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Research Letter

Chemical Examination and Hair Growth studies on the Rhizomes of *Hedychium spicatum* Buch.-ham

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ABSTRACT: The hexane extract of the rhizomes of *H. spicatum* yielded two known compounds, pentadecane, and ethyl p-methoxycinnamate. The structures of these compounds were established by spectroscopic data (UV, IR, GC, ^{1}H and ^{13}C NMR, Mass) and comparison with an authentic compounds. The crude extract, fractions and one of the isolated compounds showed hair growth property.

KEYWORDS: *Hedychium spicatum*, rhizomes, pentadecane, hair growth activity.

INTRODUCTION

Hedychium spicatum (Zingiberaceae), also known as spiked Ginger Lily is employed in the preparation of Abir, a fragrant coloured powder used during the Holi festival. The rhizomes possess strong aromatic odour and bitter camphoraceous smell. The rhizomes of the plant have been used in the preparation of cosmetic powders used for promoting hair growth. The rhizomes are also considered to have insect-repelling properties and are used for preservating clothes. The rhizomes are stomachic, carminative, stimulant and tonic, and are used in dyspepsia in the form of powder or decoction.[1] The rhizomes are much used in veterinary medicine.[2] The prior literature on *Hedychium spicatum* reveals that the cosmetic composition containing this plant extract regulates the firmness, tone or structure of skin or regulate wrinkles.[3] The compositions containing extract of *Hedychium spicatum* are useful for treating *Tinea* infections by topical application.[4] The ethanolic extract of rhizomes of *H. spicatum* possessed anti-inflammatory and analgesic activity. The anti-inflammatory activity was found in the hexane fraction and the compound hedychienone was found responsible for such activity and the analgesic activity was found in benzene fraction.[5] The cinnamic acid ester, obtained from the extracts of *H. spicatum* and *Alpinia galanga* and the same has been patented for natural sunscreen property.[6] The essential oil extracted from the rhizomes of *H. spicatum* was evaluated for *in-vitro* pediculicidal activity at 1, 5 and 25% level. At all the three concentrations, the essential oil showed more significant activity than 1% permethrin based product.[7] Previous reports on this plant occurring in different regions yielded, furanoditerpenoids,[8] terpenoids,[9-10] steroids[11] and aromatic esters.[1] However, no information was available on the preparation of an appropriate selective extract or fraction of the plant and its efficacy directed towards promoting hair growth or retarding hair fall or isolation of hair growth active compounds based on bioassay. In continuation of our interest on the isolation of biologically active molecules from medicinal plants for personal care applications,[12-21] we have undertaken the chemical examination of the rhizomes of *H. spicatum*. The present study describes the isolation of two known compounds, pentadecane (**1**) and an aromatic ester, ethyl p-methoxycinnmate (**2**) and hair growth studies of crude hexane extract, fractions and active compound.

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MATERIALS AND METHODS

General

Melting points reported are uncorrected. UV spectra were recorded on Shimadzu UV spectrophotometer. IR spectra were recorded on a Shimadzu IR prestige 21. GC spectra were recorded in Shimadzu GC-17A capillary GC. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 400 in $CDCl_3$ with TMS an internal standard and the chemical shifts being represented in parts per million (ppm, δ values). GC-MS mass spectrum on a Jeol SX 102/DA 6000 mass spectrometer. Column chromatography was performed on silica gel (100-200 mesh, Acme synthetic chemicals, Mumbai, India). Fractions and purity of the compounds were monitored by analytical thin layer chromatography (TLC) and the spots were visualized by exposure to iodine vapour or 5% sulphuric acid in methanol followed by heating the plate at

110ºC for 5 min. The TLC was performed on pre-coated silica gel plates (aluminium sheets 20X20 cm, silica gel 60 F_{254} plates of Merck KGaA, Germany). All solvents and reagents used were of analytical grade obtained from Merck. Pentadecane was obtained from M/s. Sigma aldrich, USA.

Plant material

The rhizomes of *Hedychium spicatum* were obtained from bazaar in December, 2007 and was authenticated by Dr. P. Santhan, botanist, M/s. Durva Herbal Centre, Chennai. A voucher specimen was deposited in M/s. CavinKare Research Centre, Chennai.

Extraction and isolation

The air dried and finely powdered rhizomes (2.2 kg) were extracted with hexane through soxhlet apparatus for 8 hrs. The dilute extract was filtered and evaporated to dryness in vacuo using a rotary evaporator at 40ºC to get crude hexane extract (33g). The crude hexane extract was submitted for hair growth studies and found to shown good hair growth.

Part of the crude hexane extract (30g) was subjected to column chromatography eluted with hexane, hexane: chloroform (1:1, 1:3) and chloroform to get corresponding fractions 4.3g (Fr. I), 15.5g (Fr. II) and 8.8g (Fr. III), respectively. All three fractions were submitted for hair growth studies along with crude hexane extract. Out of three, fraction I showed good hair growth promotion activity. Part of the fraction I, 1.0g was subjected to normal silica gel chromatography followed by repeated silver nitrate impregnated column chromatography with hexane: chloroform (95:5) yielded colorless compound **1** (130mg). Compound **2** (1.6 g) obtained from fraction II as colorless solid which was further crystallized in hexane to afford colorless crystalline compound.

Compound 1: Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (6H, s), 1.26 (26H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (C-1,15), 22.9 (C-2,14), 29.3 (C-3,13), 29.6 (C- 4 to12).

Compound 2: Colorless crystals; mp = 49-50ºC; IR (KBr): 2931, 1711, 1605, 1512, 1250, 1150, 830 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (1H, d, J = 16.0 Hz), 7.45 (1H, d, J = 8.8Hz), 6.88 (1H, d, J = 8.8Hz), 6.29 (1H, d, J = 16.0Hz), 4.25 (2H, q, J = 7.1Hz), 3.82 (3H, s), 1.32 (3H, t, J = 7.1Hz) ; ^{13}C NMR (100 MHz, $CDCl_3$): δ167.5, 161.1, 144.2, 129.8, 129.4, 127.3, 115.8, 114.4, 114.4, 60.5, 55.4, 14.3.

Hair growth promotion activity

The hair growth promotion activity was studied by using *in vivo* animal model[15],[22].

Animals: Female Wistar rats weighing 120-150 g, from Dr. MGR Janaki College, Chennai were used for hair growth study. Based on the guidelines of the ethical committee of the college, the animals were maintained in a clean cage and were provided with food and water ad libitum. The floor mat husk in each cage was removed and laid afresh on daily basis.

Hair growth activity *in vivo*

The hair on the dorsal portion of the body of each animal was depilated using a standard, commercially available depilatory cream. After removal of the hair, the skin was cleaned with distilled water and wiped with surgical spirit. Four centimeter square area in the depilated dorsal skin was marked with permanent ink marker. The animals which showed any skin irritant response to the depilatory were removed from the experiment and new animal was replaced.

The rats were divided into 3 groups of 6 animals each. Group 1 animals were served as negative control without any treatment. The negative control comprised of the vehicle for application (only) without having any active extract/fraction/compound. Group 2 animals were applied 50 micro liters of commercial 2% Minoxidil solution in the pre defined area. The group 3 animals were applied samples (extract/fractions/compounds) prepared in liquid paraffin at 2%. The quantity of the solution used for the experiment was 50 micro liters per 4 cm sq area per animal. The application of the Minoxidil and the test samples were continued for 30 days. The observations such as hair growth initiation time in days and hair growth completion time in days were recorded for all the animals on daily basis. The hair growth initiation time was defined as the presence of new hair in the treated site of 4 cm sq area. The hair growth completion time was defined as complete filling of hair in the treated site of 4 cm sq area in each animal which become indistinguishable from the adjacent untreated portion of the body. The average of hair growth initiation time and hair growth completion time was calculated for each group along with control animals. The untreated control for hair growth initiation time (HGIT) is 10 days and hair growth completion time (HGCT) is 30 days. The percentage reduction in hair growth completion time (% Reduction in HGCT) for the treatment is calculated by the formula given below. The results of hair growth activity are shown in [Table 1].

$$\text{Calculation} = \frac{\text{HGCT in untreated control} - \text{HGCT in test sample}}{\text{HGCT in untreated control}} \times 100$$

Table 1: Comparison of *in-vivo* hair growth promotion activity

Extract/ Fraction/ Compound	Hair growth initiation Time (HGIT in days)	Hair growth completion Time (HGCT in days)	% Reduction in time
Hexane extract	8	20	33
Fraction 1	8	20	33
Pentadecane	7	21	30
Minoxidil	6	16	47
Untreated control	10	30	0

RESULTS AND DISCUSSIONS

The initial screening of the hexane extract of the rhizomes of *H. spicatum* showed positive response in hair growth promotion activity. The bioassay guided purification of the hexane fractions of the rhizomes of *H. spicatum* repeated chromatography with a silica gel and re-crystallization with solvents furnished pentadecane and ethyl p-methoxycinnamate. The structure of the compounds were elucidated on the basis of UV, IR, GC, ^{1}H and ^{13}C NMR and Mass spectral data and comparison with an authentic samples.

The hair growth promotion activity of pentadecane showed good reduction in hair growth time, where as minoxidil, a positive control showed an excellent activity in the standard method but it had other side effects[23]. Even though the plant is being used in the preparation of hair oils, so far no reports on the compounds responsible for hair growth promotion activity.

The compound **1** was readily recognized as hydrocarbon by its preliminary spectral data. Its molecular formula was established as $C_{15}H_{32}$ by GC-MS, M^+ 212. Its IR and UV spectra showed no characteristic peaks. Its proton spectrum showed only two peaks: methyl at δ 0.86 (s) and methylene at δ 1.26 (s). Its carbon spectrum showed the presence of four signals. It showed methyl and methylene carbons. Its mass spectrum showed m/z value 212. Based on the spectral data the compound has been identified as pentadecane.[24] To confirm further the compound, pentadecane has been purchased from M/s. Sigma-Aldrich, USA and analyzed by GC along with compound **1**. The retention time of both the compounds were exactly matching with each other. Thus, the compound **1** has been established as pentadecane.

The compound **2** was identified as colorless crystals from hexane: chloroform, mp:49-50°C. It was readily recognized as aromatic acid ester based on its preliminary spectral data. Its molecular formula was established as $C_{12}H_{14}O_3$ by GC-MS, M^+ 206. The IR spectrum showed the presence of an ester peak at 1711cm^{-1} in the molecule. Its proton spectrum showed the presence of four aromatic protons at δ 7.45 (2H, d, J = 8.8Hz) and 6.88 (2H, d, J = 8.8Hz), one aromatic methoxyl group at δ 3.82 (3H, s), two double bond protons each showed as doublet at δ 7.62 and 6.29 (J = 16.0Hz), one oxymethylene group at δ 4.25 (2H, q, J = 7.1Hz), one methyl at δ 1.32 (3H, t, J = 7.1Hz). Based on the aromatic proton integration, the molecule has 1,4 di-substitution patteren. The two olefinic protons showed large coupling constant indicates that these two protons are in trans position. The carbon spectrum showed total of 12 carbons including ester carbonyl at δ 167.5. Out of twelve, eight double bond carbons at δ 161.1, 144.2, 129.8, 129.4, 127.3, 115.8, 114.4, 114.4, of which six aromatic and two olefinic carbons. It also showed one methoxy carbon at δ 55.4, one oxy methylene carbon at δ 60.5 and one methyl carbon at δ 14.3. By revealing the literature, the spectral data of the compound **2** is exactly matching with those of previously reported values. So, the compound **2** has been identified as ethyl p-methoxycinnamate.[24-25]

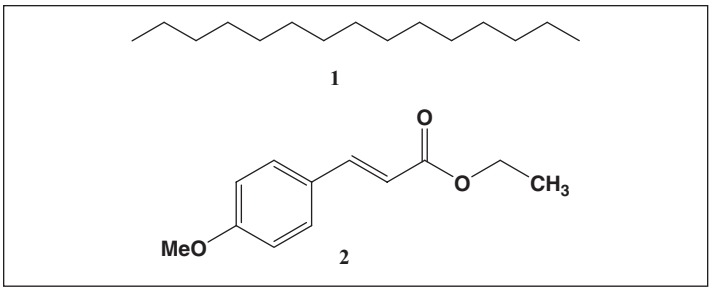

Figure 1: Compounds from *H. spicatum*

The results of hair growth promotion (Table 1) showed that crude hexane extract was required less time than pure compound, pentadecane. It is worth mentioning that many crude extracts or active fractions are showing better activity than individual compounds.

CONCLUSION

To our best knowledge, the present study is the first report of the isolation of active compound from *Hedychium spicatum* for hair growth studies.

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