



Phytochemical Studies on Indian Trumpet tree for the evaluation of its sustainable utilization in Herbal drug industry

Sulaiman C.T.¹, Sharafunnisa A.M.² and Indira Balachandran¹

¹Phytochemistry Division, Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala, India

²Department of Biochemistry, University of Calicut, Kerala, India

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Abstract: The herbal industry is posing substantial threat due to over harvesting or unscientific harvesting in the wild and habitat destruction in the form of deforestation. In the near future, many species may be totally unavailable for the use of industry due to dwindling resources. Conservation and sustainable use of the habitats of medicinal plants are imperative for ensuring continued availability of genuine herbs used to address the health needs of the majority of the world's population. The aim of the current studies was to evaluate the possibilities of sustainable utilization of an important medicinal plant, *Oroxylum indicum*, in medicine manufacture by comparing the phytochemical pattern of various parts like bark, leaf and flower with respect to its root which may lead to finding a suitable substitute for root. The quantitative estimations of major group of phytochemicals such as phenolics and flavonoids were done spectrophotometrically. The chemical profiles were compared using chromatographic techniques like TLC and HPLC. On evaluating the chemical pattern and quantitative estimation of major group of phytochemicals, it was found that the root of *O. indicum* can be substituted by its stem bark.

Key Words: *Oroxylum indicum*, Phenolics, Flavonoids, TLC, HPLC.

Introduction

Demand for medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side-effects and easily available at affordable prices. There are estimated to be over 7800 manufacturing units in India. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. While the demand for medicinal plants is growing, some of them are increasingly being threatened in their natural habitat (Anonymous, 2000; Muhammad & Awaisu, 2008).

About 90% of medicinal plants used by the industries are collected from the wild. While over 800 species are used in production by industry, less than 20 species of plants are under commercial cultivation. Over 70% of the plant collections involve destructive

harvesting because of the use of parts like roots and the whole plant in case of herbs. This poses a definite threat to the genetic stocks and to the diversity of medicinal plants if biodiversity is not sustainably used. Several medicinal plants have been assessed as endangered, vulnerable and threatened due to over harvesting or unskillful harvesting in the wild and habitat destruction in the form of deforestation (Anonymous, 2000; Sasidharan & Muraleedharan, 2003).

Oroxylum indicum (L.) Kurz, commonly known as Indian trumpet tree, has been used as a traditional medicine in Asia in ethnomedicinal systems for the prevention and treatment of several diseases (Dev *et al.*, 2010). It contains flavonoids like chrysin, baicalein and Oroxylin-A (Raghu *et al.*, 2013; Joshi *et al.*, 2014). Various parts of *O. indicum* have been reported to possess anticancer, antioxidant, hepatoprotective, antibacterial, analgesic and



gastro-protective activities (Tripathi *et al.*, 2001; Anonymous, 2001). It is used for dropsy, cough, sprains, neuralgia, hiccough, asthma, bronchitis, anorexia, dyspepsia, flatulence, colic, diarrhea, dysentery, strangury, gout, vomiting, leucoderma, wounds, rheumatoid arthritis and fever (Dinda *et al.*, 2015; Chen *et al.*, 2003).

O. indicum which is known as *Shyonaka* in Ayurveda is mentioned in various classical texts of Ayurvedic System of Medicines. It is extensively used as an important ingredient of *Dasamula* which is a compound decoction of 10 roots used in the treatment of remittent fever, otorrhoea, bronchitis, leucoderma, diarrhoea, inflammation and in acute rheumatism (Bisht *et al.*, 2011).

The present study outlines the concept of plant part substitution, evaluation of possibilities of substituting the root of plant with other parts of the same plant (Sulaiman & Balachandran, 2013). The studies were carried out in *O. indicum* to evaluate the possibilities of using other parts like bark, leaf or flower instead of root which will help sustainable utilization.

Material and Methods

Plant Material

Different parts of *O. indicum* were collected from premises of CMPR, Arya Vaidya Sala, Kottakkal, Kerala, India and were authenticated by Plant Systematics and Genetic resources Division of Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal, Kerala, India.

Chemicals

Chemicals used in the study included: (+) - Folin-Ciocalteu reagent, Gallic acid (>98.0% purity by HPLC) and Quercetin (>98.0% by HPLC) were procured from Sigma Chemicals Co. (Bangalore, India). All other chemicals employed were of standard analytical grade from Merck India.

Extraction

The various parts collected such as root, bark, leaf and flower were shade dried and

pulverized. Five gram each of the different parts was extracted successively with various solvents such as n-hexane, chloroform and methanol using reflux extraction method. Two gram each of the various parts was separately extracted with water for High pressure Liquid Chromatographic (HPLC) analysis.

Estimation of total Poly phenolic compounds

Polyphenols such as phenolics and flavonoids were estimated spectrophotometrically. The total phenolic content (TPC) was determined using Folin-Ciocalteu reagent (Singleton & Rossi, 1965; Sulaiman & Balachandran, 2012). TPC was expressed as gallic acid equivalents (GAE) in mg / g of sample. Total flavonoid content (TFC) was measured by aluminium chloride colorimetric assay (Zhishen *et al.*, 1999) and expressed as mg quercetin equivalents (mg EQ).

Thin layer chromatographic profile (TLC)

TLC profiling was done on pre-coated silica gel 60F₂₅₄ TLC plate (Merck India). The mobile phase was standardized as toluene and ethyl acetate in the ratio of 8:2 for hexane and chloroform extracts. Methanolic extracts were developed using Toluene: Ethyl acetate: Methanol in the ratio 8:2:1. Mobile phase for water extracts was standardized as Butanol, glacial acetic acid and water in the ratio 5:1:1. The chromatogram was developed in a saturated chromatographic chamber (Camag, Switzerland). The developed plate was visualized under UV at 254 nm and 366 nm and in visible light after derivetizing with Anisaldehyde sulphuric acid reagent followed by heating at 105°C for 5 minutes.

High Performance Liquid Chromatography Analysis (HPLC)

The water extracts of various plant parts were subjected to HPLC analysis using Agilent 1200 Preparative High Pressure Liquid Chromatographic system equipped with prep pump, a Rheodyne injector, Diode

Array Detector in combination with Chem32, Chemstation software. Gradient elution was performed with acetonitrile (solvent A) and 0.1% ortho-phosphoric acid (solvent B) in a binary gradient flow by increasing the concentration of solvent B; 0-5 min 40%; 5-10 min 50%; 10-15 min 60%; 15-20 min 70%. The PDA signal was recorded at 260 nm.

Results and Discussion

Total Phenolics and total Flavonoids (TPC & TFC)

The phenolic and flavonoid contents of different parts such as root, bark, leaf and flower were estimated spectrophotometrically (Table.1). Total polyphenolics were found to be more in all other parts comparing to root. The highest phenolic and flavonoid content was observed for leaf, 24.5 and 19.75 respectively. The TPC and TFC of root were observed as 6.12 and 3.25 respectively. Comparing to the root, bark also showed higher polyphenolics. The F/P value of bark and root showed that the flavonoid content is more in the bark than root. The major class of compounds such as phenolics and flavonoids were almost comparable in both root and bark. The F/P ratio can be used for the assessment of individual specificity of flavonoids towards the total polyphenolic contents (Sulaiman & Balachandran, 2012; Marinova *et al.*, 2005).

Table 1 Total polyphenolic contents of different parts of *O. indicum*

| Plant part | Total phenolics | Total flavonoids | F/P |
|------------|-----------------|------------------|------|
| Root | 6.12 | 3.25 | 0.53 |
| Bark | 6.87 | 4.80 | 0.69 |
| Leaf | 24.50 | 19.75 | 0.80 |
| Flower | 9.75 | 4.82 | 0.49 |

Thin Layer Chromatographic analysis (TLC)

TLC profiles were developed for various extracts like n-hexane, chloroform, methanol and water with different parts such as root,

bark, leaf and flowers. The chromatograms were visualized and documented on irradiating under 254 nm, 366 nm and in visible light after derivetizing with specific TLC spraying reagents. The R_f values for each separated band were recorded.

The chromatogram of n-Hexane extract of various parts is presented in Figure 1. On evaluating under 254nm, Root showed only a band at 0.51. Major bands were observed at 0.25 and 0.51 for bark. Leaf extract showed bands at 0.19, 0.25, 0.47 and 0.63. Flower showed a single band at 0.89. The band seen at 0.51 was found to be common in both root and bark and compound with R_f 0.25 is common for bark and leaf. Under 366nm, Root, Bark and flower showed only one band with green fluorescence at R_f 0.70. In the case leaf, red fluorescent bands were observed at 0.03, 0.41 and 0.62. After derivetizing with Anisaldehyde sulphuric acid reagent followed by heating at 105°C for 5 minutes, both root and bark showed a light pink colored band at 0.39 and a blue coloured band at 0.53. Leaf showed major bands at 0.09 (light pink), 0.21 (pink), 0.24 (light blue), 0.34 (light blue), 0.39 (light pink), 0.53 (blue), and 0.60 (pink). Flower extract showed major bands at 0.02, 0.06, 0.09, 0.24, 0.39, 0.53, 0.60 and 0.67.

The TLC profile of hexane extract of root showed maximum similarity with its bark. No previous reports are available on comparative TLC profile of hexane extract of *O. indicum*.

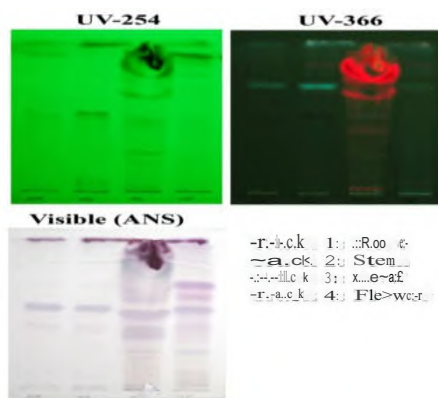


Fig. 1 TLC Profile of n- hexane extracts of *O. indicum*

Chloroform extracts of different parts of *O. indicum* showed more number of bands when compared to that of hexane extracts (Fig. 2). At 254 nm, both root and bark extracts showed chemical quenching at 0.19, 0.45, 0.51, and 0.87. Leaf extract showed bands at R_f 0.03, 0.06, 0.10, 0.19, 0.26, 0.45, 0.51, 0.68, 0.74 and 0.87. Flower showed bands at 0.45, 0.55, and 0.87. Among the separated compounds, the bands corresponding to 0.45 and 0.87 were found in all the four parts.

On evaluating under 366nm, various fluorescent bands were observed at 0.19 (dark green), 0.50 (dark green), 0.78 (blue) and 0.85 (blue) for both root and bark. A green fluorescent band was observed in root at 0.02. Leaf extract showed fluorescence at 0.03 (red), 0.06 (red), 0.10 (blue), 0.13 (red), 0.19 (dark green), 0.26 (dark green), 0.33 (red), 0.41 (red), 0.46 (red), 0.50 (dark green), 0.54 (red), 0.56 (red), 0.61 (red), 0.66 (red) and 0.73 (red). Major bands were observed at 0.02 (green), 0.16 (red), 0.78 (blue) and 0.85 (blue) for flower extracts. Among these compounds corresponding to R_f 0.19 and 0.50 are common for root, bark and leaf and 0.78 and 0.85 are common for root, bark and flower.

After derivatizing with Anisaldehyde sulphuric acid reagent followed by heating at 105°C for 5 minutes, root showed two bands at 0.03 (grey) and 0.52 (light blue). Bark showed bands at 0.03 (grey) and 0.20 (orange). Leaf showed different clouted bands with R_f 0.03 (grey), 0.05 (greenish blue), 0.09 (light blue), 0.15 (light blue), 0.26 (blue), 0.35 (blue), 0.52 (light blue), 0.62 (light pink), 0.69 (light pink), 0.74 (light green) and 0.81 (dark blue). In the case of flower, bands at 0.03 (grey), 0.52 (light blue), 0.62 (light pink) and 0.69 (light pink) were observed.

The band with R_f 0.03 was present in all the four parts and band with R_f 0.52 was common for root, leaf and flower. TLC profile of chloroform extract also showed that both root and bark are almost similar in their chemical pattern.

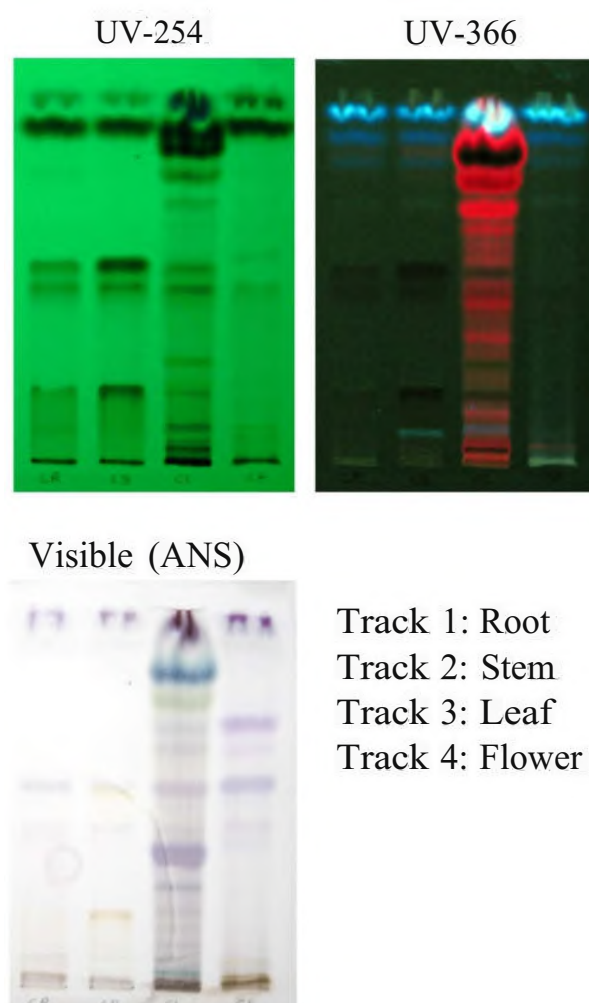


Fig. 2 TLC Profile of chloroform extracts of *O. indicum*

The TLC profile of methanolic extracts is presented in Fig.3. Under 254nm, both root and bark showed similar banding pattern with chemical quenching at 0.06, 0.23, 0.40, 0.67 and 0.73. Leaf showed bands at 0.10, 0.14, 0.20, 0.33, 0.40, 0.67 and 0.73. Major bands were observed for flower extract at 0.10, 0.14, 0.23, 0.40 and 0.67. The compounds with R_f 0.23, 0.40 and 0.67 are common in all the parts analysed. On 366 nm, both root and bark showed fluorescence at 0.39, 0.70 and 0.87. The bands showed in the root with R_f 0.10, 0.28, 0.49 and 0.59 were found to be absent in bark.

On the basis of methanolic extract, bark showed some difference in number of bands in comparison with root.

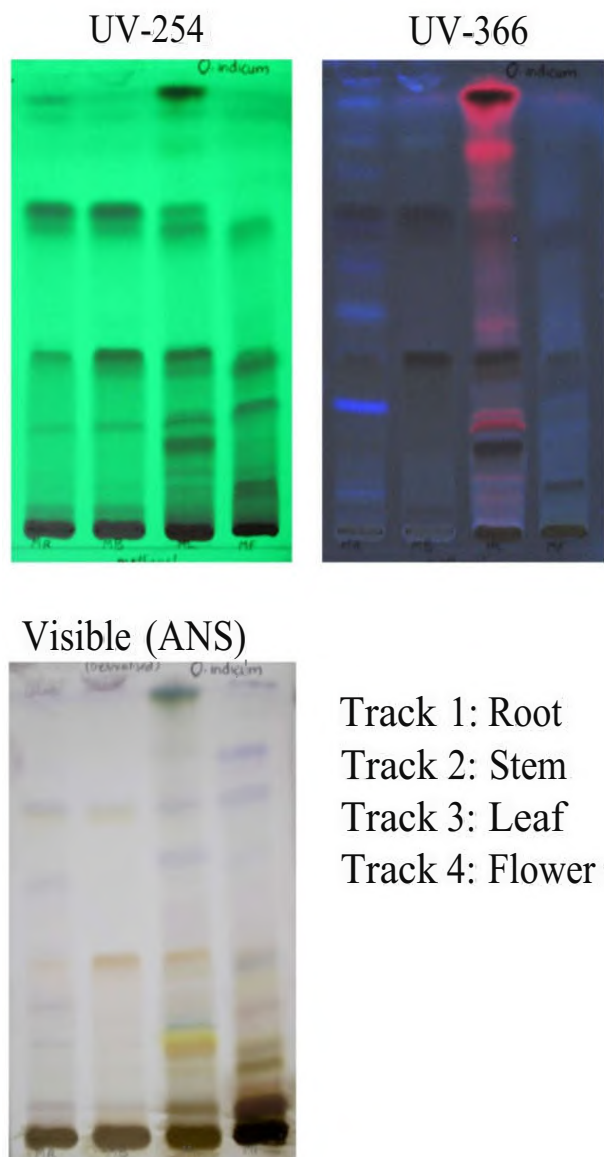


Fig. 3. TLC Profile of methanol extracts of *O. indicum*

Comparative TLC profiling of aqueous extract showed that the chemical pattern of all the parts analysed are almost similar in their chemical profiles (Fig. 4). Maximum matching was observed for root and stem bark with almost similar band intensity. The comparative chemical profiles of water extracts is an important tool for the evaluation of possibilities of using other parts of the plant

instead of root, as aqueous extract is being used for herbal drug manufacturing.

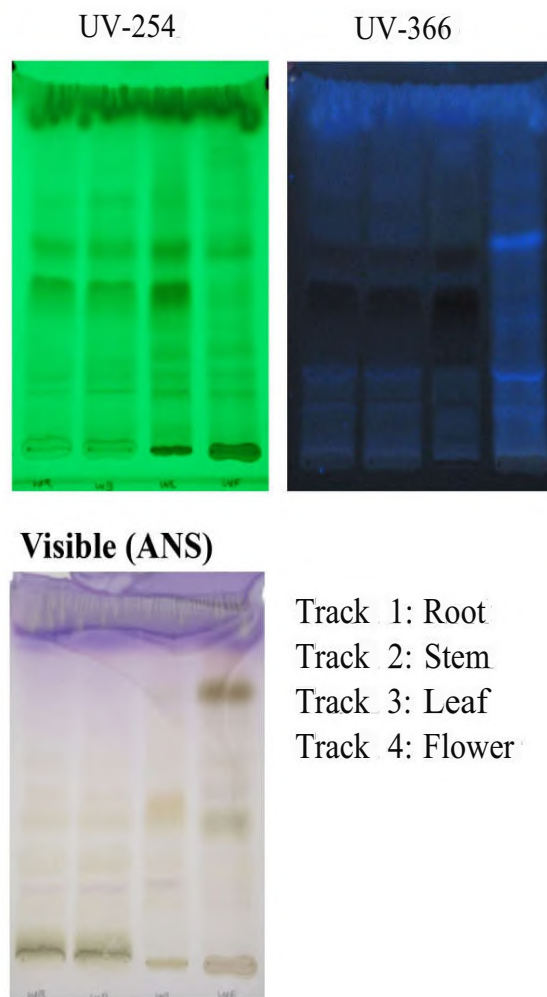


Fig. 4. TLC Profile of aqueous extracts of *O. indicum*

On the basis of TLC profiling of aqueous extract, both root and stem bark of *O. indicum* showed similar chemical pattern. Leaf and flower also showed some similarities in their chemical pattern for aqueous extract. However, maximum matching was observed for root and bark with respect to the number of bands and band intensities.

High Performance Liquid Chromatography (HPLC)

Liquid chromatography is a sensitive and accurate tool widely used for the quality

assessment of plant extracts and is currently the method of choice for both qualitative and quantitative analysis of herbal extracts. HPLC profiles were developed for the aqueous extracts of root, bark, leaf and flower. The profile of root extracts showed major peaks at R_t 2.272, 2.677, 3.041, 3.188, 3.484, 3.833, 4.238, 4.582, 4.819, 5.122, 5.410, 5.932, 6.484, and 7.272. The bark extracts of *O indicum* showed peaks at R_t 2.244, 2.650, 3.002, 3.472, 4.128, 4.694, 5.060, 5.269, 5.552, 5.884, 6.388, 6.449, 7.307, 7.906, 8.399, 11.495, and 12.572. The leaf extracts showed peaks at R_t 2.542, 3.000, 3.539,

3.673, 3.831, 4.004, 4.169, 4.402, 4.736, 5.029, 5.271, 5.543, 5.869, 6.450, 7.001, 7.878, 8.384, 11.419, and 12.505. The aqueous extracts of flower showed peaks at R_t 1.782, 2.243, 2.611, 2.929, 3.145, 3.486, 4.053, 4.552, 4.777, 5.215, 5.838, 6.391, 7.207, 8.349, and 8.752.

The HPLC profiles of root and Bark showed similar peaks (Fig 5). The number of peaks and peak area percentage were found to be comparable. The overlaid chromatogram showed super imposing peaks which reveals the phytochemical similarity of the both part

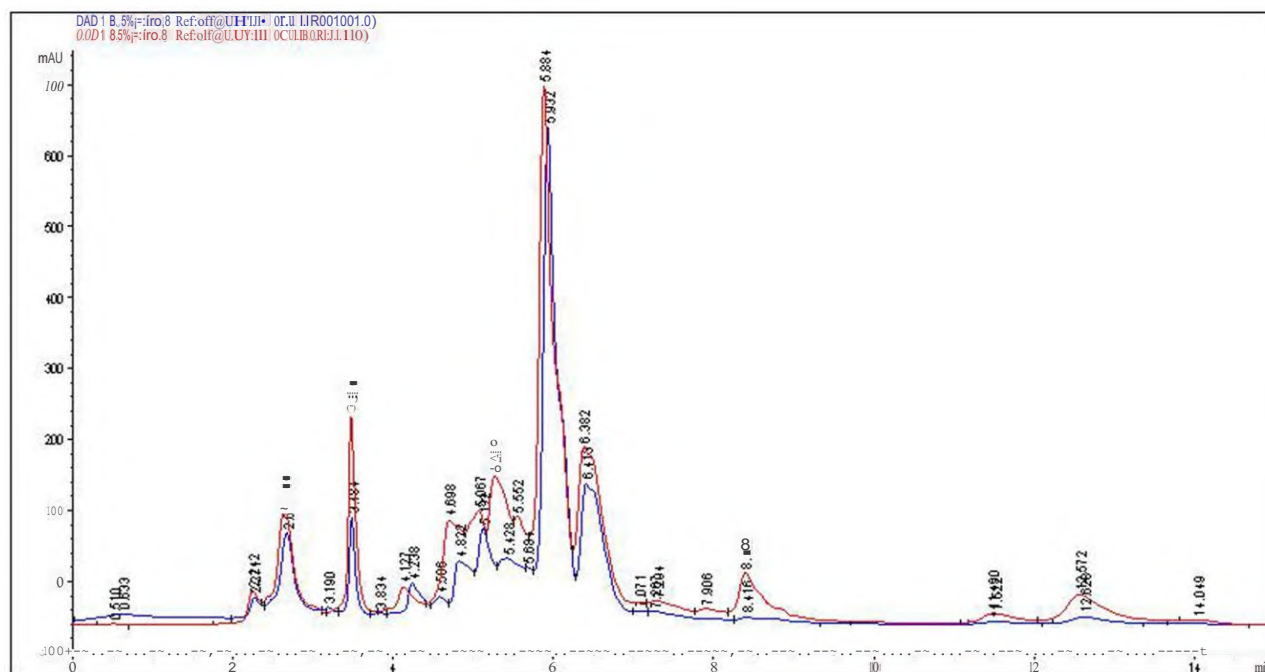


Fig. 5. Overlaid HPLC chromatogram of root and Bark of *O. indicum*

The HPLC profile also support the TLC data, the major peaks separated in the root were also found in bark extract. The area percentage of major peaks was also showed almost similar which indicates the quantitative similarities in their individual chemical constituents.

Conclusion

The management of traditional medicinal plant resources has become a matter of urgency. The concept of plant part substitution

may contribute to conserve endangered medicinal plants and help in sustainable utilization. In this present study, comparative phytochemical analyses were done in different parts such as root, stem bark, leaf and flowers of *Oroxylum indicum*, an important medicinal plant belonging to *Dasamoola* group where root is the official part for medicine manufacture. A strategy which would satisfy the requirements of sustainable harvesting would be the substitution of root with other parts of the



same plant if they are found to be phytochemically similar. The present study delineates the concept of plant part substitution in *O. indicum* to evaluate the possibility of substituting the root with its stem bark which may help sustainable harvesting.

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