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Standardization And Invitro Antioxidant Activity Of Olax Scandens

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Abstract

During recent days human beings are prone to various diseases and are requiring serious hospitalization and treatment. The majority of treatment employs high tech equipment or synthetic drugs which have notable side effects and adverse effects. To overcome these effects, use of herbal medicines or those drugs derived from natural origin is suggested and preferred. But the problem with natural drugs is the ambiguity in the potency of action repetitiveness of the results which might be due to various factors starting from collection to storage and shipping. These changes in the crude drug due to the environmental and other factors reduce the reliability on herbal drugs for effective treatment of disease inspite of their safer use. So standardization of crude drugs plays a key role in ensuring the quality and efficacy of the drug. So in the present study Olxscandens plant had been standardized in terms of its microscopy characters, physicochemical constants, phytochemical analysis and invitro antioxidant activity was investigated by taking quercetin as standard. Results suggested the bark of the plant can be extracted with highest yield using ethanol as solvent. Microscopy studies revealed the presence of cork, phelloderm, phloem fibres, xylem tissue, and calcium oxalate crystals. Heavy metal contents are under acceptable limits. Phytochemical evaluation suggested the crude drug contains phenols, flavonoids, glycosides, alkaloids etc. Total phenol content was determined and the ethanol extract contained 172mg of quercetin equivalents of total phenols. Invitro antioxidant activity proved that the ethanol extract was comparatively similar in inhibiting the free radicals generated due to superoxides, hydroxyl and peroxidation.

Keywords: Olax, standardization, antioxidant activity, phenols, extraction

Introduction

Disease treatment using drugs has been most important aspect of medicine and almost 80% of world population is still dependent on herbal remedies to treat illness. In the present scenario, the demand for herbal products is growing exponentially throughout the world, and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value. Therefore, quality control for the efficacy and safety of herbal products is essential (WHO, 2000). World Health Organization (WHO) stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles. It is now realized that herbal medicines are safe, free from adverse effects (Chopra et al., 1956).

As commercialization of the herbal medicine has happened, assurance of safety, quality and efficacy of medicinal plants and herbal products has become an important issue. The herbal raw material is prone to a lot of variation due to several factors, the important ones being the identity of the plants and seasonal variation. The steps are taken by WHO not only help to make the quality of herbal products but also to safeguard the adverse effects of the herbals too (Ahmed et al., 2015).The present study includes the study of Microscopical characters, determination of physico-chemical constants, preliminary phytochemical studies, fluorescence analysis, and quantitative analysis of phytochemical constituents and invitro antioxidant activity of the successive extractives of the powder of bark of Olaxscandens.

Materials and methods

Chemicals and Reagents

All Chemicals solvents and reagents used in the study were of analytical grade and were procured from Rankem, Mumbai and Himedia Laboratories Ltd., Mumbai.

Plant material
Fresh bark of O*laxscandensRoxb* was collected local area in Chennai, Tamil Nadu during February. The plant species was identified and authenticated by duly by a certified botanist and a voucher specimen of the identified plant species was deposited in the library for future reference.

Microscopical studies
The plant specimens for the proposed to study were collected. They were fixed with formalin and were processed properly to get casted into paraffin dyes (Johansen, 1940). Thin sections were cut using rotary microtome and stained using reagents (O'Brienetalet al., 1964). They were mounted on a microscope for observing the plant parts. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. Descriptive terms of the anatomical features are given as in the standard anatomy books (Esau, 1964).

Quantitative microscopy
Quantitative microscopy of the transverse sections and bark powder were performed to determine the size and dimensions of tissues, cells and cell contents (WC Evans, 2009).

Physico chemical constants
The plant parts were dried at room temperature for 5 days, properly ground and the powder is passed through sieve no. 80. It was evalauted for physical constants like moisture content, ash values, extractive values (Kokate et al., 1995) and flourescent analysis (Kokoshi et al., 1958). The total fibre content, Swelling index and foaming index of the bark were determined (WC Evans, 2009). The heavy metal content was estiamted in standard procedure as described by Raina et al. (Raina et al., 2015)

Phytochemical studies
Extraction
The plant material (1.5 kg bark) was air-dried under shade, coarsely powdered (Sieve no. 40) and defatted with petroleum ether (60-80 ºC) using Soxhlet apparatus by successive solvent extraction method with chloroform, Ethyl acetate, Ethanol and Aqueous Ethanol (lin et al., 2016, Avinash et al., 2012).The extracted sample was evaporated to dryness using rotary vacuum evaporator. The final yield of the extract was calculated per dry weight of powdered bark.

Priliminary phyto chemical screening
The dried extracts were tested for alkaloids, carbohydrates, glycosides, phenols, steroids, flavoniods, gums and mucilages, proteins, volatile oils, fixed oils and fats and saponins (Harbone, 1998; IP, 1966).

Fluorescence analysis of extracts
The petroleum ether, chloroform, ethyl acetate, ethanol andhydroethanol extracts of bark of *Olaxscandens* were subjected to fluorescence analysis in short UV (254 nm), long UV (365 nm) and visible light.

Ether soluble extractive
The fixed oils were extracted from powder using the continuous Soxhlet extraction technique with petroleum ether for 3 h. The extracts were filtered and concentrated under reduced pressure at 40°C (Messaoud&Boussaid, 2011).

Total volatile oil content
The essential oils were isolated from 100 g of powder by hydro distillation for 3 h, using a Clevenger-type apparatus, extracted with ethanol, and dried (anhydrous Na_2SO4) according to the Standard method (Prakash et al., 2006)

Determination of Total Phenol Content

For the extraction of the phenolic component, the fat-free sample was boiled with 50 ml of ether for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for color development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths (Edoga et al., 2005).

In vitro antioxidant activity

The in vitro antioxidant activity of the Ethanol extract was carried out using the following three assay methods in accordance with previously reported procedures with minor modifications (Li et al., 2017).

Superoxide radical scavenging activity

The reaction mixture containing phosphate buffer (0.5 mL, 100mM, pH 7.4), 1.0 mL of NADH (0.4mM), 1.0 mL of NBT (0.156mM), 0.1 mL of PMS (0.06mM) and 3 mL of the TAY/standard drugs (Quercetin& Gallic acid)of various concentrations (10- 50 µg/mL, in 90% ethanol). After incubation at 25 ºC for 1 h, the absorbance of the reaction mixture was measured at 560 nm against an appropriate blank to determine the quantity of formazan formed (More &Makola, 2020).

Hydroxyl radical scavenging activity

The reaction mixture containing 0.1 mL of 2-deoxy-2- ribose (10mM), 0.33mL of phosphate buffer (50mM, pH 7.4), 0.1 mL of FeCl3 (0.1 mM), 0.1 mlethylenediamine tetra-acetic acid (EDTA) (0.1mM), 0.1 mL of H_2O_2 (mM), 0.1 mL of ascorbic acid (1mM) and 1.0 mL of various concentrations (5-50 µg/mL) of the TAY/ standards (Quercetin& Gallic acid).After incubation for 45 min at 37 ºC, 1.0 mL of 2.8% (v/v) TCA, and 1.0 mL of [thiobarbituric acid, TBA, 0.5% (v/v) in 0.025 mol/L NaOH solution containing 0.2% (w/v) of butylated hydroxyl anisole, BHA] were added in the reaction mixture, and the mixture was incubated at 95ºC for 15 min to develop the pink chromogen. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution (Sabir et al., 2020).

Lipid peroxidation scavenging activity

Reaction mixture (0.5 mL) containing rat liver homogenate (0.1 mL, 25% w/v) in Tris-HCl buffer (40 mM, pH 7.0), KCl (30 mM), FeCl3 (0.16 mM) and ascorbic acid (0.06 mM) was incubated for 1 h at 37 ºC in the presence and absence of the isolated flavonoid /standard drugs at various concentrations (50- 250 µg/mL). The lipid peroxide formed was measured by TBARS formation (Karthik et al., 2019). For this incubation mixture 0.4 mL was treated with sodium dodecyl sulphate (8.1%, 0.2 mL), TBA (0.8%, 1.5 mL) and acetic acid (20%, 1.5 mL, pH 3.5). The total volume was then made upto 4.0 mL by adding distilled water and kept in a water bath at 100 ºC for 1 h. After cooling, 1 mL of distilled water and 5.0 mL of a mixture of n-butanol and pyridine (10:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The butanol-pyridine layer was removed and its absorbance at 532 nm was measured to quantify TBARS.

In all the three above methods, the %inhibition of scavenging activity was calculated using the following equation.

$$Percent\ inhibition\ (\%) = (A_{control} - A_{test})/A_{control} \times 100 \quad (1)$$

Where, Acontrol is the absorbance of the control and Atestrepresents the absorbance of a test substance (Ethanol Extract / standard drug).

Statistical analysis

Tests were performedin triplicate and valueswere obtained as mean±SEM of three independent studies. Quercetinwas used as standard drug.

RESULTS

Anatomy of the bark

The bark is black colour and shows thick ridges and furrow. The ridges are semicircular thick bodies with multi compound periderm (fig 1.1). These are several tangenital their periderm bands alternating

with either cortical tissue or secondary phloem which is compressed and crushed alternating with the periderm. These are gelatinous type, sclerenchyma layers; each periderm layer consists of outer layers of phellem and inner layer of phelloderm. The phellem cells are suberised dead cells and phellem cylinder is protective function. The phelloderm cells are living cells with cellulose walls. The phelloderm is living tissue (fig 1.2). The tangenital bands periderm is upto 4 or more layers (fig. 2). Each cylinder periderm are dark and reddish in colour. In the earliest periderm zones are very thick and highly wavy and cleaved. In the secondary phloem region, these are numerous horizontal wide thick blocks gelatinous fibres ; in between the fire block are seen crushed and collapsed phloem tissues.

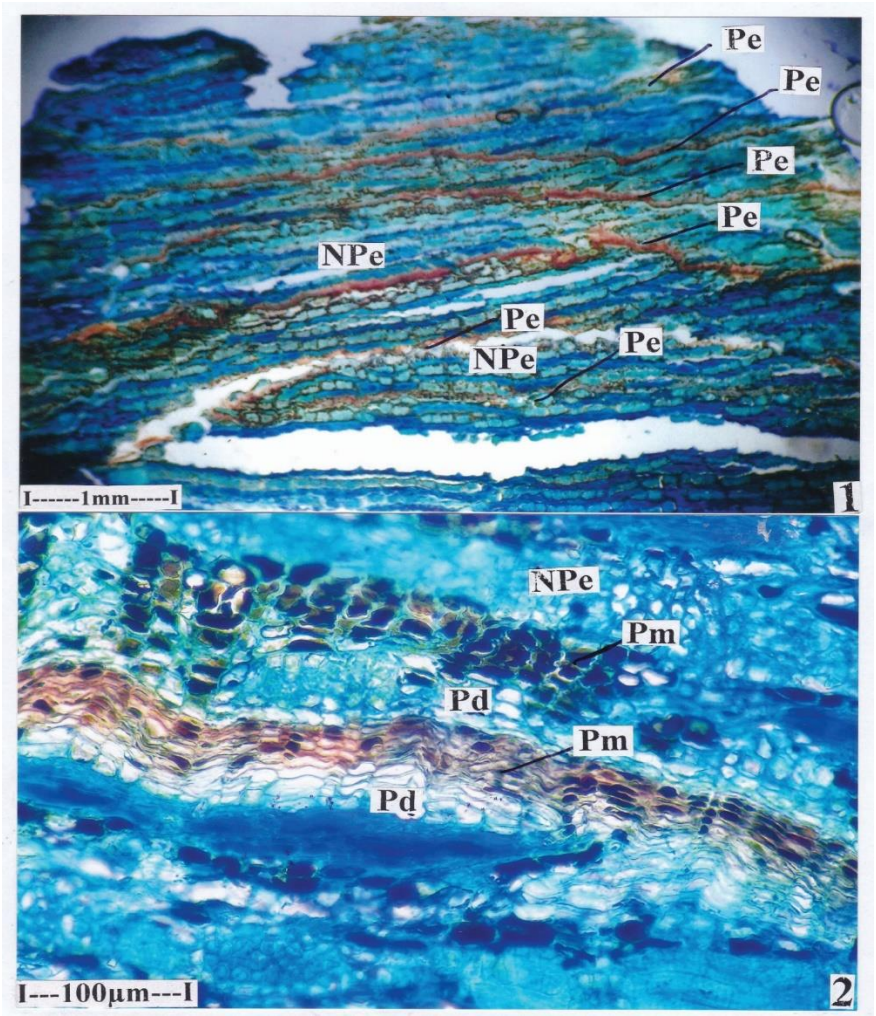


Fig 1:T.S. of olax bark; 1.1. T.S of Bark showing entire periderm and non-periderm tissues; 1.2. A single periderm cylindrical enlarged.
NPe- Non-Periderm Tissue Pd - Phelloderm, Pm - Phellem, Pe – Periderm

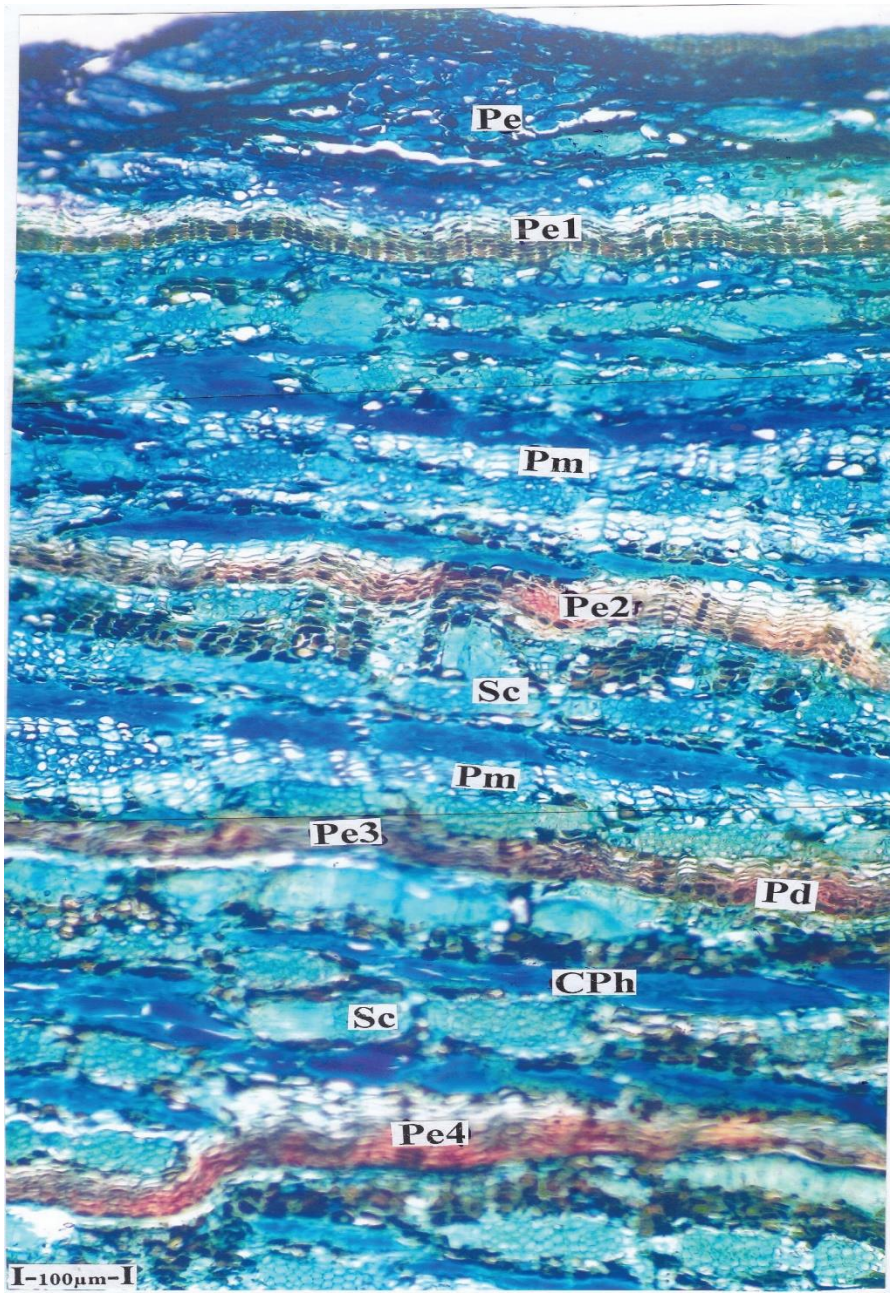


Fig 2 :- T.S of Bark showing successive cylinders of periderm called rhydidome. Pe1 - Pe 4: Successive cylinders of Periderm, Cph: Collapsed Phloem, Sc: Sclerides, Pe: Periderm, Pm: phellem, Pd: phelloderm.

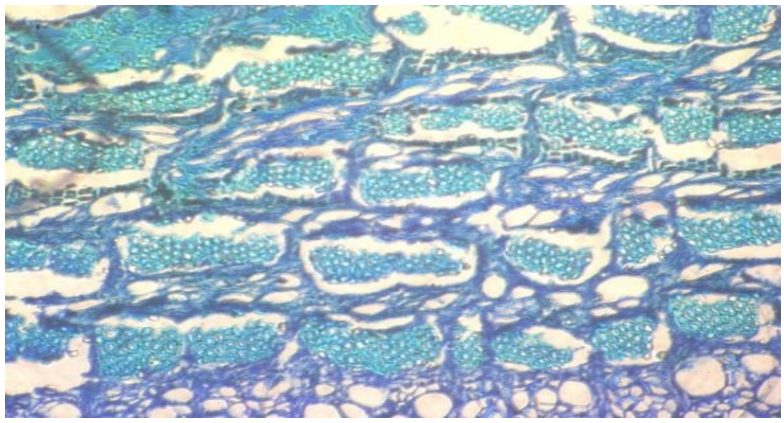


Fig 3 :- T.S of Bark showing successive cylinders of phelloderm and sclerides.

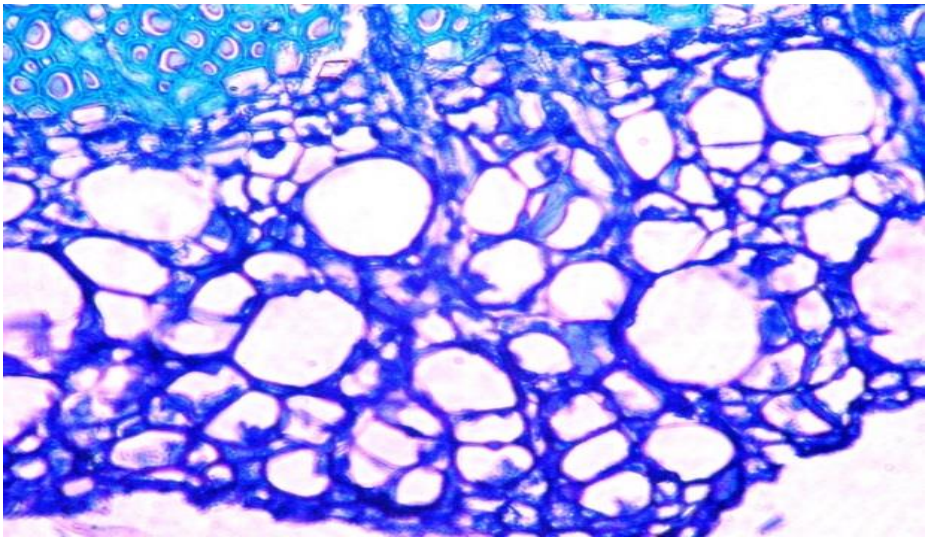


Fig:4:- TS of collapsed phloem with successive cylinders as sclereids blocks

Secondary phloem

Secondary phloem constitutes the inner part of the bark. It includes outer much thicker part while the inner part. The outer wider part consists of collateral phloem, dilated parenchyma and phloem rays. Collapsed phloem is formed by crushing phloem by diluted parenchyma cells and phloem rays in addition outer and greater of the bark and xylem tissue. The collapsed appears as dark, irregular and branched lines. The non-collapsed phloem has infact sieve elements and companion cells. Phloem parenchyma cells are not much dilated. The sieve elements are polygonal in outline, thin walled and have small companion cells attached along corner of the sieve elements (5.1, 5.2).

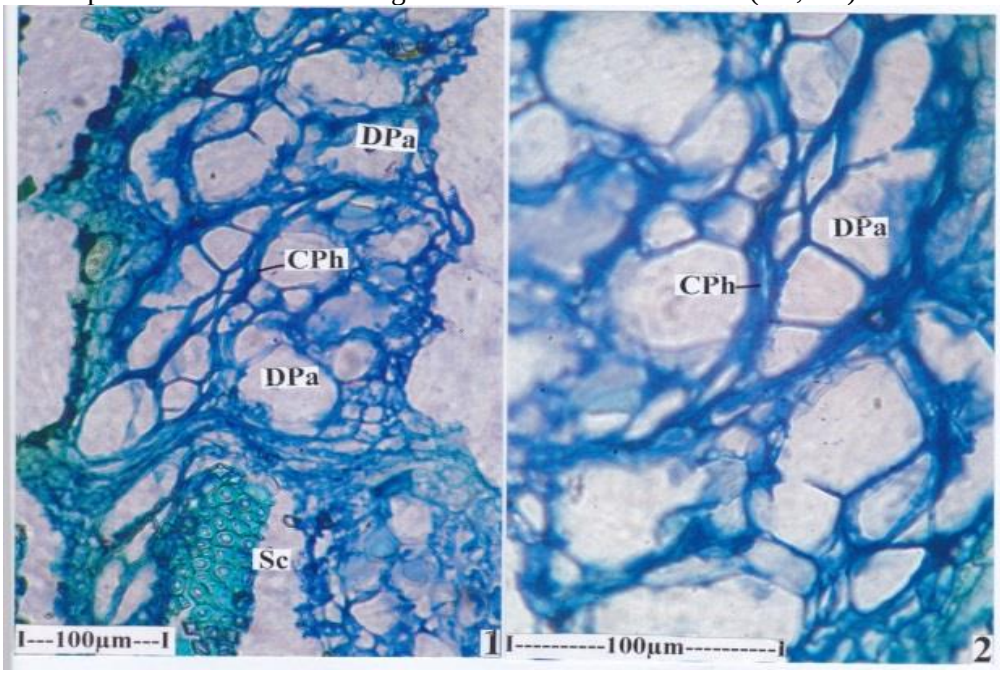


Fig:5: 1 and 2:-Ts of bark showing collapsed sieve elements and dilated parenchyma
Cph-collapsed phloem; Dpa---dilated parenchyma, sc- sclereids

Non-collapsed phloem

Internal collapsed phloem occurs a normal zone of intact non collapsed phloem (fig. 6.1, 6.2). This tissue is next outside the secondary xylem and vascular combination. The non-collapsed phloem consists of thicker walled polygonal sieve elements associated with companion cells which are small and occurs physiologically linked with sieve elements and undilated parenchyma cells (fig.6.2). The sieve elements are 30 micro grams wide and companion cells are 10 micro grams wide.

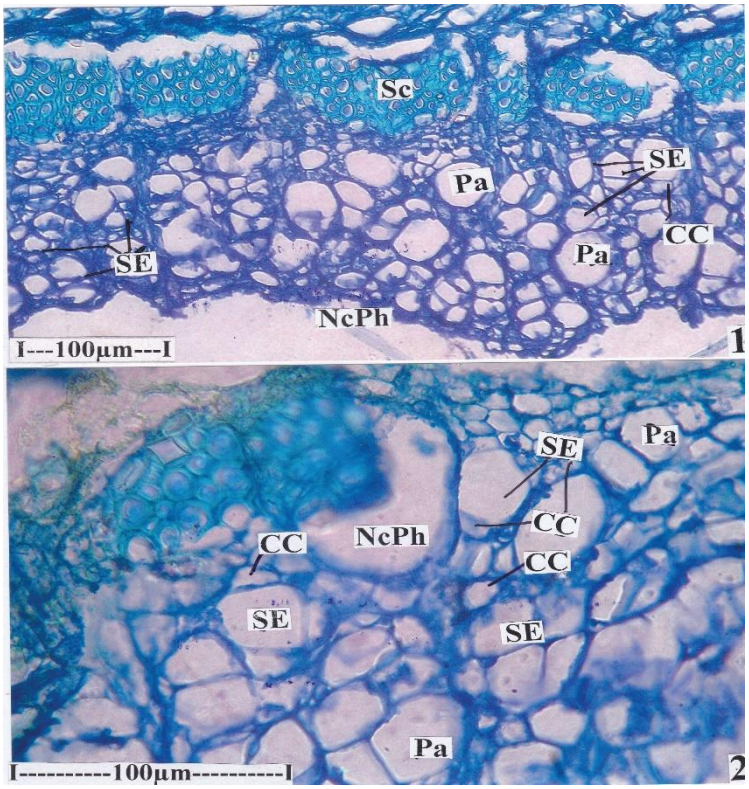


Fig:6: 1, 2:-Ts of Bark showing non-collapsed phloem tissue and sclereids block.
Cc-companion cell; Ncph-non-collapsed phloem: se-sieve element, pa-parenchyma, sc- sclereids

Tangenital longitudinal section (TLS) view of phloem

In TLS view, phloem shows phloem rays, phloem fibres (fig. 7, 8) and phloem parenchyma (fig. 9.2). Sometimes, sieve elements are visible with end wall sieve plate (fig. 9.1). The phloem rays mostly multi seriate very high, non-storied and hetero cellular. The rays have two types of cells: the cells in the middle part of the rays are polygonal and squarish, not elongated. These cells are called procumbent cells. The cells at upper and lower ends of the rays are vertically elongated and conical in shape; these are called upright cells (fig. 8.2). The seriatives of the rays is invariable: they are mostly multi seriate having many verticle rows of cells (fig. 7,8,9). These are also biseriate, having two vertical files of cells,(fig. 7.2) and single vertical rows of cells called uniseriate rays (fig. 7.2). Phloem parenchyma cells are rectangular and vertically elongated; they occur in vertical strands (fig. 9.2). The sieve element is wide and thick walled having oblique sieve plates (fig. 9.1).

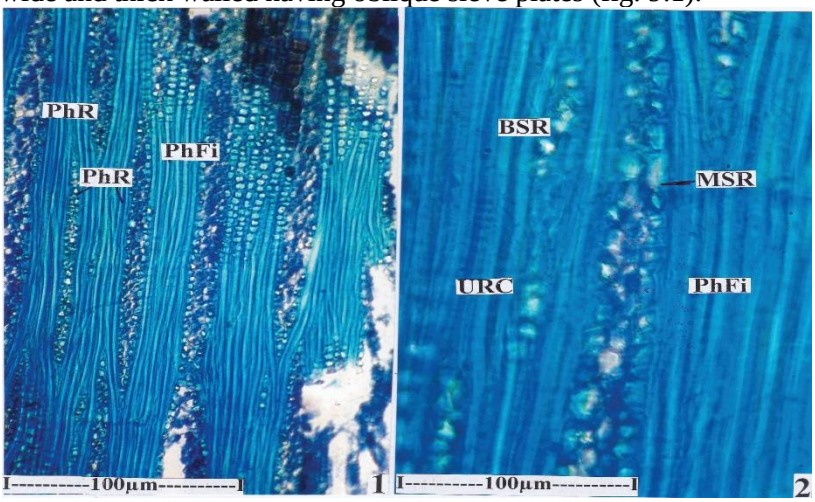


Fig:7: 1, 2;- tangential longitudinal section of phloem showing spindle shaped phloem rays and compact vertical lines of phloem fibres.
Bsr-Biseriate Ray; MSR-multi-seriate ray; phfi—phloem fibres; phR-PhloemRay's.; Procumbent cell; urc—upright cell

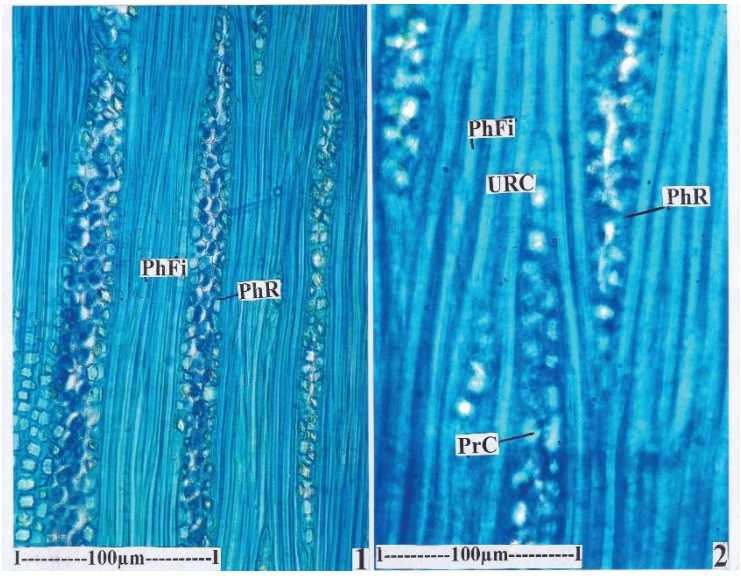


Fig:8: 1: TLS view of phloem Ray's. 2:--phloem Ray's showing Hetero cellular structure.
Phfi—phloem fibres; phR—phloem Ray's; prc-procumbent cell,.Urc-uprightcell

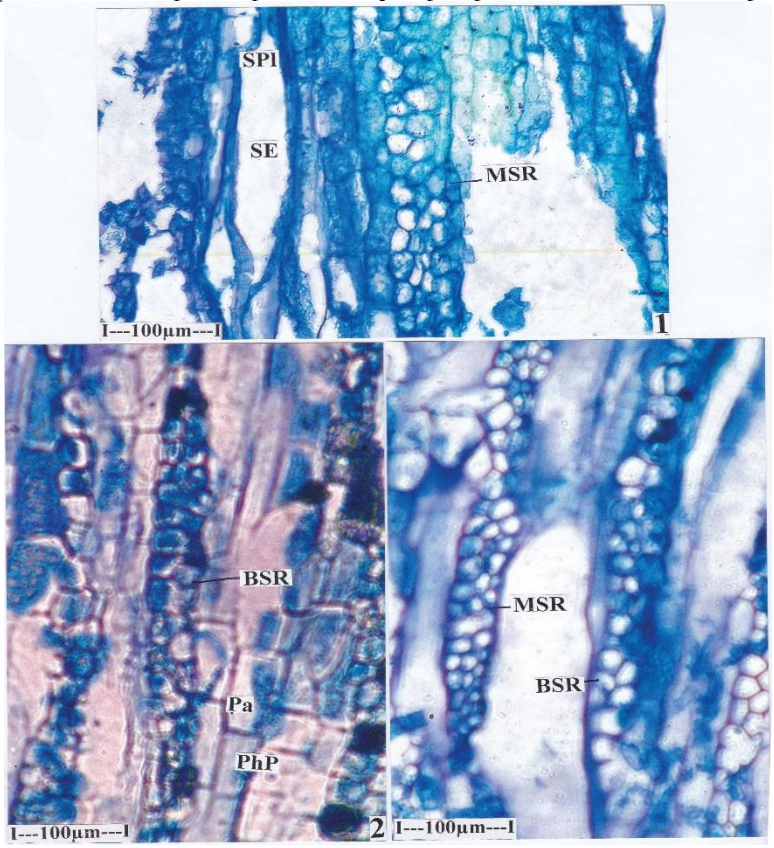
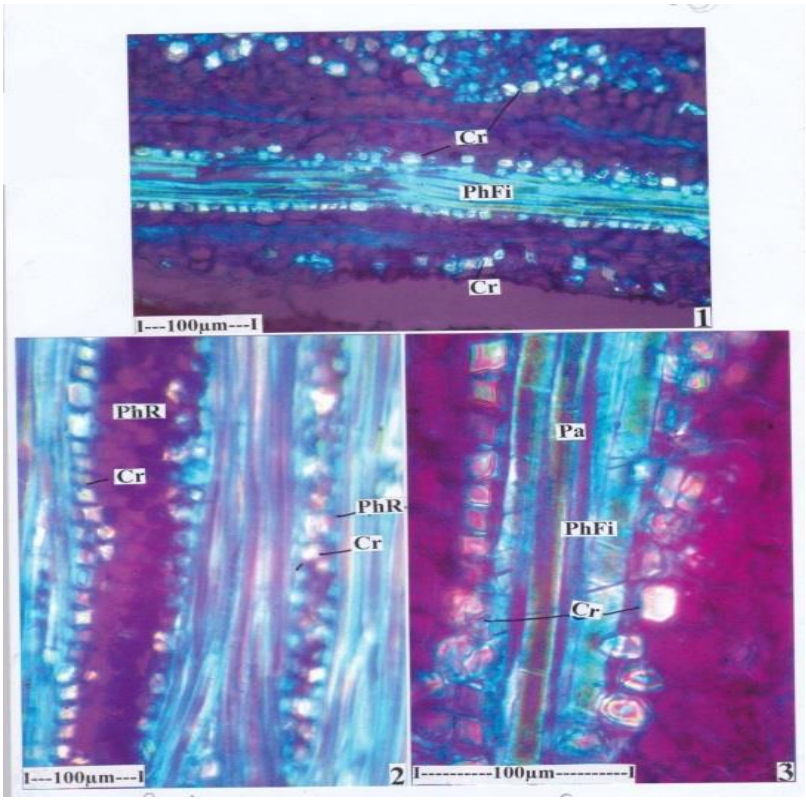


Fig:9: 1:--TLC view of phloem showing multi-seriate ray and sieve elements. 2;--Bi-seriate Ray and vertical strand of parenchyma 3:--multi seriate ray and Biseriateray.
Bsr-Bi-seriate Ray: MSR-multi-seriate ray; se-sieve elements, spl-sieve plates, paparenchyma

Crystals

Calcium oxalate crystals are abundant in the phloem. The crystals are exclusively prismatic type with polyhedral shape (fig. 10.1,2,3). The occur along the margin of the rays and fibres within the ray cells.



Fig;10: 1:-Distribution of prismatic calcium oxalate crystals on other side of the fibre 2:-distribution of crystals along lateral sides phloem ray 3:-crystals distributed along the phloem fibres and phloem parenchyma cells. cr-crystal; phFi-phloem fibres; phR-phloem ray

Radial Longitudinal Section(RLS) of Phloem Olax: Bark

In the view of RLS view phloem rays are seen in the horizontal position resembling the bricks of a wall. The cells are many layers comprising of many horizontal position. (fig.11.1, 11.2, 12). The cell wall of the rays is thin and some of the cells possess dark tannin. The rays cell in the middle portion of the ray are square. Some of them are horizontally oriented. These cells are called as procumbent cells. They located at the upper and lower ends of the ray are vertically elongated and they are called upright cells. Due to the presence of two types of cells these rays are called as heterocellular rays.

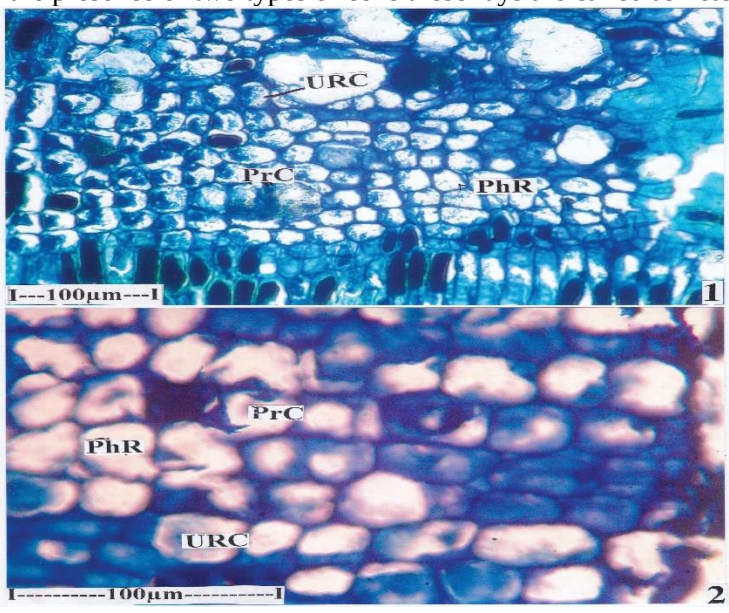


Fig:11: 1,.2:-RLS of phloem ray showing horizontal layer of cell with Central procumbent and operate cell. phR-phloem ray; PRC-procumbent cell; urc-upright

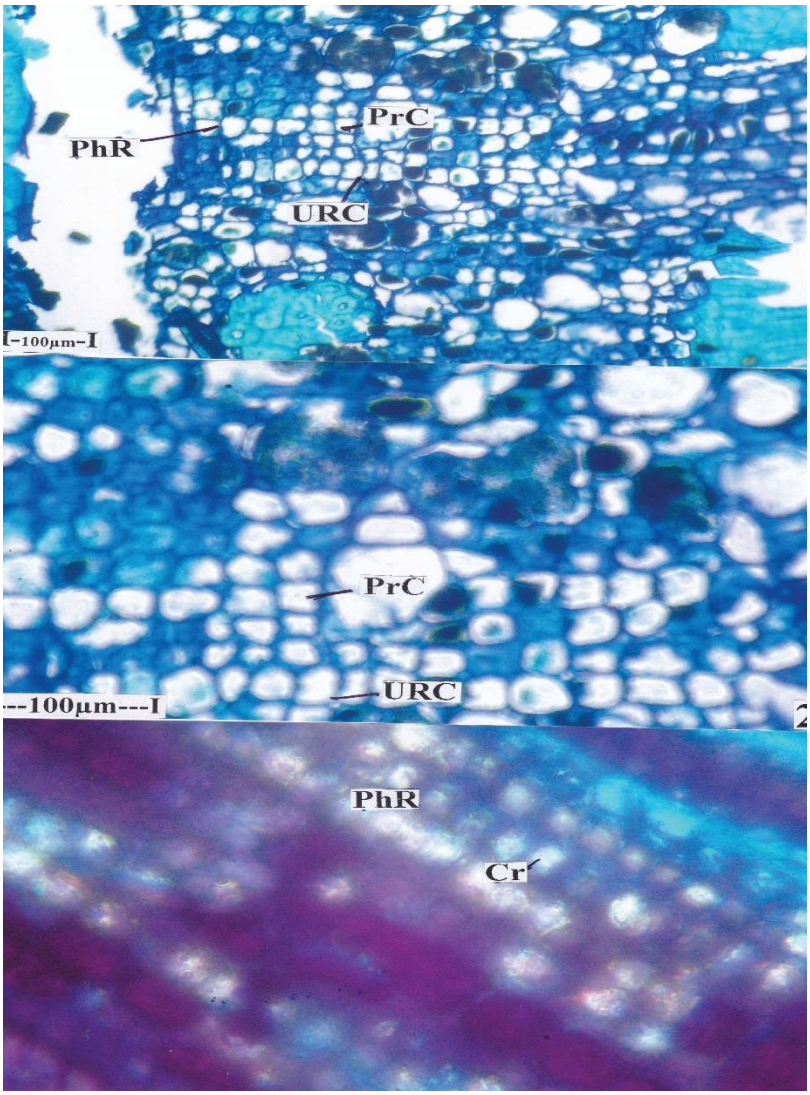


Fig:12: 1:-RLs view of phloem ray 2:-- RLS view showing procumbent and upright cell. 3:-- distribution of calcium oxalate crystals along the phloem Ray's
Cr-crystals, phR-phloem ray, prc-procumbent cell, urc-upright cell

Powder Microscopic Studies:

The powder preparation of the bark shows the following components as seen under the microscope. Fibres are common in the powder. These Fibres are long, thin cells with tapering ends (fig: 13,14). The narrow Fibres with lignified thick wells and narrow cell lumen (fig:13.1). The narrow Fibres are 1.1 µm long and 1 µm thick. A part from narrow Fibres, these are slightly wide Fibres (fig:13.2). The wide fibres are 500µm long and 15 µm thick. The wide fibres have the wells and so the wide fibres are short measuring 400 µm long and 30 µm width. Calcium Oxalate crystals are common in the powder (fig:15.1 and 2).They are prismatic type with polygonal shape (fig: 16.1 and 16.2). The crystals are seen scattered in the powder.

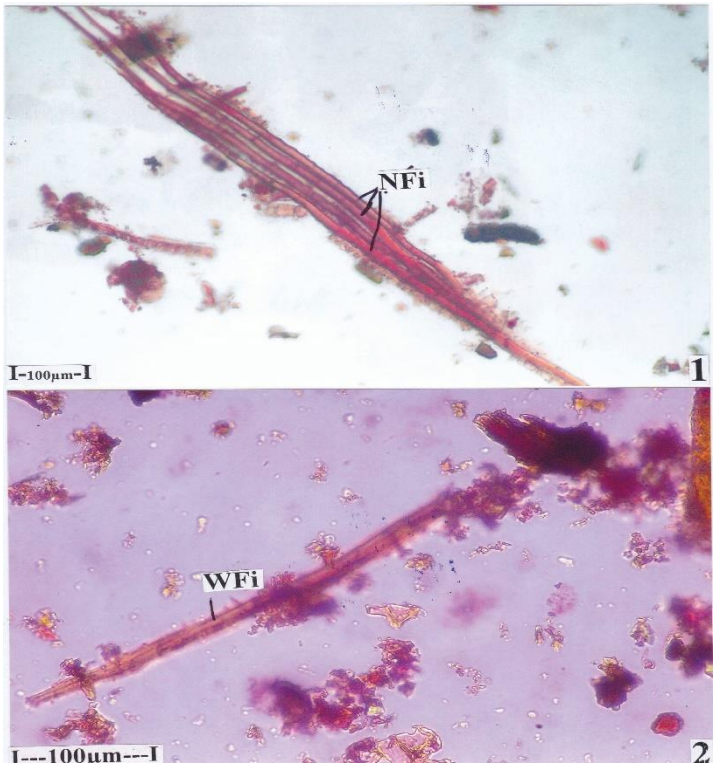


Fig:13: 1:-bark fibre as seen in Powder preparation of the bark (narrow fibres) 2:--single wild fibres. Nfi-narrow fibres; wfi-wild fibres

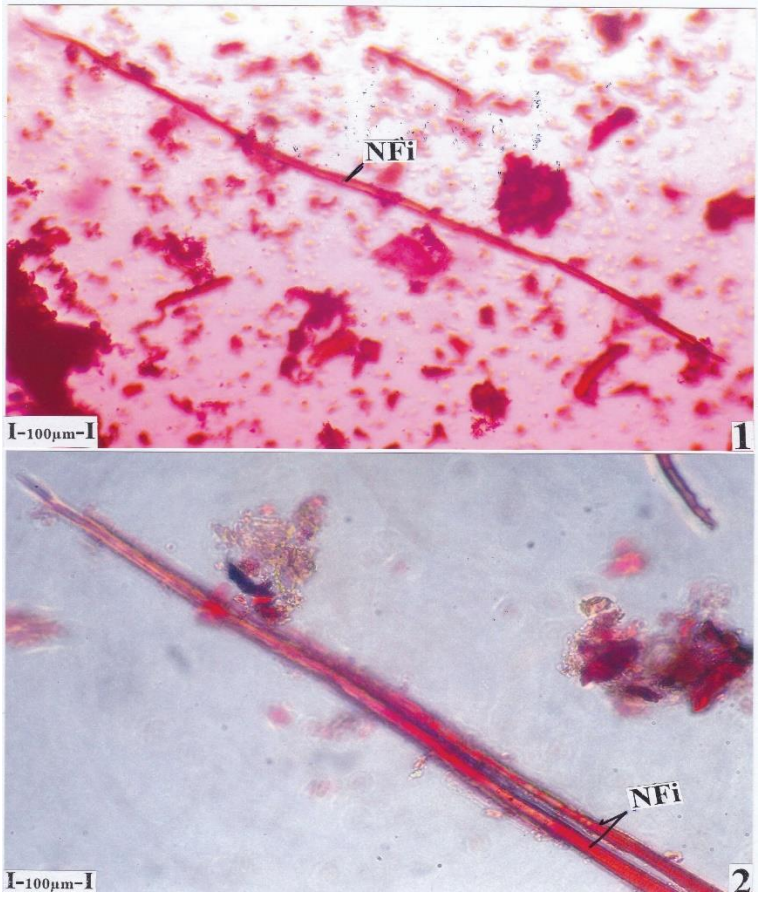


Fig14: 1: A single narrow fibre 2: A area as narrow fibres
Nf—nerve fibres

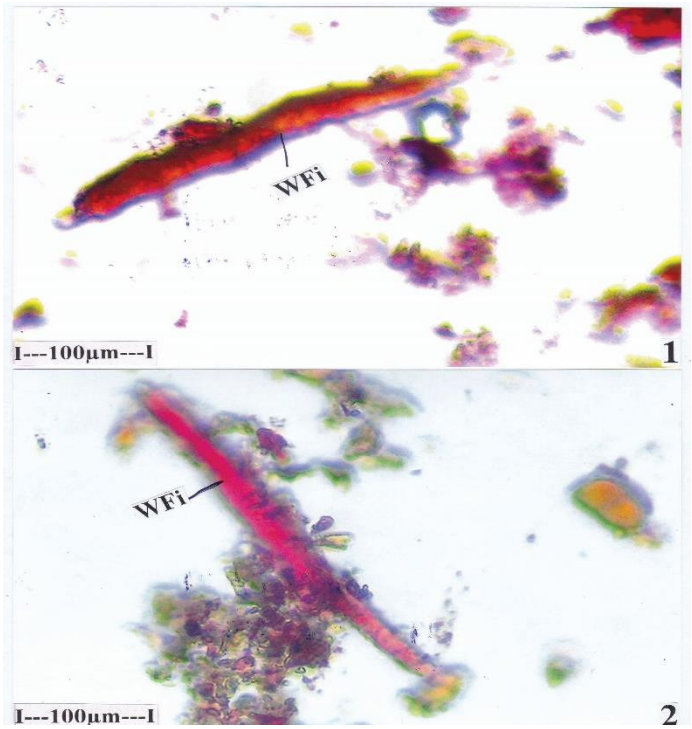


Fig:15.1&2:--short white fibres
wfi-wild fibres

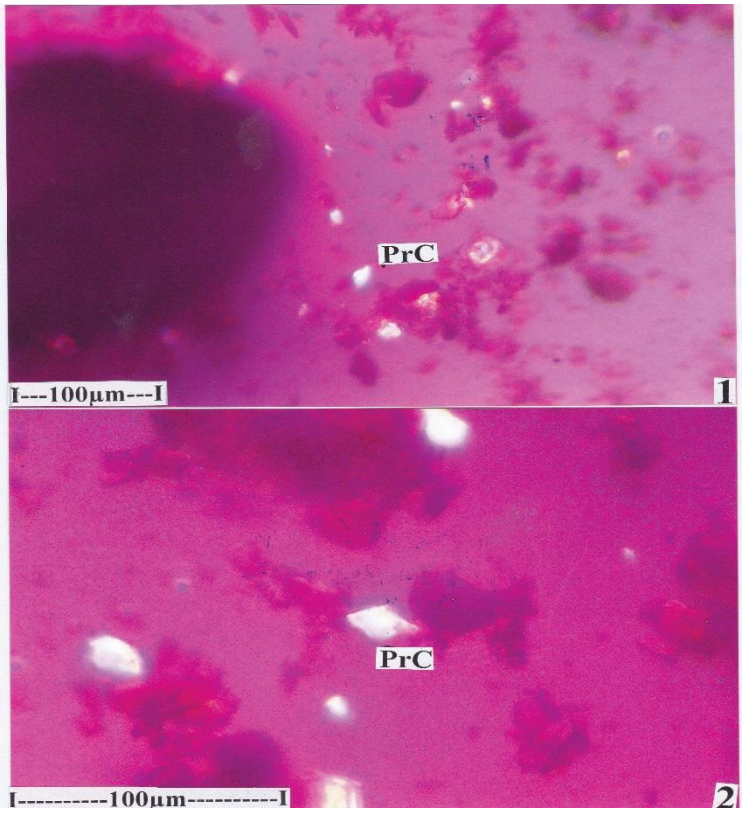


Fig16:1&2:--calcium oxalate prismatic crystals as seen in Bark powders
Prc-prismatic crystals

Quantitative Microscopy

The results of the Quantitative microscopy studies of different tissues are mandatory for the identification and separation of plant from the allied species of impurities belonging to other plants or earthy matter. So in this way the crude drugs can be distinguished from other plants by using the optical micrometric microscopy study.

Table 1: Quantitative Micrometric Microscopy of Bark of Olaxscandens

S. No.	Type of cell	Size (µm)
1	Pericyclic fibres	524.2±78.5
2	*Cork cells*	9.6±2.5
3	Calcium oxalate crystals	21.5±4.9
4	Phloem fibres	739.1±195.8 (length) 18.6±3.2 (diameter)
5	Companion cells	15.7±3.6
6	Sclerides	89.8±17.3
7	Phelloderm	91.2±10.5

Physicochemical constants

The Physicochemical constants of the bark powder like ash values, moisture content, Heavy metal content and crude fibre content were illustrated in table 2 and 3. The crude fibre content of powdered bark was found to be 15.7%w/w.The Swelling index of the bark of Olaxscandens was found to be 20.7% and foaming index was found to be 96.9%.

Table 2. Physicochemical parametres of the leaves of O*laxscandensRoxb*

S. No.	Parameters	Average % W/W
1.	**Ash values** a) *Total ash* *b) Acid insoluble ash* *c) Water soluble ash* *d) Sulphated ash*	 4.8±0.6 2.7±0.5 1.4±0.2 0.2±0.1
2.	**Moisture content** *Loss on drying*	10.3±2.3
3.	Total fiber content	15.7±4.8
4.	Swelling index	20.7±3.2
5	Foaming index	96.9±5.4

Values were expressed as Mean ±SD

Table 3. Heavy metal content in the crude drug powder

Heavy metal	Quantity ppm	Limits ppm (WHO, 2007)
Lead	2.5±0.6	10
Cadmium	BQL	0.3
Chromium	0.5±0.1	2
Nickel	1.3±0.7	ND
Arsenic	0.6±0.2	3
Mercury	BQL	1

Values were expressed as Mean ±SD

Table 4. Extract parameters of bark of O*laxscandensRoxb*

Solvent	Extractive value %w/w	Colour	Odour	Consistency
Pet. Ether soluble Extractive	7.65	Black green	Characteristic	greasy
Chloroform soluble extractive	0.74	Dark green	Characteristic	greasy
Ethyl acetate soluble Extractive	4.29	Black green	Characteristic	greasy
Ethanol soluble Extractive	22.46	Black brown	Characteristic	non greasy
Aq Ethanol soluble Extractive	19.08	Brown	Characteristic	sticky non greasy

Table 5. Extractive valuesof bark of O*laxscandensRoxb*with different solvents

Extraction solvent	Extractive value %
Alcohol soluble extractive value	26.6±3.9
Water soluble extractive value	27.1±5.1
Ether soluble non volatile extractive value	7.8±2.5
Volatile extractive value	0.6±0.3

The results of flourescent analysis and priliminary phytochemical screening was given in table 6 and 7. Priliminary phyto chemical screening revealed the presence of polyphenols, alkaloids, steroids, carbohydrates, proteins, gums and mucilages, saponins etc.

Table 6. Fluorescent analysis of bark powder of O*laxscandensRoxb*

Treatments	Observations		
	Day light	Long UV	Short UV
Powder as such	Green	Dark green	Dark green
Powder + 1N Sodium Hydroxide (aqueous)	Brownish green	Brown	Fluorescent green
Powder + 1N Sodium Hydroxide (Methanolic)	Green	Brown	Dark green
Powder + 1N Sulphuric acid	Green	Brownish green	Dark green
Powder + 1N Nitric acid	Orange red	Pale brown	Fluorescent green
Powder + 1N Hydrochloric acid	Green	Golden brown	light green
Powder + Acetic acid	Green	Deep orange	Fluorescent green
Powder + Iodine	Brownish green	Golden brown	Fluorescent dark green
Powder + Ferric chloride	Light green	Brown	Fluorescent dark green
Powder + Potassium hydroxide (methanolic)	Light green	Golden brown	Light green

Table 7. Preliminary phytochemical screening of various extracts of O*laxscandensRoxb*

Sl. No.	Test	Pet. Ether	Choloform	Benzene	Methanol	Water
1.	Carbohydrates	-	-	-	+	+
2.	Alkaloids	-	+	-	+	+
3.	Glycosides	-	-	-	+	+
4.	Tannins	-	-	-	+	+
5.	Steroids	+	-	-	-	-
6.	Triterpenoids	-	-	-	+	-
7.	Volatile oils	-	-	-	-	-
8.	Fats and fixed oils	-	-	-	-	-
9.	Flavanoids	-	-	-	+	+
10.	Polyphenols	-	-	-	+	+
11.	Saponins	-	-	-	+	+
12.	Aminoacids	-	-	-	+	+
13.	Gums and mucilages	-	-	-	-	+

"+" represents **Presence** **"-"** represents **Absence**

Total phenolic content

The total phenolic content in the ethanolic and aq ethanol extracts of the leaves of Olaxscandens was estimated by Quercetin equivalents. The total phenolic content was found to be 172 and 155mg quercetin equivalents per g weight of extract respectively. The phenols present in the extract might counter act various disorders that arise due to the oxidative free radicals.

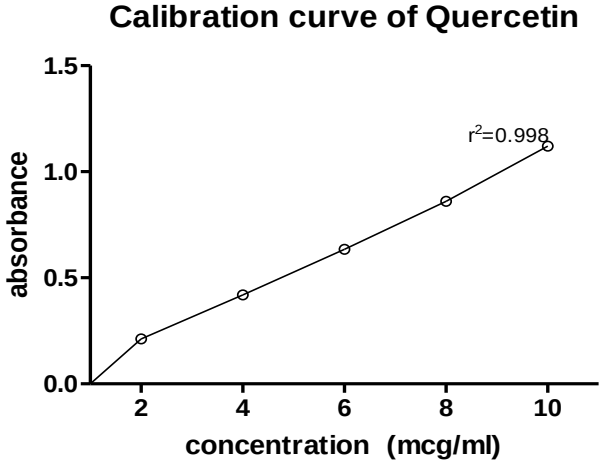


Fig 17: Standard curve of Quercetin

In vitro antioxidant activity

Superoxide radical scavenging activity

Ethanol extract showed superoxide radical scavenging activity in a concentration dependent manner.At the concentration of 50 µg/mL, Ethanol extract exhibited 93.34±0.43% of scavenging activity, while, quercetinshowed 93.85 ± 0.36% of scavenging activity at the same concentration. The percent inhibitory activity of Ethanol extract was found significant (p<0.05) as compared to the standard drug, quercetin.

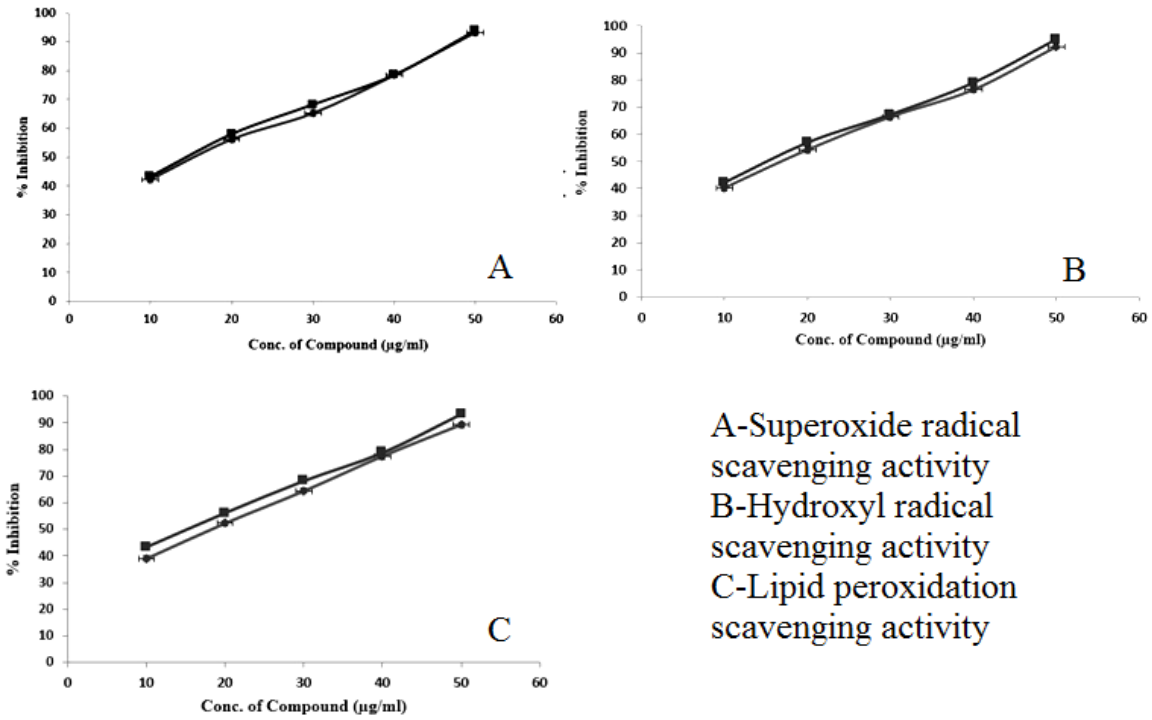


Fig 18–Invitro antioxidant activity of Ethanol extract of O*laxscandensRoxb*.
Values are mean ± SEM of three replicate experiments. %scavenging activity of Ethanol extract statistically significant at $p<0.05$, compared to Quercetin (standard).

Hydroxyl radical scavenging activity

The percentinhibition of hydroxyl radicals was92.34±0.43%for Ethanol extract at the concentration of 50µg/mL.The inhibitory activity of Ethanol extract was comparable with that of the standard drug, Quercetin (94.85 ± 0.32%) at the same concentration. The percent scavenging activity of Ethanol extract was statistically significant ($p<0.05$) as compared to Quercetin (Fig 18).

Lipid peroxidation scavenging activity

LP induced by Fe2+/ascorbate in rat liver homogenate was found to be inhibited by Ethanol extract in a concentration dependant manner and a considerable amount of lipid peroxidation inhibitory effect was observed by 89.19±0.22%, while, Quercetin inhibited by 93.48±0.35%, at the concentration of 50 µg/mL (Fig 18). Test results were considered statistically significant ($p<0.05$) as compared to the standard drug, Quercetin.

CONCLUSION

Microscopy studies revealed the presence of cork, phelloderm, phloem fibres, xylem tissue, and calcium oxalate crystals. Heavy metal contents are under acceptable limits. Phytochemical evaluation suggested the crude drug contains phenols, flavonoids, glycosides, alkaloids etc. Total phenol content was determined and the ethanol extract contained 172mg of quercetin equivalents of total phenols. Invitro antioxidant activity proved that the ethanol extract was comparatively similar in inhibiting the free radicals generated due to superoxides, hydroxyl and peroxidation.

REFERENCES

1. Ahmed, Salman &Hasan, Mohtasheem. (2015). Standardization of crude drugs: A precise review. World Journal of Pharmaceutical Research. 4. 155-174.
2. Anonymous. The Indian pharmacopoeia;2nd ed. New Delhi, India: Govt. of India publication; 1966. pp. 33–4.
3. Avinash Kumar Reddy, G., TrilokMitra, M., Shilpa, T., Shabnam, S., SatishBabu, K., Jyothi M Joy. 2012. Variation of Phenols, Flavonoids and Antioxidant Potential in Various Parts of

4. Foeniculumvulgare on Drying. International Journal of Chemical and Pharmaceutical Sciences. 3(1):74-79.
5. C.K. Kokate, K.R. Khandelwal, A.P. Pawar, S.B. Gokhale, Practical Pharmacognosy, NiraliPrakashan, 1, 1995, 11 – 19.
6. Chase CR, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of Identification. J American Pharmaceutical Sciences. 1969;38:324.
7. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. 2nd ed. Delhi, India: Council of Scientific & Industrial Research; 1956. p. 173.
8. Easu, K. 1964. Plant Anatomy John Wiley and sons. New York. Pp.767. Easu, K.1979. Anatomy of seed Plants.John Wiley and sons. New York. Pp. 550.
9. Edeoga OH, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005;4:685–8.
10. G.J. Kokoshi, J.R. Kokoshi, F.J. Sharma, Fluorescence of powdered vegetable drugs under ultra violet radiation, J. Amer. Pharm. Assn 38(10) (1958), pp. 715-717.
11. https://doi.org/10.1038/s41598-020-63491-w
12. J.B. Harborne, Methods of Extraction and Isolation, in: Phytochemical methods, third ed., Chapman and Hall, London, 1998, pp. 60-66.
13. Johansen D.A. 1940 Plant MicrotechniqueMcGraw Hill Book Co; New York Pp.523.
14. Lin, L. J., Huang, X. B., &Lv, Z. C. (2016).Isolation and identification of flavonoids components from Pterisvittata L. SpringerPlus, 5(1), 1649.https://doi.org/10.1186/s40064-016-3308-9
15. Maurice D. Awouafack, Pierre Tane and Hiroyuki Morita (August 23rd 2017). Isolation and Structure Characterization of Flavonoids, Flavonoids - From Biosynthesis to Human Health, Goncalo C. Justino, IntechOpen, DOI: 10.5772/67881. Available from: https://www.intechopen.com/chapters/54524
16. Messaoud C, Boussaid M. Myrtuscommunis berry color morphs: A comparative analysis of essential oils, fatty acids, phenolic compounds, and antioxidant activities. ChemBiodivers. 2011;8:300–10.
17. More, G.K., Makola, R.T. In-vitro analysis of free radical scavenging activities and suppression of LPS-induced ROS production in macrophage cells by Solanumsisymbriifolium extracts. Sci Rep 10, 6493 (2020).
18. O'Brien, P.Feder N. and Mc Cull M.E. 1964. Polychromatic staining of plant cell walls by toulidine blue-O protoplasma; 59:364-373.
19. Prakash O, Kasana VK, Pant AK, Zafar A, Hore SK, Mathela CS. Phytochemical composition of essential oil from seeds of Zingiberroseum Rosc. and its antispasmodic activity in rat duodenum. Ethnopharmacol. 2006;106:344–7.
20. Rania Dghaim, Safa Al Khatib, HusnaRasool, Munawwar Ali Khan, "Determination of Heavy Metals Concentration in Traditional Herbs Commonly Consumed in the United Arab Emirates", Journal of Environmental and Public Health, 2015, 6 pages, 2015. https://doi.org/10.1155/2015/973878
21. Sabir, S.M., Abbas, S.R., Shahida, S. In-Vitro antioxidant, anti-lipid peroxidative activities and In-Silico study of Terminaliachebula bioactive compounds. ClinPhytosci 6, 83 (2020).https://doi.org/10.1186/s40816-020-00233-5
22. V. P. Karthik, Punnagai, Parepalli Suresh, Darling Chellathai David. In Vitro Hydrogen Peroxide Scavenging Activity and Alpha Amylase Inhibitory Activity of Croton tiglium extract. Research J. Pharm. and Tech. 2019; 12(6):3045-3047. doi: 10.5958/0974-360X.2019.00515.8
23. WC Evans. Pharmacognosy, 16th edition, London, W.B. Saunders Company Ltd., 2009; pp. 541-570
24. World Health Organization. (2000). World Health Organization-WHO.
25. Ya Li, Sha Li, Sheng-Jun Lin, Jiao-Jiao Zhang, Cai-Ning Zhao, Hua-Bin Li. 2017. Microwave-Assisted Extraction of Natural Antioxidants from the Exotic Gordoniaaxillaris Fruit: Optimization and Identification of Phenolic Compounds. Molecules. 22(1481):1-16. DOI: 10.3390/molecules22091481