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Research Article

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Isolation, characterization and antioxidant activity of dodecyl-*p*-coumarate from *Ipomoea sepiaria*

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ABSTRACT

Ipomoea sepiaria Koenig in Roxb. Hort. Beng., commonly known as Lakshmana, is an important medicinal plant used for the treatment of burning sensation, general debility & sterility in women, hyperdypsia, constipation, diabetes and leucorrhea by the ethnic groups in India. In the present study two compounds ISPE–2 and ISPE–5 were isolated from the methanol extract of the whole plant (MEIS). Isolated compound (ISPE–2) was quantified in MEIS by high performance thin layer chromatography (HPTLC). Both MEIS and ISPE–2 were screened for in–vitro antioxidant activity by measuring DPPH (1, 1–diphenyl–2–picryl–hydrazil) radical, superoxide radical and H_2O_2 (hydrogen peroxide) scavenging capacity. Chloroform: methanol (98: 2) fraction yielded a white crystalline compound ISPE–2 (250 mg) and chloroform: methanol (95: 5) fraction yielded a white amorphous powder, ISPE–5 (20 mg). Quantity of ISPE–2 in MEIS was found to be 9.25 %. Both MEIS and ISPE–2 were found to have antioxidant activity, but activity of ISPE–*2 (*IC_{50} *>100) was found significantly low as compared to MEIS (*IC_{50} *<100*). *On the basis of spectroscopic data and physical properties, structure of ISPE–2 and ISPE–5 were confirmed as dodecyl–p–coumarate and β–sitosterol respectively. Since antioxidant activity of isolated compound ISPE–2 is low as compared to the activity of extract from which it is isolated, hence antioxidant activity of MEIS is not only due to the presence of* ISPE–*2 but might be due to the presence of several other compounds.*

Keywords: Dodecyl–*p*–coumarate; *I. sepiaria*; β–sitosterol; DPPH.

INTRODUCTION

I. sepiaria (Convolvulaceae) known as *Lakshmana* in Sanskrit, distributed throughout the India, Ceylon, Malaya and Formosa. It is a glabrous or hairy perennial herb. Stems of the plant are slender and twining. Leaves are 1–3 inch long, shape–ovate, acute apex, entire margin, cordate base and bear brownish to purplish patches towards the centre, wide sinus and rounded lobes. Flowers are arranged in the form of umbelliform with long peduncled cymes [1].

Tubers of *I. sepiaria* are sweet, cooling, alterative, uterine tonic and aphrodisiac. It is used to cure the '*tridosa*' and ulcers in Ayurvedic system of medicines. *I. sepiaria* is considered as a good antidote in arsenic poisioning [2]. The plant is also used for the treatment of burning sensation, general debility & sterility in women, hyperdypsia [3], laxative [4] and diabetes [5] by the ethnic groups in India. Root powder of *I. sepiaria* along with rice water is used for the treatment of leucorrhea by the tribes of Nalgonda District of Andhra Pradesh [6]. In Ayurvedic texts I. *sepiaria* is reported as miraculous drug and used for getting male child (*Pumsavana karma*), when the leaves juice of the plant installed in the right nostril by the pregnant women during the 2nd and 3rd month of pregnancy [7]. *I. sepiaria* is reported to have good antibacterial and antifungal activities [8–9]. Literature survey revealed that *I.*

sepiaria is an important medicinal plant but isolation and characterization of phytoconstituents has not been reported till the date. In the present study an attempt was made for the isolation and characterization of phytoconstituents from *I. sepiaria.* Both extract and isolated compound were screened for *in–vitro* antioxidant activity.

EXPERIMENTAL SECTION

Plant material

Whole part of *I. sepiaria* was collected from the medicinal garden of Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur during the month of October to December, 2012. The identification of the plant material was done at the Botanical survey of India, Howrah, West Bengal, India (Plant identification Letter: CNH/104/2012/Tech. II/950) as *Ipomoea sepiaria* Koenig in Roxb. Hort. Beng. (Convolvulaceae). For future reference a voucher specimen (No. PRL–03) of the plant material has been deposited in the Department of Medicinal Chemistry, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

Preparation of plant extract

Whole part of *I. sepiaria* was collected and shade dried until a constant weight obtained. The plant material was then pulverized in the form of coarse powder and sieved by 20 # sieve. The coarse powdered drug (2.0 Kg) was subjected to Soxhlet extraction using petroleum ether (5.0 L) as solvent for 72 h. The marc obtained after defatting process (1.6 Kg) was subjected to Soxhlet extraction using methanol (3.0 L) as solvent for 72 h. Methanol extract obtained was concentrated under reduced pressure in rotary evaporator (Perfit India, Pvt. Ltd, Ambala) below 60 ºC temperature to obtain the concentrated methanol extract of *I. sepiaria* (MEIS). The remaining traces of solvent were removed from the extract by storing in vacuum desiccator for several days to afford a dry residue (45 g).

Column chromatography of methanol extract

MEIS (12 g) was subjected to column chromatography using silica gel 80–120 # as adsorbent and eluted in gradient manner with different proportion of petroleum ether: benzene, benzene: chloroform and chloroform: methanol. Fractions were collected in 100 mL quantity in conical flask. Chloroform: methanol (98: 2) fraction yielded a white crystalline compound ISPE–2 (250 mg) which was crystallized in ethanol. Chloroform: methanol (95: 5) fraction yielded a white amorphous powder which was purified several times with ethanol to afford compound ISPE–5 (20 mg).

Quantitative estimation of ISPE–*2*

Quantitative estimation of ISPE–2 was performed by HPTLC using Camag–HPTLC instrument equipped with Linomat V sample applicator, Camag TLC scanner 3, Camag TLC visualizer and winCATS 4 software for data interpretation. Silica gel 60G F_{254} (Merck, Mumbai, India) plates (20 cm × 10 cm) was used as stationary phase for the quantitative analysis. Stock solutions of ISPE–2 (50 µg/mL) and MEIS (1.5 mg/mL) were applied on the plates for making the bands of 8.0 mm wide, 30.0 mm apart from each other and 10 mm apart from the bottom edge of the plates. Chromatographic plates were developed in ascending order up to a height of 80 mm at temperature 28 ± 2 °C using chloroform: methanol (9.9: 0.1) as mobile phase in Camag glass twin-trough chamber. After the development, plates were dried in hot air oven at 60°C temperature and scanned at 254 nm.

In–vitro antioxidant activity of MEIS and ISPE–*2*

Free radicasl scavenging activity

Free radicles scavenging activity of MEIS and ISPE–2 were performed by measuring the DPPH radical scavenging capacity as described by Mensor *et al* [10] with slight modifications. Briefly, different dilutions (25–200 µg/mL) of MEIS and ISPE–2 were prepared in methanol. To 2.5 mL of each dilution, 1.0 mL of 0.3 mM freshly prepared DPPH solution in methanol was added and allowed to react in dark at room temperature for 30 min. Absorbance of the resulting solutions were measured at 518 nm. Blank solution of each sample was prepared by adding 1 mL of methanol to 2.5 mL of sample solution, while negative control was prepared by adding 1.0 mL of 0.3 mM DPPH solution to 2.5 mL of methanol. Ascorbic acid prepared in the similar manner was used positive control.

All the estimation was performed in triplicate and the percentage DPPH scavenging activities of MEIS, ISPE–2 and ascorbic acid were determined using the formula-

$$\% \text{ radical scavenging activity} = [(A_c - A_s) / A_c] \times 100 \quad \text{.....equation (1)}$$

Where A_s = Absorbance of sample (extract or reference standard) and A_c = Absorbance of negative control.

Superoxide radical scavenging activity

Superoxide radical scavenging activity of MEIS and ISPE–2 was performed by alkaline DMSO (dimethyl sulfoxide) method as described by Elizabeth and Rao [11]. Alkaline DMSO (1 mL) was added to the reaction mixture containing 0.1 mL of nitro blue tetrazolium (NBT) (1 mg/mL solution in DMSO) and 0.3 mL of the various concentrations of the MEIS and ISPE–2 prepared in methanol. Ascorbic acid prepared in the similar way as test samples was used as positive control. Absorbance of the resulting solutions was measured at 560 nm. All estimation was performed in triplicates and the percentage inhibition was calculated using equation (1).

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of MEIS and ISPE–2 was performed as per the method described by Jayaprakasha *et al* [12]. A solution of H_2O_2 (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). 1 mL of various concentrations of MEIS and ISPE–2 prepared in methanol were added with 2 mL of H_2O_2 prepared in PBS. The absorbance of resulting solutions were measured at 230 nm after 10 min. Ascorbic acid prepared in the similar way as test samples was used as positive control. All estimations were performed in triplicates and the percentage inhibition was calculated using equation (1).

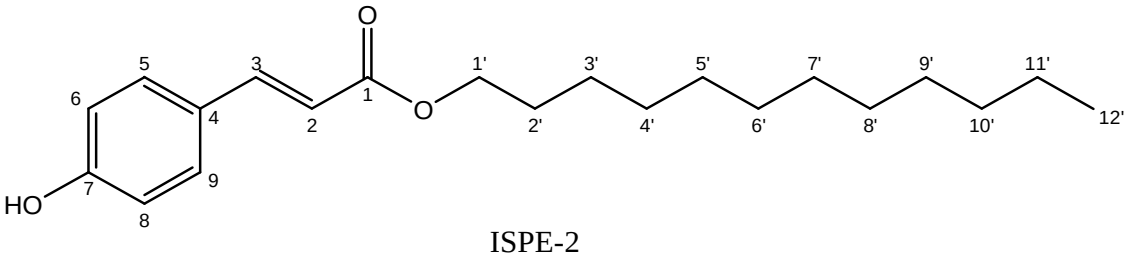
Statistical analysis

All the results were expressed as mean ± S.E.M (n=3) and the analysis of variance was done by one way ANOVA followed by Dunnett post hoc tests. The difference was considered to be significant when $p<0.05$.

RESULTS AND DISCUSSION

Characterization of ISPE–2

UV (MeOH) λ_{max}: 208, 312; mp 74–75 ºC; IR (KBr) Cm^{-1} : 3387 (O–H stretch), 2922 (C–H stretch), 2847, 1676 (trans C=C stretch), 1597, 1513 (aromatic ring system), 1466, 1272, 1028 (C–O–C stretch), 834, 722; ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.61 (1H, d, *J*=15.9 Hz, *trans* H–3), 7.40 (2H, d, *J*=8.4 Hz, H–5,9), 6.84 (2H, d, *J*=8.4 Hz, H–6,8), 6.28 (1H, d, *J*=15.9 Hz, *trans* H–2), 5.98 (1H, s, 7–OH), 4.18 (2H, t, *J*=6.6 Hz, H–1′), 1.67 (2H, m, H–2′), 1.23–1.06 (18H, br s, H–3′ – H–11′), 0.86 (3H, t, *J*=6.6 Hz, H–12′); ^{13}C–NMR ($CDCl_3$, 75 MHz); δ 168.2 (C–1), 158.1 (C–7), 144.8 (C–3), 130.2 (C–5,9), 127.3 (C–4), 116.1 (C–6,8), 115.7 (C–2), 65.1 (C–1′), 32.6 (C–10′), 29.9–26.2 (29.92, 29.89, 29.82, 29.76, 29.59, 29.52, 28.94, 26.20) (C–2′–C–9′), 22.9 (C–11′), 14.4 (C–12′); ESI–MS (positive ion mode) m/z (% rel. int.): 333 $[M+1]^+$ (10), 332$[M]^+$ (6), 329 (80), 317 (15), 311 (25), 301 (100), 288 (15), 279 (20), 255 (25).



ISPE-2

Figure 1 Structure of ISPE–2

^{13}C–NMR spectra of ISPE–2 showed the presence of 21 carbon atoms. DEPT–135 and DEPT–90 spectra indicate the presence of 1 primary carbon, 11 secondary carbons, 6 tertiary carbons and 3 quaternary carbons in the compound. A broad band in the IR spectra at 3387 $cm^{-1}$and ^{1}H–NMR signal at δ 5.98 indicate the presence of –OH group in the compound. Two doublet signals at δ 7.40 (*J*= 8.4 Hz) and δ 6.84 (*J*= 8.4 Hz) in the ^{1}H–NMR spectra indicating the presence of p–substituted benzene ring system. ^{1}H–NMR signals at δ 7.61 (d, *J*=15.9 Hz) and 6.28 (d, *J*=15.9 Hz,) showed the presence of *trans* double bond in the compound. Further a triplet peak at δ 0.85 and a singlet broad peak between δ 1.23–1.06 showed the presence of a methyl group and a long chain of CH_2. ^{13}C–NMR at δ 168.2 showed the presence of a conjugated carbonyl group in the compound. On the basis of spectroscopic data the structure of ISPE–2 was confirmed as dodecyl–*p*–coumarate. Though the dodecyl–*p*–coumarate has been previously isolated from *I. carnea*, this is the very first report for the isolation of dodecyl–*p*–coumarate from this plant.

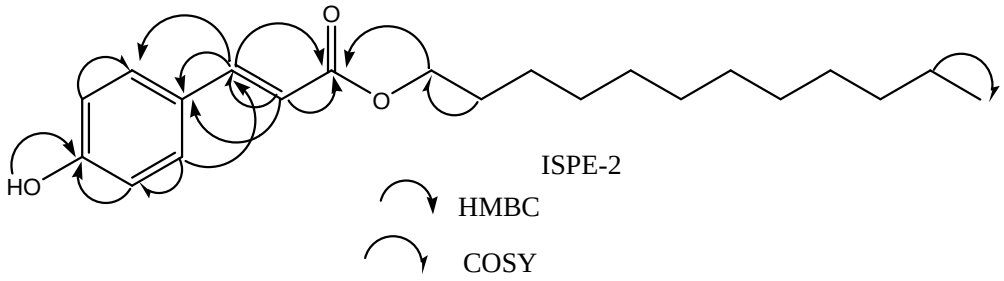


Figure 2 HMBC and COSY correlations of ISPE–2

Characterization of ISPE–*5*

Compound ISPE–5 identified as β–sitosterol on the basis of co–TLC with standard sample of β–sitosterol (R_f value: 0.6, Chloroform: methanol 90:10) and its melting point (137-138ºC). This is the first report for the isolation of β–sitosterol in this plant.

Quantitative estimation of ISPE–*2*

The quantification of ISPE–2 in MEIS was performed by HPTLC by scanning at 254 nm. Quantity of ISPE–2 in MEIS was found to be 9.25 % w/w while 0.28 % % w/w in whole part of dried plant material (Figure 3). The quantity of dodecyl–*p*–coumarate in MEIS was found to be 9.25 %.

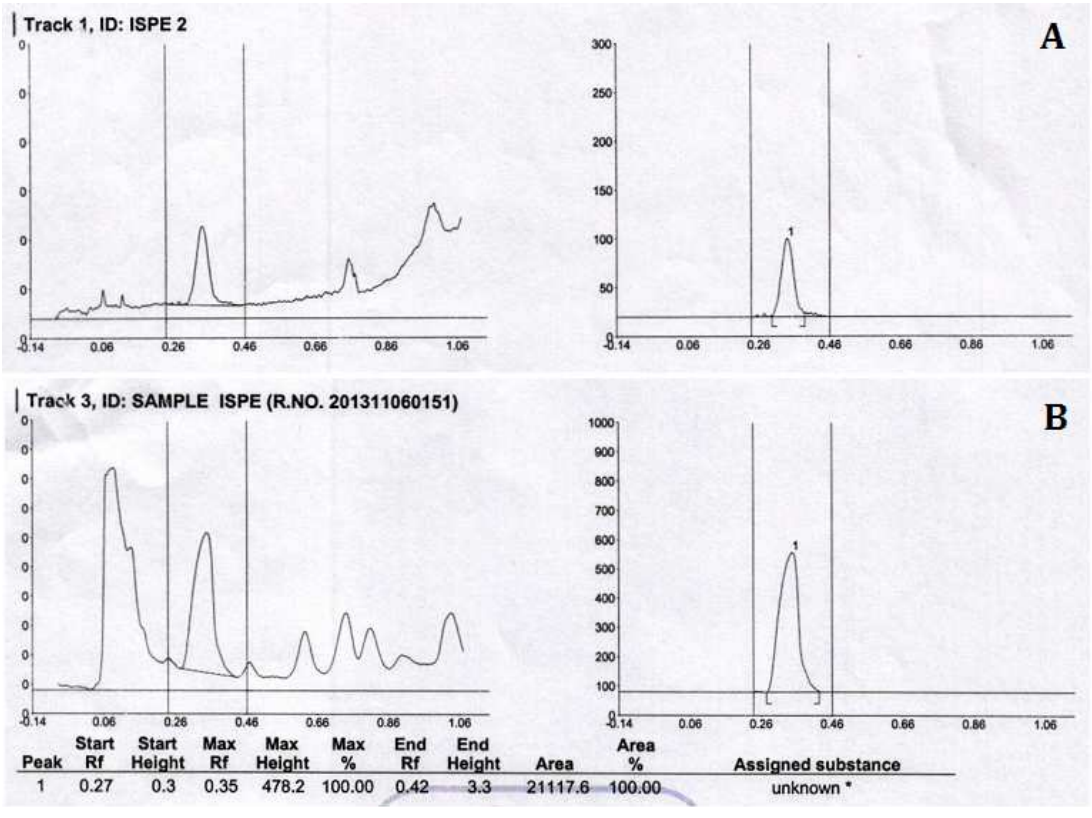


Figure 3 HPTLC quantification of ISPE–2 in MEIS. (A) Standard peak of pure ISPE–2 (B) ISPE–2 peak present in MEIS

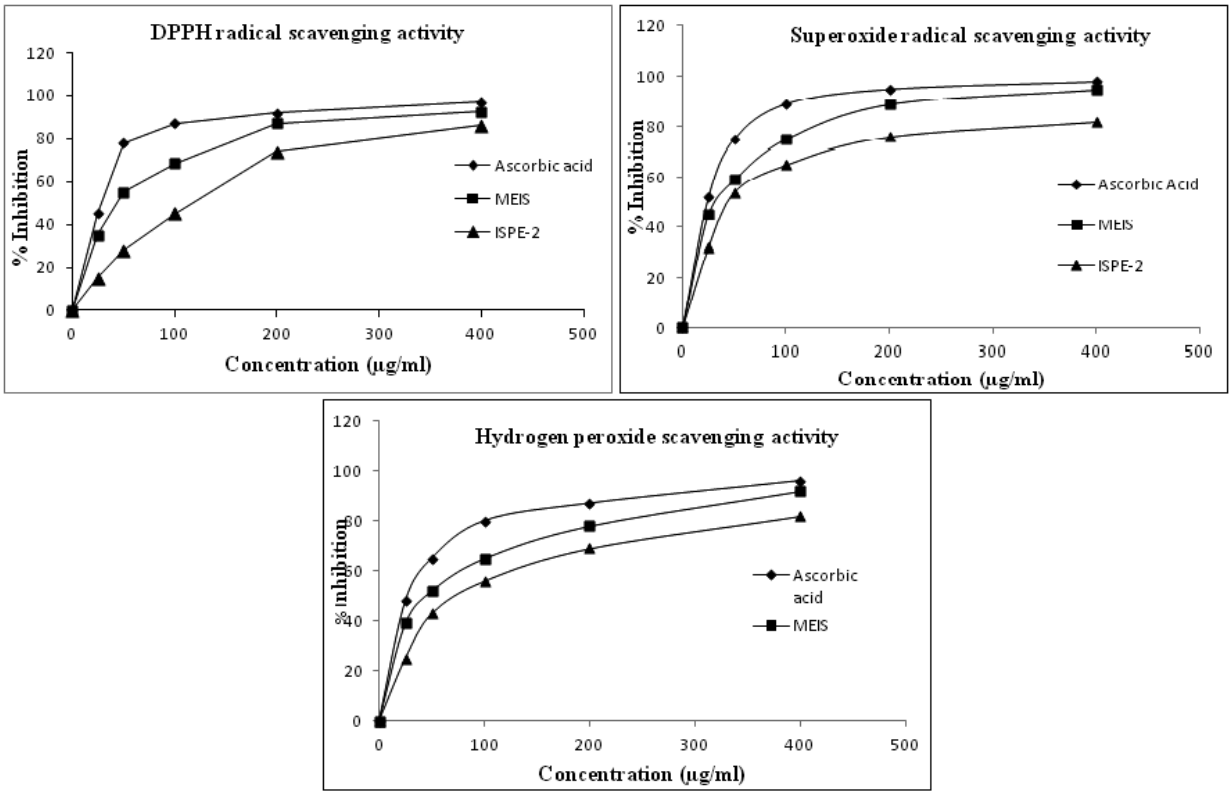


Figure 4 Radical scavenging activity of MEIS, ISPE–2 and ascorbic acid

***In–vitro* antioxidant activity**

DPPH is widely used to evaluate the antioxidant property of compounds as well as plant extracts. In DPPH assay, antioxidant substances reduce the stable DPPH radical to a yellow color diphenyl-picrylhydrazine chromogen, which shows the maximum absorbance of 518 nm [13]. Hydrogen peroxide, though not a free radical but play a crucial role in the oxidative stress [14]. Superoxide anion is a primary reactive oxygen species (ROS) and generated in the human body by auto oxidative processes. Further it leads to the generation of several secondary ROS [15]. The results of DPPH free radical, superoxide radical scavenging and H_2O_2 scavenging activity were represented in Table 1 and Figure 4. MEIS showed DPPH radical superoxide radical and H_2O_2 scavenging activity with IC_{50} value 95.07 ± 1.50, 71.38 ± 2.50 and 98.27 ± 1.67 respectively. Antioxidant activity of ISPE-2 was found significantly low (IC_{50} value 174.99 ± 2.02, 122.38 ± 2.76 and 153.24 ± 1.96 respectively) as compared to MEIS. Ascorbic acid showed significantly high antioxidant activity (IC_{50} value 33.46 ± 0.87, 23.05 ± 1.23 and 36.49 ± 0.92 respectively) as compared to both MEIS as well as compound ISPE–2.

Table 1 Antioxidant activity of MEIS, ISPE–2 and ascorbic acid

IC_{50} value in µg/mL required for scavenging the free radical			
	DPPH radical	Superoxide radical	H_2O_2 radical
Ascorbic acid	33.46 ± 0.87	23.05 ± 1.23	56.49 ± 0.92
MEIS	95.07 ± 1.50[a]	71.38 ± 2.50[a]	98.27 ± 1.67[a]
ISPE-2	174.99 ± 2.02[ab]	122.38 ± 2.76[ab]	153.24 ± 1.96[ab]

All results are expressed as Mean ±S.E.M. Statistical comparison was determined by one way ANOVA followed by the Dunnett post hoc tests.
[a]P <0.05, statistically significant compared to ascorbic acid; [b]P <0.05, statistically significant compared to MEIS.

Phenolic compounds are widely distributed in plants, act as natural antioxidants [16]. *p*–coumarates are natural hydroxycinnamic acid derivatives existing in grapes, barley, navy beans, tomatoes, carrots, garlic, honey, wine and vinegar [17–18]. *p*–coumaric acid are reported to have good antioxidant activity and are bereaved to reduce the risk of stomach cancer [19–20]. Antioxidant activity (DPPH radical, superoxide radical and H_2O_2 scavenging activity) of MEIS and dodecyl–*p*–coumarate (ISPE–2) was estimated and compared with antioxidant activity of ascorbic acid.

Though the quantity of ISPE–2 in MEIS is high yet ISPE–2 (IC_{50} >100) exhibited significantly low antioxidant activity as compared to MEIS (IC_{50} < 100) and ascorbic acid (IC_{50} < 100).

CONCLUSION

Therefore the result obtained indicates that antioxidant activity of MEIS is not only due to the presence of ISPE–2 but might be due to the presence of several other compounds along with dodecyl–*p*–coumarate. Further low antioxidant activity of dodecyl–*p*–coumarate as compared to *p*–coumaric acid mighty be due to the presence of saturated long chain alkyl.

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