IJPSR (2021), Volume 12, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 January 2020; received in revised form, 30 April 2020; accepted, 11 May 2020; published 01 February 2021

PHYTOCHEMICAL INVESTIGATION AND *IN-VITRO* ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL FROM RHIZOMES OF *HEDYCHIUM SPICATUM*

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Keywords:

Antimicrobial activity, Essential oil, Ethyl p-methoxycinnamate, *Hedychium spicatum*, GCMS

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ABSTRACT: The chemical configuration and antimicrobial activity of *Hedychium spicatum* rhizome based essential oil was studied, which is also commonly known as Kapoorkachri, grown grown wildlyacross Karnataka Karnataka, India. Thirty-eight compounds were detected from the rhizome based essential oil by GCMS. The main compound was characterized as Ethyl p-methoxy-cinnamate (50.1%), Ethyl cinnamate (26.22%), and Eucalyptol (5. 68%). The anti-bacterial activity was performed by the Broth dilution method. Oil-DMSO solution in seven dilutions was developed starting from 100%, 50%, 25%, 12.5%, 6.25%, 3.12% and 1.56% against five selected human pathogenic organisms which comprised of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. Anti-microbial activity against these pathogens were found to be 25%,25%,25%, 50% and 1.56% respectively. The oil showed positive inhibitory effects on organisms compared to the reference standard of Ciprofloxacin and Amphotericin B.

INTRODUCTION: The antimicrobial activity of volatile oils has received attention in recent years in parallel with advances in customary approaches of protecting the health of mammals and food against the presence of pathogenic microorganisms. Thus, the probe on the antimicrobial activity of plant extracts and essential oils against different pathogens have been performed globally [1]. In the last few years, there have been many efforts to find out the efficacy and potency of different essential oils for their role as a substitute for synthetic preservatives in food and pharmaceuticals [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(2).853-58

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(2).853-58

The essential oils of the plants are the main aromatic components with multiple therapeutic efficacies. They are also known as volatile oils due to the fact that they rapidly vaporize when exposed to the atmosphere at room temperature.

In general, essential oils consist of a blend of different types of molecules. Essential oils are extracted from seeds, roots, stems, barks, leaves, fruits, fruit rinds, flowers, or resins of different plants. The essential oils have a synergistic action with preservatives to preserve food which is stored for an extended period of time [3]. The medicinal nature of essential oils from certain therapeutic plants is due to the presence of hydrophobic secondary metabolites and volatile components, which are mainly composed of terpenes and phenyl propanoids [4]. *Hedychium spicatum* belonging to the family Zingiberaceae, commonly known as Kapoorkachri is useful in the treatment of

bronchitis, indigestion, eye disease, blood purification, and inflammation [5]. It is a perennial rhizomatous plant, growing in diverse habitat regions of the west Himalayas. Rhizomes are light greyish green, slanting with erect inflorescence, leafy shoot 20-50 cm high [6].

As a part of our research, we have performed physicochemical analysis of rhizome, GCMS characterisation of essential oil, and checked its anti-microbial efficacy against common selected human pathogenic organisms. The study focuses on antimicrobial efficacy of this indigenous oil, which can be used as a preservative in cosmetic products.

MATERIALS AND METHODS:

Plant Materials: The freshly collected rhizomes of *Hedychium spicatum* (Batch number. NPD/ 670/ 2015) were purchased from the local market of Bangalore, Karnataka. The sample was identified as well as authenticated by Dr. Kannan, botanist, Department of Pharmacognosy, The Himalaya Drug Company (Bangalore, Karnataka). The rhizomes of *Hedychium spicatum* were shade dried and kept in a sealed LDPE poly bag.

Source of Bacterial Culture: The bacterial cultures of *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), and Yeast; *Candida albicans* (ATCC10231) were procured from ATCC, Virginia, USA. In contrast, *Bacillus subtilis* (NCTC 10400) was procured from England. The cultures were maintained in the laboratory by frequently subculturing into Soybean casein digest agarmedia (Himedia, Mumbai, India).

Physico-chemical Analysis of Raw Material:

Total Ash: 2 g of *Hedychium spicatum* rhizomes was air-dried, powdered, and weighed in a previously weighed silica crucible and incinerated, gently at first, and gradually increased the temperature to 675 ± 25 ºC until free from carbon.

Water Soluble Extractive Value: 5 g of the rhizome was air-dried, coarsely powdered, and macerated with 100 ml of the chloroform-water mixture in a closed flask for 24 h, continuously shaken for 6 hours, and allowed to stand for 18 hours. It was rapidly filtered; preventing any loss of solvent, 25 ml of the filtrate was dried completely in a tared flat bottomed shallow dish at 105 ºC and weighed. The percentage of water-soluble extractive value was calculated.

Alcohol Soluble Extractive Value: 5 g of the rhizome was air-dried, coarse powdered, and macerated with 100 ml of absolute alcohol in a closed flask for 24 h. It was continuously shaken for 6 h and kept still for 18 h. It was rapidly filtered; preventing any loss of solvent, 25 ml of the filtrate was dried completely in a tared flat bottomed shallow dish at 105 ºC and weighed. The percentage of alcohol-soluble extractive value was calculated.

Loss on Drying: The sample was cut by shredding about 3 mm in thickness, and 10 g of the sample was kept in a tared evaporating dish. After keeping the drug in the tared evaporating dish, it was dried at 110 ºC for 5 h and weighed. The weighing and drying were continued for about an hour interval until the difference between two successive weights corresponded to not more than 0.25%.

Volatile Oil Content: 10 g of the *Hedychium spicatum* rhizomes were weighed accurately and grinded using a mixer to obtain coarse powder (30-60 mesh). The coarse powder was taken in a 1000 mL flat bottomed flask, and about 600 mL of water was added. The plant material was distilled for 8 hours using a Clevenger apparatus. The oil was partitioned to remove water, and a trace amount of moisture was removed using anhydrous sodium sulphate. The pure oil is decanted to the amber-colored glass vial and stored in room temperature.

TLC Fingerprint: 1 g of sample was weighed in a 250 ml flat bottomed flask. 10 ml of methanol was added and refluxed at 80 ºC ± 2 ºC for about 35 minutes. The extract was passed through Whatman no.1 Filter paper. 15 µl of the filtered extract was spotted in pre-coated thin layer silica plate 60 F 254, 10 × 10 cm, and developed using a solvent system of Toluene: Ethyl acetate (75:25).

The dried plate was visualized under UV 254 nm and 366 nm using a UV cabinet. The plate was derivatized using vanillin sulphuric acid reagent; it was heated at 105 ºC for about 10 min and visualized under white light.

GC-MS Analysis: The essential oil was analyzed using Bruker scion 456 GC-MS equipped with ZB

5 MS column (0.25 mm ID × 30 m L, the thickness of 0.25 microns). The *H. spicatum* essential oil was diluted in ethyl acetate and about 2.0 µL of this solution was injected into column. Helium was used as carrier gas at a flow rate of 1.0 mL/ min. The injector temperature was programmed at 280 °C. The Initial temperature was maintained at 90 ºC for 2 min, raised to 150 ºC at 5 ºC/min maintained for 4 min, then to 280 ºC at 10 ºC/min maintained for 5 minutes. The quantitative data (percentage composition) was determined by normalization.

The mass detector was functionalized in electronic ionization mode (-70 eV). The oven temperature program and injection procedure were the same as above. Transfer line temperature and filament lamp was maintained at 280 °C and 230 °C, respectively. The components were discovered by comparison of their mass spectra with those from the Wiley Registry of Mass Spectral Data 7 and linear retention indices (LRI) as well as compared to literature data [8].

Antimicrobial Activity:

Broth Dilution Method: Microorganisms were inoculated into a liquid growth media in the presence of varied concentrations of an anti-microbial agent. This method was performed to investigate the minimal inhibitory concentration (MIC) of oil against selected pathogens. Anti-biotics; Amphotericin B at 50 µg/ml was used as a standard for yeast, and Ciprofloxacin at 50 µg/ml was used for bacteria. The procedure involves preparation and dilution of the antimicrobial agent in a liquid growth medium poured in tubes. 200 µl of 100% *Hedychium spicatum* oil was taken as control. The oil samples were diluted with Muller Hilton Broth (MHB) media (M391, Himedia, USA) at different concentrations in the 96-well microtiter plate. Beginning with 100%, 50%, 25%, 12.5%, 6.25%, 3.12% and 1.56 % respectively.

MHB was used as a negative control. The saline suspension was used for the colony-forming count of microorganisms; microbial suspension with MHB is used as an experimental test solution. Microbial suspension with antibiotic was taken as the reference standard. Each well was inoculated with 10 µl of microbial inoculums prepared with a similar medium after dilution of standardized microbial suspension, which was made up to 10^4 cfu/ml of 24 h culture. After that, the mixture was incubated without agitation under suitable conditions at 35 ºC for 24 to 48 h. The UV absorbance was checked at 580 nm for control and test samples. All the experiments were carried out in triplicates.

RESULTS AND DISCUSSION:

Chemical Configuration of Essential Oil: The chemical configuration of the oils obtained from *Hedychium spicatum* rhizome is given in **Table 1**. The total essential oil content obtained from rhizomes was found to be 2.96% v/w. The water extractive value and alcohol extractive values were found to be 11.98% w/w and 3.61% w/w, respectively.

The TLC fingerprint profile of *Hedychium spicatum* oil was developed in Toluene: Ethyl acetate solvent system in the ratio (75:25) as depicted in **Fig. 1** at white light and different UV wavelengths of 254 and 366 nm, respectively. Thirty-eight components were identified by GC-MS as described in **Table 2**.

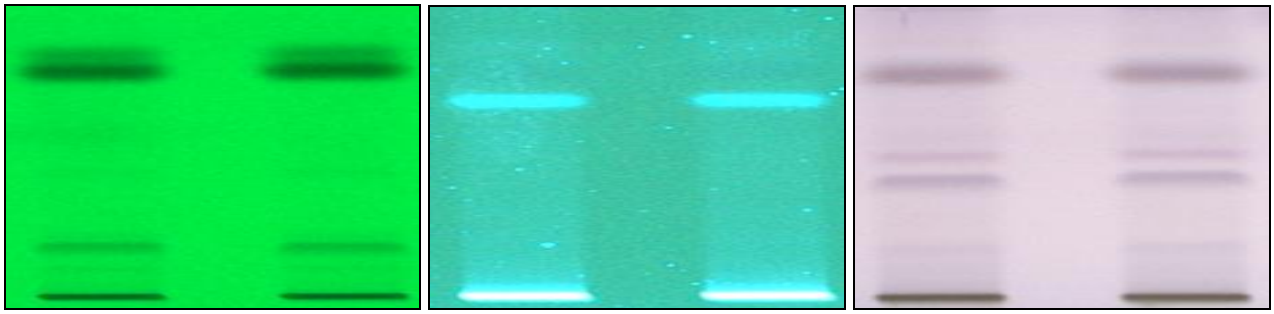
TABLE 1: PHYSICOCHEMICAL ANALYSIS OF *HEDYCHIUM SPICATUM* RHIZOMES

Test Parameters	Observation
Description	Off white to light brown colorrhizome
Total ash	6.35 %w/w
Water soluble extractive value	11.98 %w/w
Alcohol soluble extractive value	3.61 %w/w
Loss on drying	11.75 %w/w
Total volatile oil content	2.96 %v/w
TLC fingerprint	Pattern developed

The main constituents were found to be Ethyl p-methoxy cinnamate (50.1%), Ethyl cinnamate (26.22%) and Eucalyptol (5.68%). Apart from that Eicosane (3.35%), Endo-Borneol (2.44%), 3-Carene (1.10%), Camphene (1.04%), α-Gurjunene (0.63%), β-Copaene (0.48%), p-Cymen-8-ol (0.38%), α-Pinene (0.37%), Verbenone (0.32%), Limonene oxide (0.28%), Isobornyl formate (0.26%), Eucarvone (0.25%), Thymol (0.25%), O-Cymene (0.22%), Longipinocarvone (0.21%), β-Pinene (0.20%), α-acorenol (0.17%), Camphor (0.15%), Caryophyllene oxide (0.14%), D-Limonene (0.14%), (1R)-(-) Myrcenol (0.14%), Cubenol (0.12%), (Z)-Pinocarveol (0.12%), p-Cymene (0.06%), Trans-α-Bergamottin (0.06%), α-

Ylangene (0.06%), Linalylanthranilate (0.05%), Pinocarvone (0.05%), (-)-Spathulenol (0.05%), α-Calacorene (0.04%), Cis-verbenol (0.04%), 3-Nitro Propionic acid (0.04%), Linalool oxide (0.04%), Rotundene (0.04%), (R)-Lavandulyl acetate (0.02%) were identified.

As reported by Prakash *et al.*, (2010), Bottini *et al.*, (1987), Nigam *et al.*, (1979), Garg *et al.*, (1977), Dixit *et al.*, (1977) and Sabulal *et al.*, (2007) have reported 1,8-cineole and α-eudesmol (17.0), β-eudesmol (12.6), (E) Caryophyllene (16.6), Linalool (18.0), Linalool (25.6) respectively [9-12].



245 nm **366 nm** **WHITE LIGHT**

FIG. 1: TLC PROFILE FOR *HEDYCHIUM SPICATUM* RHIZOMES

TABLE 2: PERCENTAGE COMPOSITION OF THE ESSENTIAL OIL OBTAINED FROM RHIZOMES OF *HEDYCHIUM SPICATUM*

S. no.	Name of the compound	Percentage
1	Ethyl p-methoxycinnamate	50.1
2	Ethyl cinnamate	26.22
3	Eucalyptol	5.68
4	Eicosane	3.35
5	Endo-Borneol	2.44
6	3-Carene	1.1
7	Camphene	1.04
8	α-Gurjunene	0.63
9	β-Copaene	0.48
10	p-Cymen-8-ol	0.38
11	α-Pinene	0.37
12	Verbenone	0.32
13	Limonene oxide	0.28
14	Isobornylformate	0.26
15	Eucarvone	0.25
16	Thymol	0.25
17	O-Cymene	0.22
18	Longipinocarvone	0.21
19	β-Pinene	0.2
20	α-acorenol	0.17
21	Camphor	0.15
22	Caryophyllene oxide	0.14
23	D-Limonene	0.14
24	(1R)-(-) Myrcenol	0.14
25	Cubenol	0.12
26	(Z)-Pinocarveol	0.12
27	p-Cymene	0.06
28	Trans-α-Bergamottin	0.06
29	α-Ylangene	0.06
30	Linalylanthranilate	0.05
31	Pinocarvone	0.05
32	(-)-Spathulenol	0.05
33	α-Calacorene	0.04
34	Cis-Verbenol	0.04
35	3-Nitro Propionic acid	0.04
36	Linalool oxide	0.04
37	Rotundene	0.04
38	(R)-Lavandulyl acetate	0.02

The oil showed significant antimicrobial activity against the tested organisms of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa,* and *Candida albicans*.

Antimicrobial Activity: Most of the essential oils contain a complex blend of components and thus have various antimicrobial properties; most of this action appears to have derived from oxygenated terpenoids, Being lipophilic compound, essential oils cross the cytoplasmic membrane and cell wall, thereby the cytotoxic activity appears to be linked to the disarray of the structures present in different layers of polysaccharides, fatty acids, and phospholipids. Due to this mechanism of action, it acts on various targets at the same time [7].

Permeability, chemical configuration, and surface charge of the outer structures of the micro-organisms mainly determined these differences; the lipophilicity of terpenes is linked with the antimicrobial mechanism [8]. Numerous reports have been reported regarding the mechanisms of anti-microbial property of the oils, and some studies have been partly elucidated.

The antimicrobial investigation of the oil was reported using the Broth dilution method. According to this method, the oil showed anti-microbial activity against the selected pathogens; *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Amphotericin B and Ciprofloxacin were kept as standards for *Candida albicans* and for all

the bacterial strains, respectively. Percent concentration showing complete inhibition against the selected pathogens; *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* were found to be 25%, 25%, 25%, 50%, and 1.56%, respectively, as mentioned in **Table 3**. Amphotericin B and Ciprofloxacin showed activity against all the organisms at 1.56% (0.31 µg).

TABLE 3: PERCENT CONCENTRATION SHOWING COMPLETE INHIBITION OF TESTED ORGANISMS

	Percentage Inhibition of tested organisms		
Organisms	***Hedychium spicatum* oil (200 µg/ml)**	**Ciprofloxacin (0.31 µg/ml)**	**Amphotericin B (0.31 µg/ml)**
Escherichia coli (ATCC 8739)	25%	1.56%	---
Staphylococcus aureus (ATCC 6538)	25%	1.56%	---
Pseudomonas aeruginosa (ATCC 9027)	50%	1.56%	---
Bacillus subtilis (NCTC 10400)	25%	1.56%	---
Candida albicans (ATCC 10231)	1.56%	---	1.56%

CONCLUSION: The *Hedychium spicatum* rhizome oil showed significant positive antimicrobial activity against all the tested organisms of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*.

The essential oil contains 50.1% of Ethyl p-methoxycinnamate and 26.22% w/w of Ethyl cinnamate as major components identified through GC-MS which is reported for its UV protection activity [13] and hair growth activity [14]. This oil can be explored further for cosmetic and pharmaceutical applications.

ACKNOWLEDGEMENT: Authors are highly thankful to Dr. Rangesh P, Chief Scientific Officer, Dr. Venugopal, Group leader, Microbiology department, and Dr. Vijayakumar M., Senior Research Scientist, Phytochemistry department, The Himalaya Drug Company, for providing necessary inputs and facilities in this research to successfully complete the experiment.

CONFLICTS OF INTEREST: The authors report no conflicts of interest during this research.

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How to cite this article:
Arumugam I, Krishnan C, Ramachandran S, Krishnan D, Das D and Thamankar V: Phytochemical investigation and *in-vitro* antimicrobial activity of the essential oil from rhizomes of *Hedychium spicatum*. Int J Pharm Sci & Res 2021; 12(2): 853-58. doi: 10.13040/IJPSR. 0975-8232.12(2).853-58.