**IJPSR (2013), Vol. 4, Issue 4** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 December, 2012; received in revised form, 19 January, 2013; accepted, 23 March, 2013

# PHYTOCHEMICAL EVALUATION AND HPTLC PROFILING OF EXTRACTS OF *SALACIA OBLONGA*

Sujata Basu, Mamta Pant and Rachana*

Department of Biotechnology, Jaypee Institute of Information Technology, A-10, Sector 62, Noida – 201307, Uttar Pradesh, India

**Keywords:**
*Salacia oblonga*, Mangiferin, Phytochemicals, HPTLC, Anti-diabetic, Anti-oxidant

**Correspondence to Author:**

**Rachana**

Department of Biotechnology, Jaypee Institute of Information Technology, A-10, Sector 62, Noida – 201307, Uttar Pradesh, India

E-mail: rachana.dr@iitbombay.org

**ABSTRACT:** Plants of medicinal importance though are quite well known among the Ayurvedic practitioners since years, yet many of them have not been standardized, validated and documented completely. Among such medicinally valuable plants, *Salacia* holds a place as an effective antidiabetic herb and many species of this plant are in use as anti-diabetics. The present study reports the phytochemical analysis of one of its species *Salacia oblonga* (SO) (roots and stems), belonging to the family Celastraceae (bittersweet), through various biochemical and chromatographic methods. TLC and HPTLC analysis of six different extracts of SO revealed numerous bands, indicating the presence of diverse groups of phytocompounds, many of them are assumed to contribute significantly to its antioxidant activity as well as, other biological activities. This type of analysis can help in fingerprint profiling of the plant and its various species. The identification and characterization of the phytocompounds can further help in finding out molecular targets/mechanism of action of the constituents of this herb that are responsible for its biological activities.

**INTRODUCTION:** The growing awareness about the side effects and complications of chemical and synthetic medicines, cosmetics and health supplements have triggered the usage of herbal products both in the Eastern and Western World. Herbal products are being used more in recent days and they are one of those substances that people use in a self-prescribed manner. *Salacia* holds a place as an effective antidiabetic herb and comprises of about 100 species, out of which, in India, *Salacia reticulata* (SR) and *Salacia oblonga* (SO) are predominantly used [1]. Unfortunately SR has been declared as an endangered species [2, 3, 4].

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Article can be accessed online on:
www.ijpsr.com

SO is distributed in the southern region of India and has been in use since several years extensively in the form of food supplements in countries like India, Japan and Korea, for controlling high blood glucose levels [5]. The root-bark of this plant is pale yellow and light brownish coloured and is used as a decoction or as powder in the treatment of diabetes and other diseases like rheumatism, gonorrhea, itches, asthma, thirst and ear diseases [6].

Moreover, the root extract has shown inhibitory activity on intestinal α - glucosidases, thereby preventing blood glucose rise in diabetics [7]. *Salacia oblonga* is also reported to possess good antioxidant and anti-inflammatory activity [6, 8]. The extract containing the active constituents Salacinol, Kotalanol, and Mangiferin have been reported to significantly lower the blood glucose levels through inhibition of the enzymes involved in glucose metabolism [9, 10].

Several studies have reported the characterization of SO up to only a certain extent. The aqueous extract of SO (dried root) was analyzed by HPLC to measure the amount of Mangiferin [11, 12]. Attempts were also made towards establishment of fractionation protocols for hot water extract of roots and stems of SO to identify its active fractions [13]. The six fractions thus obtained were analyzed through $^{1}H$ NMR, $^{13}C$ NMR, DEPT *etc*. and one of its fraction was detected as the active constituent Mangiferin.

In comparison to other fractions, this fraction caused a greater 2-deoxy-D-glucose uptake in L6 myotubes and the extent of stimulation was comparable with the pure compound Mangiferin. In another study, the active phyto-compounds Salacinol and Kotalanol along with nine other sugar related components were isolated from the water soluble portion while, a new triterpene, Kotalagenin 16-acetate along with known diterpenes and triterpenes were isolated from the Ethyl acetate portion [10].

The present study is an attempt to perform phytochemical analysis of various extracts of SO for the presence of various types of compounds with respect to its main active constituent Mangiferin. This aims towards further isolation of various active constituents to characterize the mechanism of action of SO, *in vitro*.

**MATERIALS AND METHODS:** Soxhlet apparatus, Water bath, TLC $60F_{254}$ (Merck), UV torch (Genei Pvt. Ltd., Bangalore, India) were the basic apparatus and instruments used for the study. Analytical grade solvents viz. Methanol, Ethanol, Chloroform, Ethyl acetate, Petroleum ether, Formic acid, Toluene, Acetone, and Glacial acetic acid were used. Mangiferin was obtained from Sigma Aldrich, India. All other reagents used for biochemical tests were of analytical grade.

**Plant material:** The crushed roots of *Salacia oblonga* (**Figure 1**) was purchased from Sami Labs, Bangalore, India. The Methanolic-aqueous extract powder of SO (roots and stems) was obtained from Natural Remedies Pvt. Ltd., Bangalore, India.

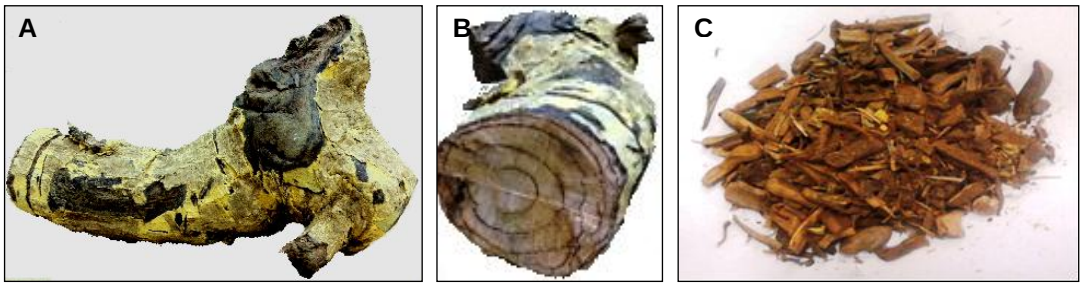



**Figure 1: Roots of *Salacia oblonga*, A: lateral view of the root; B: T.S. of root showing the ring pattern of SO; C: crushed roots of SO**

**Extraction and Isolation:** 2 g of crushed SO roots were subjected to successive separate Soxhlet extractions using a series of solvents of increasing polarity starting from Petroleum ether, Chloroform, Ethyl acetate, and Methanol respectively. The ethanol and water extract of SO was prepared by boiling 2 g of the root powder in water bath at 70 - 80ºC for 6 – 8 h.

All the extracts were filtered through Whatman no.1 filter paper and then air-dried [14, 15, 16, 17]. They were further used for various phytochemical analysis and TLC profiling. The methanolic-aqueous extract powder of SO was reconstituted in methanol by boiling for 12 min in water bath at 65ºC.

**Phytochemical evaluation of SO extracts:** The Ethanolic, Methanolic, and Water extracts of the SO were evaluated for the presence or absence of various phytoconstituents like Carbohydrates (Molisch's test, Benedict's test, Fehling's test), Proteins and Amino acids (Millon's test, Biuret test, Keller Killiani test), Sterols and Triterpenoids (Salkowski's test), Alkaloids (Mayer's test, Dragendroff's test, Wagner's test), Phenolic compounds (Ferric chloride test, Zinc-hydrochloride reduction test), Flavonoids (Zinc-hydrochloride reduction test, Alkaline reagent test), Tannins (Gelatin test, Ferric chloride test), Anthocyanins (tests at different pH, Amyl alcohol test), and Anthraquinone derivatives (Borntrager's test) respectively [18, 19].

**Thin Layer Chromatography (TLC) and HPTLC analysis:** TLC and HPTLC was performed using aluminium pre-coated TLC sheet (Merck, 12 cm x 10 $cm^2$; 0.2 mm thick) to confirm the presence of active phenolic constituent Mangiferin along with separation of numerous phytocompounds in six different SO extracts with an aim for further molecular level studies [20, 21, 22]. The chromatographic system used was HPTLC- DESAGA Applicator AS 30, 230 V, with HPTLC Densitometer CD 60, 230V, with Windows® software ProQuant®. The solvent system Toluene: Acetone: Glacial acetic acid (9: 1: 0.5) was used in order to achieve a good resolution and development of polar and non-polar spots of phytoconstituents present in the plant extract.

The mobile phase was allowed to run through the adsorbent phase up to 3/4$^{th}$ of the plate. On visualizing the separation of solvent front after a definite time period, the sheets were removed from the TLC chamber and air-dried at room temperature. The scanning of the spots were done visually, UV exposure (254 nm and 366 nm) with or without staining with Permanganate, Potassium dichromate, Phosphomolybdic acid, Anisaldehyde-sulphuric acid stains, and Iodine vapour. The HPTLC plates were developed and the chromatograms were obtained respectively.

## RESULTS AND DISCUSSION:

**Biochemical analysis of SOE:** The need to identify the wide variety of phytocompounds of SO and their implication in the medicinal world led us to conduct the present study. The extraction method adopted in this study for the six crude extracts of SO gave the percentage yields of 14.15% (Water extract), 9.6% (Ethanol), 8.8% (Methanol), 11.2% (Ethyl acetate), 6.2% (Chloroform), and 6.3% (Petroleum ether), respectively. The yield of Mangiferin was highest in the Methanolic (1.2%), Ethanolic (1.18%), and Ethyl acetate (1.28%) extracts.

Among several secondary metabolites, qualitative phytochemical screening of the Ethanolic, Methanolic and Aqueous extracts of roots of SO revealed the presence of Carbohydrates, Sterols, Phenolic compounds, Alkaloids, Flavonoids, and Tannins as shown in Table 1. The Ethanolic, Methanolic, and Aqueous extracts showed absence of Proteins and Amino acids, Anthocyanins and Anthraquinone derivatives. The Methanolic - aqueous SO (root and stem) powder was reconstituted in 2% HCl and it indicated the presence of Alkaloids.

**Table 1: Phytochemical screening of various solvent extracts of *Salacia oblonga***

| Extracts | Carbohydrates | Proteins and Amino acids | Sterols and Triterpenoids | Alkaloids | Phenolic compounds | Flavonoids | Tannins | Anthocyanins | Anthraquinone derivatives |
|---|---|---|---|---|---|---|---|---|---|
| Ethanol | + | - | + | - | + | + | ++ | - | x |
| Methanol | + | - | + | - | + | + | ++ | - | x |
| Water | + | - | - | - | - | + | + | - | x |
| Hydroalcoholic extract + 2% HCl | x | x | x | ++ | x | x | x | x | x |

(+) = positive; (-) = negative; (++) = abundant; x = not analyzed

**TLC and HPTLC analysis of SOE:** The phytocompounds of SO were analyzed through TLC and HPTLC, using two solvent systems [Ethyl acetate: Glacial acetic acid: Formic acid: Water (100: 11: 11: 26) for TLC analysis; Toluene: Acetone: Acetic acid (9: 1: 0.5) for HPTLC analysis]. TLC was performed for the marker compound Mangiferin (5 µg), Methanolic and Ethanolic extracts of SO (10 µg each) powder, by using the first solvent system. TLC and HPTLC analysis was adopted since it allows analysis of many samples in parallel. These methods are simple, economical, and the latter also gives a high sample throughput. As shown in **Figure 2** (A = under visible light; B = under UV 254 nm light; C = under UV 365 nm light), TLC profile of Methanolic and Ethanolic extracts of SO confirmed the presence of Mangiferin. Although, in Figure 1C, a light yellow band (above orange band for Mangiferin) were observed in both the extracts, indicating the presence of an unknown compound.

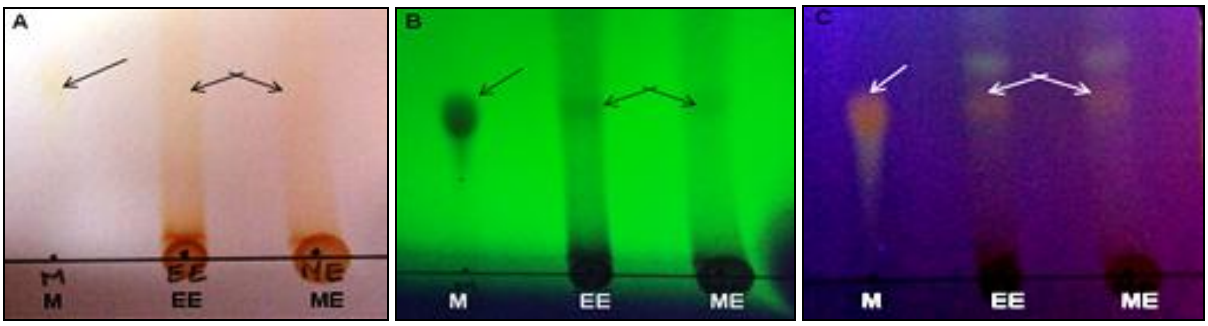



**Figure 2: TLC analysis of Mangiferin (M), Ethanolic extract (EE) and Methanolic extract (ME) of *Salacia oblonga* (A – under visible light, B – under UV 254 nm light, C – under 365 nm light)**

On confirmation of the presence of polar compound Mangiferin in both the extracts, we looked forward for detection of other polar and non-polar phytoconstituents of SO in six different extracts (Methanolic, Ethanolic, Ethyl acetate, Chloroform, Petroleum ether, and Aqueous). HPTLC plates were developed using the second solvent system (Toluene: Acetone: Acetic acid). The HPTLC profiles of these extracts of SO are shown in Figure 3A - 3F. 50 $\mu$g of each of the six extracts of SO were loaded in separate lanes on the TLC plate. The developed TLC plates were further exposed to UV 254 nm and 366 nm light as well as, different stains to observe various bands corresponding to different phytocompounds in the plant.

**Observation under UV light (254 nm and 366 nm):** As, it can be seen from **Figure 3A – 3F**, the band corresponding to the marker compound Mangiferin gave a peak with $R_f$ value 0.12 in all the seven lanes in the densitometric scan (**Figure 4A – 4G**). Exposure of the spotted and developed TLC and HPTLC plates to UV 254 nm showed the presence of numerous organic compounds as dark and light bands in a green background (Figure 3A).

The Methanolic and Ethanolic extracts showed greatest similarity in band pattern, indicating the extraction of similar types of phytocompounds in almost equal quantities in both. The Ethyl acetate extract showed maximum number of bands as compared to other extracts. The mobile phase Toluene: Acetone: Glacial acetic acid (9: 1: 0.5) gave a good resolution of marker compound Mangiferin with $R_f$ value 0.14 (TLC) and 0.12 - 0.13 (HPTLC) in the six extracts of *Salacia*. UV 254 nm light exposed TLC plate showed three unique bands with approximate $R_f$ values of 0.24 (Ethanolic, Ethyl acetate, and Chloroform extracts) (Figures 3C - 3E), 0.39 (Chloroform and Petroleum ether extracts) (Figures 3E and 3F), and 0.83 (Ethanolic, Methanolic, and Chloroform extracts), respectively (Figure 3B, 3C and 3E).

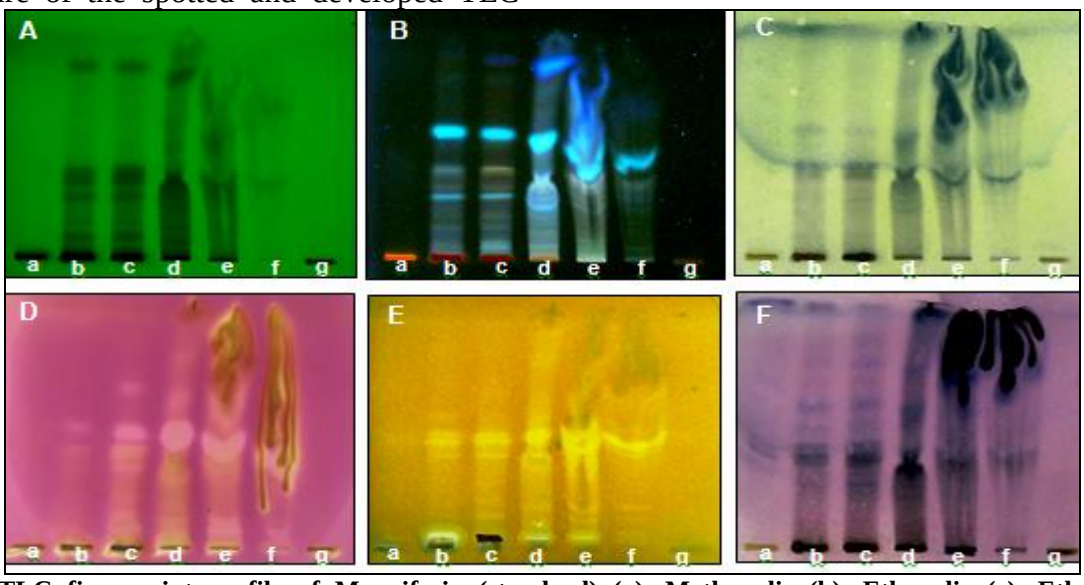



**Figure 3: HPTLC fingerprint profile of Mangiferin (standard) (a), Methanolic (b), Ethanolic (c), Ethyl acetate (d), Chloroform (e), Petroleum ether (f), and Water (g) extracts of *Salacia oblonga* on exposure to UV light 254 nm (A) and 366 nm (B), Phosphomolybdic acid stain (C), $KMnO_4$ stain (D), $K_2Cr_2O_7$ stain (E), and Anisaldehyde-sulphuric acid stain (F) in the solvent system Toluene : Acetone : Glacial acetic acid (9 : 1 : 0.5)**

UV 366 nm exposure of the developed TLC and HPTLC slides revealed multi-coloured bands with varying intensities (Figure 3B). It corresponds to several polar and non-polar compounds, which show light and dark orange, light yellow, fluorescent green, and fluorescent sky blue color bands, respectively. The $R_f$ values of spots obtained during TLC, corresponding to a varied range of phytoconstituents in six SO extracts are tabulated in **Table 2**.

**TABLE 2: $R_f$ VALUES FOR DIFFERENT TYPES OF PHYTOCONSTITUENTS OBTAINED BY TLC**

| SOE extracts | UV 254 nm | UV 366 nm | Anisaldehyde reagent |
|---|---|---|---|
| Mangiferin | 0.14 | 0.14 | 0.14 |
| Aqueous | 0.14; 0.20; 0.43; 0.53; 0.58 | 0.14; 0.20; 0.43; 0.53; 0.58 | 0.14; 0.36; 0.58; 0.65 |
| Ethanolic | 0.14; 0.20; 0.43; 0.53; 0.58; 0.88 | 0.14; 0.20; 0.43; 0.53; 0.58; 0.65 | 0.14; 0.36; 0.41; 0.58; 0.65; 0.73 |
| Methanolic | 0.14; 0.20; 0.43; 0.53; 0.58; 0.88 | 0.14; 0.20; 0.43; 0.53; 0.58; 0.65 | 0.14; 0.36; 0.41; 0.58; 0.65; 0.73 |
| Ethyl acetate | 0.14; 0.20; 0.25; 0.36; 0.43; 0.53; 0.58; 0.88 | 0.14; 0.20; 0.43; 0.53; 0.58; 0.65; 0.88 | 0.14; 0.36; 0.58; 0.65; 0.73 |
| Chloroform | 0.14; 0.20; 0.36; 0.43; 0.53; 0.58; 0.88 | 0.14; 0.43; 0.65; 0.88 | 0.14; 0.36; 0.58; 0.73 |
| Petroleum ether | 0.53; 0.58; 0.88 | 0.11; 0.14; 0.53; 0.58; 0.65 | 0.14; 0.58; 0.60; 0.65; 0.73 |

It is known that, most polycyclic aromatic hydrocarbons with extended conjugated π-electrons are intensely fluorescent. It is also reported that, few of the saturated organic and inorganic molecules also exhibit intense fluorescence [23]. So, there are possibilities of presence of many saturated and unsaturated compounds in SO as depicted in our data. The TLC and HPTLC slides showed intense fluorescent bluish-green bands in five SO extracts, thin bluish-green bands with decreasing intensity from Methanolic to Petroleum ether extract lane, and a very intense blue band in the Ethyl acetate and Chloroform extracts, respectively. UV 366 nm exposed plate showed a single light yellow band of $R_f$ value 0.11 in Petroleum ether extract only. Our data is quite distinct from the fluorescence analysis data of root powder of *Salacia chinensis* [24], thereby proving the uniqueness in composition of phytocompounds in SO extract powder (root and stem).

The exposure of the TLC plate to iodine vapor is among the oldest methods for visualization of organic compounds. This, followed by further exposure to UV light revealed the presence of many unsaturated and aromatic compounds as yellowish-brown bands, fluorescent green and blue bands, as well as few dark bands. The difference in $R_f$ values of Mangiferin and other compounds in the TLC and HPTLC slides might be due to several factors like temperature, starting spot width, and sample loading technique.

**Observation after staining:** Compounds, which are not normally visible and also do not exhibit intense native fluorescence during UV exposure, can be chemically derivatized to yield characteristic colored bands or spots. For above said purpose, individual developed TLC plates were stained with four different stains separately: Phosphomolybdic acid stain (Figure 3C), $KMnO_4$ stain (Figure 3D), $K_2Cr_2O_7$ stain (Figure 3E), and Anisaldehyde-sulphuric acid stain (Figure 3F), respectively.

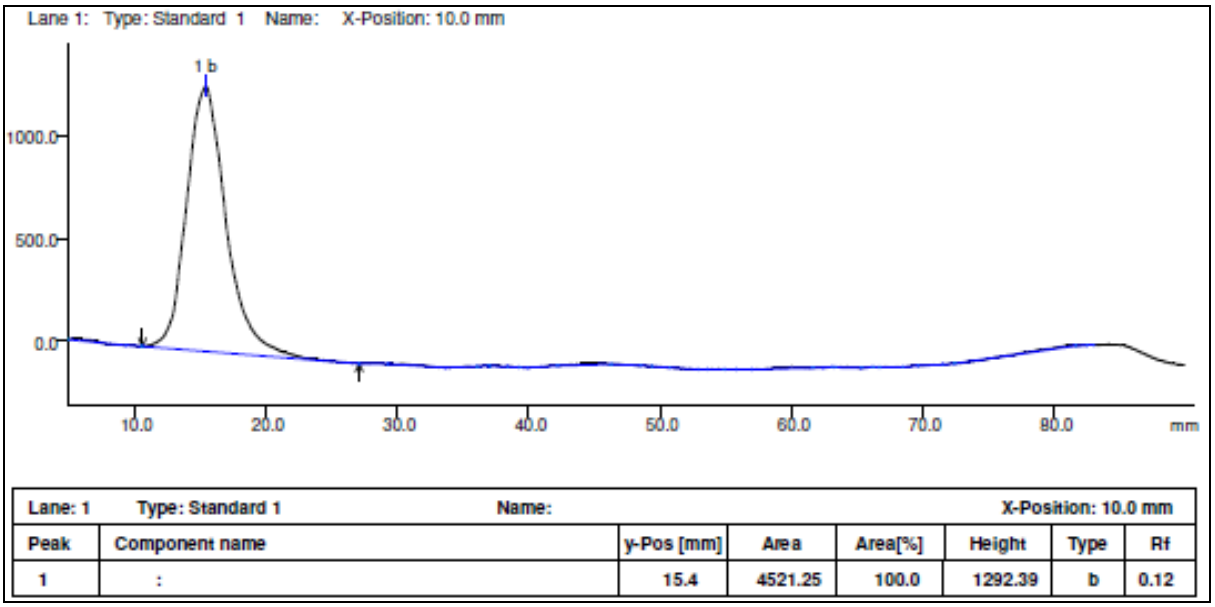
The TLC plate stained with Phosphomolybdic acid showed dark bluish green to grey bands in a light pale green background under visible light. Its pattern is almost similar to that in Figure 3A.

The TLC plate stained with Potassium Permanganate stain showed yellow and yellowish-brown bands in a light pink background indicating the presence of unsaturated alkenes, alkynes, amines, and alcohols in the extracts. It showed two distinct light yellow bands just above the sample application point in Ethanolic, Ethyl acetate, and Chloroform extracts similar to Figure 3E but not as Figure 3C.

The dichromate stained TLC plate on exposure to UV 254 nm showed distinct fluorescent yellow bands near to the solvent front, indicating towards less polar compounds in the Ethanolic, Methanolic and Ethyl acetate extracts. UV 366 nm exposure of the same plate showed a single intensely fluorescent blue band just below the solvent front.

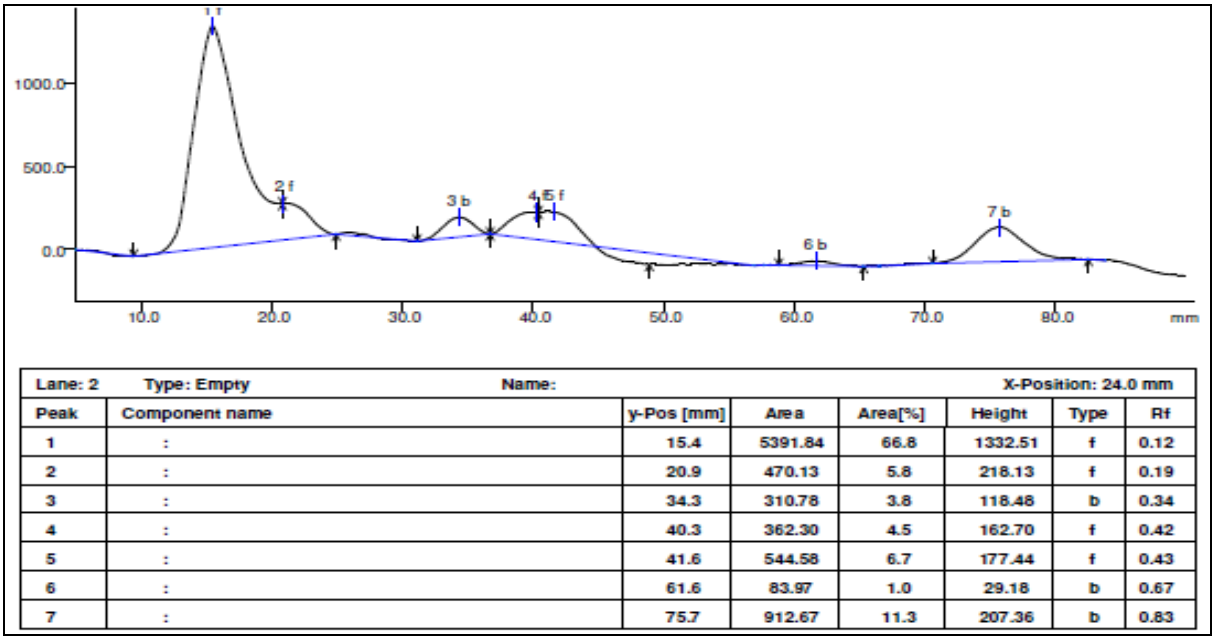
The Anisaldehyde-sulphuric acid staining showed multi-coloured bands ranging from pink, violet, grey, to bluish grey thus, indicating the presence of Phenolics, Steroids and other compounds in the extracts. Exposure of the plate to UV 366 nm showed a unique orange band present in all the extracts (in decreasing order of intensity) along with the marker compound Mangiferin. The Anisaldehyde staining of the TLC plate showed the presence of more phytocompounds in the Aqueous extract of SO which could not be detected by UV exposure alone.

**Comparative analysis of the HPTLC chromatogram:** A comparative analysis of the HPTLC chromatogram of six different SO extracts were done with the standard compound Mangiferin (**Figure 4A – 4G**). Lane 1 showed a single peak for the marker compound Mangiferin (peak area of 4521.25, $R_f$ value of 0.12). Mangiferin was detected in all the six SO extracts, with concentration sequence as follows: Ethyl acetate > Methanolic > Ethanolic > Chloroform > Water > Petroleum ether extracts. The Ethyl acetate extract showed extraction of maximum number of polar and non-polar phytocompounds while the Aqueous extract showed band for a single polar and non-polar compound with $R_f$ values 0.12 and 0.79. Lane 2 (Figure 4B) showed seven different peaks wherein Mangiferin has the highest peak area of 5391.84 ($R_f$ value of 0.12) and a peak height of 1332.51.




| Lane: 1 | Type: Standard 1 | Name: | | | | X-Position: 10.0 mm | |
|---|---|---|---|---|---|---|---|
| Peak | Component name | y-Pos [mm] | Area | Area[%] | Height | Type | Rf |
| 1 | : | 15.4 | 4521.25 | 100.0 | 1292.39 | b | 0.12 |

**FIGURE 4A: LANE 1: CHROMATOGRAM OF MANGIFERIN (MARKER COMPOUND)**

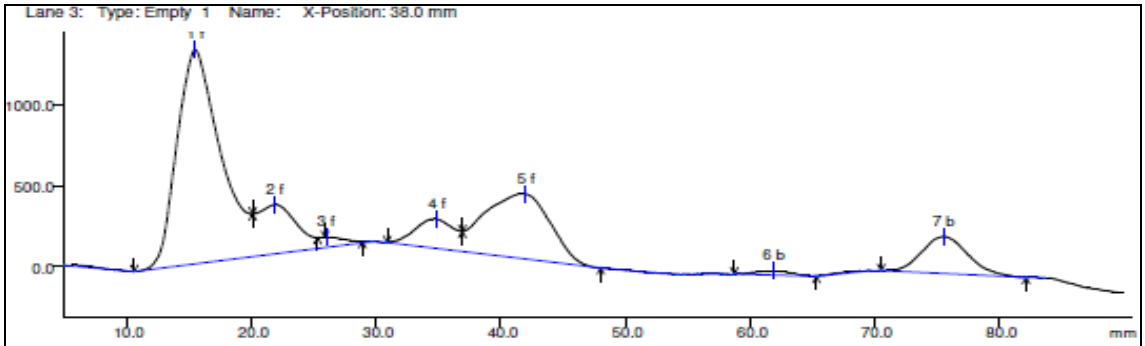



| Lane: 2 | Type: Empty | Name: | | | | X-Position: 24.0 mm | |
|---|---|---|---|---|---|---|---|
| Peak | Component name | y-Pos [mm] | Area | Area[%] | Height | Type | Rf |
| 1 | : | 15.4 | 5391.84 | 66.8 | 1332.51 | f | 0.12 |
| 2 | : | 20.9 | 470.13 | 5.8 | 218.13 | f | 0.19 |
| 3 | : | 34.3 | 310.78 | 3.8 | 118.48 | b | 0.34 |
| 4 | : | 40.3 | 362.30 | 4.5 | 162.70 | f | 0.42 |
| 5 | : | 41.6 | 544.58 | 6.7 | 177.44 | f | 0.43 |
| 6 | : | 61.6 | 83.97 | 1.0 | 29.18 | b | 0.67 |
| 7 | : | 75.7 | 912.67 | 11.3 | 207.36 | b | 0.83 |

**FIGURE 4B: LANE 2: CHROMATOGRAM OF METHANOLIC EXTRACT OF SO**

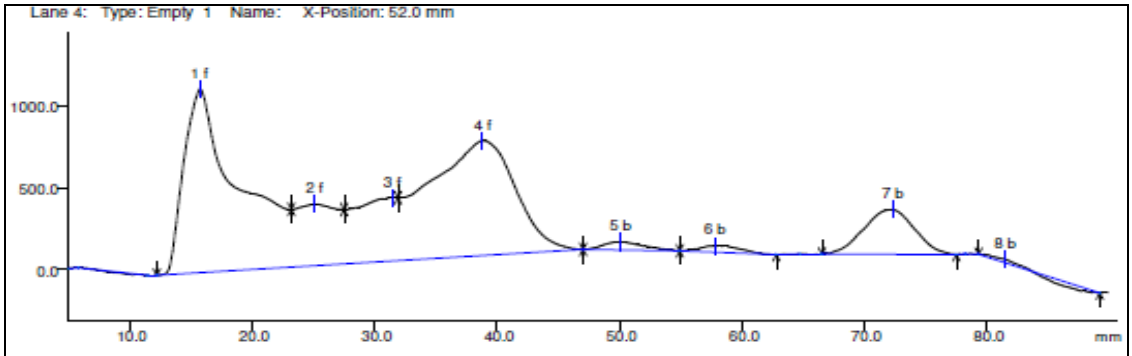
Lane 3 (Figure 4C) also showed seven different peaks, with Mangiferin (peak 1) having the highest peak area of 5305.63 and a height almost similar to Methanolic extract of SO.

The Ethanolic extract showed a single band ($R_f$ value 0.25) distinct from the Methanolic extract. Peak 2 with $R_f$ value 0.20 is present in higher concentration in Ethanolic extract as compared to Methanolic extract of SO. The peaks with $R_f$ values 0.25 (Ethanolic), 0.24 (Ethyl acetate), and 0.22 (Chloroform) (Figure 4C, 4D, and 4E) might be compounds with almost similar structures and chemical properties, but the phytoconstituents could not be identified as SO has vast number of phytocompounds that remains unidentified yet. The peaks with $R_f$ values 0.42, 0.43 (both Methanolic), 0.44 (Ethanolic), and 0.45 (Chloroform) also indicates almost identical phytocompounds. Lane 4 (Figure 4D) showed maximum number of peaks. The peaks with $R_f$ values 0.13 and 0.40 have the highest peak areas of 5770.46 and 5788.69 respectively, thereby indicating its presence in higher concentrations in this extract.




| Lane: 3 | Type: Empty | Name: | | | | X-Position: 38.0 mm | |
|---|---|---|---|---|---|---|---|
| Peak | Component name | y-Pos [mm] | Area | Area[%] | Height | Type | Rf |
| 1 | : | 15.5 | 5305.63 | 49.8 | 1324.70 | f | 0.12 |
| 2 | : | 21.9 | 1101.94 | 10.4 | 304.78 | f | 0.20 |
| 3 | : | 26.0 | 107.33 | 1.0 | 62.85 | f | 0.25 |
| 4 | : | 34.9 | 654.05 | 6.1 | 179.89 | f | 0.35 |
| 5 | : | 42.0 | 2408.64 | 22.6 | 400.86 | f | 0.44 |
| 6 | : | 61.9 | 88.90 | 0.8 | 26.01 | b | 0.67 |
| 7 | : | 75.5 | 977.54 | 9.2 | 228.43 | b | 0.83 |

**FIGURE 4C: LANE 3: CHROMATOGRAM OF ETHANOLIC EXTRACT OF SO**




| Lane: 4 | Type: Empty | Name: | | | | X-Position: 52.0 mm | |
|---|---|---|---|---|---|---|---|
| Peak | Component name | y-Pos [mm] | Area | Area[%] | Height | Type | Rf |
| 1 | : | 15.7 | 5770.46 | 35.3 | 1121.62 | f | 0.13 |
| 2 | : | 25.1 | 1506.25 | 9.2 | 376.20 | f | 0.24 |
| 3 | : | 31.4 | 1560.92 | 9.6 | 387.23 | f | 0.31 |
| 4 | : | 38.8 | 5788.69 | 35.5 | 701.41 | f | 0.40 |
| 5 | : | 50.1 | 182.62 | 1.1 | 48.13 | b | 0.53 |
| 6 | : | 57.8 | 151.59 | 0.9 | 38.40 | b | 0.62 |
| 7 | : | 72.3 | 1307.09 | 8.0 | 273.36 | b | 0.79 |
| 8 | : | 81.5 | 60.86 | 0.4 | 21.36 | b | 0.90 |

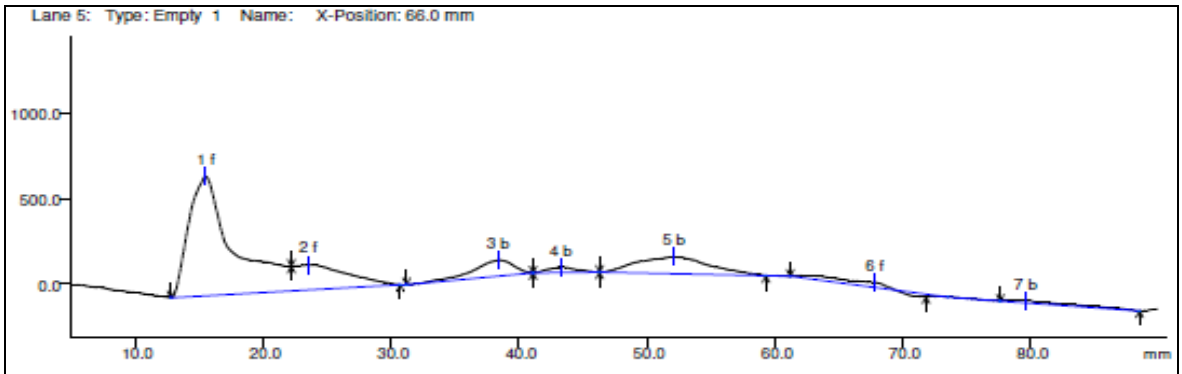
**FIGURE 4D: LANE 4: CHROMATOGRAM OF ETHYL ACETATE EXTRACT OF SO**

The peak with $R_f$ value 0.79 (Ethyl acetate and Water extracts) (Figure 4D and 4G) showed much higher concentration in the former. Two highly non-polar phytocompounds have been detected in lesser concentrations in the Ethyl acetate and Chloroform extracts with $R_f$ values 0.90 and 0.88 (Figure 4D and 4E), respectively.

Lane 5 (Figure 4E) showed the presence of seven different peaks, with Mangiferin present in almost half the concentration as compared to Ethanolic, Methanolic, and Ethyl acetate extracts, respectively.

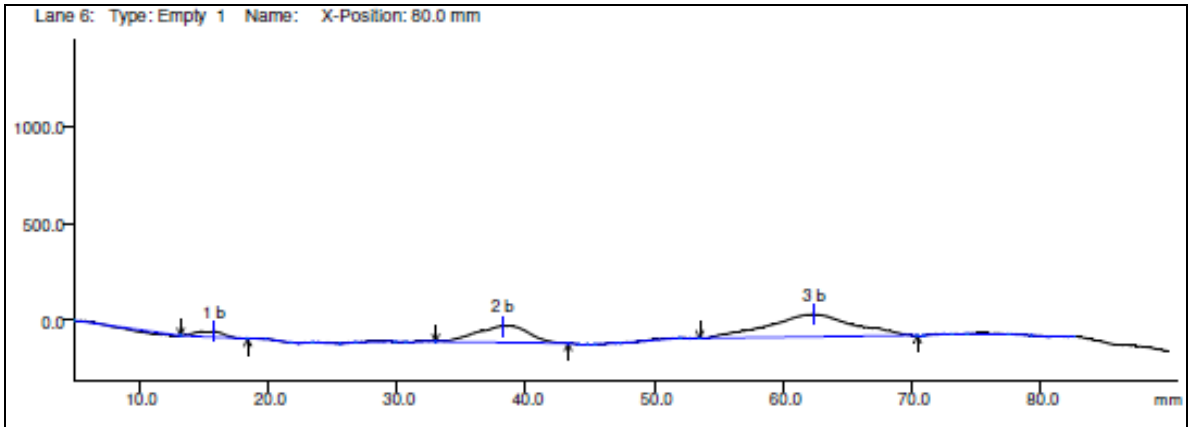
Lane 6 (Figure 4F) showed three peaks only, wherein the marker compound is present in least concentration (peak area of 74.70 and a peak height of 29.29) as compared to five other extracts of SO.

The peaks with $R_f$ values 0.67 (Methanolic and Ethanolic), and 0.68 (Petroleum ether extract) showed comparatively higher concentration in the latter. Lane 7 (Figure 4G) showed the least number of peaks, showing poor extraction. The marker compound Mangiferin ($R_f$ value of 0.12) has a lesser area of 1675.62 in this Aqueous extract.



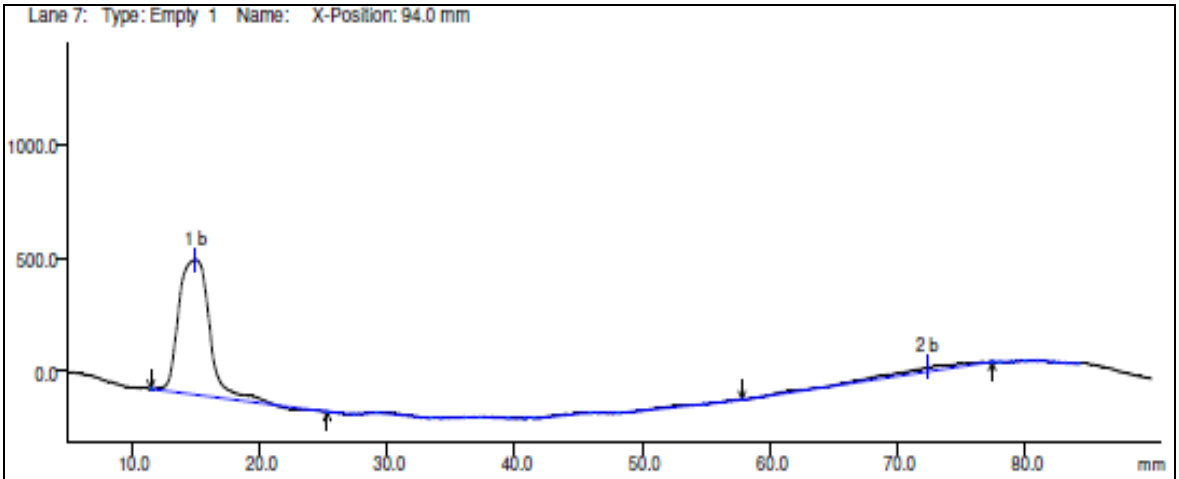

| Lane: 5 | Type: Empty | Name: | | | | X-Position: 66.0 mm | |
|---|---|---|---|---|---|---|---|
| Peak | Component name | y-Pos [mm] | Area | Area[%] | Height | Type | Rf |
| 1 | : | 15.5 | 2807.22 | 58.2 | 699.26 | f | 0.12 |
| 2 | : | 23.5 | 696.57 | 14.5 | 147.69 | f | 0.22 |
| 3 | : | 38.4 | 352.17 | 7.3 | 93.98 | b | 0.39 |
| 4 | : | 43.3 | 82.53 | 1.7 | 31.77 | b | 0.45 |
| 5 | : | 52.1 | 641.96 | 13.3 | 98.57 | b | 0.55 |
| 6 | : | 67.8 | 155.30 | 3.2 | 28.29 | f | 0.74 |
| 7 | : | 79.6 | 84.80 | 1.8 | 13.76 | b | 0.88 |

**FIGURE 4E: LANE 5: CHROMATOGRAM OF CHLOROFORM EXTRACT OF SO**




| Lane: 6 | Type: Empty | Name: | | | | X-Position: 80.0 mm | |
|---|---|---|---|---|---|---|---|
| Peak | Component name | y-Pos [mm] | Area | Area[%] | Height | Type | Rf |
| 1 | : | 15.8 | 74.70 | 5.5 | 29.29 | b | 0.13 |
| 2 | : | 38.2 | 394.65 | 29.2 | 84.76 | b | 0.39 |
| 3 | : | 62.4 | 884.30 | 65.3 | 116.24 | b | 0.68 |

**FIGURE 4F: LANE 6: CHROMATOGRAM OF PETROLEUM ETHER EXTRACT OF SO**




| Lane: 7 | Type: Empty | Name: | | | | X-Position: 94.0 mm | |
|---|---|---|---|---|---|---|---|
| Peak | Component name | y-Pos [mm] | Area | Area[%] | Height | Type | Rf |
| 1 | : | 15.0 | 1675.62 | 91.3 | 596.69 | b | 0.12 |
| 2 | : | 72.3 | 160.12 | 8.7 | 19.31 | b | 0.79 |

**FIGURE 4G: LANE 7: CHROMATOGRAM OF WATER EXTRACT OF SO**

**CONCLUSION:** Plants contain several bioactive secondary plant metabolites that elicit pharmacological or toxicological effects in human beings and animals [25]. Due to natural variability, the qualitative and quantitative composition of the plants may vary considerably. This preliminary study may help detect many unidentified phytocompounds, which can be used to characterize SO samples obtained from various geographical locations. This can also help to characterize various types of SO extracts for further therapeutic use. Among the several types of phytocompounds present in *Salacia* species, the flavones and flavonols with 7-hydroxyl and/or catechol moiety at the B ring has been reported to exhibit strong aldose reductase inhibitory activity [26].

Depending on the structure of flavonoids, UV 365 nm exposure of the TLC plate yields bands of dark yellow, green, or blue fluorescence respectively [27]. The active moieties of the flavonoids might be one of the contributors for characteristic fluorescent bands in TLC and HPTLC analysis. Besides this, the Methanolic and Ethanolic extracts showed a very close band pattern during HPTLC, indicating possibility of almost similar pharmacological modes of action of these two extracts if, given for the treatment of various diseases. The presence of known active constituents like Mangiferin (a xanthone glucoside) and several others in various SO extracts indicates its use with pharmacological relevance in reducing blood glucose levels or in bringing about significant changes in the blood glucose metabolism related signaling pathways through specific target approach [28].

Further study with various standard compounds is necessary for identification of these fluorescent and non-fluorescent active constituents. Currently, this type of analysis, particularly TLC and HPTLC, is used for fingerprinting of a particular species/variety of various plants, thereby establishing the fingerprint profile of the plants. This can further help in standardization of the plant/extract/product for quality control studies.

Our study also provides an option for further research into additional studies on other plant parts of *Salacia oblonga* to detect the presence or absence of the phytoconstituents and study their pharmacological and commercial use, with a broader perspective of whether the plant parts actually work synergistically in reducing blood sugar to normal levels. The difference might lie in the concentrations of phytocompounds present within-plant parts, or different varieties/species grown at different places at different time period.

The present study therefore, is an attempt to establish the phytoconstituent profile of SO, which may further help to compare and standardize this plant for several formulations and identification purpose.

It is further needed to characterize individual bands obtained in different extracts with the identifications of other marker compounds for the plant *Salacia*.

**ACKNOWLEDGEMENT:** We express our thanks to Dr. R. S. Gaud (Dean), Dr. Nancy Pandita, Mrs. Geeta Pai and Mr. Jay Savai of Dept. of Pharmacy, NMIMS University, Mumbai, India for their kind support in providing their Central Instrument facility for HPTLC analysis of the plant extracts.

## REFERENCES:

1. Paarakh PM, Patil LJ, Thanga SA: Genus *Salacia*: A comprehensive review. J Nat Rem 2008; 8:116–131.
2. Singh AB: Medicinal plant survey of Dumka, Hazaribagh and Gumla districts. Forest Resources Survey Division, Jharkhand, Ranchi; 2002-2003; 25-26.
3. Pullaiah T and Naidu CK: Antidiabetic plants in India and herbal based antidiabetic research. Regency publications: New Delhi, 2003: 272.
4. Prabhuji SK, Rao GP, Patil SK: Recent advances in Medicinal Plants Research. 2005; 63.
5. Singh A and Duggal S: Salacia spp: Hypoglycemic principles and possible role in diabetes management. Integr Med Aug/Sep 2010; 9: 4.
6. Ismail TS, Gopalakrishnan S, Begum VH, Elango V: Anti-inflammatory activity of *Salacia oblonga* Wall. and Azima tetracantha Lam. J. Ethnopharmacol 1997; 56:145-152.
7. Matsuda H, Yoshikawa M, Morikawa T, Tanabe G, Muraoka O: Antidiabetic constituents from *Salacia* species. J Tradit Med 2005; 22:145–153.
8. Krishnakumar K, Augusti KT, Vijayammal PL: Hypoglycaemic and anti-oxidant activity of *Salacia oblonga* Wall. extract in streptozotocin induced diabetic rats. Indian J Physiol Pharmacol 1999; 43:510-514.
9. Ghavami A, Johnston BD, Jensen MT, Svensson B, Pinto BM: Synthesis of nitrogen analogues of salacinol and their evaluation as glycosidase inhibitors. J Am Chem Soc 2001; 123: 6268–6271.
10. Matsuda H, Murakami T, Yashiro K, Yamahara J, Yoshikawa M: Antidiabetic principles of natural medicines. IV. Aldose reductase and alpha-glucosidase inhibitors from the roots of *Salacia oblonga* wall. (Celastraceae): structure of a new friedelane- type triterpene, kotalagenin 16-acetate. Chem Pharm Bull 1999; 47:1725–1729.
11. Li Y, Peng G, Li Q, Wen S, Huang TH, Roufogalis BD, Yamahara J: *Salacia oblonga* improves cardiac fibrosis and inhibits postprandial hyperglycemia in obese zucker rats. Life Sci 2004; 75:1735–1746.
12. He L, Qi Y, Rong X, Jiang J, Yang Q, Yamahara J, Murray M, and Li Y: The Ayurvedic medicine *Salacia oblonga* attenuates diabetic renal fibrosis in rats: suppression of angiotensin II/AT1 signaling. eCAM 2009; 1-13.
13. Giron MD, Sevillano N, Salto R, Haidour A, Manzano M, Jimenez ML, Rueda R, and Lopez-Pedrosa JM: *Salacia oblonga* extract increases glucose transporter 4-mediated glucose uptake in L6 rat myotubes: Role of mangiferin. Clin Nutr 2009; xxx:1–10.
14. Rafiq M and Mitra SK: Purified methanolic extract of *Salacia reticulata* Wight. ameliorates insulin resistance and metabolic alterations in rats fed high fructose diet. Int J Pharmacol 2010; 6:386-392.
15. Ramamoorthy J, Vanathy Meera R, Venkataraman S, Devi P: Phytochemical investigation and anti-inflammatory activity of *Salacia reticulate*. J Chem Pharm Res 2010; 2:618-625.
16. Sellamuthu PS, Muniappan BP, Perumal SM, Kandasamy M: Antihyperglycemic effect of mangiferin in streptozotocin induced diabetic rats. J Health Sci 2009; 55:206-214.
17. Chakraborty DD, Ravi V, Chakraborty P: Phytochemical evaluation and TLC protocol of various extracts of *Bombax ceiba* Linn. Int J Pharm Sci Res 2010; 1: (Suppl) 66-73.
18. Khan AM: Pharmacognostic and phytochemical investigation of the leaf of *Bergenia ciliate* and evaluation of the same for antilithiatic and antioxidant activities. Dissertation submitted to the Rajiv Gandhi University of Health Sciences, Karnataka, Bangalore, December 2005.
19. Ameh SJ, Obodozie OO, Inyang US, Abubakar MS, Garba M: Quality control tests on *Andrographis paniculata* Nees (Family: Acanthaceae) – an Indian 'Wonder' plant grown in Nigeria. Trop J Phar Res 2010; 9:387-394.
20. Dholwani KK, Saluja AK, Bhatt S, Chauhan N: Pharmacognostical profiling of roots of *Salacia prenoides* L. J Phcog 2009; 1:233-237.
21. Vandana and Arora S: Comparison of TLC fingerprint profile of different extracts of *Embelia ribes*. Int J PharmTech Res 2010; 2:2438-2440.
22. Hepsibah PTA, Rosamma MP, Prasad NBR, Kumar PS: Standardization of the Ayurvedic medicine Pindataila. Ancient Science of Life 1996; XV: 222-225.
23. Wehry EL: Handbook of Instrumental Techniques for Analytical Chemistry, Chapter 26, Prentice Hall PTR, 1997; 510-511.
24. Deokate UA and Khadabadi SS: Phytopharmacological aspects of *Salacia chinensis*. J Phcog Phytother 2012; 4:1-5.
25. Bernhoft A: Bioactive compounds in plants – benefits and risks for man and animals, Proceedings from a symposium held at The Norwegian Academy of Science and Letters, Oslo. 13 – 14 November 2008.
26. Matsuda H, Morikawa T, Toguchida I, Yoshikawa M: Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. Chem Pharm Bull 2002; 50:788-795.
27. Anderson YM and Markham KR: Flavonoids: Chemistry, Biochemistry, and Applications, CRC Press; 2006; 11.
28. Liu H, Wang K, Tang Y, Sun Z, Jian L, Li Z, Wu B, Huang C: Structure elucidation of *in vivo* and *in vitro* metabolites of Mangiferin. J Pharm Biomed Anal 2011; 55:1075–1082.

**How to cite this article**:
Basu S, Pant M and Rachana: Phytochemical evaluation and HPTLC profiling of extracts of *Salacia oblonga*. *Int J Pharm Sci Res* 2013; 4(4); 1409-1418.