnumakkara, A. B., Ichikawa, H., Anand, P., Mohankumar, C. J., Hema, P. S., Nair, M. S. and Aggarwal, B. B. (2008). Coronarin D, a labdane diterpene, inhibits both constitutive and inducible nuclear factor-κB pathway activation, leading to potentiation of apoptosis, inhibition of invasion, and suppression of osteoclastogenesis. Molecular Cancer Therapeutics, 7:3306-3317.

Lin, G., Li, P., Li, S. L., Chan, S. W. (2001). Validation method of chromatographic fingerprinting for *Camelia sinensis*. Journ. Chromatography A 935:321-338.

Reukngam, N., Chimnoi, N., Khunnawutmanotham, N. and Techasakul, S. (2014). Antimicrobial activity of coronarin D and its synergistic potential with antibiotics. BioMed Res. Inte., 2014, Article ID 581985, 8 pages.

Ray, A., Dash, B., Sahoo, S., Sahoo, A., Jena, S., Kar, B., Chatterjee, T., Ghosh, B. and Nayak, S. (2016). Development and validation of an HPTLC method for estimation of coronarin D in *Hedychium coronarium rhizome*. Acta Chromatographica, DOI: 10.1556/1326.2017.29.3.11.

Kaomongkolgit, R., Jamdee, K., Wongnoi, S., Chimnoi, N. and Techasakul, S. (2012). Antifungal activity of coronarin D against Candida albicans. Oral Surgery, Oral medicine, Oral Pathology and Oral Radiology 114:61-66.

Morikawa, T., Matsuda, H., Sakamoto, Y., Ueda, K. and Yoshikawa, M. (2002). New farnesane-type sesquiterpenes, hedychiols A and B 8, 9-diacetate, and inhibitors of degranulation in RBL-2H3 cells from the rhizome of *Hedychium coronarium*. Chem. and Pharmaceu. Bull. 50:1045-1049.