Research Article

PHARMACOGNOSTIC EVALUATION OF STEM AND ROOT OF *SALACIA OBLONGA* WALL

Anuradha Upadhye*, Lourelle Dias, GP. Pathak and Mandar N. Datar

Biodiversity and Palaeobiology group, Agharkar Research Institute, Pune - 411 004, Maharashtra, India.

ABSTRACT

In Traditional Indian Medicine three species of genus *Salacia* viz. *S. oblonga* Wall., *S. chinensis* L. and *S. reticulata* Wight are known as 'Saptarangi'. There is no detailed pharmacgonostic profile of *S. oblonga;* hence study of its stem and root was carried out. Macroscopic characters were documented. Microscopically stem and root showed distinct stone cells and red colouring matter. Total and water soluble ash were higher in root (1.57±0.012) while stem showed higher acid insoluble ash (0.26±0.03). Methanolic extract was higher in root (0.092) and stem (0.057) than other solvents. Primary metabolites: stem showed higher total carbohydrates (71.381±5.1) while root showed higher total proteins (10.561±0.5). Secondary metabolites were higher in root (Polyphenols - 0.79±0.04, Tannins - 0.12±0.0005). Antioxidant activity was observed to be more in the root which could be due its higher concentration of polyphenols. These developed parameters may prove significant in identification of the crude drug 'Saptarangi'.

Key Words: *Salacia oblonga*, Pharmacognosy, Proximate and phytochemical analysis, Antioxidant activity.

INTRODUCTION

Genus *Salacia* L. belongs to Family Celastraceae with approximately 145 species distributed worldwide of which 16 species are found in India (Mabberley,D.J. 2008; https://indiabiodiversity.org; http://www.plantsoftheworldonline.org). It is a climbing shrub, globally distributed in Indian peninsula, Thailand, and the Philippines. In India, it is mainly distributed along Western Ghats of Maharashtra, Goa, Karnataka, Kerala, coastal region and semi-evergreen patches of Southern Western Ghats (Paarakh, *et al*. 2008).Out of the known species of *Salacia* observed in India *S. chinensis* L., *S. oblonga* Wall. and *S. reticulata* Wight. Are widely used in Ayurvedic System of Traditional Indian Medicine under the accepted name 'Saptarangi' (Deokate, *et al*.2012; Musini, *et al*. 2015; Tandon, *et al*. 2012). Extensive work has been done on pharmacognostic studies of *S. chinensis* L. and *S. reticulata* Wight. In crude drug market mixture of root and stem, pieces are sold (personal observation) which makes the establishment of the identity, a difficult task. *S. oblonga* has been extensively used against a wide array of disorders, Type-2 diabetes and obesity (Chawla, *et al*. 2013), haemorrhoids, wound healing capacity, leucorrhoea, leprosy, skin diseases, hyper-hydrolysis, hepatopathy (https://indiabiodiversity.org). It is also used as anti-microbial, anti-oxidative, anti-inflammatory, nephroprotective, anti-mutagenic (Giron, et al. 2009).

Though *S. oblonga* is a proven resource of medicine, information on its pharmacognostic standardization is meagre. It is a climbing shrub (Fig.1) with lenticellate branches and several elliptic-oblong leaves, flowers axillary, greenish-yellow, fruits orange in colour, globose or somewhat pyriform, seeds embedded in the pulp (Singh, *et al*. 2001). In the crude drug market stem and or root pieces are sold as drug and available morphology-based taxonomic characters fail to prove the exact identity of the crude drug.

Hence in the present study stem and root of *S. oblonga* were studied using pharmacognostic parameters. Macroscopic and microscopic parameters will be helpful in identification of the crude drug. The proximate analysis will be useful for selection of quality material. The study will be helpful to crude drug users such as

pharmaceuticals and traditional medico-practitioners.

MATERIALS AND METHODS

Plant collection and authentication

The stem and root samples of *S. oblonga* Wall. were collected from Amboli, Western Ghats of Maharashtra during September 2015 (Fig. 1). The crude drug was deposited at Agharkar Herbarium of Maharashtra Association (AHMA) repository as per standard and Voucher deposition numbers S/B-49, R-237 were assigned for future reference (Fig. 2 & Fig. 3).

Chemicals and instruments

All the reagents and chemicals of S. D. Fine (analytical grade) were used for the analysis. Compound microscope (Olympus CX 31) was used for microscopic studies. Photo-documentation was done using microscope camera using Toupview software. Muffle furnace (Tempo make) and Moisture balance (Sartorius MA 45) were used for proximate analysis. Spectrophotometer (Labindia Analytical. UV 3000) was used for quantitative assays.

Pharmacognostic studies

Macroscopic and organoleptic characters were studied and observations on shape, size, colour, texture, fracture were documented for the stem and root pieces as per standard quality control parameters. Microscopic observations were carried out on the hand sections preserved in 50% glycerol as per standard protocol (Mukherjee,P. 2002).

Proximate analysis

Moisture analysis of fresh stem and root samples was carried out using automated Sartorius moisture balance and expressed in % Loss. Ash analysis was performed to check the purity of the samples and values were expressed as % w/w. Extractive analysis was performed and values were expressed in percent w/w extractive. These dried extracts were used for further quantitative studies (Mukherjee,P. 2002).

Quantification of primary metabolites

Quantification of carbohydrates was done using Anthrone method and values were expressed as mg/ml. Quantification of proteins was done using Lowry's method and values were expressed as mg/ml (Sadasivam, *et al*. 1996).

Quantification of secondary metabolites

Quantification of total Polyphenols was done using Folin-Ciocalteu method and values were expressed as mg/ml. Quantification of total Tannins was done using Folin-Denis method and values were expressed as mg/ml (Sadasivam, *et al*. 1996).

Antioxidant activity

Antioxidant activity was screened using two universal tests i.e. DPPH and ABTS. Both the tests were carried out as per Shirwaikar A*. et al.*

RESULTS

Macroscopy

Macroscopic and Organoleptic characters were documented for both stem and root in Table 1.

Microscopy

Stem: The section is circular in outline. Outermost 4-7 layers of cork cells are rectangular in shape, thick-walled, continuous. Cortex is made up of 5-20 celled thin walled rectangular to polygonal parenchymatous cells. Most of these cells are filled with oval simple or 2-3 celled compound starch grains. In the cortex circular or oval thick walled lignified simple stone cells are randomly distributed. Phloem occurred in irregular patches and phloem fibres are with blunt ends (Fig. 4 & 5)

Root: The section is circular in outline. Outermost 2-3 layers of cork cells rectangular, thick walled and discontinuous. Cortex is made up of 10 celled rectangular to polygonal thin-walled parenchymatous cells. Cells filled with oval simple starch grains. The cortex consists of lignified simple stone cells uniformly distributed. Phloem a comparatively broad zone of irregular phloem patches, phloem fibres with blunt or pointed ends and highly thick-walled with a very narrow lumen (Fig. 6&7).

Proximate Analysis

Parameters such as moisture content, total ash, acid insoluble ash, water-soluble ash and extractive values (petroleum ether, chloroform, acetone, methanol and water extracts) were determined. The results are presented in Table 2.

Quantitative studies

Quantitative analysis of primary (total carbohydrates, total proteins, and total fats) and secondary (polyphenols and tannins)

metabolites was carried out. The results presented in Table 3.

Antioxidant analysis

In DPPH and ABTS assays, the root showed higher antioxidant activity than that of the stem; but lesser than that of the standard (Ascorbic acid and Trolox).The results presented in Table 4.

DISCUSSION

Botanical drugs have characteristic features which have been used to establish the identity, quality and purity of a concerned drug which can be carried out using various pharmacognostic studies and phytochemical screening methods (Chenthurpandy, *et al*. 2009). Macro, micro, and physiochemical standards are considered as the identifying parameters to standardize and authenticate the drug. Plants rich in secondary metabolites like polyphenols, tannins, alkaloids, etc. are responsible for defensive properties (Arora, *et al*. 2017). Microscopy is one of the significant tools which help to differentiate genuine drug from its contaminant. In the stem and root of *S. oblonga* presence of stone cells, red colouring matter and arrangement of phloem cells are main distinguishing features (Fig. 5&7).Total ash and water extract values were observed higher in root indicating the presence of some water-soluble inorganic matter. Methanolic extract of root showed higher value this corresponds to the higher content of phytoconstituents. Secondary metabolites were higher in the root, this proved its high biological activity and hence confirms its utility as a crude drug.

Antioxidants are those compounds that are known to prevent the oxidative destruction of essential metabolites such as lipids, proteins, and nucleic acid (Balik, *et al*. 2008). Most of the known naturally occurring antioxidants are derived from plants (Chua, *et al*. 2008). They are known for their therapeutic effects, multiple mechanisms of action and non-toxic nature (Padmanabhan, *et al*. 2012). It is known theory that plants which show the higher amount of polyphenols also show higher antioxidant activity (Chua, *et al*. 2008). The root of *S. oblonga* has higher antioxidant activity as compared to the stem which is in accordance with the results of total phenols and tannins.

CONCLUSION

The current study provides information regarding pharmacopoeial parameters such as identification, authentication, and purity of root and stem drug of *S. oblonga.* Further exploration of the detailed chemical constituents in *S. oblonga* may contribute in the development of new life-saving drugs.

ACKNOWLEDGEMENTS

The authors are grateful to Director, Agharkar Research Institute Pune for providing the facility to conduct the experimental work & Rajiv Gandhi Science &Technology Commission, Mumbai for funding.

Table 1: Macroscopic and Organoleptic characters of *S. oblonga* Wall (stem and root)

CHARACTERS	Stem (Fig. 2)	Root (Fig. 3)
Shape	Cylindrical	Cylindrical
Size	L: 23-34 cm W: 2-5 cm	L: 33-35 cm W: 7-9 cm
Inner surface	Pale yellow coloured, distinct cambium rings observed, fracture hard splintery	Pale yellow to pink coloured, longitudinally striated, fracture hard
Outer surface	Ash grey coloured, with scattered moss or lichen patches, randomly scattered lenticels present, fracture fibrous	Yellowish brown coloured, greenish yellow when scraped, randomly scattered lenticels on a primary root, fracture fibrous

Table 2: Proximate analysis of *S. oblonga*Wall (stem and root)

	Stem	Root
Proximate Analysis – Ash Analysis		
Total ash	0.78±0.03*	0.0078±0.00007*
Water soluble ash	0.11±0.02*	0.75±0.11*
Acid-insoluble ash	0.26±0.03*	0.21±0.023*
Proximate Analysis – Extractive Analysis (in gm/ml)		
Petroleum ether	0.011*	0.012*
Chloroform	0.011*	0.015*
Acetone	0.046*	0.039*
Methanol	0.057*	0.092*
Water	0.014*	0.025*
* n=3, values expressed as mean ± SEM		

Table 3: Quantitative analysis of *S. oblonga* Wall (stem and root)

	Stem	Root
Quantitative studies – Primary metabolites (mg/0.1 ml)		
Moisture (% L)	4.16*	6.82*
Total carbohydrate	71.381±5.1*	57.796±1.9*
Total Protein	7.175±0.3*	10.561±0.5*
Quantitative studies – Secondary metabolites (mg/µl)		
Polyphenols	0.16±0.02*	0.79±0.04*
Tannins	0.03±0.001*	0.12±0.0005*
* n=3, values expressed as mean ± SEM		

Table 4: Screening of Antioxidant activity (stem and root)

EXTRACT (MeOH)	*IC50* in µg/ml	
	DPPH	ABTS
Stem	38.54 ± 0.05*	67.81 ± 0.75*
Root	26.6 ± 0.22*	42.1 ± 0.14 *
Standard	8.0 ±0.04*	37 ±0.21*
* n=3, values expressed as mean ± SEM		

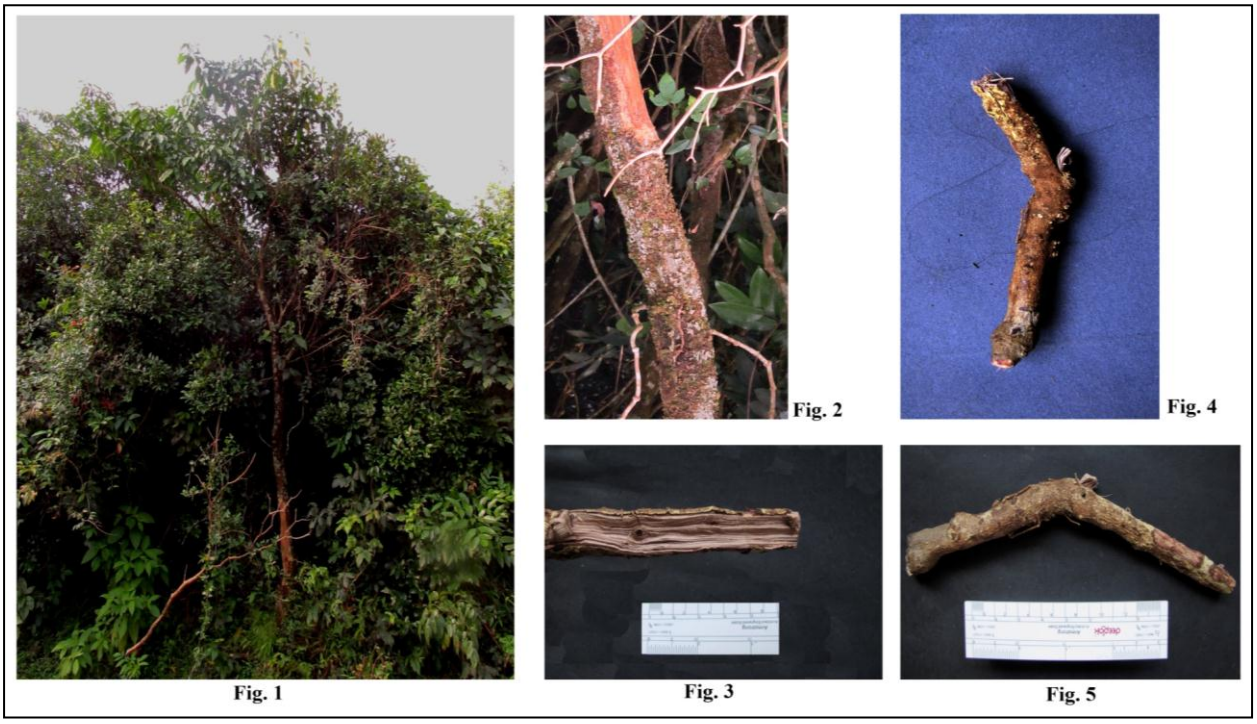

Fig. 1 Fig. 2 Fig. 3 Fig. 4 Fig. 5

Fig. 1 – 5: *Salacia oblonga* Wall. – Habit and Habitat, Fresh sample (Stem), Dried sample (Stem), Fresh sample (Root), Dried sample (Root).

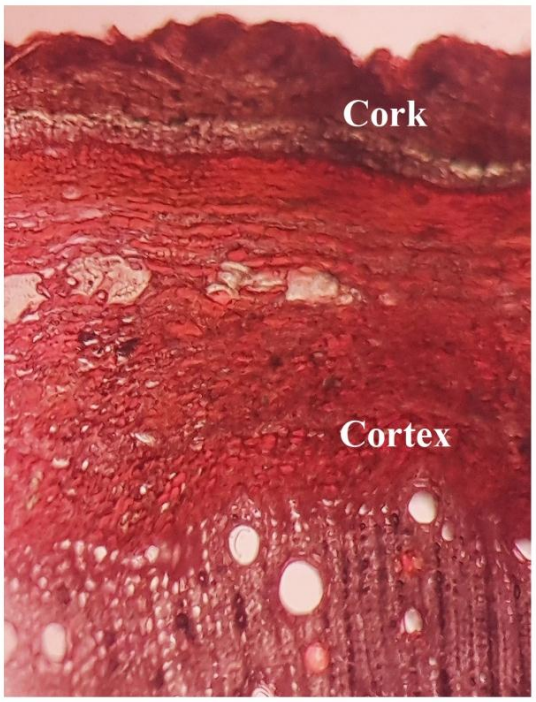

Fig. 6

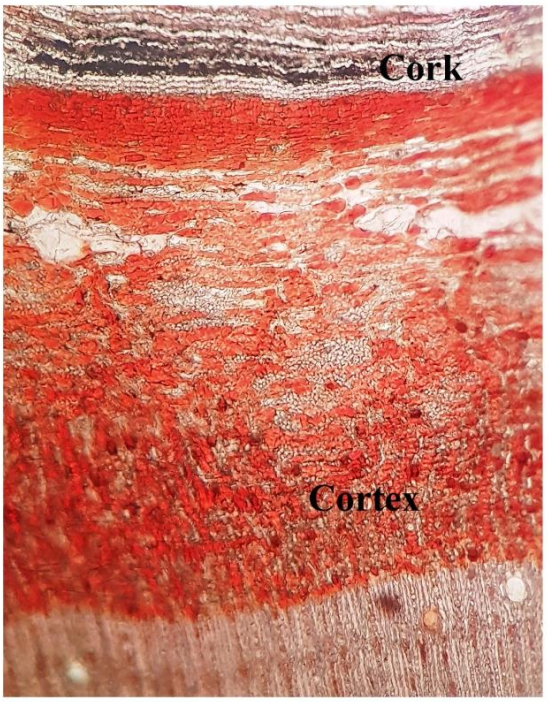

Fig. 7

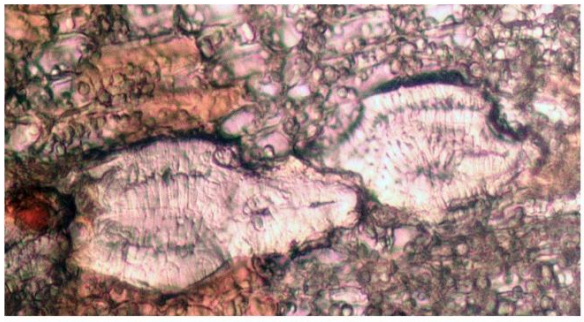

Fig. 8

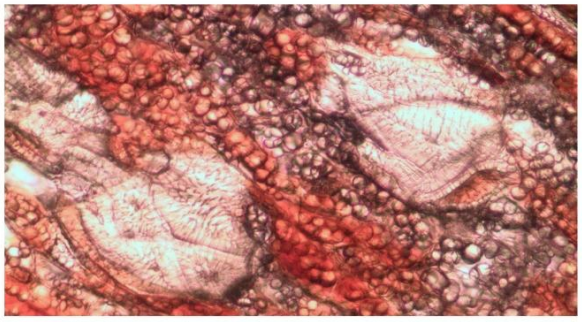

Fig. 9

Fig. 6 – 9: *Salacia oblonga* Wall. – Microscopy (Stem – Overview), Microscopy (Stem – Stone cells), Microscopy (Root – Overview), Microscopy (Root – Stone cells).

REFERENCES

1. Arora, M., Kaur, G., Kahlon, P.S., Mahajan, A. & Sembi, J.K. 2017. Pharmacognostic Evaluation & Antimicrobial Activity of Endangered Ethnomedicinal Plant *Crepidium acuminatum* (D. Don) szlach. *Phcog.J.* 9(6):56-63.
2. Chawla, A., Singh,S. & Sharma, A.K. 2013. *Salacia oblonga* Wall: A review on its Pharmacognostic, Phytochemical and Pharmacological Aspects. *IJRPBS.*4(4):1215-1228.
3. Chenthurpandy, P., Kalidass, C. & Mohan, V.R. 2009. Pharmacognostical Investigation of *Hiptage benghalensis* (L.) Kurz. (Malpighiaceae). *Phcog J.*1(2):103-105.
4. Deokate, U.A. & Khadabadi, S.S. 2012. Phytopharmacological aspects of *Salacia chinensis*. *JPT.*4(1):1-5.

5. Giron, M.D., Sevillano,N., Salto,R., Haidour, A., Manzano, M., Jimenez,M.L., Rueda.R, & Lopez-Pedrosa, J.M. 2009. *Salacia oblonga* extract increases glucose transporter 4-mediated glucose uptake in L6 rat myotubes: Role of mangiferin. *Clinical nutrition*. 28:565-574.
6. http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:331924-2#children
7. https://indiabiodiversity.org/species/list?offset=0&sort=lastUpdated&order=desc&startsWith=AZ&taxon=175418&classification=265799&isDeleted=false&total=0&sGroup=829&view=grid
8. Mabberley,D.J. 2008. *Mabberley's Plant Book, Portable Dictionary of Plants, their classification, and uses*. 3rd Edn. (Cambridge University Press).
9. Mukherjee, P. 2002. *Quality control of herbal drugs: An approach to evaluation of Botanicals*. Business Horizons.New Delhi.
10. Musini, A. & Giri,A. 2015. *Salacia oblonga* Wall: An endangered plant of immense pharmaceutical values. *JOCPR*.7(5):1125-1129.
11. Paarakh, P.M., Patil, L.J. & Thanga, S.A. 2008. Genus *Salacia*. A Comprehensive Review. *Jrnl. of Natural Remedies*. 8(2):116-131.
12. Sadasivam, S. & Manikam, A. 1996. Biochemical Methods, 2nded. New Age International (P) Limited Publishers. New Delhi.
13. Shirwaikar, A., Prabhu, K.S. & Punitha, I.S.R. 2006. *In vitro* antioxidant studies of *Sphaeranthusindicus* (Linn). *IJEB*.44:993-996.
14. Singh, N.P., Lakshminarsimhan, P., Karthikeyan,S. & Prasanna, P.V. 2001.Flora of Maharashtra State, Dicotyledons, Vol 2.Botanical Survey of India, Calcutta.
15. Tandon,N. & Sharma, P. 2012. Quality Standards of Indian Medicinal Plants Volume 10, (Medicinal Plants Unit, Indian Council of Medicinal Research, New Delhi).334.
16. Balik, J., Kyselakova, M., Vrchotova, T.J., Kumsta,M., Veverka,J., Hic,P., Totusek,J. & Lefnerova, D. 2008. Relations between polyphenols content and antioxidant activity in vine grapes and leaves. *Czech J. Food Sci*. 26:25-32.
17. Chua,M.T., Tung,Y.T. & Chang, S.T. 2008. Antioxidant activities of ethanolic extracts from the twigs of *Cinnamomum osmophloeum*. *Biosource Technology*.99:1918-1925.
18. Padmanabhan,P. & Jangle, S.N. 2012. Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal plants and their combinations. *IJPSDR.* 4(2):143-146.