

PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *Ailanthus excelsa* *Roxb.* AND *Oroxylum indicum* (L) Benth. Ex. Kurz. (SYONAK) USED IN INDIAN SYSTEM OF MEDICINE

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ABSTRACT

There is a huge demand for Ayurvedic plants all over the world and it has become a subject of deep research globally. Syonak is one of the ingredients of *Dashamoola* and is used in a large number of formulations in Ayurveda. Apart from its use as a part of *Dashamoola*, *Syonaka* is used as a single drug both internally and externally in inflammation, rheumatoid arthritis and various other ailments. It is one of the ingredients in many important Ayurvedic formulations, such as *Dasamoolarishtam*, *Dasamoola rasayanam*, *Amrutarishtam*, *Chyavanaprasha* and others. The proposed research study is an attempt to evaluate pharmacognostic, phytochemical and pharmacological profile of *Ailanthus excelsa* Roxb. and *Oroxylum indicum* (L) Benth. Ex. Kurz. The common names of both of these species as mentioned in ayurvedic literature is Syonak which is a matter of controversy. Root bark and Stem bark of both the plants is selected for the study as these parts are commonly used as a part of several Ayurvedic formulations. Phytochemical analysis revealed higher content of flavonoids and phenols in both root bark and stem bark of Syonak B compared to Syonak-A whereas alkaloids and glycosides content is found to be higher in both the parts of Syonak-A compared to Syonak-B. Antiageing activity is found to be higher and more consistent in Syonak-B compared to Syonak-A samples.

Keywords: *Dashmoola*, *Oroxylum indicum*, *Ailanthus excelsa*.

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INTRODUCTION

Ayurveda is the oldest health care system in the world, the history of which can be traced back to 4000 BC.¹ A large number of the population in developing countries are dependent on traditional practitioners.² Syonaka has been an important herb in Ayurvedic and traditional medical system for several years.³ It is used as such and also as a component of many poly-herbal preparations in Ayurveda. It is also a component of subgroup Brihatpanchmoola of *Dashmoola*, which is a very important fixed-dose combination being used in a varied number of ayurvedic formulations like Mahanaryan tail, Chywanprash, *Dasamoolarishta* etc.⁴ The root/ stem bark possess anti-inflammatory properties, used in treating urticaria, asthma jaundice, sore throat, laryngitis, diarrhoea, gastralgia, erythema, dysentery, measles and possess antiallergic properties.^{5,6} However, the botanical identity of Syonaka is subject to debate. Bhavprakashnighantu described *Ailanthus excelsa* as 'Aralu' and Syonaka is mentioned as its synonym.^{7,8} Amarkosh aralu mentioned "shyonak and tintuk" as same plant.⁹ Nighantu writers have mentioned it as *Oroxylum indicum* (Bignoniaceae). Adarsha Rajniguntakar Nighantu states that out of two Syonakas, one is aralu and other is

tintuk.¹⁰ This paper presents pharmacognostic, phytochemical and pharmacological studies of root bark/stem bark of *Oroxylum indicum* and *Ailanthus excelsa*. Antiageing activity has been selected for the study as both species are good sources of phenolic compounds which possess antioxidant properties and can be used as a good antiageing plant source. Antiageing activity has been explored for the first time in these species. Presently, root bark/stem bark of *Oroxylum indicum* is being used as a part of Brihatpanchmoola.

EXPERIMENTAL

Collection and Identification of Raw Materials

Stem bark, Root Bark, Leaves and Young roots of *Oroxylum indicum* and *Ailanthus excelsa* were received from the Bioresource Development Group, Dabur Research and Development Centre against Voucher no.'s DRDC-1262-BRD/OI and DRDC-1263-BRD/AI. The plant materials were identified by Dr. G.P. Kimothi and Dr. C.S. Rana, Taxonomist, Dabur Research and Development Centre, Sahibabad, Ghaziabad.

Chemicals/ Reagents

HPLC grade solvents were used and reagents used were of analytical grade. Milli-Q Academic A10 water purification system (Millipore, France) was used for water purification.

Pharmacognostical Procedures

Each sample was assigned a sample code as per a pre-designed procedure. The sample was dried further to reduce the moisture content to 8-10% and divided into 3 portions *v.i.z.* for pharmacognostic and physico-chemical study, for extraction purposes and rest for sample retention and referencing.

Macroscopic Study

The samples were examined to observe the macroscopic characters first with the naked eye, followed by visualization with the help of a lens (10X). Photographs were obtained for recording the macroscopic features.

Microscopic Study

The method adopted for the study is microtomy. The instrument used is microtome capable of cutting a section at a preset thickness by sliding the block into a cutting tool, usually a blade or knife, which was attached to the machine.¹¹ Leica RM 2245 model microtome was used for microtoming purposes.

Physicochemical Studies

The fine powdered samples were analyzed for physico-chemical parameters¹² and screened for the presence of various secondary metabolites *v.i.z.* alkaloids, glycosides, total phenols and flavonoids.¹³

Preparation of Extracts

Aqueous extract of the plant materials was prepared using the decoction process. Around 200 g of the dried crushed plant materials were taken in different vessels, respectively and a sufficient quantity of double distilled water was added and proceeded further for extraction. The solution obtained was then concentrated and filtered. It was finally dried and collected in bottles and stored in a refrigerator for further testing and evaluation.

TLC Fingerprinting

Around 1 g of extract was taken, added 5 ml of methanol, sonicated for 10 minutes and then the volume was made up to 10 ml in a volumetric flask with methanol and used as a test solution. Ethyl acetate: Formic acid: Acetic acid: Water (50:5.5:5.5:13) was used as a solvent system for TLC development. The solvent system was developed inhouse after several trials to obtain the best resolution of spots.

HPLC Studies

The samples were evaluated for oroxylin content. HPLC method was developed to obtain precise results.¹⁴ The study was carried out on a Shimadzu high-performance liquid chromatography system (Model SPD-M20A) with diode array detector (230 V), model LC-30 AD pumps, model SIL-30 AC autosampler unit and a CTO-20 A thermostat was controlled by Shimadzu Lab solutions software. The separation of

compounds was achieved using a C18 column. Mobile phase used was 0.1 %TFA: ACN (60:40 v/v). Pump was set at isocratic mode with a flow rate of 1.0 mL/min. The selected injection volume was 20 μ L for all samples taking detection wavelengths at 274 nm.

Pharmacological Evaluation

Anti-ageing Activity

Senescence is characterized by a stable growth arrest that limits the proliferation of damaged cells.^{15,16} There are several biomarkers associated with cellular senescence. β -Galactosidase is one such biomarker that is widely used to detect cellular senescence in vitro. Senescence-associated β -galactosidase (SA- β -gal) activity can be studied in skin keratinocytes or skin fibroblasts. The signs of aging happen all over the body and especially on human skin.¹⁷ Oxidative stress induced by chemical agents such as t-BHP and H₂O₂ results in an increase in beta-gal activity and leads to aging. Inhibition of this activity indicates anti-aging properties. Hence, the present study evaluated the anti-aging effects of test items via inhibition in β -Galactosidase activity in skin fibroblast cells, HFF-1.

RESULTS AND DISCUSSION

Natural products might be potential drugs for humans and also these products and analogs can act as intermediates for the synthesis of useful drugs.¹⁸ As per the results of the present study, stem and root barks of Syonak A and B can be differentiated on macro-microscopic characters (Table-1 and 2, Fig.-1 to 4).

Table-1: Comparative Macroscopic Characteristics of Syonak-A and Syonak-B

Syonak -A		Syonak B	
Root Bark	Stem Bark	Root Bark	Stem Bark
It appears pale to dark bright yellowish in color. They have many longitudinal fissures throughout the length. The outer surface can be easily peeled off from the wood portion of the root.	The bark pieces are curved or slightly channeled; 1-1.5 cm thick; outer surface somewhat rough, faintly longitudinally striated, at places faintly longitudinally cracked with rounded or transversely elongated lenticels, greyish brown; inner surface finely longitudinally striated, at places very smooth, pale yellowish-white. Fracture outer granular, inner splintery; taste bitter and astringent; odor characteristic.	Cream yellow to yellowish-brown cut pieces. 10-16 cm in length, 2-3 cm in width and 3-5 mm in thickness. The external surface is rough, fractures with cracks.	Grey colored 4-8 cm in length, 1-3 cm in width, 4-8 mm cut pieces, flat or channeled with warty cracks. External surface has fissures. Rough external surface with longitudinal and transverse cracks with fractures

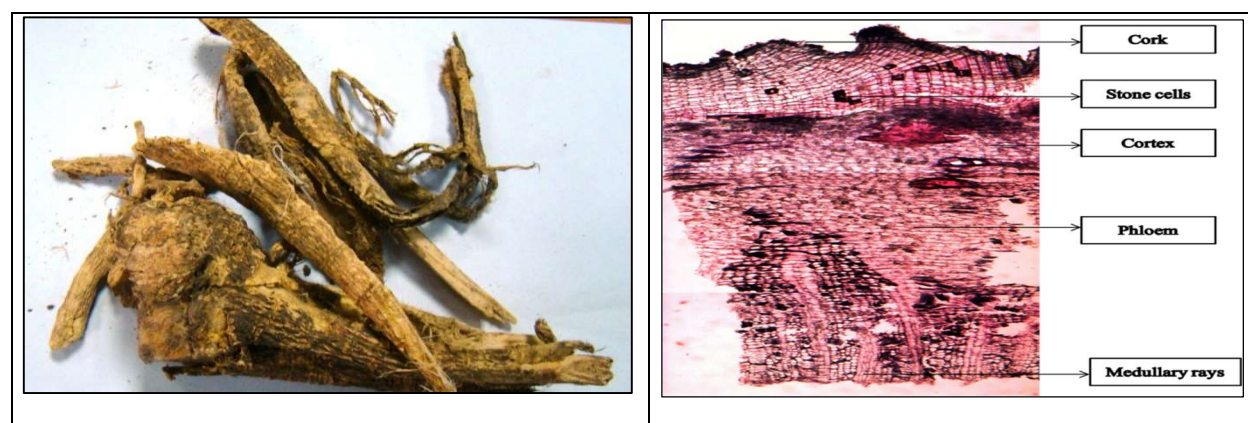


Fig.-1: Morphological and Histological Features of Root Bark-Syonak-A

Table-2: Microscopic Studies of Syonak-A and Syonak-B

Syonak -A		Syonak B	
Root Bark	Stem Bark	Root Bark	Stem Bark
Bark consists of 10-12 layers outer cork cells with stone cells widely distributed. The presence of cambium can be noticed. Phelloderm consists of parenchymatous cells along with starch cells evenly distributed among them. Uniseriate to multiseriate medullary rays can be seen, which are homogenous and radially elongated.	TS of the bark show multilayered cork and parenchymatous cortex traversed by groups of stone cells, arranged tangentially or vertically. Phloem almost occupying a two-thirds area of the section traversed by medullary rays, stone cells and plenty of calcium oxalate crystals traversing throughout parenchymatous cells of the cortex and phloem region. Medullary rays are multiseriate, rarely uni- to biseriate.	Cork consists of parenchyma and stone cells. Stone cells are of varied shapes and sizes. Sieve elements, parenchyma and stone cells are present in secondary phloem. Fibers are present in groups. Tri to octaseriate medullary rays is present in the inner region.	Cork is composed of 15-20 layers of elongated rectangular cells. Parenchyma and stone cells are present in phelloderm. Fibers are arranged in concentric rings and in groups.

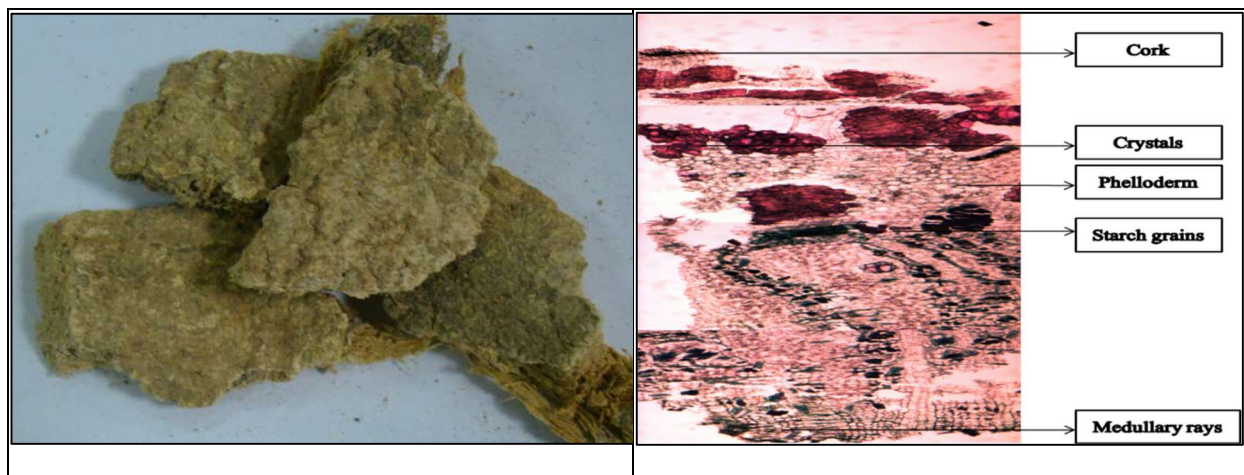


Fig.-2: Morphological and Histological Features of Stem bark-Syonak-A

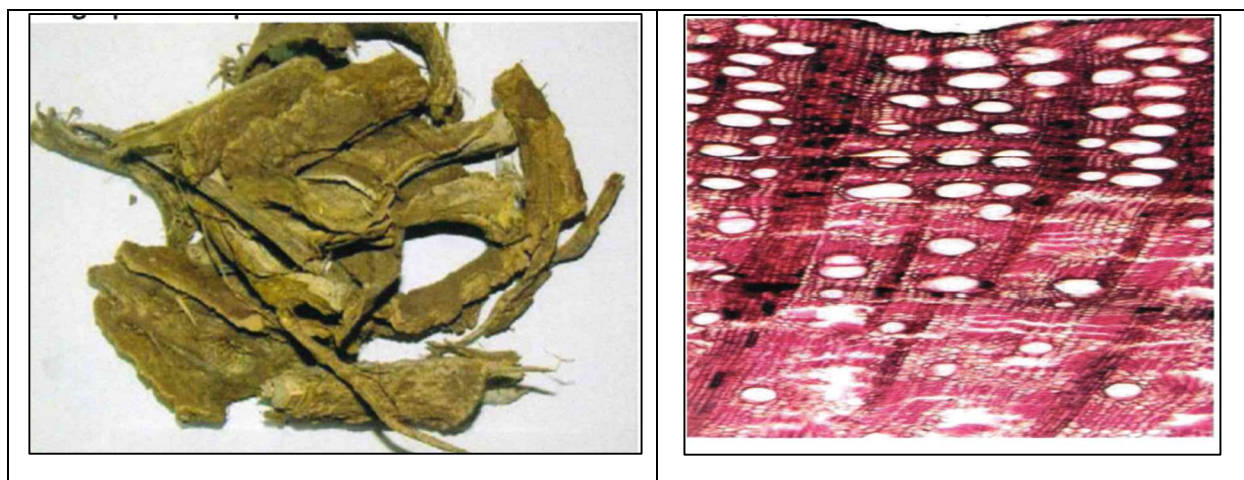


Fig.-3: Morphological and Histological Features of Root Bark-Syonak-b

A distinct unpleasant odor was found in Syonak-A, whereas Syonak- B has no such odor. Syonak A has a bitter and astringent taste, whereas Syonak B has an only bitter taste. Stem bark and root bark of Syonak-B showed comparatively higher alcohol and water-soluble extractive values than Syonak-A. Extractive values determine the amount of active constituents soluble in particular solvents and gives an idea about the nature of constituents present in a sample. There is a slight difference in ash values observed in both samples. Ash values give an idea about the inorganic materials present in the sample. Total ash gives an idea about the total inorganic materials present, whether from the plant tissue itself and external contamination.

Acid insoluble ash basically measures the presence of silica in the sample. Loss of drying is an important parameter as an excess of moisture in a drug encourage microbial growth. Loss on drying is found to be slightly higher in Syonak B samples compared to Syonak A samples. A comparatively higher flavanoid and phenolic contents were observed in root bark and stem bark of Syonak-B than Syonak-A. On the other hand, Syonak-A has higher alkaloids and glycosides content compared to Syonak-B (Table-3). Some common, as well as some distinct spots, are observed in both the species in HPTLC fingerprinting (Fig.-5).

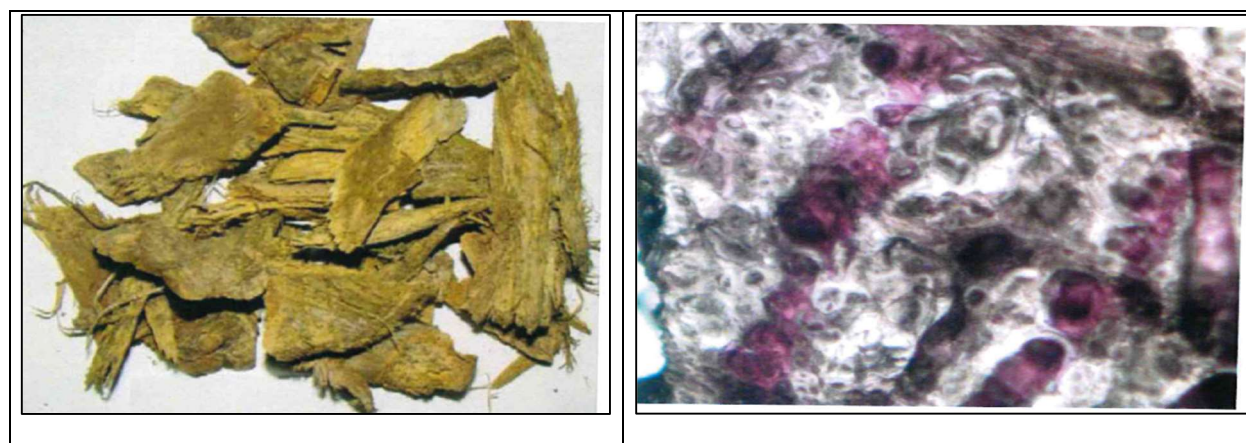


Fig.-4: Morphological and Histological Features of Stem Bark-Syonak-B

Oroxylin content is found to be higher in both the stem bark and root bark of syonak-B compared to syonak-A in HPLC studies (Fig.-6). Antiageing activity is also found to be higher and more consistent in Syonak-B compared to Syonak-A samples (Fig.-7). The Comparative macroscopic characteristics of Syonak-A and Syonak-B are listed in Table-1. The microscopic studies of Syonak-A and Syonak-B are listed in Table-2. The results of various physicochemical observations of Syonak-A and Syonak-B are given in Table-3.

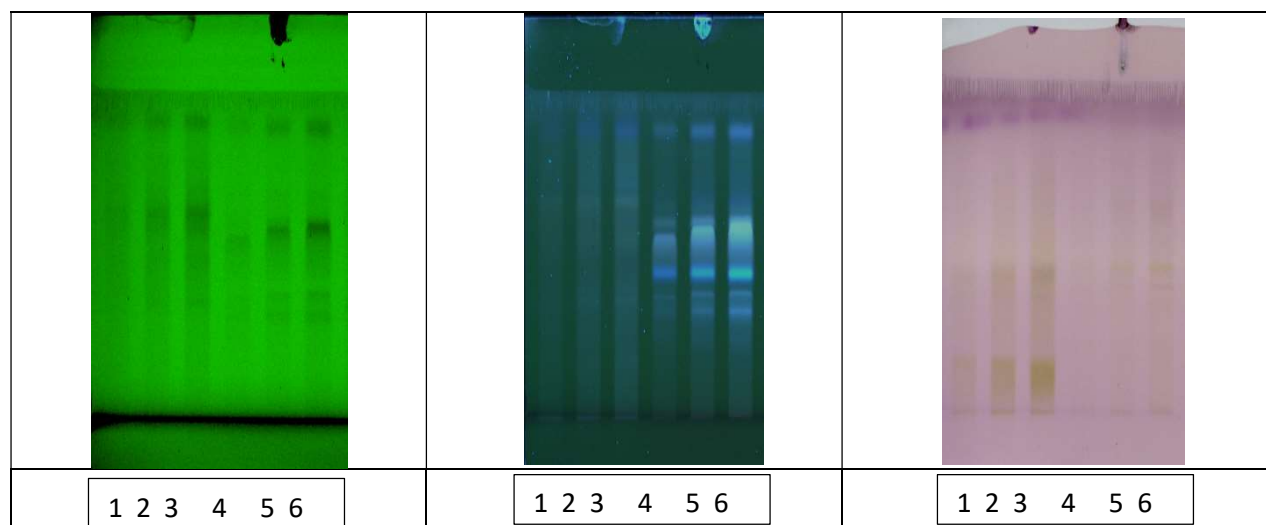


Fig.-5: Comparative Fingerprinting of extracts of Syonak B and Syonak A

1 Root bark of syonak-B; 2,3 Stem bark (syonak B); 4 Root Bark (Syonak-A); 5,6 Stem Bark (Syonak-A)

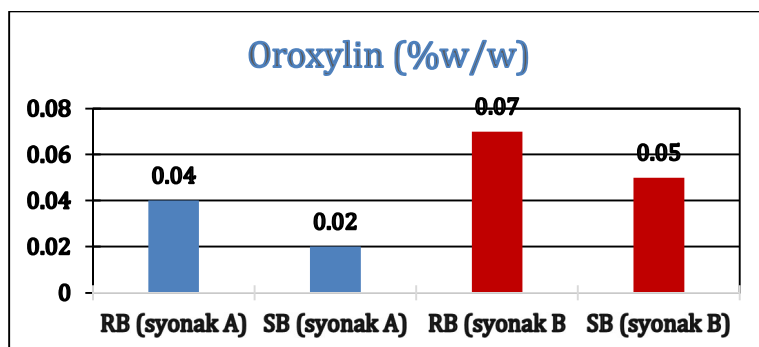


Fig.-6: Data showing comparative Oroxylin Content in Syonak B and Syonak-A by HPLC

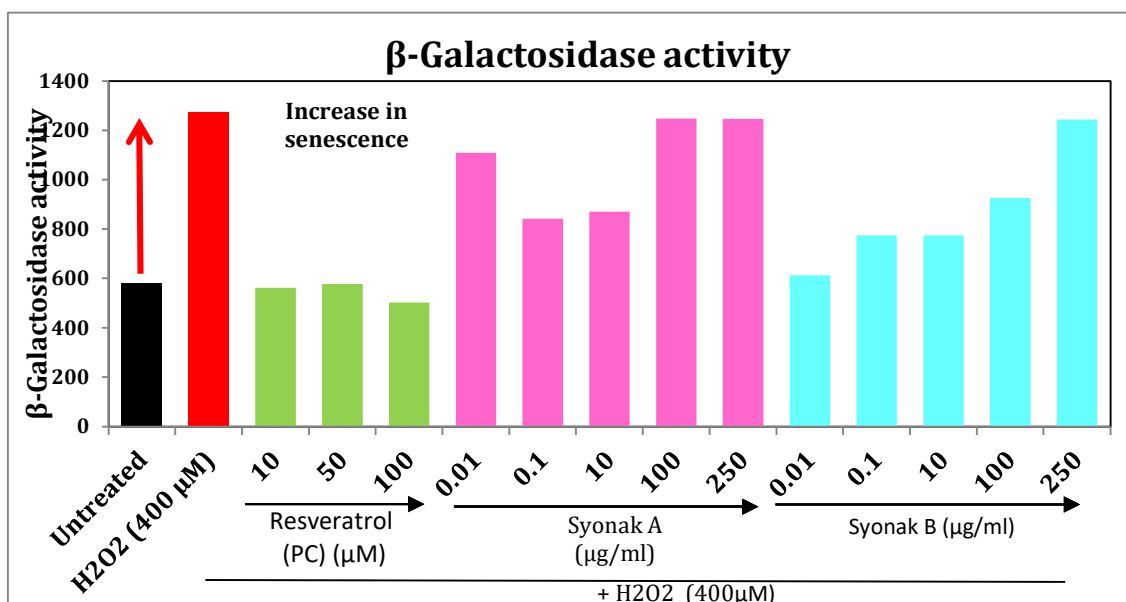


Fig.-7: Comparative β-galactosidase Activity in Extracts of Syonak-B and Syonak-A

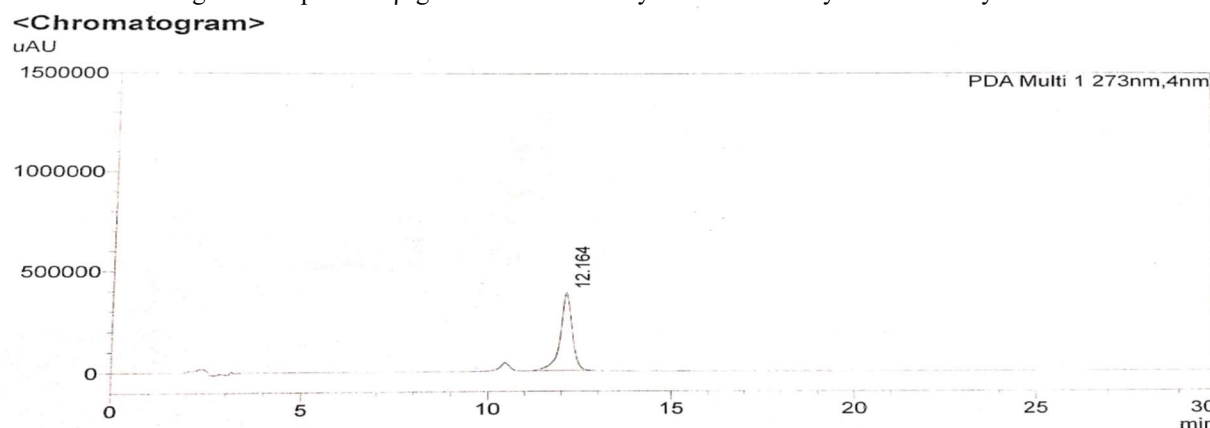


Fig.-8: HPLC Chromatogram of Oroxylin in Syonak

Table-3: Physicochemical Observations of Syonak-A and Syonak-B (Standard Deviation= ± 5%)

	Root Bark		Stem Bark	
	Syonak-A	Syonak-B	Syonak-A	Syonak-B
LOD (%w/w)	4.2	5.3	4.2	6
Total ash	11.5	10.5	7.5	9.5
Acid insoluble ash	8.2	2	2.6	3.1

oluble extractive	7.8	20.2	7.2	23.5
Alcohol soluble extractive	0.5	2.3	0.62	3.2
Flavanoids	1.2	6.5	1.2	3.5
Phenols	0.3	4	0.15	4
Alkaloids	2.5	0.52	1.3	0.55
Glycosides	0.5	0.2	0.6	0.36

CONCLUSION

Two botanical plants commonly named Syonak in literature were included in this study viz ‘*Ailanthus excelsa* Roxb. of family Simaroubaceae is named Syonak-A’ and ‘*Oroxylum indicum* L. Benth ex. Kurz of the family Bignoniaceae is named Syonak-B’. Basis the observations on antiageing activity, it may be concluded that *Oroxylum indicum* is more effective as per the data obtained. Pharmacognostic, phytochemical and chromatographic studies will be helpful for quality evaluation and for setting the monographs of these drugs.

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