

## Hepatoprotective activity of terpenoids and terpenoid fractions of *Scoparia dulcis* L

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### SUMMARY

*Scoparia dulcis* L. is widely used in the traditional system of medicine for treating liver ailments. In the present study the terpenoids and terpenoid fractions isolated from 1:1:1 petroleum ether, diethyl ether and methanol (PDM) extract of *Scoparia dulcis* L. were tested for their *in vitro* 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Selected samples from the assay were further tested for their *in vitro* hepatoprotective activity against CCl<sub>4</sub> induced hepatotoxicity in freshly isolated rat hepatocytes. In the *in vitro* antioxidant study, fractions 7, 11, 13, 14, and 15 and PDM extract show the DPPH radical scavenging activity. The phytochemical screening of all these fractions show the presence of terpenoids. In the *in vitro* hepatoprotective study all these fractions and the PDM extract significantly prevent the CCl<sub>4</sub> induced changes in the aspartate aspartate amino transferase, alanine amino transferase and alkaline phosphatase levels ( $p < 0.05$ ). The above results are comparable with the standard, silymarin. The results of the study indicate that, the PDM extract of *Scoparia dulcis* L. possesses potential hepatoprotective activity and this may be attributed to its free radical scavenging potential, which in turn may be attributed to the presence of terpenoids.

**Key words:** *Scoparia dulcis* L.; Hepatoprotective activity; Carbon tetrachloride

### INTRODUCTION

The liver is a versatile organ which is responsible for the metabolism of chemicals and for the regulation of internal chemical environment. Hepatotoxicity may be caused by thousands of synthetic chemicals, drugs, bacteria, fungi, plants and animal toxicants. These agents cause liver

damage either by themselves or by getting converted to toxic metabolites (Victor, 1952; Valmane, *et al.*, 1974; Carlo, 1979; Gupta, 1983). The most common hepatic disorders are viral hepatitis, non-alcoholic fatty liver disease (NAFLD), drug-induced hepatotoxicity, and alcoholic fatty liver disease. In India, it is reported that about 1% of the population is infected with hepatitis C and 2 - 4% with hepatitis B virus. NAFLD is the most prevalent liver disease, affecting up to 24% of patients in the general population and up to 74% of those with obesity. The prevalence of this disease is likely to continue

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to rise, paralleling the increasing global prevalence of diabetes and obesity (Furquan, 2007). Although, liver has a tremendous ability for regeneration, acute liver illnesses often leads to serious chronic sequelae such as, chronic hepatitis, cirrhosis and even carcinoma.

Herbal drugs play a major role in the treatment of hepatic disorders. A number of medicinal plants and their formulations are widely used for the treatment of these disorders. *Scoparia dulcis* L (family: Scrophulariaceae) is a glabrous under shrub with small white flowers commonly found on the waste lands and fallow fields. This plant is widely used in the traditional system of medicine for treating liver ailments (Kirtikar and Basu, 1988; Yoganarasimhan, 1996; Parrotta, 2001). Phytochemical screening carried out by earlier workers on the plant has revealed the presence of 3 different types of diterpenoids; labdane-type (scopadulcic acids A, B, C, and scopadiol) scopadulan-type (scopadulcic acids A and B, and scopadulciol) and aphidicolin - type (scopadulin) (Hayashi, 1998). It is also reported to contain 7-O-methyl scutellarein, a flavonoid and amellin, an antidiabetic compound (Ramesh *et al.*, 1979).

Our earlier studies with the aqueous, 70% alcoholic and 1:1:1 petroleum ether, diethyl ether and methanol extracts of this plant, has shown significant hepatoprotective activity in rodents models of acute liver damage and cirrhosis (Praveen *et al.*, 2008; Praveen *et al.*, 2009). In the present study an effort has been made to isolate the active compounds and the fractions containing terpenoids and to screen them for their *in vitro* hepatoprotective activity against CCl<sub>4</sub> induced toxicity in freshly isolated rat hepatocytes.

## MATERIALS AND METHODS

### Drugs and chemicals

Silymarin was a gift sample from Micro Labs, Hosur, India. Aspartate amino transferase (ASAT) and alanine amino transferase (ALAT), alkaline

phosphatase (ALP) and total protein (TP) kits were from RANDOX Laboratories Ltd. United Kingdom. Collagenase, insulin and dexamethasone were from Sigma chemical Co, St Louis, USA. Ham's F 12 was from Hi-Media laboratories, Mumbai. All other chemicals and reagents used were of analytical grade.

### Preparation of PDM extract

The terpenoid extract of *Scoparia dulcis* L. was prepared using petroleum ether (40 - 60 °C), diethyl ether and methanol as reported earlier (Manirudin and Jasmin, 1990). Authenticated (voucher no. BUB12005) whole plant was collected from the campus of Manipal College of Pharmacy, Udupi, India. The whole fresh plant was dried under shade at room temperature for seven days and reduced to coarse powder (sieve No.10/40). This powder was used for the preparation of petroleum ether, diethyl ether and methanol (PDM) extract by soxhlet extraction method. The dried powder (125 g) was extracted three times with 2.5 liters of PDM at 50 - 55 °C for 24 h. The extract obtained was concentrated at 50 °C for 12 h.

### Isolation of terpenoids and terpenoid fractions from PDM extract

PDM extract (25 gm) was mixed with 25 gm of silica gel (60 to 120 mesh size). The column was packed with silica gel (60 to 120 mesh size) ten times to the weight of dry crude extract using petroleum ether by wet packing method. The adsorbed crude drug was subjected to the prepared column. The column was eluted initially with petroleum ether, followed by petroleum ether and diethyl ether (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100), then by diethyl ether: methanol. 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100), in a gradient manner. Each fraction of 250 ml was collected and concentrated. Purification of isolated compounds was carried out by washing and recrystallization.

### Characterization of isolated compounds

The melting points of the isolated compounds were determined in open capillaries and are uncorrected. The temperatures were expressed in °C and are uncorrected. The IR spectra of compounds were recorded on Perkin-Elmer Infrared 283 FTIR spectrometer by KBr pellet technique and are expressed in  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR spectra were recorded on Bruker DRX 300 (300 MHz, FT NMR) spectrophotometer using TMS as an internal standard and  $\text{CDCl}_3$  and  $\text{DMSO-d}_6$  as solvents. The chemical shifts are expressed in  $\delta$  ppm and the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartlet, m = multiplet. Mass spectrum was obtained using LC MS (Schimadzu 2010 EV) under electro spray ionization (ESI) technique.

### 1, 1-Diphenyl-2-picrylhydrazyl [DPPH] scavenging assay

The assay was carried out in 96 well micro titre plates. The extract (100  $\mu\text{l}$ ) and the standard (100  $\mu\text{l}$ ) were serially diluted with double distilled water. DPPH (100 mM, 100  $\mu\text{l}$ ) solution was added to all the wells except the blank and incubated at room temperature for 20 min. The absorbance was measured at 490 nm using ELISA reader (BIORAD - 550).

*In vitro* hepatoprotective activity of PDM extract and its fractions

The *in vitro* hepatoprotective activity of the PDM extract and its selected fractions were carried out using freshly isolated rat hepatocytes. The rat liver cells were isolated as per the modified procedure of Seglen (1976). A male Wistar rat (250 g) was given insulin (500 IU/animal, i.p.) and anaesthetized with ketamine and xylazine injection (ketamine 83 mg/ml, xylazine 16.7 mg/ml, dose 0.75 ml/kg, i.p.). The abdomen of the rat was cleaned with 70% alcohol and cut opened to expose the portal vein. The portal vein was isolated and a cannula was inserted and secured with the help of a silk thread. The inferior vein cava was cut open before starting the perfusion. The perfusion was started with 100 ml

warm (37 °C) calcium free HEPES buffer containing 1% bovine serum albumin at the flow rate of 10 ml/min, then with 50 ml of TPVG (Trypsin, Phosphate buffer saline, Versine, Glucose), followed by calcium free HEPES buffer containing 0.075% collagenase and 4 mM calcium chloride.

After perfusion, the liver lobes were removed and transferred into a sterile conical flask containing calcium free HEPES buffer, the tissue was dispersed gently and the cell suspension was stirred with the help of a magnetic stirrer for 5 min to release the hepatocytes into the solution. The cell suspension was filtered through nylon mesh (250  $\mu$ ) and the filtrate was centrifuged at 1000 rpm for 15 min. The resulting cell pellet was gently re-suspended in calcium free HEPES buffer. This washing procedure was repeated three times. The cell viability was determined by the trypan blue dye exclusion method. The isolated hepatocytes were cultured in Ham's F12 medium, supplemented with 10% new born calf serum, antibiotic,  $10^{-6}$  M dexamethasone and  $10^{-8}$  bovine insulin. The cell suspension was incubated at 37 °C in a humidified incubator under 5%  $\text{CO}_2$ .

$\text{CCl}_4$  induced hepatotoxicity assay was carried out, after an incubation for 24 h, the hepatocytes were exposed to the fresh medium containing  $\text{CCl}_4$  (1%) along with or without various concentrations of test samples and standard, silymarin (n = 6). After 60 min of  $\text{CCl}_4$  challenge the media was analyzed for aspartate amino transferase (ASAT), alanine amino transferase (ALAT), alkaline phosphate (ALP) and total proteins (TP) using commercial kits in Merck auto analyser.

### Phytochemical analysis of PDM extract and its active fractions

The PDM extract and its active fractions were analyzed for the presence of phytochemicals by using standard methods (Tyler *et al.*, 1981). It was then subjected to HPTLC analysis (CAMAG, Muttenz, Switzerland) using precoated TLC plates of silica gel 60  $\text{F}_{254}$  (Merck, Darmstadt, Germany). A solution of PDM extract and its active fractions (5 mg/ml)

were spotted using Linomat IV applicator. TLC was developed using chloroform methanol (9:1) as solvent system. After development, the TLC plate was dried and scanned using CAMAG HPTLC scanner III, integrated with WIN CATS software (Version 4.06) and the peaks were recorded at wavelength of 254 nm.

### Statistical analysis

The data was represented as mean  $\pm$  S.E.M. Results were analyzed statistically by one-way ANOVA followed by Dunnett's multiple comparison test using Prism software (Version 4). The minimum level of significance was set at  $p < 0.05$ .

## RESULTS

### Preparation of PDM extract

The soxhlet extraction of 500 g of the plant material with 1:1:1 petroleum ether, diethyl ether, and methanol, yielded 35 g of greenish brown residue (yield 7% w/w).

### Isolation of terpenoids and terpenoid fractions from PDM extract

The gradient elution of the PDM extract with petroleum ether, diethyl ether, and methanol resulted in 34 fractions. Recrystallization of fractions F3 (1), F3 (2) eluted with petroleum ether and diethyl ether (80: 20), resulted in the isolation of compounds 1 and 2, respectively. Recrystallization of fraction F4 (1) and F4 (2) eluted with petroleum ether and diethyl ether (70: 30), resulted in the isolation of compounds 3 and 4, respectively.

### Characterization of isolated compounds

Analytical data for compound 1: mp 125 - 127 °C (Crystal, white solid). IR (KBr,  $\text{cm}^{-1}$ ): 3472 (O-H stretching), 2919 (C-H aliphatic stretching), 1707 (C=O, stretching), 1472 (C=C), 1362 (C-O stretching).  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  0.9 - 1.2 ( $\text{CH}_3 \times 3$ , s), 1.2 - 2.0 ( $\text{CH}_2 \times 8$ , m), 2.3 (CH, t), 3.41 ( $\text{CH}_2$ , s), 5.5 (=CH-, t). MS (m/z): 286, 259, 191, 152, 69. Based on the above

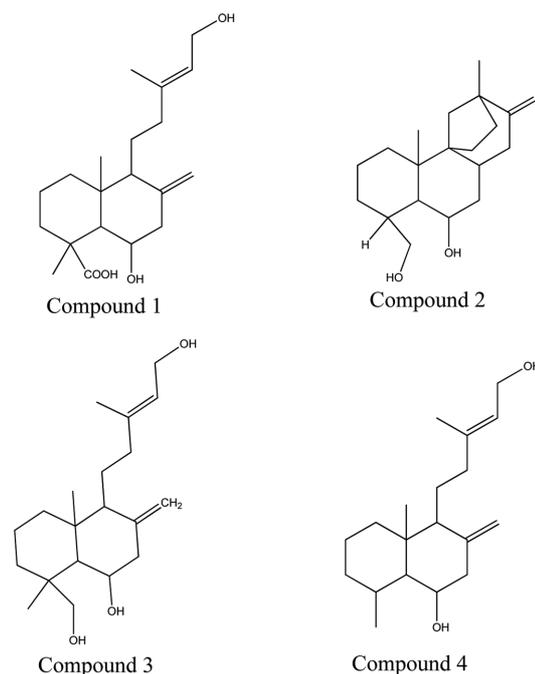


Fig. 1. Proposed structures of the isolated compounds.

data and the data from literature, the structure shown in Figure 1 is proposed for compound 1.

Analytical data for compound 2: mp 87 - 89 °C (Crystal, white solid). IR (KBr,  $\text{cm}^{-1}$ ): 3312 (O-H stretch), 2944 (C-H aliphatic stretching), 1716 (C=O, stretching), 1463 (C=C, stretching), 1030 (C-O, stretching).  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  5.2 (OH, d), 3.14 (OH, s), 1.2 - 2.16 ( $\text{CH}_2$ , CH), 0.7 - 1.0 ( $\text{CH}_3$ ). Mass fragments (m/z) 259, 241, 229, 135. Based on the above data and the data from literature, the structure shown in Figure 1 is proposed for compound 2.

Analytical data for compound 3: mp 135 - 138 °C (Crystal, white solid). IR (KBr,  $\text{cm}^{-1}$ ): 3426 (OH, stretching), 2958 (C-H, aliphatic stretching), 1464 (C=C, stretching), 1051 (C-O).  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  0.7 ( $\text{CH}_3 \times 3$ , s), 1.12 - 2.08 ( $\text{CH}_2 \times 8$ , CH  $\times$  3), 5.0 (CH=, t), 5.35 ( $\text{CH}_2$ =, s). Mass fragments (m/z) 303, 289, 84, 43. Based on the above data and the data from literature, the structure shown in Figure 1 is proposed for compound 3.

Analytical data for compound 4: mp 63 - 66 °C, IR (KBr,  $\text{cm}^{-1}$ ): 3452 (OH, stretching), 2942 (C-H

**Table 1.** Effect of PDM extract and its fractions on DPPH radical scavenging activity

	IC 50 ( $\mu\text{g/ml}$ )
PDM extract	169.5 $\pm$ 0.62
Fraction 7	157.2 $\pm$ 1.47
Fraction 11	352.1 $\pm$ 3.50
Fraction 13	75.0 $\pm$ 1.33
Fraction 14	135.6 $\pm$ 4.66
Fraction 15	127.8 $\pm$ 2.59
Vitamin C	9.4 $\pm$ 0.70

Values are mean  $\pm$  SEM, n = 3

aliphatic, stretching), 1686 (C=C-CH<sub>3</sub>), 1452 (C=C), 1033 (C-O, stretching). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  0.7 - 1.0 (CH<sub>3</sub> x 3, s), 1.1 - 1.8 (CH<sub>2</sub> x 5, CH, m), 1.9 (CH<sub>2</sub>, m), 2.3 (CH<sub>2</sub>, m), 3.0 (OH, t), 3.1 (OH, d), 4.58 (CH=), 4.7 (CH=). Mass fragments (m/z) 292, 259, 248, 207, 189, 95, 81, 43. Based on the above data, the structure shown in Figure 1 is proposed for compound 4.

### 1, 1-Diphenyl-2-picrylhydrazyl [DPPH] scavenging assay

The results of the *in vitro* antioxidant activity of the PDM extract, its fractions and isolated pure compounds are given in the Table 1. Among the listed samples and the PDM extract, F7 (40:60, petroleum ether and diethyl ether), F11 (100% diethyl ether), F13 (80:20, diethyl ether and methanol), F14 (70:30, diethyl ether and methanol) and F15 (60:40, diethyl ether and methanol) showed *in vitro* antioxidant activity against DPPH free radicals. All these samples scavenged the DPPH free radical in a dose dependent manner. Among these, fraction 13 showed the most potent antioxidant activity with an IC<sub>50</sub> value of 75.0  $\pm$  1.33 (Mean  $\pm$  SD), which is comparable to the standard, Vitamin C, which showed an IC<sub>50</sub> value of 9.4  $\pm$  0.7 (Mean  $\pm$  SD).

**Table 2.** Effect of PDM extract and its fractions on CCl<sub>4</sub> intoxicated freshly isolated rat hepatocytes

Group	Treatment	Concentration	ASAT (u/l)	ALAT (u/l)	ALP (u/l)	TP (g/dl)
Group 1 (Normal)	Vehicle	0.5% DMSO	43.5 $\pm$ 1.5	60.1 $\pm$ 4.0	13.4 $\pm$ 1.2	1.2 $\pm$ 0.03
Group 2 (Control)	Vehicle	0.5% DMSO	312.2 $\pm$ 7.2	125 $\pm$ 5.8	28.4 $\pm$ 0.9	0.85 $\pm$ 0.02
Group 3	Silymarin	20 $\mu\text{g/ml}$	66.2 $\pm$ 4.6*	78.6 $\pm$ 3.9*	16.4 $\pm$ 0.6*	0.91 $\pm$ 0.03
Group 4	PDM extract	63 $\mu\text{g/ml}$	88.0 $\pm$ 2.8*	81.2 $\pm$ 4.2*	17.5 $\pm$ 0.8*	0.94 $\pm$ 0.05
		31 $\mu\text{g/ml}$	96.8 $\pm$ 3.6*	85.6 $\pm$ 3.6*	20.4 $\pm$ 0.4*	0.93 $\pm$ 0.06
		16 $\mu\text{g/ml}$	112.5 $\pm$ 4.5*	105.6 $\pm$ 4.6*	20.5 $\pm$ 0.8*	0.85 $\pm$ 0.02
Group 5	Fraction 7	63 $\mu\text{g/ml}$	71.5 $\pm$ 2.5*	62.3 $\pm$ 2.1*	15.5 $\pm$ 0.6*	0.92 $\pm$ 0.05
		31 $\mu\text{g/ml}$	82.3 $\pm$ 2.6*	75.6 $\pm$ 2.3*	18.4 $\pm$ 0.9*	0.84 $\pm$ 0.06
		16 $\mu\text{g/ml}$	88.4 $\pm$ 2.5*	98.6 $\pm$ 4.5*	21.5 $\pm$ 0.8*	0.85 $\pm$ 0.02
Group 6	Fraction 11	63 $\mu\text{g/ml}$	127.5 $\pm$ 6.6*	82.8 $\pm$ 3.5*	19.5 $\pm$ 0.8*	0.82 $\pm$ 0.04
		31 $\mu\text{g/ml}$	123.4 $\pm$ 5.8*	94.3 $\pm$ 4.1*	23.8 $\pm$ 0.7*	0.86 $\pm$ 0.03
		16 $\mu\text{g/ml}$	160.5 $\pm$ 5.9*	102.2 $\pm$ 3.8*	24.1 $\pm$ 0.6*	0.85 $\pm$ 0.02
Group 7	Fraction 13	63 $\mu\text{g/ml}$	68.5 $\pm$ 2.1*	71.6 $\pm$ 2.9*	14.5 $\pm$ 0.9*	0.94 $\pm$ 0.04
		31 $\mu\text{g/ml}$	72.6 $\pm$ 3.1*	85.6 $\pm$ 2.3*	16.6 $\pm$ 0.4*	0.83 $\pm$ 0.05
		16 $\mu\text{g/ml}$	78.9 $\pm$ 2.8*	95.3 $\pm$ 2.5*	19.6 $\pm$ 0.9*	0.81 $\pm$ 0.03
Group 8	Fraction 14	63 $\mu\text{g/ml}$	128.4 $\pm$ 9.1*	82.5 $\pm$ 3.2*	16.5 $\pm$ 0.4*	0.88 $\pm$ 0.04
		31 $\mu\text{g/ml}$	132.4 $\pm$ 8.1*	89.6 $\pm$ 2.5*	18.7 $\pm$ 0.5*	0.89 $\pm$ 0.05
		16 $\mu\text{g/ml}$	152.6 $\pm$ 8.8*	95.6 $\pm$ 4.6*	19.8 $\pm$ 0.9*	0.87 $\pm$ 0.03
Group 9	Fraction 15	63 $\mu\text{g/ml}$	85.6 $\pm$ 5.6*	82.5 $\pm$ 4.9*	18.2 $\pm$ 0.8*	0.84 $\pm$ 0.04
		31 $\mu\text{g/ml}$	99.6 $\pm$ 6.5*	95.8 $\pm$ 6.8*	19.1 $\pm$ 0.4*	0.86 $\pm$ 0.04
		16 $\mu\text{g/ml}$	105.5 $\pm$ 7.5*	99.7 $\pm$ 3.9*	19.8 $\pm$ 0.6*	0.85 $\pm$ 0.02

All the groups except group 1 received 1% CCl<sub>4</sub> treatment

Values are mean  $\pm$  SEM, n = 6, \*: P < 0.05 when compared to group 2

**In vitro hepatoprotective activity of PDM extract and its fractions**

The results of the *in vitro* hepatoprotective activity of the PDM extract, and its fractions are given in the Table 2. All the fractions and PDM extract at the tested concentrations (63, 31 and 16 µg/ml) showed significant dose dependent protection against CCl<sub>4</sub> induced elevation in the ASAT, ALAT and ALP ( $P < 0.05$ ). No significant effect on CCl<sub>4</sub> induced changes were, however, observed in TP levels. Standard, silymarin at concentration of 20 µg/ml significantly prevented the CCl<sub>4</sub> induced elevation in

the ASAT, ALAT and ALP ( $P < 0.05$ ). Silymarin also showed no significant effect on TP levels.

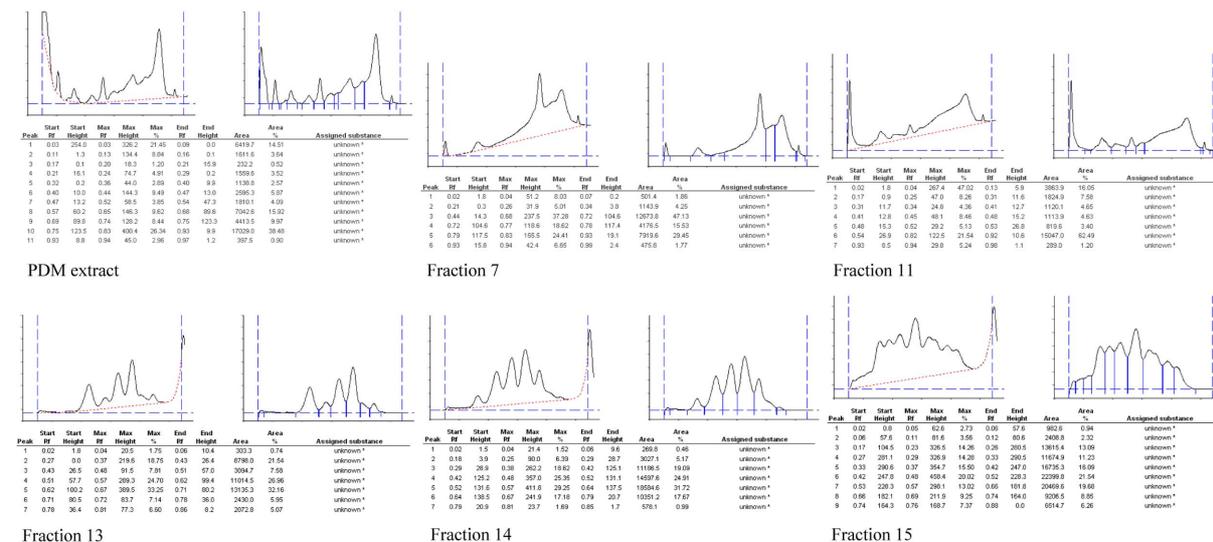
**Phytochemical analysis of PDM extract**

The qualitative phytochemical analysis of PDM extract and its active fractions showed the presence of terpenoids and phytosterols (Table 3). The HPTLC densitometry chromatogram of PDM extract and its active fractions at 254 nm are given in Figure 2. PDM extract shows eleven well resolved peaks, with R<sub>f</sub> values of 0.03, 0.13, 0.20, 0.24, 0.36, 0.44, 0.52, 0.65, 0.74, 0.83, and 0.94. Fraction 7,

**Table 3.** Results of qualitative phytochemical analysis of PDM extract and its active fractions

S. No.	Name of the test	Results					
		PDM extract	Fraction 7	Fraction 11	Fraction 13	Fraction 14	Fraction 15
1	Alkaloids	-	-	-	-	-	-
2	Carbohydrates	-	-	-	-	-	-
3	Saponins	-	-	-	-	-	-
4	Phytosterols	+	+	+	+	+	+
5	Tannins	-	-	-	-	-	-
6	Flavonoid	-	-	-	-	-	-
7	Terpenoids	+	+	+	+	+	+
8	Phlobatannins	-	-	-	-	-	-
9	Glycosides	-	-	-	-	-	-
10	Tannins	-	-	-	-	-	-

+: present, -: absent



**Fig. 2.** HPTLC densitometry chromatogram of PDM extract and its active fractions at 254 nm.

shows six well resolved peaks with Rf values of 0.04, 0.26, 0.68, 0.77, 0.83, and 0.94. Fraction 11, shows seven well resolved peaks with Rf value of 0.04, 0.25, 0.34, 0.45, 0.52, 0.82, and 0.94. Fraction 13, shows seven well resolved peaks with Rf values of 0.04, 0.37, 0.48, 0.57, 0.67, 0.72, and 0.81. Fraction 14 shows seven well resolved peaks with Rf values of 0.04, 0.25, 0.38, 0.48, 0.57, 0.67 and 0.81 and Fraction 15 shows nine well resolved peaks with Rf values of 0.05, 0.11, 0.23, 0.29, 0.37, 0.48, 0.57, 0.69, and 0.76.

## DISCUSSION

In the present study, 34 different fractions and four pure compounds were obtained from the PDM extract by column chromatography. The isolated compound 1 is similar to diterpenoid, Scoparic acid A, Compound 2 is similar to diterpenoid, dulcinol and Compound 3 is similar to diterpenoid, scoparinol, as been reported by earlier workers (Manirudin and Jasmin, 1990). All the isolated structures do not possess any aromatic rings. The possible reasons could be the benzoic ester got cleaved during the process of extraction and isolation. Compound 4 is found to be a novel compound, based on the available analytical data. However, further analytical data is required to confirm its complete structure.

The *in vitro* antioxidant activity study of these isolated fractions and pure compounds confirmed that, only fractions 7, 11, 13, 14 and 15 have DPPH scavenging potential and all other fractions and pure compounds fail to scavenge DPPH radical. Among these fractions showing DPPH scavenging activity, fraction 13 show the highest potency (Table 1). In the *in vitro* hepatoprotective study, using freshly isolated rat hepatocytes, all the fractions (selected from the antioxidant study) and the PDM extract show significant protection against CCl<sub>4</sub> induced changes in the ASAT, ALAT and ALP levels. Fraction 13 also show the highest activity against CCl<sub>4</sub> induced damage, when

compared to all other fractions and PDM extract. The preliminary phytochemical screening of the PDM extract and its fractions 7, 11, 13, 14 and 15 show the presence of terpenoids (Table 3). The results of the present study once again confirms our earlier reports on the hepatoprotective activities of the aqueous and alcoholic extracts, and PDM extract of this plant against CCl<sub>4</sub> induced liver cirrhosis in rats and CCl<sub>4</sub> induced acute liver injury in mice, respectively (Praveen *et al.*, 2008; Praveen *et al.*, 2009).

The hepatotoxic effect of CCl<sub>4</sub> known to be due to its metabolite CCl<sub>3</sub><sup>o</sup>, a free radical that alkylates cellular proteins and other macromolecules (Albano, 1982; Richard, 1983; Trevor, 1984). Several compounds belonging to the class of terpenes have been reported earlier to possess antioxidant properties, including monoterpenes hydrocarbons (Mycene, terpinolene, pinene), oxygenated monoterpenes (Nerol, geraniol, linalol, thymol), sesquiterpene hydrocarbons (Humulene, valencene, calarene), oxygenated sesquiterpenes (trans-trans-farnesol, farnesol, farnesyl acetate, guaiol), diterpene hydrocarbons (Phytol, abetine), and tetraterpene hydrocarbons (Caratenoids) (Giuseppe and Maria, 2000; Bysheng *et al.*, 2002; Grabmann, 2005). The PDM extract of *Scoparia dulcis* L. has been reported to contain three labdane-type diterpenes designated as scoparic acid A, B and C, scopadulcic acid A and B, scoparinol and dulcinol (Manirudin and Jasmin, 1990). In the present study three terpenoids similar in structure to that of scoparic acid A, dulcinol and scoparinol were isolated and tested for their *in vitro* antioxidant activity. All these compounds, however, show no *in vitro* free radical scavenging potential, whereas fractions 7, 11, 13, 14 and 15 show good *in vitro* antioxidant activity and the phytochemical analysis of these fractions show the presence of terpenoids. In addition, all these fractions also show significant *in vitro* hepatoprotective activity. Based on the above results it may be concluded that, the *in vivo* (Praveen *et al.*, 2008) and *in vitro* hepatoprotective activity of the PDM extract may be attributed to its

free radical scavenging capability, which in turn may be attributed to its terpenoid constituents.

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