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HPTLC and reverse phase HPLC methods for the simultaneous quantification and *in vitro* screening of antioxidant potential of isolated sesquiterpenoids from the rhizomes of *Cyperus rotundus*

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ABSTRACT

Three sesquiterpenoids solavetivone, aristolone and nootkatone were isolated from the acetone extract of *Cyperus rotundus* by silica gel column chromatography and identified by spectral studies. Solavetivone has been isolated for the first time from the species. Simple, sensitive and selective HPTLC and HPLC methods with ultraviolet detection (245 nm) were developed and validated for the simultaneous quantification. HPTLC method was validated in terms of their linearity, LOD, LOQ, precision, accuracy and compared with RP-HPLC-UV method. Among the three sesquiterpenoids isolated, nootkatone possessed the highest radical scavenging potential (IC_{50} 4.81 μg/ml) followed by aristolone (IC_{50} 5.28 μg/ml) and solavetivone (IC_{50} 6.82 μg/ml) by DPPH radical scavenging assay. Total antioxidant activity against phosphomolybdenum reagent was also studied. The methods described in this paper were able to identify and quantify sesquiterpenoids from the complex mixtures of phytochemicals and could be extended to the marker based standardization of polyherbal formulations containing *C. rotundus*.

1. Introduction

Antioxidants are health beneficial compounds which react with an excess of reactive oxygen and nitrogen species under 'oxidative stress' conditions, thereby preventing related diseases such as cell ageing, cardiovascular diseases, mutagenic changes and cancerous tumor growth [1]. The growing interest in the substitution of synthetic antioxidant by natural ones has fostered research on plant sources and the screening of raw materials for identifying new antioxidants. Recently, natural foods and food-derived components have received a great deal of attention because they are safe and not perceived as medicine; some of these are known to function as chemopreventive agents against oxidative damage [2].

Cyperus rotundus L. (Family: Cyperaceae; *C. rotundus*) rhizomes were grown throughout the world. It is well-known for its traditional medicinal uses described for exerting anti-inflammatory [3], antipyretic, analgesic [4], antidiarrheal [5] and antimalarial effects [6,7]. In ancient Indian medical literature (Ayurveda, Charaka Samhita – 3000 B.C.), *C. rotundus* tubers were listed together with nine other plant species such as lekhania drugs, which were capable of 'de-fatting' adipose or muscular tissues [8]. The plant has long been used as a folk medicine and as a food. Despite the bitter taste of the tubers, they are edible and have nutritional value. Extracts of *C. rotundus* were reported to lower glycemia in a rodent model of diabetes [9]. Most of these effects were attributed to the presence of sesquiterpenes in the rhizomes of *C. rotundus* [7,10]. *C. rotundus* is also considered as a worst weed because of its widespread distribution and difficult to control [11]. This weed is commonly present in tomato and pepper fields and its interference has been known to reduce yields in a number of crops [12].

In this paper, we report the isolation, identification and quantification by HPTLC and HPLC of three sesquiterpenoids from the rhizomes of *Cyperus rotundus* and elucidate the total antioxidant and radical scavenging potential of the purified compounds by *in vitro* assays.

2. Materials and methods

2.1. Chemicals

Ammonium molybdate from Merck, Mumbai, India and sodium phosphate from Alfa Aesar, India were purchased. HPLC grade solvents were obtained from Rankem India Ltd. 2,2′-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, *etc.* were obtained from Sigma–Aldrich Chemicals (St Louis, MO, USA). All other reagents used were of standard analytical grade.

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2.2. Spectral analysis

IR spectra were taken with Alpha FT-IR, Bruker Optics. NMR spectroscopy was performed on a Bruker DPX NMR spectrometer operated at 500 MHz for ^{1}H and 125 MHz for ^{13}C and DEPT using $CDCl_3$ as solvent (Merck, Mumbai, India). Mass spectra were recorded using JEOL JMS 600 H mass spectrometer. UV spectrum and absorbance were measured using a Shimadzu 1601 UV-VIS spectrophotometer (UV 1601, Kyoto, Japan).

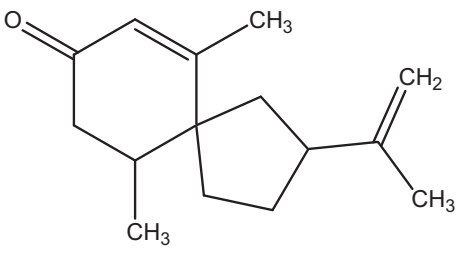
2.3. Plant materials and extraction

2.3.1. Plant materials

The rhizomes of *Cyperus rotundus* (5 kg) were obtained from a registered medicinal plant vendor in Trivandrum and the plant was identified and authenticated by the approved taxonomist of the Tropical Botanical Garden and Research Institute (TBGRI), Palode, Thiruvanathapuram, Kerala, India. A voucher specimen (No. 034/2011) has been deposited in the herbarium for further use. The rhizomes were cleaned and dried in the air oven at a temperature of 50 °C.

2.3.2. Extraction and isolation of sesquiterpenoids

The air dried rhizomes (500 g) of *C. rotundus* were extracted with acetone at room temperature (27 °C) which after removal of solvent under reduced pressure yielded 30 g of extract. This extract was subjected to gradient elution with 100–200 mesh silica gel column chromatography using the solvents hexane–ethyl acetate (100:0–0:100) to give 195 fractions which were grouped into fraction pools based on similarities in TLC. From this, third fraction (1.91 g) was purified further by column chromatography using hexane–ethyl acetate with increasing polarities which yielded **1** (52 mg) as colorless oil. The fifth fraction (726 mg) on further purification by column chromatography yielded **2** (56 mg) and **3** (326 mg) in pure form. The structures of all isolated compounds were identified by the interpretation of their spectral data *viz.*, UV, IR, HR-MS, ^{1}H, ^{13}C and DEPT NMR, as well as by comparison of their spectral data with those reported in the literature (Fig. 1).



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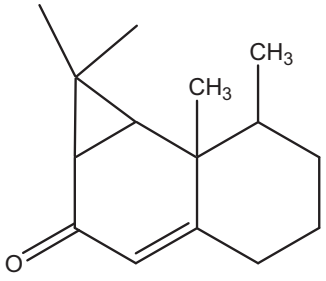

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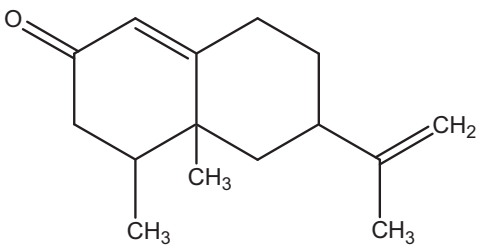

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Fig. 1. Chemical structure of (1) solavetivone, (2) aristolone and (3) nootkatone.

2.4. Preparation of sample solutions

A reference stock solution with a concentration of 1 mg/ml of isolated compounds was prepared in methanol. Various concentrations of the solutions (0.1–1 μg/ml) were prepared with appropriate dilution of the stock solution for HPTLC and HPLC analysis. The solutions were filtered through 0.45 μm PTFE filter prior to analysis.

2.5. HPTLC instrumentation

Silica gel HPTLC plates (Kieselgel 60 F 254, 20 cm × 20 cm, 0.2 mm thickness, Merck, Darmstadt, Germany) were washed with methanol before use and kept at 60 °C for 30 min for the analysis. The samples (5 μl) were spotted in the form of bands of width 6 mm by means of a Camag Linomat V (Switzerland) fitted with a Hamilton microliter syringe. A constant application rate of 0.1 μl/s was employed and the space between the two bands was maintained as 5 mm.

The plates were developed in an ascending manner with 30% hexane–ethyl acetate solvent system in a presaturated development chamber. The plates were dried in an air oven after development at a temperature of 50 °C and scanned using a TLC Scanner 3 (Camag) in absorbance–reflectance mode. The radiation source used for analysis was a deuterium lamp emitting a continuous UV spectrum between 200 and 300 nm. The slit dimension was kept at 5 mm × 0.45 mm and 10 mm/s scanning speed was employed. Analysis was performed in an air-conditioned room maintained at 22 °C and 65% relative humidity. Various concentrations of isolated compounds and extract were analyzed a minimum of three times. Data processing was performed with WinCATS planar chromatography manager software (version 1.4.3). UV spectra of the isolated compounds were obtained using Camag TLC Scanner 3 and purity of the HPTLC band was established.

2.6. HPLC instrumentation

The integrated high performance liquid chromatography was performed on a Waters liquid chromatography equipped with a Rheodyne injector and a Waters 2487 (M/s Waters GESMBH, Hietzinger Hauptstrasse 145, A 1130, Vienna, Austria, Europe) UV detector. The system is interfaced with a personal computer for data acquisition and control (Millennium). The separation of compounds was made on a μ Bondapak C_{18} column (150 mm × 4.6 mm, 5.0 μm) at room temperature. The mobile phase used was a mixture of acetonitrile–water (60:40) pumped at a flow-rate of 1 ml/min and the sample was chromatographed at a series of wavelength from 200 to 300 nm. All solvents and mobile phases were of HPLC grade, and water was purified on a Millipore Milli-Q system.

2.7. Method validation

Comparison between retention time and spectra of the peaks ascertained the specificity of the method. By comparing the spectral levels at three different levels, *i.e.*, start, apex and end positions assessed the peak purity of the chromatogram. Linearity of HPLC and HPTLC methods were obtained by determining the detector responses against a series of varying concentrations of isolated compounds. Five analyses per concentration were conducted and calibration plots were constructed.

Limits of detection (LOD) and quantification (LOQ) of the methods were calculated using the equations LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$ where σ is standard deviation of response and S is the slope of calibration curve. The limit of detection is the lowest concentration of an analyte in a sample that can be detected but cannot be used for the quantification as it falls below the linear range and limit of quantification is the lowest concentration of the analyte in a sample that can be quantified with acceptable precision and accuracy under the conditions of operation.

Repeatability was determined by the measurement of instrumental, inter and intra-assay precision. Instrumental precision was measured by scanning the same spot of a single concentration seven times. The repeatability or intra-assay precision was studied by analyzing repeatedly, in the same laboratory and on the same day, at three concentrations. Intermediate precision included the analysis of the same three extracts and each of them analyzed three times a day over three days by different analyst. The results of repeatability and intermediate precision are expressed as % RSD.

Accuracy of the methods was determined by standard addition techniques. Known amounts of isolated compounds in a range of low, medium and high concentration were added to preanalyzed samples and analyzed under the optimized conditions. Addition experiments for each concentration were performed in triplicate and the accuracy was calculated as the % of analyte recovered. Three analyses per concentration were performed and mean ± SD was determined. Robustness of the method was determined by introducing small changes in certain chromatographic parameters and expressed in terms of % RSD.

2.8. Antioxidant capacity by phosphomolybdenum method

Antioxidant capacity by phosphomolybdenum reagent was evaluated by the method of Prieto et al. with slight modifications [13]. An aliquot of 0.3 ml of sample solution (from 1 mg/ml) was combined with 3 ml of reagent solution (0.6 M conc. H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate in 100 ml distilled water) in test tubes. The tubes were capped and incubated at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured. Antioxidant capacities of solavetivone, aristolone, nootkatone and acetone extract were expressed as ascorbic acid equivalents (100 g of sample).

2.9. Radical scavenging assay using DPPH radical

The radical scavenging efficacy of isolated compounds was evaluated in terms of purple colored DPPH radical spectrophotometrically [14]. Different installments of the sample solution (2–10 μg/ml) were mixed with 1.5 ml of methanolic solution of DPPH radical and the reaction mixture was covered and kept for 30 min in dark. The degree of decrease of purple color indicates the radical scavenging potential of the added substance. After 30 min incubation, absorbance was measured at 517 nm against control. Gallic acid served as the standard. The antioxidant capacity of samples to scavenge DPPH radical was calculated by the equationscavenging effect (%) $= \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$where A_{control} is the absorbance of blank sample (containing each reagent sample solution) and A_{sample} is the absorbance of samples. The percentage radical scavenging activity was plotted against concentration to obtain 50% inhibition of the drug scavenging DPPH radical.

2.10. Statistical analysis

The experimental results are expressed as the mean ± standard deviation of three parallel measurements. Statistical analyses were performed with Origin Pro 8 and the results were subjected to one way analysis of variance and the significance of differences between sample means was calculated. $P \leq 0.05$ was considered significant.

3. Results and discussion

3.1. Isolation of sesquiterpenoids

Column chromatographic purification of acetone extract of *C. rotundus* rhizomes on silica gel afforded three sesquiterpenoids solavetivone, aristolone and nootkatone (Fig. 1).

3.1.1. Structure elucidation of sesquiterpenoids

Solavetivone was obtained as colorless mobile oil. The molecular formula of the compound was deducted as $C_{15}H_{22}O$ on the basis of HRFABMS at m/z 219.65 $[M+H]^+$ (cal. 218.34) classified this compound as a sesquiterpene and this was supported by ^1H, ^{13}C and DEPT NMR data. The IR spectrum revealed an α, β unsaturated carbonyl group at 1711 cm^{-1} and terminal methylene groups at 2919, 1600 and 901 cm^{-1}. No hydroxyl group was observed. The ^1H NMR spectrum showed a secondary methyl group (δ 1.009, d, J 6.0 Hz, 3H), a methyl group attached to β position of α, β unsaturated carbonyl group (δ 1.77, brs, 3H), an isopropenyl group (δ 1.69, brs, 3H and δ 4.77, brs, 2H), and an olefinic methine (δ 5.62, brs, 1H). The UV spectrum had λ_{MeOH} 245 nm which was consistent with the enone system. This is the first report of a vetispirane derivative, solavetivone in *C. rotundus*. Solavetivone has been isolated for the first time as a phytoalexin and a major stress metabolite produced by infected potato tubers [15] and later as a minor volatile component of tobacco leaves [16].

Aristolone, an aristolane type sesquiterpenoid was obtained as clear oil and crystallized with hexane. Aristolone has a hydro naphthalene skeleton with a ketone group at C-10 position and two methyl groups at C-4 and C-5 positions. The molecular formula of the compound was deducted as $C_{15}H_{22}O$. Molecular weight calculated for aristolone was 218.34 and the obtained was 219.46 $[M+H]^+$. The IR spectrum showed an α, β unsaturated carbonyl group at 1711 cm^{-1}. Terminal methylene groups were observed at 2920, 2853, 2385 and 2351 cm^{-1}. ^1H spectrum showed an olefinic proton at δ 5.68 as an unresolved multiplet. Furthermore, at δ 1.04 a pair of doublets could be attributed to the cyclopropyl hydrogen at C-2 and a further doublet at δ 1.88 was assigned to the cyclopropyl proton at C-11. The three tertiary methyl groups were clearly evident at δ 0.962, 0.965 and 1.18 while the secondary methyl group appeared at δ 0.84. Cytotoxicity against selected cancer cells and anti-inflammatory effects of aristolone were determined by *in vitro* [17].

Nootkatone, a eudalenoid sesquiterpene was obtained as pale oil with sweet smell. The molecular formula of the compound was deducted as $C_{15}H_{22}O$ and was confirmed on the basis of spectral data such as IR, ^1H, ^{13}C, DEPT NMR and HRFABMS. UV spectrum shows an absorption peak at 245 nm (Fig. 2). FABMS shows a molecular ion peak at 219.76 $[M+H]^+$ (cal. 218.34). IR spectrum shows characteristic peaks at 1711 cm^{-1} indicating an α, β unsaturated carbonyl group. Terminal methyl groups were appeared at 2921,

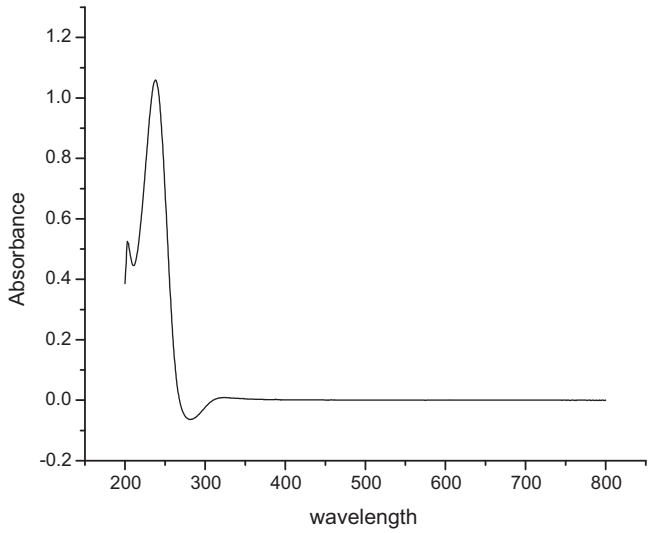

Fig. 2. UV spectrum of nootkatone.

2852 and 1556 cm^{-1}. ^1H NMR spectrum showed an olefinic proton at δ 5.69 as a sharp singlet. A secondary methyl group was observed at δ 1.046 as a doublet and a tertiary methyl group at 1.10 as a singlet. An isopropenyl group was observed at 1.70 as a singlet. Nootkatone exhibits antiplatelet properties and is considered as a AChE (acetyl cholinesterase) inhibitor. Human AChE inhibitors may find use as safe chemotherapy drugs for Alzheimer's disease and Myasthenia–Gravis like syndrome [18]. Nootkatone metabolites show antiproliferative activity toward cancer cell lines A549 and HL-60 [19].

3.2. Optimization of HPTLC chromatographic conditions

Various concentrations of solavetivone, aristolone and nootkatone along with acetone extract of *C. rotundus* were spotted in an HPTLC plate and developed with mobile phases of different polarities. The mobile system of 30% hexane–ethyl acetate resulted in sharp, symmetric and well resolved peaks at a wavelength of 245 nm and an R_f value of 1.37 for solavetivone, 1.09 for aristolone and 0.94 for nootkatone. Since the R_f values of solavetivone and aristolone are close, they seem to be merged in the chromatogram. The HPTLC chromatograms of the compounds recorded at 245 nm is shown in Fig. 3. The calibration curve was found to be linear. Peak area and concentration were subjected to linear regression analysis to calculate the calibration equation and correlation coefficients. The peaks corresponding to solavetivone, aristolone and nootkatone in the HPTLC profile of acetone extract was identified by comparing its R_f values and spectrum. In plant material, the amounts of solavetivone, aristolone and nootkatone were present in 0.2, 0.4 and 0.5% respectively.

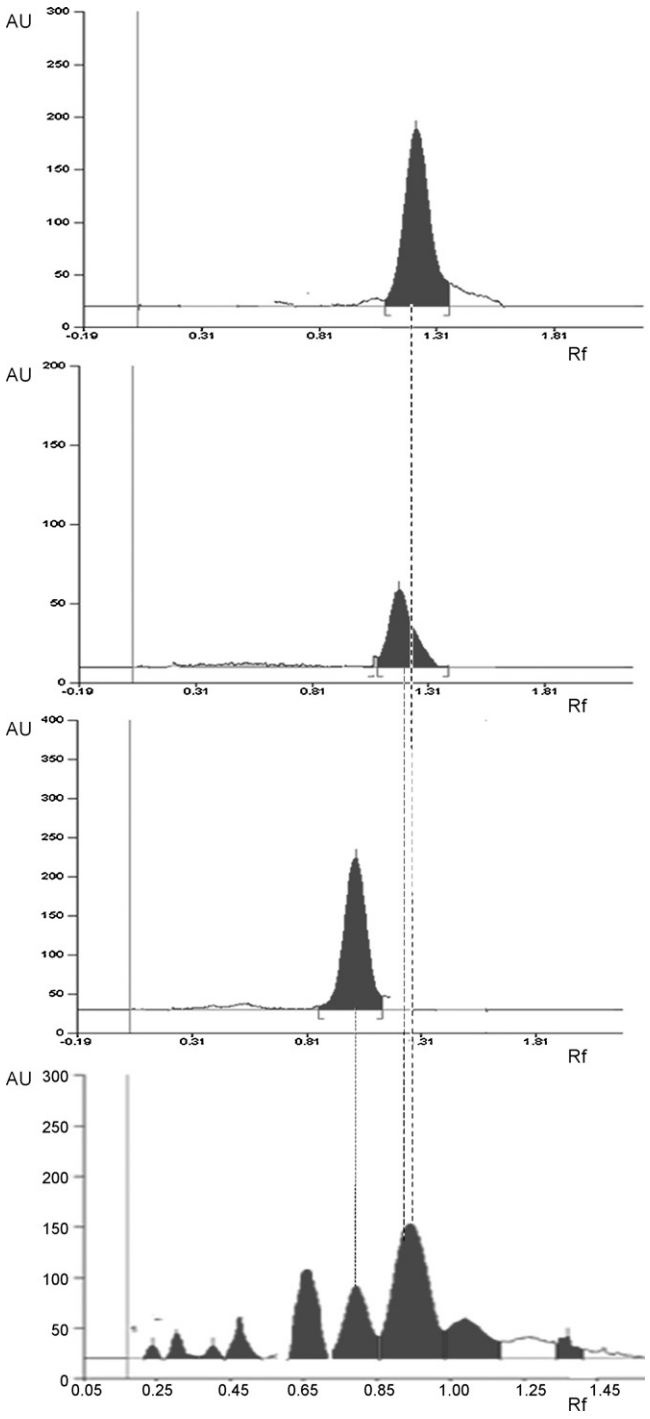

Fig. 3. HPTLC chromatograms of solavetivone, aristolone, nootkatone and acetone extract at 245 nm.

3.3. RP-HPLC-UV analysis of solavetivone, aristolone and nootkatone

A simple isocratic program was used to elute three sesquiterpenoids in a single run within a reasonable period of time. In order to get reproducible retention time, prior to next injection, the column was solvent conditioned by passing the initial solvent through the column until the baseline stabilized. Acetonitrile and water mixtures with varying ratios were tried as mobile phases using reverse phase column for profiling the isolated compounds and acetone extract. An isocratic mobile phase of acetonitrile–water (60:40) was optimized so as to obtain a complete sesquiterpenoid profile. The spectra of sesquiterpenoids were obtained by using a UV detector. As maximally efficient detection can be obtained by selecting the wavelength where the compound has the maximum absorption and the compounds were detected at a wavelength of 245 nm. With this optimized conditions solavetivone was found to elute at 10.51 min, aristolone at 8.87 min and nootkatone at 8.78 min as a symmetric and well resolved peak (Fig. 4). The amounts of solavetivone, aristolone and nootkatone were present in 0.2, 0.4 and 0.5% respectively in plant material. The method selectivity was assessed by evaluating the similarity (≥95%) between UV spectra at start, middle and end of the compound peaks.

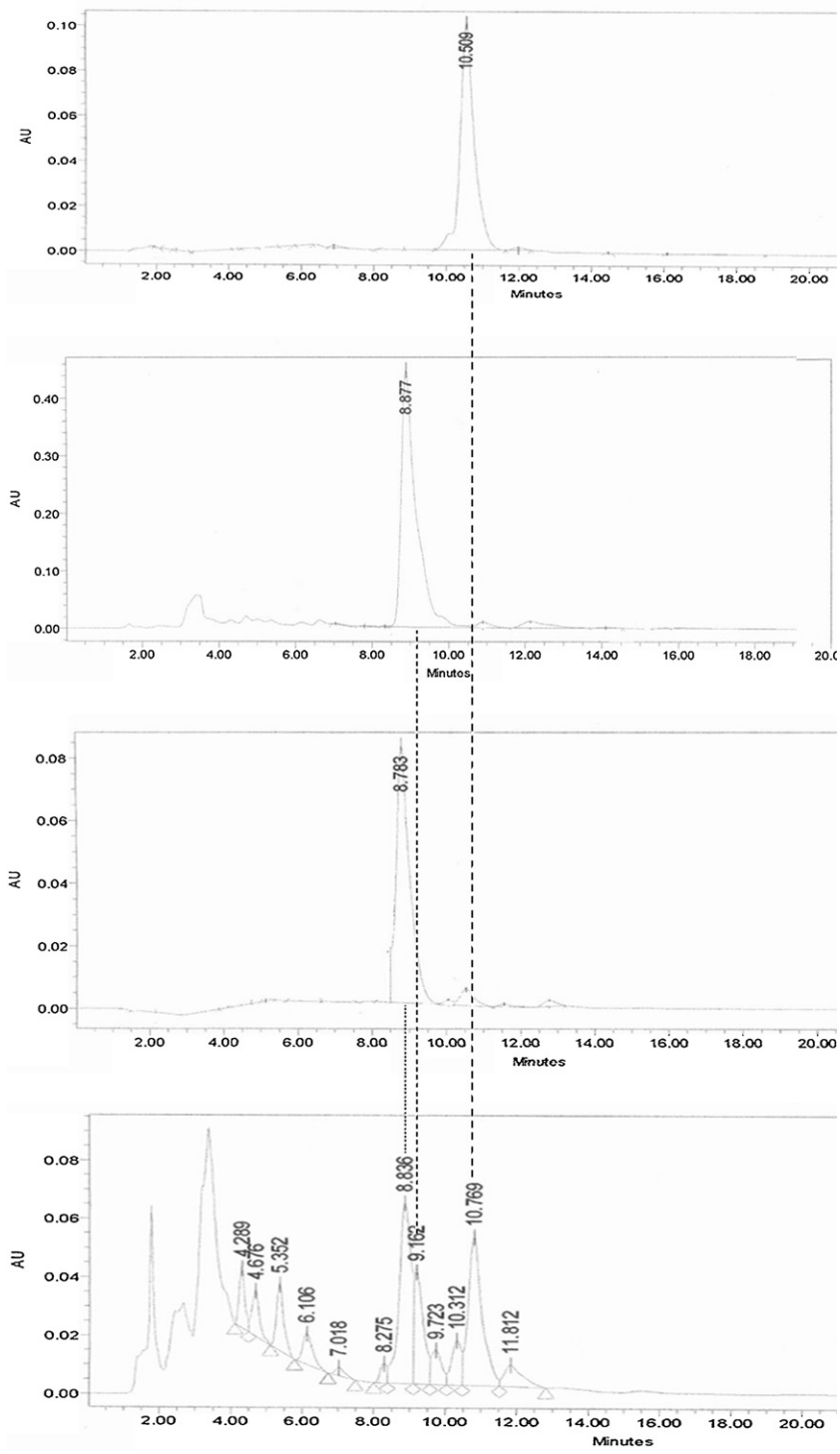

Fig. 4. HPLC chromatograms of solavetivone, aristolone, nootkatone and acetone extract at 245 nm.

3.4. Validation of methods

The developed HPTLC and HPLC methods were validated in terms of their linearity, limit of detection and quantification, precision, accuracy and robustness.

3.4.1. Linearity

Linearity between the detector responses and concentration of solavetivone, aristolone and nootkatone in HPTLC and HPLC (0.1–1 μg/ml) were evaluated. All the isolated compounds show a correlation coefficient within the range of $r = 0.99$. The amounts in plant material, correlation coefficient, LOD and LOQ of the methods, *etc.* are shown in Table 1.

3.4.2. Precision

Instrumental, intra and interassay precision are shown in Table 2 in terms of % RSD. The values were in acceptable range.

Table 1
Amount in plant material, linearity and limits of detection and quantification of HPTLC and HPLC analysis of solavetivone, aristolone and nootkatone.

Parameters	Solavetivone		Aristolone		Nootkatone	
	HPTLC	HPLC	HPTLC	HPLC	HPTLC	HPLC
Amount present in plant material	0.2%	0.2%	0.4%	0.4%	0.5%	0.5%
Regression equation[a]	$y = 1660 + 2562x$	$y = 55479.98 + 1080000x$	$y = 156.79 + 2808.72x$	$y = 12446400x - 2114330$	$y = 292.27 + 450.98x$	$y = 20070800x - 2857550$
Linear range (μg/ml)	0.5–1	0.1–1	0.5–1	0.5–1	−0.5	0.1–0.5
Correlation coefficient (r)	0.9846	0.9889	0.9809	0.9910	0.9914	0.9984
LOD (μg/ml)	1.18	0.6	0.495	1.68	0.229	1.61
LOQ (μg/ml)	3.6	2.02	1.5	5.1	0.696	4.88

[a] y = peak area, x = amount of substance added (μg).

Table 2
Precision (RSD %).

Precision	Solavetivone		Aristolone		Nootkatone	
	HPTLC	HPLC	HPTLC	HPLC	HPTLC	HPLC
Instrumental	0.76	0.99	0.98	1.01	1.01	1.23
Repeatability	0.92	1.38	1.14	1.84	1.13	1.71
Intermediate precision	1.67	1.67	1.96	1.49	1.97	1.58

3.4.3. Accuracy

To verify the accuracy of the method, recovery studies were performed by the method of addition of known amounts of isolated compounds to extract solution. Single concentration of each compound was added to the previously analyzed extract solution and the recovery was calculated and it was repeated three times. The accuracy was then calculated from the test results as the percentage of recovery of low, medium and high concentrations and the data are shown in Table 3. It can be seen that the proposed method has an adequate degree of accuracy for the determination of sesquiterpenoids.

3.4.4. Robustness

For HPTLC method, robustness was calculated by introducing small changes in certain chromatographic conditions, *i.e.*, by varying time from spotting to chromatography and from chromatography to scanning, composition, amount of mobile phase (±10%), temperature (±2 °C), dimension of HPTLC plate, development chamber (10 cm × 10 cm and 20 cm × 20 cm) and saturation time (±5 °C). The variations in HPTLC analysis because of these changes were ≤1.7 (% RSD) only. For assessing the robustness of HPLC method, variations in flow rate of mobile phase (±10%), column temperature (±2 °C) and detection wavelength (±2 nm) and value obtained for the robustness was ≤1.4 (% RSD) and is within the range required for the analysis by HPLC.

3.5. HPTLC versus HPLC

The reliability of HPTLC densitometric analysis was verified by analyzing five independently prepared acetone extracts of *C. rotundus* simultaneously by HPTLC and HPLC methods. Each sample was analyzed in triplicate and mean values were compared by matched pair Student's T-test. The observed T value (T_{obs}) was calculated by,

$$T_{obs} = \frac{|\bar{d}|}{\sqrt{\sum d_i^2 - 1/n\left(\sum d_i\right)^2/n(n-1)}}$$

where d_i is the difference between two pairs of measurements for the same observation i.

For five pairs of analysis the T_{obs} was 1.71 which was lower than the value obtained from Student's distribution table, T (95, 5, 4) = 2.78 for a risk factor of 5%. The results showed that there is no statistically significant difference between HPTLC and HPLC analytical methods.

3.6. Total antioxidant activity by phosphomolybdenum method

The antioxidant capacity of the compounds was measured spectrophotometrically through phosphomolybdenum method, which was based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate Mo (V) compounds with a maximum absorption at 695 nm. A higher absorbance indicates higher antioxidant activity. The antioxidant activities of isolated compounds and extract ranked in the order nootkatone > acetone extract > aristolone > solavetivone on the basis of absorbance. The result shows that 1 g of nootkatone posses 15.83 mg of ascorbic acid acitivity, 1 g acetone extract gives only 15.25 mg of ascorbic acid activity, 1 g of aristolone shows 6.17 mg of ascorbic acid activity and 1 g of solavetivone shows 2.8 mg of ascorbic acid activity. From this result, nootkatone posses more antioxidant potential. The results showed that the sesquiterpenoids were effective antioxidants. In phosphomolybdenum assay hydrogen and electron transfer occurs from antioxidant to Mo (VI) complex. The data presented here indicated that the antioxidant activity of sesquiterpenoids may act in a similar fashion as reductones by donating the electrons and reacting with free radicals to convert them to more stable product and terminate free radical chain reaction.

3.7. DPPH radical scavenging activity

DPPH• assay is routinely practiced for the assessment of free radical scavenging potential of an antioxidant molecule and

Table 3
Accuracy.

Parameter	Excess of solavetivone added (%)						Excess of aristolone added (%)						Excess of nootkatone added (%)					
	HPTLC			HPLC			HPTLC			HPLC			HPTLC			HPLC		
% Recovery	10	30	100	10	30	100	10	30	100	10	30	100	10	30	100	10	30	100
	99.8	101.7	103.6	104.6	100.6	101.8	99.2	102.4	104.8	101.2	99.8	102.8	100.8	102.7	105.6	99.9	103.4	102.9

considered as one of the standard and easy colorimetric methods for the evaluation of antioxidant properties of pure compounds. This method has been used extensively to predict the antioxidant activity because of the relatively short time required for analysis. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm [20]. Radical scavenging potential of the isolated compounds and extracts were compared with standard gallic acid. A low value of IC_{50} or a high percentage of radical scavenging activity indicates strong antioxidant activity. Among the three isolated compounds, the most potent radical scavenger was nootkatone (IC_{50} 4.81 μg/ml), followed by aristolone (IC_{50} 5.28 μg/ml) and finally solavetivone (IC_{50} 6.82 μg/ml) as the least active compound. Acetone extract also show an antioxidant potential with an IC_{50} value of 4.19 μg/ml compared with standard gallic acid (IC_{50} 0.483 μg/ml).

4. Conclusions

To the best of our knowledge this is the first study on the HPTLC and HPLC quantification and validation of three sesquiterpenoids solavetivone, aristolone and nootkatone from the rhizomes of *Cyperus rotundus*. Statistical findings prove that the methods are selective and repeatable for the analysis of biologically active components. Solavetivone has been reported for the first time from the rhizomes of *C. rotundus*. This work also demonstrates the comparative antioxidant potential by *in vitro* manner. The findings obtained in this study clearly demonstrate that rhizomes of *C. rotundus* possess highly potent antioxidant molecules. So this study is of great interest to both pharmaceutical and food industries because of their valuable use as natural additives to replace toxic synthetic food additives and can be used to control oxidative stress and related diseases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2012.05.042.

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