

**PHYSICOCHEMICAL, PHYTOCHEMICAL AND HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY ANALYSIS OF THE ROOT BARKS OF *ONOSMA ECHIOIDES***AMBREEN SHOAIB<sup>1</sup>, HEFAZAT HUSSAIN SIDDIQUI<sup>1</sup>, BADRUDDEEN<sup>1</sup>, ALEZA RIZVI<sup>1</sup>, RAKESH KUMAR DIXIT<sup>2\*</sup><sup>1</sup>Department of Pharmacology, Faculty of Pharmacy, Integral University, Lucknow, Uttar Pradesh, India. <sup>2</sup>Department of Pharmacology, King George Medical University, Lucknow, Uttar Pradesh, India. Email: Kumardixitakesh94@gmail.com

Received: 17 May 2017, Revised and Accepted: 12 June 2017

**ABSTRACT**

**Objective:** *Onosma echioides* (OE) L., family Boraginaceae, is most recurrently used remedial herb, which is extensively dispersed all over India. It is used as purgative, bladder obstacle, liver chaos, kidney obstruction, sciatic pain, and gout. The study was meant to carry out the physicochemical, phytochemical, and high-performance thin layer chromatography (HPTLC) analysis on root bark of OE.

**Methods:** The physicochemical parameters were carried out as per the World Health Organization guideline. The preliminary chemical test was performed as per pharmacopeia and conventional methods. Camag HPTLC system equipped with TLC autosampler 4 applicator, TLC scanner 3 and win CATS 1.4.4. Software was used for HPTLC analysis of plant extract with standard, i.e., shikonin. The extracts were developed using toluene:ethyl acetate:formic acid (5:4:0.3) as a mobile phases using standard procedures and scanned under ultraviolet 254 nm and 366 nm.

**Results:** OE is composed of sterols, tannins, alkaloids, flavonoids, glycoside, saponin, and carbohydrates. The plant was found to be free from adulteration and contamination. The fluorescence characteristics of leaf powder were studied both in visible light and ultraviolet light (254 nm and 365 nm) after treatment with various reagents. The TLC plate showed several spots at different R<sub>f</sub> when viewed under 254 nm, 366 nm. The HPTLC profile showed several peaks that indicated the presence of various phytochemicals along with its active constituent, i.e., shikonin.

**Conclusion:** The study provides referential information for the standardization of the plant. The HPTLC profile will help in authentication and standardization of the plant.

**Keywords:** *Onosma echioides*, High-performance thin layer chromatography, Phytochemical screening, Standardization.

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**INTRODUCTION**

*Onosma echioides* (OE) L., family Boraginaceae, is most recurrently used remedial herb, which is extensively dispersed all over India, Nepal, Pakistan, China, and Afghanistan [1]. It is commonly known as Maha rangi, Ratan jot and Maharanga [2-4]. Ratan jot is traditionally used in Indian food and lends its red color to some versions of the curry dish like Rogan Josh. The leaves of OE are widely used as a purgative, bladder obstacle, liver chaos, kidney obstruction, pain, and gout [5]. Its root pulp is utilized as an antiseptic for wounds [6], bruises and eruptions [7]. The previous study has reported the anthelmintic and alexipharmic activity of OE extract [1]. A study has revealed defending character of OE in croton oil urge skin malignancy and oxidative damage in Swiss mice [5]. This effect was could be owing to active constituents of shikonins and alkannins present in plant extract. Interestingly, these compounds also reported to promote wound healing in an albino rat model [8].

Recently, various extraction techniques are urbanized for the development of herbal components and its pharmacological activities [9]. Shikonin and alkannin are enantiomeric natural dyes; active components presents in the root bark of OE, which possess immense application in pharmaceuticals, cosmetics and food colorants along with their other derivatives, such as deoxyshikonin, acetylshikonin, and hydroxyalkanin [10].

In this study, the physicochemical parameters of the plant were studied, and the n-hexane, chloroform, methanol, and aqueous extracts of the dried root bark of OE were subjected to preliminary phytochemical screening and high-performance thin layer chromatography (HPTLC) analysis.

**METHODS****Plant collection and authentication**

Dried root bark of OE (crude form) was procured from the authenticated licensed shop from Lucknow, India. The authenticity of plant material was confirmed from the text report of National Botanical Research Institute, Lucknow, India, and a reference specimen (voucher no. NBRI/CIF/397/2013) was deposited. The dried plant material was coarsely powdered for the determination of ash values, extractive values, preliminary phytochemical investigation and HPTLC analysis as per standard methods.

**Plant extraction**

Sequential extraction of dried root bark of OE was performed by refluxed on water bath for 6 hrs at 60°C using different solvents on the basis of increasing polarity, viz., n-hexane, chloroform, and methanol. Rotary evaporator (BUCHI Rotavapor R-205, Switzerland) was used to concentrate the filtrates obtained under vacuum at 40°C. The extractive yields of n-hexane, chloroform, methanol, and aqueous extracts were calculated.

**Physicochemical parameters**

Several parameters were assessed such as extractive values in different solvents, fluorescent analysis, total ash, acid-insoluble ash, water-soluble ash, foreign matter, and moisture content using standard methods. Calibrated digital pH meter was used to measure the pH of 1 and 10% aqueous extracts [11,12].

**Preliminary phytochemical screening**

Preliminary phytochemical screening of n-hexane, chloroform, methanol, and aqueous was carried out for the detection of various compounds using standard procedures [13-15].

### TLC and HPTLC analysis

Slurry of silica gel G was prepared in distilled water and poured over glass plates to form a thin layer. The prepared plates were air dried for setting and then kept in an oven at 100-120°C (30 minutes) for activation. The extracts were dissolved in respective solvents and spotted over an activated plate (1 cm above from the bottom). The spotted plates were kept in a previously saturated developing chamber containing mobile phase, and allowed to run 3/4<sup>th</sup> of the height of the prepared plate 20. The plates were air dried, and number of spots were noted and Rf value was calculated [16].

The instrument employed was Camag HPTLC system (Muttentz, Switzerland) equipped with a sample applicator TLC Linomat 5 Application (Camag) with win CATS software version 1.4.4. The plate was developed using different solvent systems for each extract in a pre-saturated Camag Twin through Chamber. The mobile phase used for developing the n-hexane extract was toluene:ethyl acetate:formic acid in the ratio of 5:4:0.3 v/v, respectively. The plate was developed, removed from the chamber and allowed to dry. The developed plate was scanned using TLC Scanner 3 and analyzed with win CATS software version 1.4.4. At  $\lambda$  max 254 nm and 366 nm using deuterium light source, the slit dimensions were 5.00 mm  $\times$  0.45 mm. The chromatograms were recorded.

### RESULTS AND DISCUSSION

#### Macroscopical characteristics

These are reddish color root having several layers which are twisted and modestly coarse (Fig. 1). Externally the roots are cylindrical, sometimes furrowed. Segments are irregularly formed approximately 6-11 cm long and 2-6 cm in diameters. It has a characteristics odor.

As the part of standardization study, the macroscopic examination of root bark was studied. Macroscopical evaluation is a technique of qualitative assessment based on the study of morphological and organoleptic characters. Macroscopical characters of root bark can serve as diagnostic characters.

#### Physicochemical analysis

The physicochemical characters of powdered drug of root bark of OE were evaluated. The extraction was done using different solvents such as n-hexane, chloroform, methanol, and water. Other parameters were also investigated, viz., ash value, acid-insoluble ash, water-soluble ash, loss on drying, fluorescent analysis, and foreign matter are presented in Table 1. Total ash is important for evaluating the purity and quality of the herb. A high ash value is indicative of contamination, substitution or adulteration. An increase in acid-insoluble ash indicates contamination with sand and soil [17]. Due to less acid insoluble value, it was found that the plant is free from adulteration and contamination of soil. The fluorescence analysis of the powdered drug of OE in various solvents was performed under normal and ultraviolet (254 nm and 366 nm) light and powdered drug reaction with different reagents were evaluated in Tables 2 and 3, respectively. The pH of 1% and 10% aqueous solution of powdered drug of OE were tabulated in Table 4.

The results showed greater extractive values in successive extraction indication the effect of polarity of solvent. Their percentages were calculated with reference to air dried drug. The percent extractives in different solvents indicated quantity and nature of constituents in the extracts. The extractive values were also helpful in determining the soluble nature of a particular constituent in a particular solvent.

The fluorescence analysis of the root bark of OE was performed using various solvents and was performed under normal and UV light.

#### Preliminary phytochemical screening

The preliminary phytochemical investigation of OE showed in Table 5, which showed the presence of sterols, flavonoids, glycosides, phenolic compounds, carbohydrates, and alkaloids.



Fig. 1: Root barks of *Onosma echioides*

Table 1: Physicochemical parameters of *Onosma echioides*

| Quantitative parameter     | Values obtained (%) w/w |
|----------------------------|-------------------------|
| n-hexane extractive        | 8.3                     |
| Chloroform extractive      | 3.6                     |
| Alcohol soluble extractive | 4.6                     |
| Water-soluble extractive   | 11.4                    |
| Total ash                  | 10.66                   |
| Acid-insoluble ash         | 4.83                    |
| Water-soluble ash          | 12.66                   |
| Loss on drying             | 4.7                     |
| Foreign matter             | 0.76                    |

Table 2: Florescent analysis of *Onosma echioides* in different solvent system

| Solvent used               | Daylight       | UV light (254 nm) | UV light (366 nm) |
|----------------------------|----------------|-------------------|-------------------|
| Benzene                    | Grayish brown  | Greenish brown    | Gray              |
| Dist. Water                | Reddish brown  | Light green       | Dark green        |
| NaOH in water              | Dark brown     | Light green       | Golden brown      |
| NaOH in CH <sub>3</sub> OH | Blackish brown | Dark green        | Black             |
| Chloroform                 | Golden brown   | Yellowish green   | Dark brown        |
| Dil. HNO <sub>3</sub>      | Brown          | Light brown       | Golden brown      |
| Acetone                    | Light brown    | Light brown       | Yellowish brown   |
| Ethyl acetate              | Light brown    | Yellow            | Yellowish green   |

Table 3: Powdered drug reaction with different reagents of OE

| Treatment                                   | Observation     |
|---|-----------------|
| Powder as such                              | Reddish brown   |
| Concentrated HCl                            | Brown           |
| Concentrated HNO <sub>3</sub>               | Brownish Yellow |
| Concentrated H <sub>2</sub> SO <sub>4</sub> | Yellowish brown |
| Glacial acetic acid                         | Brownish red    |
| Benzene                                     | Grayish brown   |
| NaOH in methanol                            | Gray            |

OE: *Onosma echioides*

**TLC**

TLC of n-hexane extract was carried out using toluene:ethyl acetate:formic acid in the ratio of 5:4:0.3, respectively, and the Rf values were recorded (Fig. 2) and depicted in Table 6. The visualizing reagent employed was anisaldehyde-sulfuric acid reagent to effect visualization of the resolved spots.

TLC is also valuable for the preliminary separation and determination of plant constituents. The chromatographic value may serve as a characteristics print for quantitative evaluation of root bark of OE.

**HPTLC of OE n-hexane extract**

HPTLC fingerprinting is a valuable tool for the evaluation of herbal medicine. This study was conducted for the quantitative determination

**Table 4: Determination of pH of OE**

| Aqueous solution (%) | pH  |
|----------------------|-----|
| 1                    | 5.0 |
| 10                   | 6.2 |

OE: *Onosma echioides***Table 5: Preliminary phytochemical screening of OE n-hexane extract**

| Tests                       | n-hexane extract of OE |
|-----------------------------|------------------------|
| Test for sterols            | +                      |
| Tannins test                | -                      |
| Flavonoids                  | +                      |
| Test protein and amino acid | -                      |
| Glycosides test             | +                      |
| Phenolic test               | +                      |
| Carbohydrate test           | +                      |
| Saponin test                | +                      |
| Alkaloids test              | +                      |

OE: *Onosma echioides***Table 6: TLC of n-hexane extract of OE**

| Test extracts | Solvent system                              | Number of spots | Rf value                    |
|---------------|---|-----------------|-----------------------------|
| n-hexane      | Toluene:ethyl acetate: formic acid: 5:4:0.3 | 5               | 0.97, 0.9, 0.82, 0.76, 0.65 |

TLC: Thin layer chromatography, OE: *Onosma echioides***Table 7: Peak table for HPTLC profile of OE n-hexane extract**

| Track 1, ID: OE |                     |                   |                   |                 |         |                   |                 |           |          |                    |
|-----------------|---------------------|-------------------|-------------------|-----------------|---------|-------------------|-----------------|-----------|----------|--------------------|
| Peak            | Start position (Rf) | Start height (AU) | Max position (Rf) | Max height (AU) | Max (%) | End position (Rf) | End height (AU) | Area (AU) | Area (%) | Assigned substance |
| 1               | 0.00                | 0.3               | 0.02              | 75.9            | 15.72   | 0.03              | 17.1            | 1022.3    | 4.26     | Unknown*           |
| 2               | 0.04                | 17.2              | 0.04              | 20.5            | 4.25    | 0.06              | 9.1             | 333.7     | 1.39     | Unknown*           |
| 3               | 0.39                | 9.3               | 0.42              | 17.4            | 3.61    | 0.44              | 15.1            | 552.1     | 2.30     | Unknown*           |
| 4               | 0.46                | 18.5              | 0.50              | 29.5            | 6.12    | 0.50              | 27.3            | 948.6     | 3.96     | Unknown*           |
| 5               | 0.61                | 37.0              | 0.69              | 165.4           | 34.27   | 0.74              | 74.9            | 11486.0   | 47.90    | Unknown*           |
| 6               | 0.74                | 74.9              | 0.79              | 145.4           | 30.12   | 0.85              | 8.0             | 9093.7    | 37.93    | Shikonin           |
| 7               | 0.86                | 3.3               | 0.88              | 28.5            | 5.90    | 0.90              | 0.2             | 540.9     | 2.26     | Unknown*           |

OE: *Onosma echioides*. \* Shows the unknown components present in plant extract**Table 8: Peak table for HPTLC profile of shikonin**

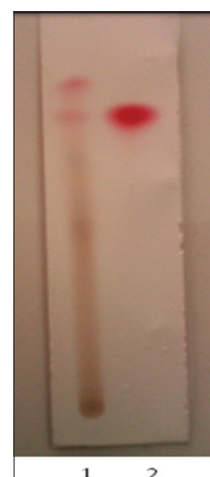
| Track 2, ID: Shikonin |                |              |              |            |        |              |            |           |        |                    |
|-----------------------|----------------|--------------|--------------|------------|--------|--------------|------------|-----------|--------|--------------------|
| Peak                  | Start position | Start height | Max position | Max height | Max %  | End position | End height | Area      | Area % | Assigned substance |
| 1                     | 0.76 Rf        | 1.3 AU       | 0.81 Rf      | 108.7 AU   | 100.00 | 0.84 Rf      | 1.5 AU     | 4937.0 AU | 100.00 | Shikonin           |

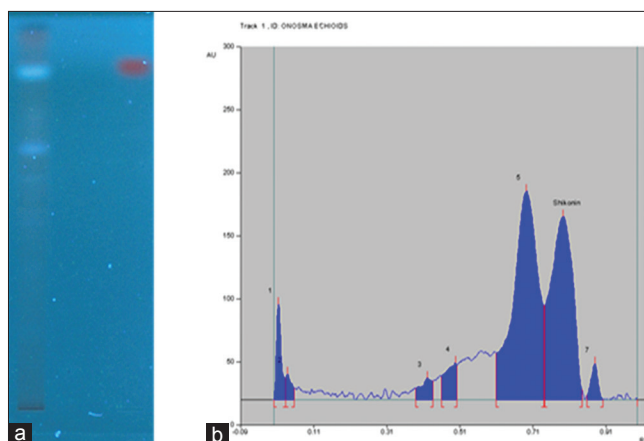
HPTLC: High-performance thin layer chromatography

of phytochemicals present in the plant extract [18]. HPTLC of n-hexane extract of OE was carried out using the same solvent as that in TLC. Toluene:ethyl acetate:formic acid in the ratio of 5:4:0.3, respectively, and the Rf values were recorded. The densitogram of n-hexane extract OE and its active constituent, i.e., shikonin was scanned and analyzed with win CATS software version 1.4.4. At  $\lambda$  max 254 nm and 366 nm using deuterium light source, the slit dimensions were 5.00 mm  $\times$  0.45 mm. The chromatograms were showed in Fig. 3 and peak table for HPTLC profile of OE was showed in Table 7. Whereas, peak table for HPTLC profile of shikonin was recorded in Fig. 4 and illustrated in Table 8, which showing the presence of shikonin in OE n-hexane extract.

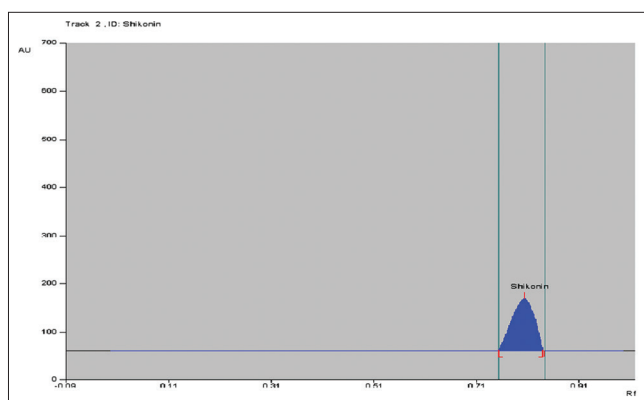
**CONCLUSION**

It can be concluded that the present study on root bark of OE can serve as an important source of information to ascertain the identity and to determine the quality and purity of plant material available in the market. These parameters are necessary for the identification of drugs. The presence of various chemical constituents in OE may be a potential cause of treatment of various disorders. The quality of the plant can be estimated by determining the physical parameters. These investigations are of great importance for carrying out the revalidation and estimation of its other pharmacological activities.

**Fig. 2: Fingerprinting profile: (1) n-hexane extract of *Onosma echioides* (2) Shikonin**



**Fig. 3: (a) High-performance thin layer chromatography fingerprinting of n-hexane of *Onosma echioides* (OE) scanned at wavelength at 366 nm and (b) densitogram of n-hexane of OE scanned at wavelength at 366 nm**



**Fig. 4: High-performance thin layer chromatography fingerprinting of shikonin the active constituent of *Onosma echioides* as a reference standard at 366 nm**

#### ACKNOWLEDGMENT

The authors are thankful to Integral University, Lucknow, for the encouragement and the facilities provided (IU/R&D/2017-MCN00094).

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