Quantification of mangiferin content in different parts of *Salacia chinensis* L., a potential anti-diabetic plant from Kerala regions of the Western Ghats

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Abstract

Salacia chinensis L., is a potential medicinal plant with high therapeutic values that has been extensively used in traditional and modern medicines to treat diabetes and obesity. The present investigation is aimed to quantify the mangiferin (an anti-diabetic molecule) content in different parts of S. chinensis distributed in the Kerala region of the Western Ghats using Reverse-Phase High Performance Liquid Chromatography (RP-HPLC). Mangiferin content was detected in all analysed plant parts. Among the different root samples analysed, the highest (2.32mg/g) mangiferin content was observed in the samples collected from Sreenivasapuram region followed by the samples collected from Kumarakom region (1.04mg/g) and Palode region (0.97mg/g). The lowest concentration (0.34mg/g) in the root was observed in the sample collected from Vattappara region.

From the analysed leaf samples, highest (2.14mg/g) content was obtained in the accession collected from Palode region followed by the samples collected from Sreenivasapuram region (1.68mg/g) and Kumarakom region (1.62mg/g). All the collected stem samples showed lower mangiferin content (0.05mg/g to 0.76mg/g). The roots as well as leaves were identified as good source of mangiferin whereas stem samples contain very low quantity of mangiferin. The present investigation opens an avenue for effective extraction of the bioactive compound for antidiabetic property.

Keywords: Anti-diabetic, HPLC, Mangiferin, *S. chinensis.*

Introduction

Salacia (family - Celastraceae), a significant genus with nutritional, medicinal and pharmaceutical implications is widely distributed in the tropical and subtropical regions. 21 species are found in India of which 15 species are known to occur in peninsular India and are distributed in the Western Ghats of Karnataka, Kerala, Tamil Nadu, Maharashtra and Southern Orissa[12]. Many species of the genus (*S. chinensis, S. oblonga, S. reticulata, S. malabarica, S. macrosperma* etc.) are woody climbers which are commonly known as Saptrangi or Ekanayakam or Ponkoranti. They have been used for years in traditional (Ayurveda) as well as modern medicine for the treatment of various diseases especially for diabetes mellitus. Roots and golden coloured stems are the vital parts of *Salacia* species used in the medicinal preparations[16,18].

S. chinensis is the most prevalent species of *Salacia*, which is extensively used in the treatment of a variety of diseases such as diabetes, arthritis, rheumatism, inflammation, leucorrhoea, fever, skin diseases, obesity, cancer, liver disorder, inflammation, venereal and bronchitis[3,7,16]. The presence of major bioactive constituents such as mangiferin, salacinol, kotalanol, morolicacid, oleanoic acid, betulinic acid and the presence of alkaloids, tannins, terpenoids, flavonoids, saponins, glycosides and coumarins[18,19,25] in the root and stem of *Salacia* species render *S. chinensis*[9,13] medicinally rich. The presence of mangiferin is attributed to the high antidiabetic potential as well as other medicinal properties in *Salacia* species.

Mangiferin is a natural C-glucosyl xanthonoid polyphenol known as a natural miracle bioactive compound involved in multi-target regulatory activities such as inhibition of protein kinase, activation of peroxisome proliferator-activated receptor-γ (PPAR-γ), inhibition of α-glucosidase, aldoreductase and pancreatic lipase. Mangiferin is also used in the treatment of various lifestyle-related disorders such as kidney[17] and liver-related disorders[16] and different types of human cancer[6]. It has been effectively used in the treatment of Alzheimer's and Parkinson's disease that prevent neurodegeneration[4] and also possess anti-HIV property[20]. In this context, the present investigation is aimed in performing a comparative study on the quantitative determination of mangiferin content in different plant parts (root, leaf and stem) of *S. chinensis* collected from Kerala regions of the Western Ghats.

Material and Methods

Plant collection and sample preparation for extraction: Different accessions of *S. chinensis* were collected from Kerala regions of the Western Ghats. Fresh samples of root, leaf and stem were cleaned under running tap water to eradicate dust, soil and other external materials. The plant samples were then nicked into small pieces and dried under shade for 3 weeks. The dried plant samples were ground to coarse powder and were stored in air tight containers till further analysis.

Extraction of plant samples: The powdered individual samples were subjected to hot Soxhlet extraction. 5g of powdered samples was soaked in 180-220ml of methanol and further extraction was carried out by continuous percolation method using a Soxhlet apparatus which continued for 72hrs with subsequent concentration of filtered extracts by rotary evaporator. A dark yellowish red concentrate for root and dark greenish concentrate for leaf and light greenish red concentrate for stem were obtained. Crude methanolic extracts were prepared using HPLC grade methanol and filtered using 0.45µm membrane filter (Millipore) for HPLC analysis.

Chemicals used for HPLC analysis: All chemicals used in these experiments were of analytical grades and were procured from Merck, Millipore and Sigma-Aldrich, U.S. including acetic acid, HPLC grade methanol, HPLC grade water.

HPLC analysis: A simple sensitive and precise Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was used for the quantification of mangiferin from different parts of *S. chinensis*[8]. The chromatographic determination was carried out on a Shimadzu HPLC system (Schimadzu LC 20 AD) composed of two pumps, rhenode injector and C18 column equipped with PDA detector (SPS-20A). For RP-HPLC analysis, the mobile phase composed of 1% acetic acid and methanol (15:85, v/v) with a flow rate of 1ml/min. Calibration graph of mangiferin was plotted by injecting different concentrations of standard in methanol at a wavelength of 254nm, with a running time of 10 min and the injection volume of 20µl using a 25µl capacity syringe (Hamilton microlite syringe). The HPLC system was made stable for 1hr before injection.

Linearity and accuracy: Five different concentrations (0.5, 1, 10, 50 and 100ppm) of mangiferin standard were prepared in HPLC grade methanol for calculating linearity and accuracy. 20µl of mangiferin standard was injected to HPLC which was observed at 254nm. Experiments were repeated three times and calibration curve was plotted with average peak area against mangiferin concentrations. Accuracy of the current study was evaluated by consecutive analysis (n=3) for three concentrations (1, 10 and 50ppm) of standard mangiferin solution carried out using the method.

Results and Discussion

In the current study, plants from different natural populations of *S. chinensis* were collected from the Kerala regions of the Western Ghats for quantifying mangifer in content in the collected plant parts (root, leaf and stem) through HPLC method (Table 1 and fig. 1). HPLC chromatogram of standard mangiferin was obtained at a retention time of 2.78min for 3 replicates (Fig. 3) and different parts of *S. chinensis* showed a retention time of around 2.7min (Figures 4-8). A linear relation with a good correlation coefficient was obtained when the peak area (y) of mangiferin was plotted against different concentrations (1, 10 and 50ppm). A correlation coefficient (r^2) of 0.999 was obtained for the calibration curve equation (Fig. 2). The results of the current investigation revealed that there is a significant diversity in the mangiferin content of all plant parts, with concentrations in various parts ranging from 0.05mg/g to 2.32mg/g.

Root and leaf samples contained highest amount of mangiferin than stem samples. The concentration of mangiferin in roots of different accessions varied from 0.34mg/g to 2.32mg/g. The root sample from Sreenivasapuram region in Thiruvanathapuram district exhibited the highest concentration of mangiferin (2.32mg/g) followed by samples from Kumarakom region in Kottayam district (1.04mg/g), Palode region in Thiruvanathapuram district (0.97mg/g) and Vandanam region in Alappuzha district (0.73mg/g). The lowest content (0.34mg/g) was observed in the root sample collected from Vattappara region, Thiruvanathapuram district.

All the analysed leaf samples contained significant quantity of mangiferin content ranging from 0.92mg/g to 2.14mg/g. The highest mangiferin content (2.14mg/g) in the leaf sample was observed in the sample collected from Palode region, Thiruvanathapuram district followed by the samples collected from Sreenivasapuram region, Thiruvanathapuram district, with mangiferin content of 1.68mg/g and Kumarakom region (1.62mg/g) in Kottayam district. Leaf sample collected from Vandanam region in Alappuzha district showed 1.20mg/g mangiferin and 0.92mg/g in the sample collected from Vattappara region, Thiruvanathapuram district.

Table 1
Qunatitative analysis of mangiferin content in different parts of *S. chinensis* collected from Kerala regions of the Western Ghats.

S.N.	Place of collection		Geographical co-ordinates			Mangiferin Concentration (mg/g)		
	Locality	District	Latitude	Longitude	Altitude (m)	Leaf	Root	Stem
1	Palode	Thiruvananthapuram	8°45'8.66"	77°1'41.23"	116	2.14	0.97	0.24
2	Sreenivasapuram		8°44'49.83"	76°44'5.50"	31	1.68	2.32	0.76
3	Vattappara		8°35'0.46"	76°58'36.96"	84	0.92	0.34	0.24
4	Kumarakom	Kottayam	9°37’55.8”	76°25’27.5”	4	1.62	1.04	0.05
5	Vandanam	Alappuzha	9°24’48.8”	76°20’59.3”	0	1.20	0.73	0.14

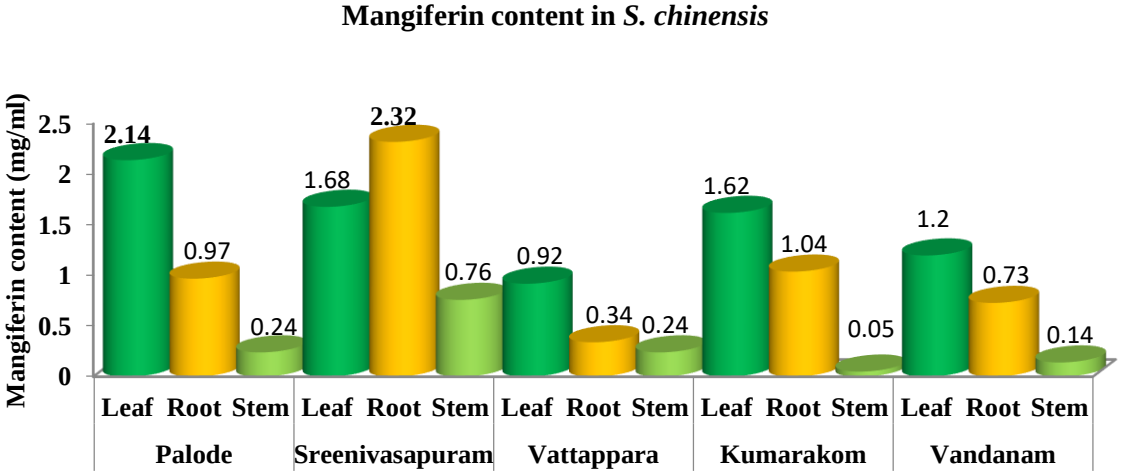


Figure 1: Mangiferin content in different parts of *S. chinensis* collected from Kerala regions of the Western Ghats

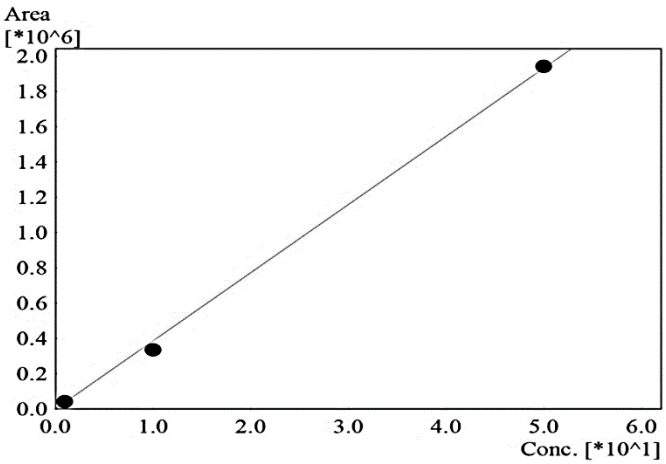
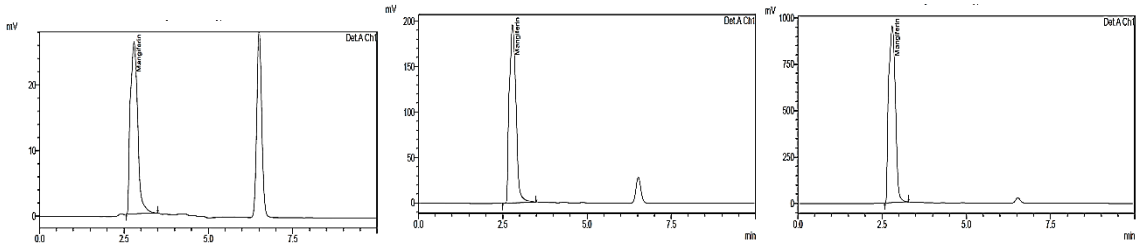
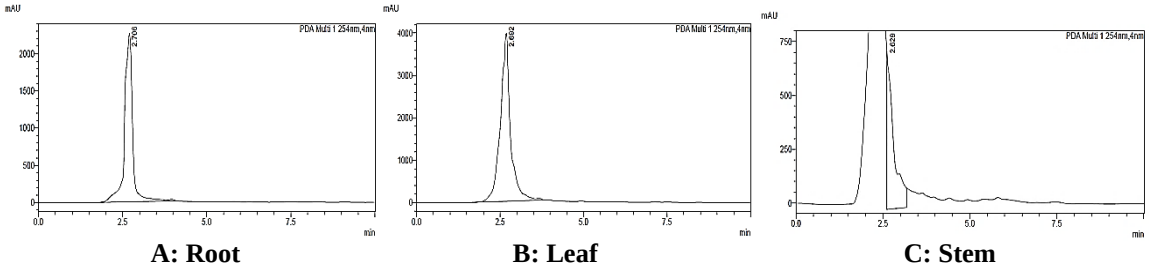


Figure 2: Calibration curve of mangiferin standard



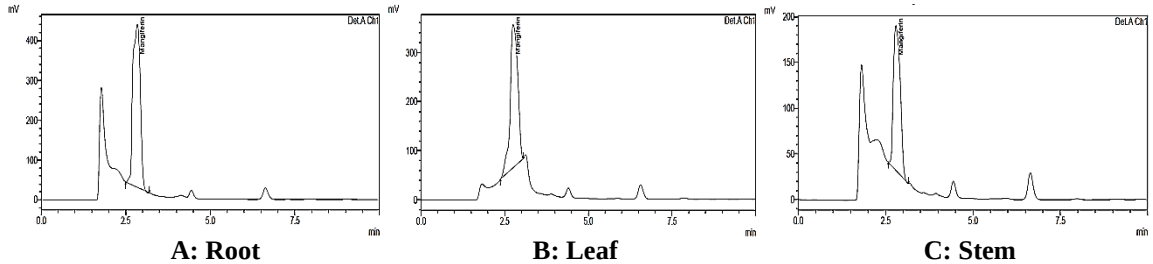
A: 0.5 ppm of mangiferin **B: 10 ppm of mangiferin** **C: 50 ppm of mangiferin**

Figure 3: HPLC chromatogram of standard Mangiferin



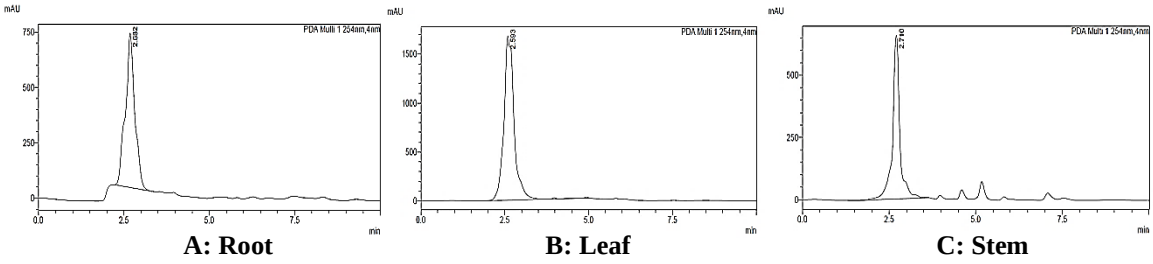
A: Root **B: Leaf** **C: Stem**

Figure 4: HPLC chromatogram of different parts of *S. chinensis* collected from Palode region



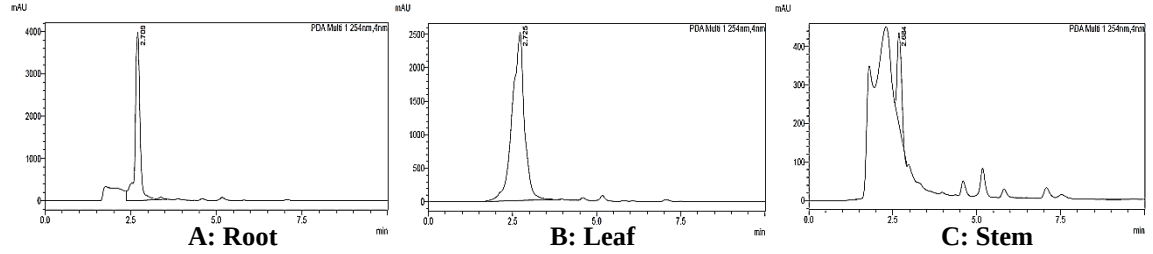
A: Root **B: Leaf** **C: Stem**

Figure 5: HPLC chromatogram of different parts of *S. chinensis* collected from Sreenivasapuram region



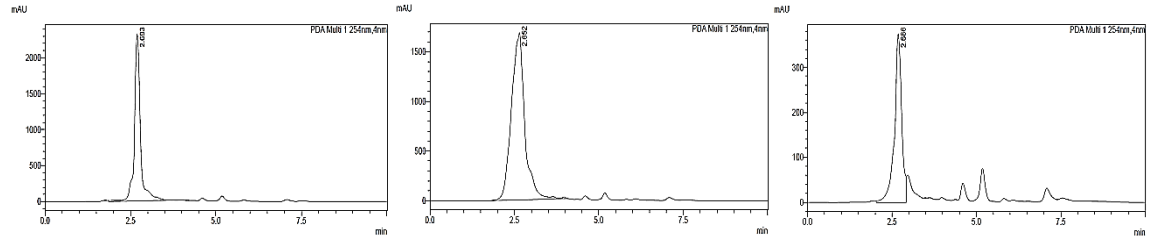
A: Root **B: Leaf** **C: Stem**

Figure 6: HPLC chromatogram of different parts of *S. chinensis* collected from Vattappara region



A: Root **B: Leaf** **C: Stem**

Figure 7: HPLC chromatogram of different parts of *S. chinensis* collected from Kumarakom region



A: Root **B: Leaf** **C: Stem**

Figure 8: HPLC chromatogram of different parts of *S. chinensis* collected from Vandanam region

Mangiferin content in the stem samples of different accessions ranged from 0.05mg/g to 0.76mg/g indicating relatively lower concentrations. The mangiferin content in the stem samples revealed 0.76mg/g, 0.24mg/g, 0.24mg/g, 0.14mg/g and 0.05mg/g in the accessions collected from Sreenivasapuram (Thiruvanathapuram dist.), Palode region (Thiruvanathapuram dist.), Vattappara region (Thiruvanathapuram dist.), Vandanam region (Alappuzha dist.) and Kumarakom region (Kottayam dist.) respectively.

Plants of *Salacia* species are extensively used in the treatment of diabetes mellitus because of the presence of major bioactive compounds such as mangiferin, salacinol and kotalanol. Nonetheless, their low toxicity, ease of availability and rich diversity of phytochemical compounds also contributed to their excessive market demand[4]. Mangiferin is considered as a "super antioxidant" and it has been found to exhibit anti-diabetic, anti-viral, anti-cancer, anti-aging, immunomodulatory, hepatoprotective and analgesic properties. Mangiferin is reported in different *Salacia* species[14,22-24] and is used in the treatment of various life style disorders such as kidney and liver-related disorders and cancer[6,16]. Mangiferin content in *Salacia* species was quantified by several techniques such as HPTLC[22], HPLC[8,14,23,24] and LC-MS[10] and these reports exhibited varying mangiferin content in the root of *S. chinensis*.

The present work provides a comparison of mangiferin content in different parts (root, leaf and stem) of *S. chinensis* collected from Kerala regions of the Western Ghats. The present analysis showed the highest mangiferin content in the root (2.32mg/g) and leaf (2.14mg/g) of *S. chinensis* collected from Thiruvananthapuram district of Kerala when compared to the mangiferin content in the root of *S.*

chinensis (1.57mg/g) collected from Pandalam region of Pathanamthitta district, Kerala[8]. Approximately 1.57mg/g (1.57%) and 1.54mg/g (1.54%) were reported in *S. chinensis* of Tamil Nadu region and Sirsi region of Karnataka respectively[21,25].

The highest mangiferin content (5.82mg/g/58.24mg/ml) in the root sample and the lowest (0.30mg/g/3.07mg/ml) in the stem sample was reported by Kamat et al[12]. More or less the same concentration of mangiferin content was reported by Jayaraj et al[8] in other species of *Salacia* like *S. brunoniana* (2.58mg/g) and *S. beddomei* (2.19mg/g) collected from Kulamavu region, Idukki district and Kakkayam region, Kozhikode district[8]. Chavana et al[1] reported very low concentration (0.39mg/g/391.8ppm) from the root samples of *S. chinensis* collected from the Amboli region of Maharshtra.

The present analysis concentrated on different accessions in Kerala regions of the Western Ghats showing 0.34mg/g-2.32mg/g mangiferin content in the root samples, 0.92mg/g-2.14mg/g in the leaf samples and 0.05mg/g-0.76mg/g in the leaf samples of *S. chinensis*. The findings indicated comparatively better concentration of mangiferin (2.14mg/g) content in the leaf samples of *S. chinensis* collected from Palode region, Thiruvananthapuram district. Variations in the amount of mangiferin content in the different samples from the various geographical locations were possibly affected by the different environmental factors.

Mangiferin is a versatile compound present in *S. chinensis* that can be used as a complimentary medicine for the treatment of various lifestyle diseases being cost-effective and considered bio-safe. We can also suggest the leaves are also a raw material source of mangiferin content for future pharmaceutical preparations. The presence of mangiferin along with kotalanol and salacinol gives *S. chinensis* an added benefit rendering it an indefeasible anti-diabetic plant.

Conclusion

Mangiferin is a xanthonoid compound, naturally occurring polyphenol with excellent pharmacological activities. The quantitative determination of mangiferin in the different parts of the *S. chinensis* collected from the Kerala regions of the Western Ghats was carried out using RP-HPLC technique. The present study revealed better (2.14mg/g) concentration of mangiferin content in the leaves of *S. chinensis.* The analysis also identified that stem, root and leaf of *S. chinensis* could be the most promising raw material for anti-diabetic drug formulations based on the mangiferin content.

In fact, the root and stem of *Salacia species* have been extensively used in traditional medicine to treat diabetes mellitus. The current investigation poses a trend that can arrest culling of the whole plant for root harvest by introducing leaf as an alternate source which has comparable mangiferin content as compared to root.

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References

1. Chavan J.J., Ghadage D.M., Kshirsagar P.R. and Kudale S.S., Optimization of extraction techniques and RP-HPLC analysis of antidiabetic and anticancer drug mangiferin from roots of 'Saptarangi' (*Salacia chinensis* L.), *J. Liq. Chromatogra & Relat Technol.*, **38(9)**, 963 **(2015)**

2. Chavana J.J., Ghadage D.M., Bhoite A.S. and Umdale, Micropropagation, molecular profiling and RP-HPLC determination of mangiferin across various regeneration stages of Saptarangi (*Salacia chinensis* L.), *Ind. Crops. Prod*., **76**, 1123 **(2015)**

3. Chawla Anshul, Thapar Shipra and Gupta Kumar Girish, Development and Validation of RP-HPLC method for the analysis of Moxifloxacin and its degradation products, *Res. J. Chem. Environ*., **27(1)**, 14 **(2023)**

4. Erten F., Orhan C., Tuzcu M., Er B., Defo Dee P.B., Sahin N. and Sahin K., *Salacia chinensis* exerts its antidiabetic effect by modulating glucose-regulated proteins and transcription factors in high-fat diet fed-streptozotocin-induced type 2 diabetic rats, *J. Food Biochem*., **44(12)**, 1 **(2020)**

5. Feng S.T., Wang Z.Z., Yuan Y.H., Sun H.M., Chen N.H. and Zhang Y., Mangiferin: a multipotent natural product preventing neurodegeneration in Alzheimer's and Parkinson's disease models, *Pharmacol. Res*., **146**, 104336 **(2019)**

6. Imran M., Arshad M.S., Butt M.S., Kwon J.H., Arshad M.U. and Sultan M.T., Mangiferin: a natural miracle bioactive compound against lifestyle related disorders, *Lipids Health Dis.*, **16(1)**, 1 **(2017)**

7. Jadhav S.Y., Das S., Patil A.M., Gadekar M., Dibara G. and Shinde S.S., Evaluation of anti-diabetic, antioxidative and proteolytic potentials of *Salacia chinens* organic extracts, *J Med. Plant. Res.*, **9(6)**, 42 **(2021)**

8. Jayaraj R., Sasidharan N., Tom B. and Muhammad A.K., Comparative Phytochemical Profiling and Quantification of Mangiferin Content in Species of *Salacia* from Southern Western Ghats of India, *J. Biol. Act. Prod. Nat*., **6(3),** 209 **(2016)**

9. Kajimoto O., Kawamori S., Shimoda H., Kawahara Y., Hirata H. and Takahashi T., Effects of a diet containing *Salacia reticulata* on mild type 2 diabetes in humans: A placebo-controlled, cross-over trial, *J. Jpn. Soc. Nutr. Food. Sci*., **53(5)**, 199 **(2000)**

10. Kaliappan I., Kammalla A.K., Ramasamy M.K., Aruna A. and Dubey G.P., LC-MS quantification of mangiferin in hydroalcoholic extract of *Salacia oblonga*, *Salacia roxburghii* and polyherbal formulation, *Int J Phytopharm.*, **4(1)**, 11 **(2014)**

11. Kamat S.G., Vasudeva R. and Patil C.G., GC-MS and LC-MS based phytochemical profiling and quantification of Mangiferin in six species of *Salacia* from the Western Ghats of India, *J. Exp. Biol*., **8(4)**, 132 **(2018)**

12. Kamat S.G., Vasudeva R. and Patil C.G., Taxonomic identity, occurrence of six species of *Salacia* and first report on chromosome numbers of the *Salacia chinensis* L. and *Salacia oblonga* Wall ex Wight and Ern Var. from Western Ghats of Karnataka (India), *Genet. Resour. Crop. Evol.*, **67(1)**, 241 **(2020)**

13. Kobayashi M., Akaki J., Yamashita K., Morikawa T., Ninomiya K., Yoshikawa M. and Muraoka O., Suppressive effect of the tablet containing Salacia chinensis extract on postprandial blood glucose, *Jpn. Pharmacol. Ther*., **38(6)**, 545 **(2010)**

14. Kumar K.A., Kumar R.M., Aruna A. and Kaliappan I., Development and validation of a RP-HPLC method for the simultaneous determination of Mangiferin, Ellagic acid and Hydroxycitric acid in polyherbal formulation, *Pharmacogn. J*., **6(3)**, 23 **(2014)**

15. Li L., Dong Y., Liu X. and Wang M., Mangiferin for the Management of Liver Diseases: A Review, *Foods*, **12(13)**, 2469 **(2023)**

16. Li Y., Huang T.H. and Yamahara J., *Salacia* root, a unique Ayurvedic medicine, meets multiple targets in diabetes and obesity, *Life. Sci*., **82(21-22)**, 1045 **(2008)**

17. Lum P.T., Sekar M., Gan S.H., Jeyabalan S., Bonam S.R., Rani N.N.I.M., Ku-Mahdir K., Soew L.J., Wu Y.S., Subramaniyan V., Fuloria N.K. and Fuloria S., Therapeutic potential of mangiferin against kidney disorders and its mechanism of action: A review, *Saudi J. Biol Sci.*, **29(3)**, 1530 **(2022)**

18. Matsuda H., Yoshikawa M., Morikawa T., Tanabe G. and Muraoka O., Antidiabetogenic constituents from *Salacia* species, *J. Trad. Med*., **22(1)**, 145 **(2005)**

19. Minh T.T., Anh N.T., Thang V.D. and Van Sung T., Study on chemical constituents and cytotoxic activities of *Salacia chinensis* growing in Vietnam, *Zeitschrift fur Naturforschung B*., **65(10)**, 1284 **(2010)**

20. Silpraist K., Seetaha S., Pongsanarakul P., Hannongbua S. and Choowongkomon K., Anti-HIV-1reverse transcriptase activities of hexane extracts from Asian medicinal plants, *J. Med. Plant Res*., **5(19)**, 4899 **(2011)**

21. Smitha G.N., Asif A.K., Joshi V.G. and Mukesh S.S., Validated HPTLC method for mangiferin in *Salacia chinensis*, *J. Pharm. Res.*, **3(5)**, 1107 **(2010)**

22. Sukumar Karuganti, Bharadwaj Anahita, Swarnabala Ganti and Muralitharan Gangatharan, Efficiency of silicate solubilizing Bacillus tequilensis SKSSB09 in enhancing drought mitigation in Zea mays L., *Res. J. Biotech.*, **18(2)**, 1 **(2023**)

23. Thanh V.N., Christopher J.S., Michael C.B. and Quan V.V., Isolation and Maximisation of Extraction of Mangiferin from the Root of *Salacia chinensis* L., *Separations*, **6(3)**, 44 **(2019)**

24. Vijaya S., Ashish H. and Nitin K., HPLC Estimation of Mangiferin in *Salacia chinensis* Linn., *Asian J. Chem*., **21(9)**, 6679 **(2009)**

25. Yoshikawa M., Nishida N., Shimoda H., Takada M., Kawahara Y. and Matsuda H., Polyphenol constituents from *Salacia* species: quantitative analysis of mangiferin with alpha-glucosidase and aldose reductase inhibitory activities, *J. Pharm. Soc. Jpn*., **121(5)**, 371 **(2001)**.

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