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GC-MS and HR-LCMS fingerprinting of various parts of *Oroxylum indicum* (L.)Vent. A comparative phytochemical study based on plant part substitution approach

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Abstract

Oroxylum indicum, popularly known as 'Syonaka', is one of the *Dasamoola* root species and an ingredient of many other herbal formulations. With a concern to conservation, the present study focuses on chromatographic analysis of phytochemicals present in various parts of this tree for finding possibilities to substitute aerial parts for root. In GC-MS analysis, 10, 12 and 18 bioactive compounds were identified respectively from root, stem and leaves. The abundance of the principal compound oroxylin and chrysin did not show much variation in root and stem. In LCMS analysis 100 different compounds were identified from various parts. Majority of the bioactive compound like cosmosin, quercitrin, ginkgolide J, rhapontin, leuteoline, hesperetin, naringenin, eriodictyol, S-4 nitrobenzyl glutathione, lecanoric acid, sennidin B, chlortetracycline, kanamycin etc. were detected in all parts. The study recommends for the use of young stem in herbal formulations instead of root, as an effective conservation strategy for this tree.

Keywords: *Oroxylum indicum*, Plant part substitution, GC-MS analysis, HR-LCMS analysis.

Introduction

Plant based formulations are used world-wide for decades, due to their better healing power and immune modulatory activity. Our traditional and modern healing therapies exploit the wide range of secondary metabolites produced by plants, which in turn lead to indiscriminate harvesting of raw drug from natural sources. This has ultimately rendered many indigenous medicinal plant species endangered. *Oroxylum indicum* (L) Vent. is an important medicinal tree of Bignoniaceae family, whose root is highly demanded in the Indian and Chinese drug market. The root of this tree is one among the 10 roots used in the top selling Ayurvedic formulation- *Dasamoolarishta* [1]. It was estimated that the yearly consumption of *Dasamoola* raw drugs in Indian herbal drug market is more than 10,000 million tonnes [2]. The root of this tree is also used in other Ayurvedic preparations like *Dhanwanthara ghrita*, *Dhanwantharishta*, *Brahma rasayana*, *Narayana taila* etc. [3, 4]. Compounds like ellagic acid, Oroxylin, Chrysin, Baicalin, were reported to be present in this tree [5, 6]. Unscientific harvesting of roots, added with poor fruit set and seed abortion has resulted in the drastic decline and disappearance of this tree in natural population [7]. It was reported that this tree has fallen under the RET (Rare Endangered Threatened) listed plants of Western Ghats of India [8]. Unavailability or scarcity of authentic medicinal plants were often coped with substituting or adulterating the original one with plant parts of inferior quality [9]. But substituting the medicinally important part with other parts of the same plant was often recommended as a sustainable harvesting strategy for slow growing species like trees so as to protect the valuable medicinal plants from destructive harvesting of underground parts. But the potential of plant part substitution varies according to the species [10]. Therefore the equivalency of the substituted part should be proved in terms of bioactivity and phytochemistry to ensure the efficacy and reliability of herbal medicines. Integration and incorporation of modern analytical methods like chromatographic and spectral techniques are authentic tools for generating fingerprints of active compounds present in crude herbal drugs. In the present study the detailed phytochemistry of root bark, young stem and leaves of *O. indicum* were unravelled using Gas chromatographic-Mass spectrometric (GC-MS) analysis and High resolution Liquid chromatographic and Mass spectrometric (HR-LCMS) analysis. The various compounds in root stem and leaves were critically compared seeking scientific reasons for using aerial parts instead of root.

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Materials and methods

Collection of plant material and preparation of extract

Root, young stem and leaves of *O. indicum* were collected from medicinal plant resource garden Kanjirapuzha, Palakkad District. The plant materials were thoroughly washed, shade dried and coarsely powdered using a mixer grinder. About 5 grams of powdered plant parts were macerated separately with 100 ml of methanol in a conical flask kept in a rotary shaker for 18 hours^[11]. The extract was filtered using whatman filter paper No.1 and collected in a petri dish. The solvent was evaporated to dryness by placing the extract inside the oven at 60 °C for 4 hour. The concentrated crude extracts were collected and stored in air tight borosil vials for further study.

GCMS analysis- Instrument specification and Operational conditions

GCMS analysis was carried out using a *Schimidzu* GC-MS Model No. QP 2010 S nonpolar chromatographic column with 30 meter length, 0.25 mm internal diameter and 0.25 µm thickness. The column operates in an electron impact mode at 70eV with helium as carrier gas. The oven was set with an initial temperature of 80 °C and final temperature 260 °C. and the ion source temperature was maintained at 200 °C. A volume of 1 µl sample was injected to the injection port in split mode with a total flow of 24 ml/ min, column flow 1 ml/ min, purge flow 3 ml/ min and a linear velocity of 36.8 cm/sec under 65 k Pa pressure. The sample was run for a total duration of 50 minutes. The mass/ charge ratio value was initially set at 50 and a final value of 500. The compounds were identified based on their mass to charge ratio and matched with standards in NIST (National institute of standards and technology) library.

HR-LCMS - Instrument specification and Operational conditions

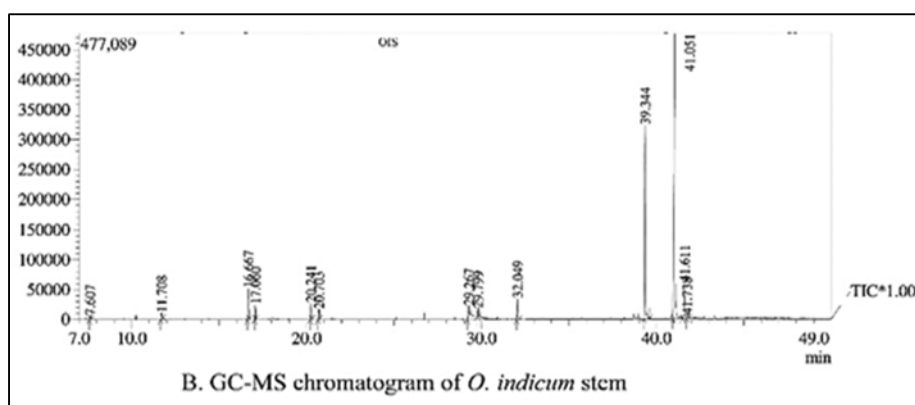
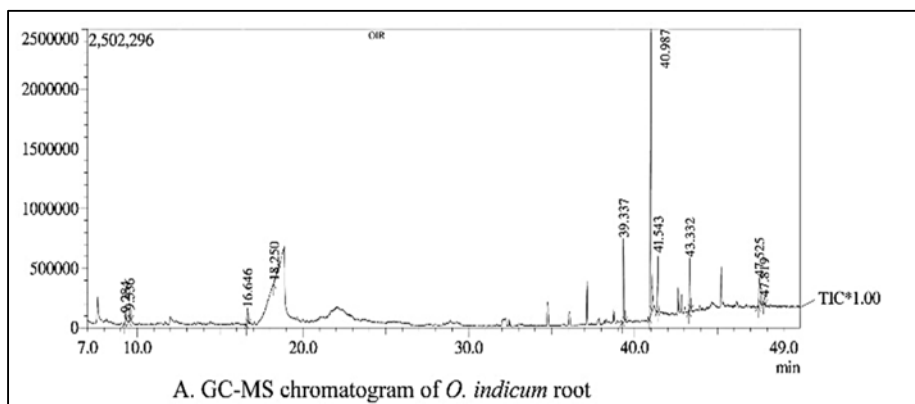
HRLC-MS analysis was performed with a Thermo fisher Scientific (Q-TOF) High resolution Orbitrap Liquid chromatogram equipped with Q extractive plus Mass spectrometer and proteome discoverer analyst version 1.42 software system. It has a Hypersil GOLD C18 column of dimension 100 x 2.1mm-3µ and dual AJS ESI (electro spray ionization) source. The instrument combines the analytical separation of HPLC and powerful detection technique of mass spectrometry and can provide high performance chromatographic separation of compounds with an m/z ratio ranging from 50 to 8000 amu, resolution 280000, scan speed of 12 Hz and mas accuracy less than 1 ppm.

The instrument was operated in a stop time mode for 30 minutes, with a gradient elution flow of 0.3 ml/minute and a pressure of 1200 bar. 95% water and 5% acetonitrile were used as solvents with a sample injection volume of 5 µl. The mass spectra of the compounds were obtained with a scan rate of 1.00 and m/z ratio ranging from 103- 1000. The LCMS data was obtained using proteome discoverer analyst version 1.42 software system. The obtained data was sorted manually to list out the parameters of various compounds.

Results and discussion

GC-MS analysis

The chromatogram pattern was almost similar for root and stem (Figure 1). But the name of compounds differed according to the variation in fragmentation pattern of compounds. The highest peak area was achieved by the principal compound oroxylin (55.61% in root and 51.65% in stem). But the quantity of oroxylin in leaf was only 4.78%. Another important flavonoid compound chrysin has a peak area of 8.85% and 6.59% in root and stem respectively, but in leaf it was below detectable level.



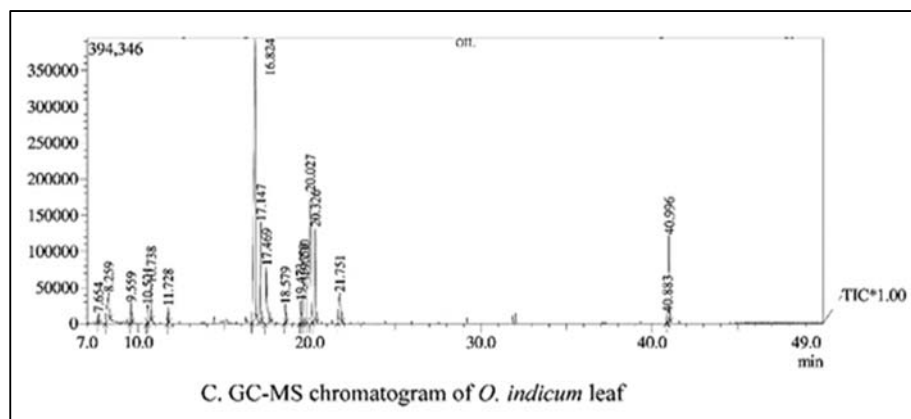


Fig 1: GC-MS chromatogram of methanolic extracts of various parts of *O. indicum*
A. root, B. stem, C. leaf

GC-MS analysis of the root of *O. indicum* revealed the presence of 10 compounds viz-Methylisopropyl nitrosamine, Pyranone, Methyl 3-Ethyl-3-Pentenoate, 1,5-Anhydro-d-talitol, Silikonfett SE 30, Oroxylin, Chrysin, Heptasiloxane hexadecamethyl, Cyclononasiloxane octadecamethyl and β -sitosterol.

Stem revealed the presence of 13 compounds which include Methylpiperidine-(R)-MTPA amide, 4-vinylphenol, 2-Cyclohexen-1-one, 3methyl-, 1-Heptanol-2,4-dimethyl-(R,R)-(+)-, Cyclobutanecarboxylic acid- octyl ester, Decanoic acid, Hexadecanoic acid, Ethyl pelargonate, Tetradecanal, 1,2-benzenedicarboxylic acid, Oroxylin, Chrysin and D,L-Phenylalanine amide.

The 18 compounds identified from leaf include 5-methyluracil, 4-methyloxazole, 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, Benzoic acid, 5-Methoxypyrrolidin-2-one, 4-Vinylphenol, Seudenone, 1,10-decane-1,1,10,10-d4-dio, Benzene-ethanol, 4-hydroxy-, Tetrahydrofuran-2-one, 5-[1-hydroxyhexyl]-, Trans-1,2-d2-1,2-dihydroxy-cyclopentane, Phenol, 4-amino-, 2-(2',2',2'-d3-ethyl)pyridine, 2-d,2-pentadecyl-1,3-dioxepane, Cyclobutanecarboxylic acid, decyl ester, 1,4-Dioxin, 2,3-dihydro-, (-)- β -caryophyllene epoxide and the principle compound Oroxylin. The details of these compounds are summarised in table 1.

Table 1: Compounds detected from various parts of *O. indicum* through GC-MS analysis

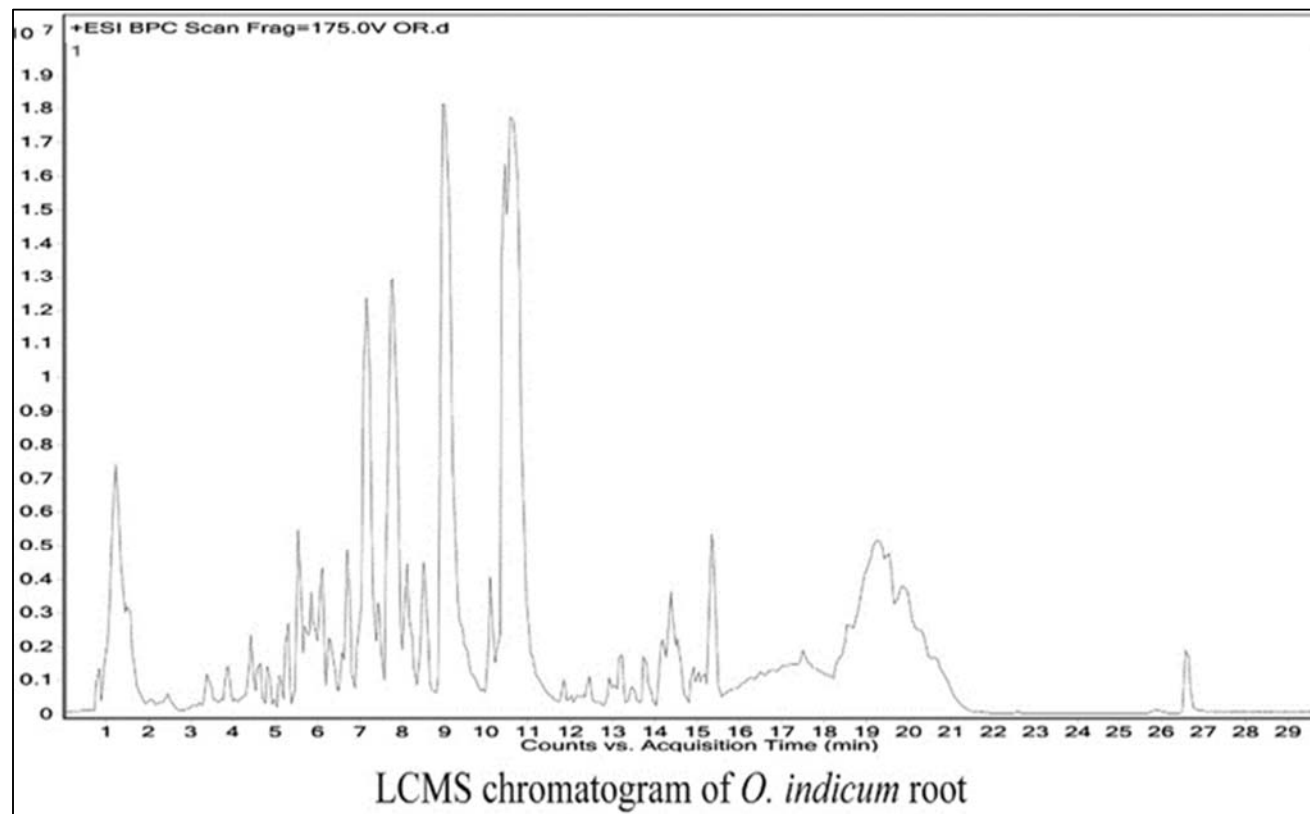
| Compounds in the methanolic extracts of root of <i>O. indicum</i> | | | | | | |
|---|----------------|---|--|---------|-------------|--------|
| Peak | Retention time | Name of Compound | Mol. Formula | Mass | Peak area % | m/z |
| 1 | 9.284 | Methylisopropyl nitrosamine | C ₄ H ₁₀ N ₂ O | 102.14 | 1.47 | 56.00 |
| 2 | 9.536 | Pyranone | C ₅ H ₄ O ₂ | 96.08 | 2.22 | 144.00 |
| 3 | 16.646 | Methyl 3-Ethyl-3-Pentenoate | C ₈ H ₁₄ O ₂ | 142.2 | 3.61 | 82.05 |
| 4 | 18.250 | 1,5-Anhydro-D-talitol | C ₆ H ₁₂ O ₅ | 164.16 | 1.79 | 57.00 |
| 5 | 39.337 | Silikonfett SE 30 (Grevels) | | | 11.49 | 73.05 |
| 6 | 40.987 | Oroxylin | C ₁₆ H ₁₂ O ₅ | 284.26 | 55.61 | 69.00 |
| 7 | 41.543 | Chrysin | C ₁₅ H ₁₀ O ₄ | 254.24 | 8.85 | 254.00 |
| 8 | 43.332 | Heptasiloxane, hexadecamethyl- | C ₁₆ H ₄₈ O ₆ Si ₇ | | 8.46 | 73.05 |
| 9 | 47.525 | Cyclononasiloxane, octadecamethyl | C ₁₈ H ₅₄ O ₉ Si ₉ | 667.4 | 5.44 | 73.05 |
| 10 | 47.818 | Beta-sitosterol (Cupreol) | C ₂₉ H ₅₀ O | 414.7 | 1.06 | 81.10 |
| Compounds in the methanolic extracts of stem of <i>O. indicum</i> | | | | | | |
| 1 | 7.607 | Methylpiperidine-(R)-MTPA amide | C ₇ H ₁₄ N ₂ O | 142.2 | 0.81 | 126.05 |
| 2 | 11.708 | 4-vinylphenol | C ₈ H ₈ O ₈ | 120.15 | 0.94 | 120.10 |
| 3 | 16.667 | 2- Cyclohexen-1-one, 3methyl- | C ₇ H ₁₀ O ₂ | 110.15 | 3.79 | 82.00 |
| 4 | 17.060 | 1-heptanol,2,4-dimethyl-(R,R)-(+)- | C ₉ H ₂₀ O | 144.25 | 1.53 | 55.00 |
| 5 | 20.241 | Cyclobutanecarboxylic acid, octyl ester | C ₁₃ H ₂₄ O ₂ | 212.33 | 1.86 | 55.05 |
| 6 | 20.703 | Decanoic acid | C ₁₀ H ₂₀ O ₂ | 172.26 | 1.35 | 73.00 |
| 7 | 29.267 | Hexadecanoic acid | C ₁₆ H ₃₂ O ₂ | 256.43 | 1.92 | 73.00 |
| 8 | 29.799 | Ethyl pelargonate | C ₁₁ H ₂₂ O ₂ | 186.29 | 0.78 | 88.05 |
| 9 | 32.049 | Tetradecanal | C ₁₄ H ₂₈ O | 832.00 | 2.56 | 71.05 |
| 10 | 39.344 | 1,2-benzenedicarboxylic acid | C ₈ H ₆ O ₄ | 166.14 | 25.37 | 149.00 |
| 11 | 41.051 | Oroxylin | C ₁₆ H ₁₂ O ₅ | 284.26 | 51.65 | 69.00 |
| 12 | 41.611 | Chrysin | C ₁₅ H ₁₀ O ₄ | 254.24 | 6.59 | 254.05 |
| 13 | 41.738 | D,l-phenylalanine amide | C ₉ H ₁₂ N ₂ O | 164.2 | 0.83 | 120.10 |
| Compounds in the methanolic extracts of leaf of <i>O. indicum</i> | | | | | | |
| 1 | 7.654 | 5-methyluracil | C ₅ H ₆ N ₂ O ₂ | 126.113 | 0.45 | 126.00 |
| 2 | 8.259 | 4-methyloxazole | C ₄ H ₅ N O | 83.09 | 3.78 | 55.00 |
| 3 | 9.559 | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | C ₆ H ₈ O ₄ | 144.12 | 1.00 | 143.95 |
| 4 | 10.521 | Benzoic acid | C ₇ H ₆ O ₂ | 122.12 | 1.10 | 84.00 |
| 5 | 10.738 | 5-Methoxypyrrolidin-2-one | C ₅ H ₉ NO ₂ | 115.13 | 4.68 | 84.00 |
| 6 | 11.728 | 4-Vinylphenol | C ₈ H ₈ O | 120.15 | 0.69 | 120.05 |
| 7 | 16.824 | Seudenone | C ₇ H ₁₀ O | 110.15 | 30.99 | 82.00 |

| | | | | | | |
|----|--------|--|--|--------|-------|--------|
| 8 | 17.147 | 1,10-decane-1,1,10,10-d4-dio | C ₁₀ H ₂₂ O ₂ | 174.28 | 5.56 | 55.00 |
| 9 | 17.469 | Benzeneethanol, 4-hydroxy- | C ₈ H ₁₀ O ₂ | 138.16 | 5.38 | 107.05 |
| 10 | 18.579 | Tetrahydrofuran-2-one, 5-[1-hydroxyhexyl]- | C ₁₀ H ₁₈ O ₃ | 186.25 | 0.80 | 101.05 |
| 11 | 19.473 | Trans-1,2-d2-1,2-dihydroxy-cyclopentane | C ₅ H ₁₀ O ₂ | 102.13 | 1.30 | 101.05 |
| 12 | 19.650 | Phenol, 4-amino- | H ₂ NC ₆ H ₄ OH | 109.12 | 3.43 | 84.00 |
| 13 | 19.700 | 2-(2',2',2'-d3-ethyl)pyridine | C ₇ H ₉ N | 107.15 | 6.81 | 109.00 |
| 14 | 20.027 | 2-d,2-pentadecyl-1,3-dioxepane | C ₂₀ H ₄₀ O ₂ | 312.5 | 18.35 | 102.05 |
| 15 | 20.326 | Cyclobutanecarboxylic acid, decyl ester | C ₁₇ H ₂₂ O ₂ | 268.4 | 6.84 | 55.00 |
| 16 | 21.751 | 1,4-Dioxin, 2,3-dihydro- | C ₄ H ₆ O ₂ | 86.09 | 3.71 | 86.00 |
| 17 | 40.883 | (-)-.Beta.-caryophyllene epoxide | C ₁₅ H ₂₄ O | 220.35 | 0.37 | 93.00 |
| 18 | 40.996 | Oroxylin | C ₁₆ H ₁₂ O ₅ | 284.26 | 4.78 | 69.00 |

HRLC-MS analysis

The HRLC-MS chromatogram of various parts of *O. indicum* were depicted in figure 2. The peaks were almost similar in root, stem and leaves. The sorted list of known compounds along with their retention time, molecular formula, mass, m/z ratio and their presence or absence in various parts were summarised in Table 2. A total of 100 different compounds were detected from various parts of this tree. Among these 13 compounds viz- Cosmosin, Leuteolin, Propanoic acid 2-hydroxy-3-[(4-hydroxy-1-naphthalenyl)oxy]-, Chlortetracycline, Hesperetin, Eriodictyol, S-(4-Nitrobenzyl) glutathione, Indol glycerol phosphate, Lecanoric acid, Naringenin, Kanamycin, Trandopril glucuronide and 3alpha,6beta,7alpha-trihydroxy -5beta-cholan-24-oic acid were detected in all parts. Root consisted a total of 41 compounds and stem consisted of 53 compounds. Root and stem shared 21 compounds in common including the above mentioned 13 compounds. The other common compounds of

root and stem are 7-Dehydrologanin tetraacetate, Quercitrin, O-Desmethyloxotolrestat, Ginkgolide J, 1-Phosphatidyl 1D myoinositol-3-phosphate, Sennidin B and Haematoxylin 11-hydroxy palmitic acid. This reveals that majority of the bioactive compounds of root are shared by stem also. Some important bioactive compounds like Cisapride, Carisoprodol, Apin, 4-(2-hydroxypropoxy)-3,5-dimethyl phenol, 7-Epiloganin tetraacetate, Naphthyl glucuronide, Hieracin, Iodovulone-I, Aesculin, Khayanthone were detected exclusively from stem. The common compounds of leaf and stem are Ketoconazole, Cefamandole naftate, Naringenin-7-O-glucoside, Oxotolrestat, Pentahydroxy flavone, Rhamnetin and 2,4-dimethyl tetradecanoic acid. Leaf consist of 50 compounds. Some important compounds like 3-indolyllactic acid, Rosmarinic acid, Salsalate, Fluvoxamine acid, Acebutol, Leukotriene E4, Oxfendazole, Evoxine, 18-Oxocortisol, Acocadene acetate, Normeperidine and Mitoxanthrone were detected only in leaf.



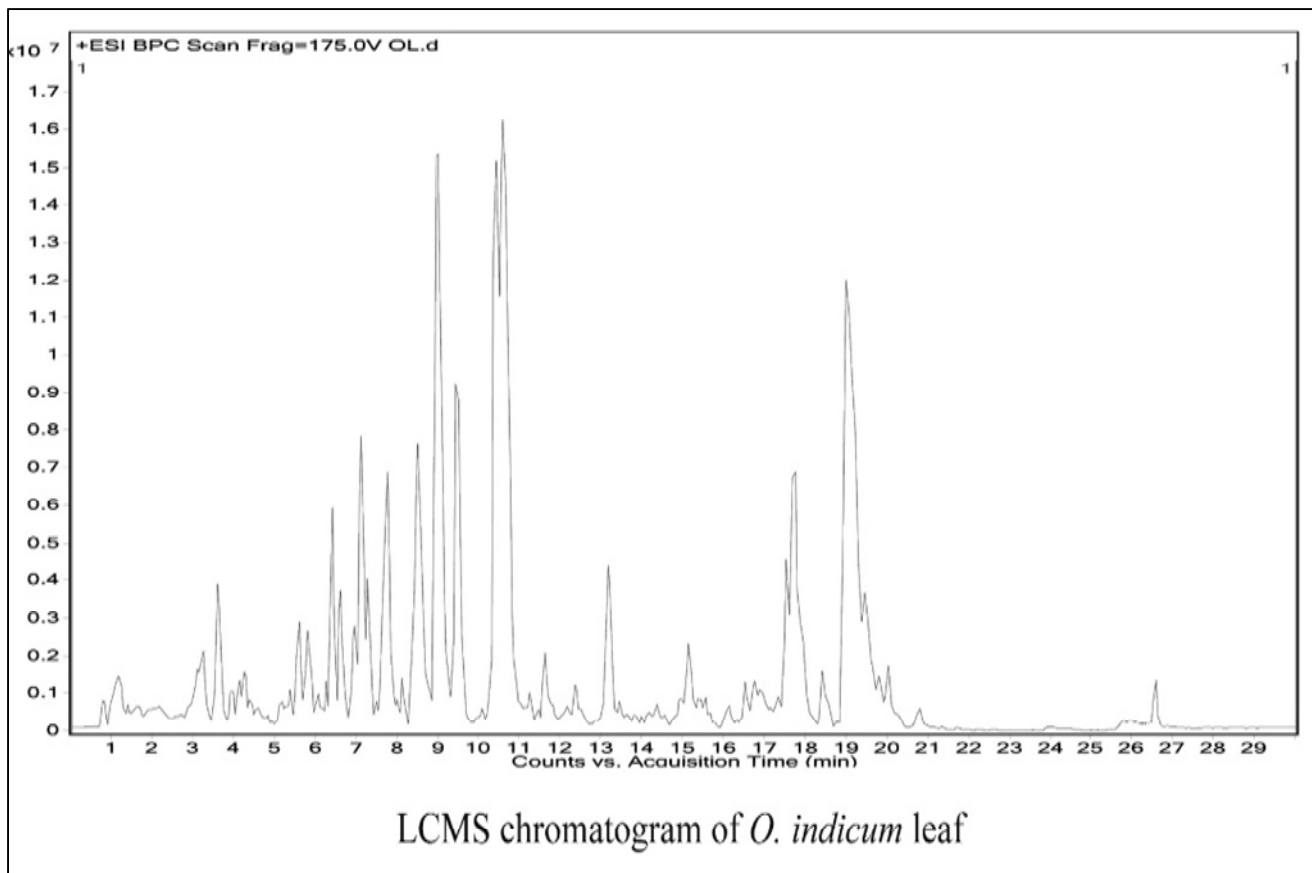
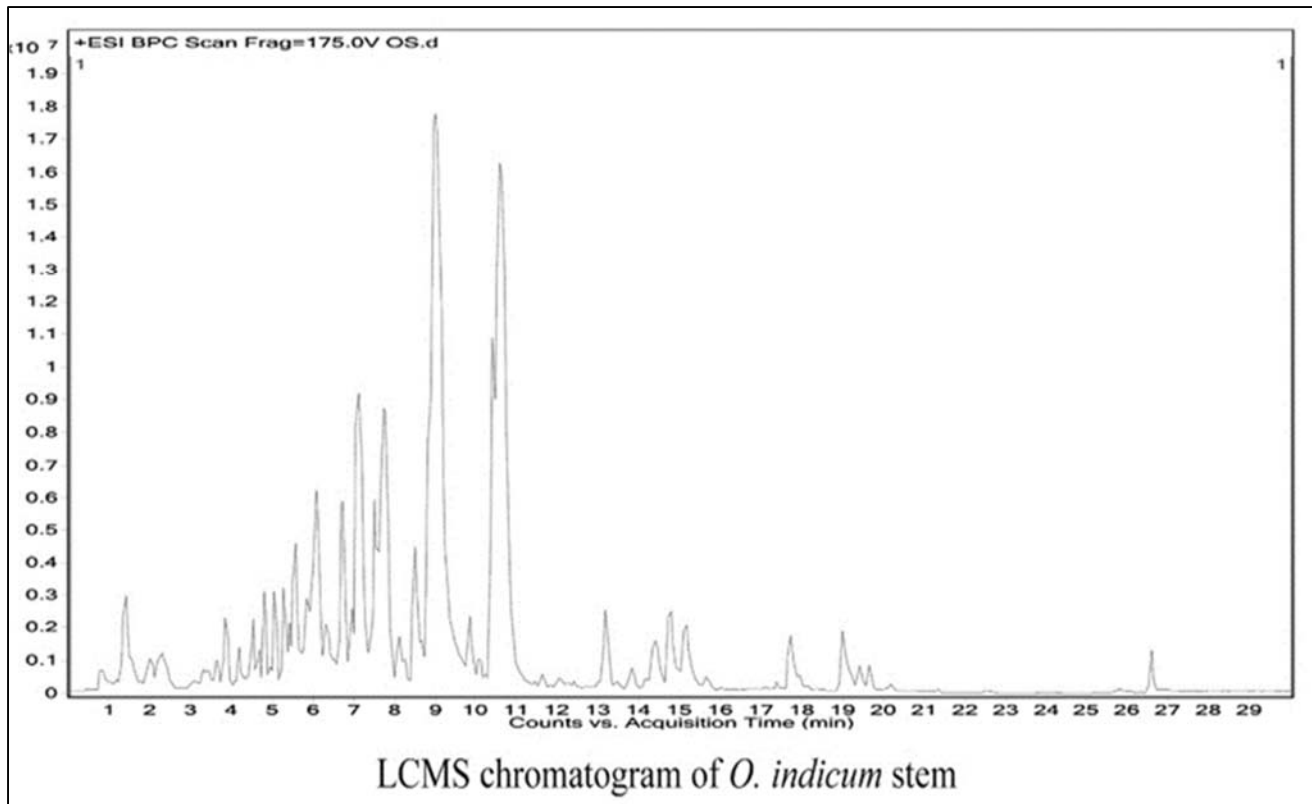


Fig 2: HR-LCMS chromatogram of methanolic extracts of various parts of *O. indicum*

Table 2: Compounds detected from the methanolic extracts of root, stem and leaf of *O. indicum* through HR-LCMS analysis

| Sl. No | R.T | Name of compound | Mol. Formula | Mass | m/z | R | S | L |
|--------|--------|--|---|----------|----------|---|---|---|
| 1 | 1.402 | Methyl N (amethylbutyryl)glycine | C ₈ H ₁₅ NO ₃ | 173.1042 | 156.1009 | + | - | - |
| 2 | 3.369 | 1,2-Benzenediol, 4-[[4-(4-fluorophenyl) 3piperidiny] methoxy]-, (3Strans)- | C ₁₈ H ₂₀ FNO ₃ | 317.1459 | 318.1532 | + | - | - |
| 3 | 4.482 | Methsuximide | C ₁₂ H ₁₃ NO ₂ | 203.0936 | 186.0902 | + | - | + |
| 4 | 5.243 | 7-Dehydrologanin tetraacetate | C ₂₅ H ₃₂ O ₁₄ | 556.1797 | 579.1688 | + | + | - |
| 5 | 5.737 | Quercitrin | C ₂₁ H ₂₀ O ₁₁ | 448.0987 | 449.106 | + | + | + |
| 6 | 6.431 | O-Desmethyloxotolrestat | C ₁₅ H ₁₂ F ₃ NO ₄ | 327.0721 | 328.0802 | + | + | - |
| 7 | 7.086 | Cosmosiin | C ₂₁ H ₂₀ O ₁₀ | 432.104 | 433.1112 | + | + | + |
| 8 | 7.113 | Luteoline | C ₁₅ H ₁₀ O ₆ | 286.0471 | 269.0435 | + | + | + |
| 9 | 7.297 | Propanoicacid,2-hydroxy-3-[(4-hydroxy-1 naphthalenyl)oxy]- | C ₁₃ H ₁₂ O ₅ | 248.0695 | 271.0587 | + | + | + |
| 10 | 7.444 | Carboxyltolmetin | C ₁₅ H ₁₃ NO ₅ | 287.0773 | 270.0747 | + | - | - |
| 11 | 7.471 | S-(4-Nitrobenzyl) glutathione | C ₁₇ H ₂₂ N ₄ O ₈ S | 442.1122 | 447.0904 | + | + | + |
| 12 | 7.59 | Chlortetracycline | C ₂₂ H ₂₃ Cl N ₂ O ₈ | 478.11 | 461.1063 | + | + | + |
| 13 | 7.646 | Hesperetin | C ₁₆ H ₁₄ O ₆ | 302.0777 | 285.0745 | + | + | + |
| 14 | 7.724 | Ginkgolide J | C ₂₀ H ₂₄ O ₁₀ | 424.1377 | 447.127 | + | + | - |
| 15 | 7.793 | Indoleglycerol phosphate | C ₁₁ H ₁₄ N O ₆ P | 287.056 | 270.0519 | + | + | + |
| 16 | 7.968 | 3-(a-Naphthoxy)lactic acid glucuronide | C ₁₉ H ₂₀ O ₁₀ | 408.1064 | 431.0955 | + | - | + |
| 17 | 8.049 | Epigallocatechin | C ₁₅ H ₁₄ O ₇ | 306.075 | 311.0536 | + | - | - |
| 18 | 8.232 | 1-Phosphatidyl 1D myoinositol-3-phosphate | C ₁₁ H ₂₀ O ₁₆ P ₂ | 470.0238 | 493.0146 | + | + | - |
| 19 | 8.282 | BICUCULLINE (+) | C ₂₀ H ₁₇ NO ₆ | 367.1042 | 368.1113 | + | - | - |
| 20 | 8.388 | Eriodictyol | C ₁₅ H ₁₂ O ₆ | 288.0624 | 271.0592 | + | + | + |
| 21 | 8.545 | N-Acetylsphingosine | C ₂₀ H ₃₉ NO ₃ | 341.2939 | 346.2726 | + | - | - |
| 22 | 8.65 | Lecanoric acid | C ₁₆ H ₁₄ O ₇ | 318.0727 | 301.0692 | + | + | + |
| 23 | 9.086 | 3-Desmethyl- deshydroxy scleroiin | C ₁₄ H ₁₂ O ₄ | 244.0748 | 249.0534 | + | - | - |
| 24 | 9.09 | Thyroacetic acid | C ₁₄ H ₁₂ O ₄ | 244.0747 | 267.0639 | + | - | - |
| 25 | 10.087 | Sennidin B | C ₃₀ H ₁₈ O ₁₀ | 538.0885 | 539.0957 | + | + | - |
| 26 | 10.24 | Pholcodine | C ₂₃ H ₃₀ N ₂ O ₄ | 398.221 | 403.1994 | + | - | - |
| 27 | 10.304 | Rhapontin | C ₂₁ H ₂₄ O ₉ | 420.1399 | 403.1368 | + | - | - |
| 28 | 10.412 | Naringenin | C ₁₅ H ₁₂ O ₅ | 272.0693 | 255.0666 | + | + | + |
| 29 | 10.478 | Haematoxylin | C ₁₆ H ₁₄ O ₆ | 302.0803 | 285.0779 | + | + | - |
| 30 | 10.591 | Indoprofen | C ₁₇ H ₁₅ NO ₃ | 281.0997 | 286.0786 | + | - | - |
| 31 | 10.654 | Genkwanin | C ₁₆ H ₁₂ O ₅ | 284.0673 | 267.0641 | + | - | - |
| 32 | 12.227 | Hematoporphyrin | C ₃₄ H ₃₈ N ₄ O ₆ | 598.277 | 581.2734 | + | + | + |
| 33 | 13.025 | 9(S)-HpOTrE | C ₁₈ H ₃₀ O ₄ | 310.2133 | 293.21 | + | - | - |
| 34 | 13.194 | Kanamycin | C ₁₈ H ₃₆ N ₄ O ₁₁ | 484.2374 | 507.2266 | + | + | + |
| | 14.073 | 5S-hydroxy-hexadecanoic acid | C ₁₆ H ₃₂ O ₃ | 272.2363 | 295.2254 | + | - | - |
| 35 | 14.234 | 11-hydroxy palmitic acid | C ₁₆ H ₃₂ O ₃ | 272.2361 | 277.2147 | + | + | - |
| 36 | 14.594 | 13-HOTE | C ₁₈ H ₃₀ O ₃ | 294.2183 | 277.2148 | + | - | - |
| 37 | 15.162 | Ambroxol | C ₁₃ H ₁₈ Br ₂ N ₂ O | 375.9812 | 358.9781 | + | - | - |
| 38 | 16.509 | GPA(18:0/22:0)[U] | C ₄₃ H ₈₅ O ₈ P | 759.579 | 782.5679 | + | - | - |
| 39 | 19.043 | Trandolapril glucuronide | C ₃₀ H ₄₂ N ₂ O ₁₁ | 606.2826 | 607.29 | + | + | + |
| 40 | 19.712 | 3alpha,6beta,7alpha-trihydroxy -5beta-cholan-24-oic Acid | C ₂₄ H ₄₀ O ₅ | 408.2863 | 391.283 | + | + | + |
| 45 | 0.802 | Sulfabenzamide | C ₁₃ H ₁₂ N ₂ O ₃ S | 276.0545 | 299.0442 | - | + | - |
| 46 | 1.226 | 3,7-Epoxy caryophyllan-6-one | C ₁₅ H ₂₄ O ₂ | 236.174 | 241.1527 | - | + | - |
| 47 | 1.939 | Carisoprodol | C ₁₂ H ₂₄ N ₂ O ₄ | 260.1719 | 243.1686 | - | + | - |
| 48 | 2.327 | Pantoic acid | C ₆ H ₁₂ O ₄ | 148.0745 | 171.0636 | - | + | - |
| 49 | 3.238 | Cisapride | C ₂₃ H ₂₉ Cl FN ₃ O ₄ | 465.1823 | 466.1893 | - | + | - |
| 50 | 5.046 | Apin | C ₂₆ H ₂₈ O ₁₄ | 564.1462 | 565.1533 | - | + | - |
| 51 | 5.385 | 4-(2-hydroxypropoxy)-3,5-dimethyl-Phenol | C ₁₁ H ₁₆ O ₃ | 196.111 | 219.1001 | - | + | - |
| 52 | 5.642 | Ketoconazole | C ₂₆ H ₂₈ C ₁₂ N ₄ O ₄ | 530.1417 | 531.1478 | - | + | + |
| 53 | 5.845 | Cefamandole nafate | C ₁₈ H ₁₈ N ₆ O ₅ S ₂ | 462.079 | 463.0853 | - | + | + |
| 54 | 5.936 | 7-Epiloganin tetraacetate | C ₂₅ H ₃₄ O ₁₄ | 558.1952 | 563.1737 | - | + | - |
| 55 | 5.945 | Naphthyl glucuronide | C ₁₆ H ₁₆ O ₇ | 320.0881 | 303.085 | - | + | - |
| 56 | 6.02 | Ethanesulfonic acid, 2- [[[(3a,5b,7a,12a)-3,7Dihydroxy -24-oxo-12-(sulfooxy) cholan 24yl]amino] | C ₂₆ H ₄₅ NO ₁₀ S ₂ | 595.2486 | 600.227 | - | + | - |
| 57 | 6.381 | 4'-Hydroxyfenoprofen glucuronide | C ₂₁ H ₂₂ O ₁₀ | 434.1192 | 417.1158 | - | + | - |
| 58 | 6.587 | Hieracin | C ₁₅ H ₁₀ O ₇ | 302.0419 | 285.0382 | - | + | - |
| 59 | 6.64 | iodovulone I | C ₂₁ H ₂₉ O ₄ | 472.122 | 477.1007 | - | + | - |
| 60 | 6.742 | Naringenin-7-O glucoside | C ₂₁ H ₂₂ O ₁₀ | 434.1201 | 417.1166 | - | + | + |
| 61 | 6.743 | Koparin 2'-methyl ether | C ₁₇ H ₁₄ O ₆ | 314.0777 | 297.0745 | - | + | - |
| 62 | 6.744 | Oxotolrestat | C ₁₆ H ₁₄ F ₃ NO ₄ | 341.086 | 342.095 | - | + | + |
| 63 | 6.905 | Methyl 7-Deshydroxy pyrogallin-4-carboxylate | C ₁₃ H ₁₀ O ₆ | 262.0487 | 285.0378 | - | + | - |
| 64 | 6.997 | Pentahydroxy flavanone | C ₁₅ H ₁₂ O ₇ | 304.0569 | 287.0535 | - | + | + |
| 65 | 7.345 | Veratricacidglucuronide | C ₁₅ H ₁₈ O ₁₀ | 358.0909 | 363.0696 | - | + | - |
| 66 | 8.154 | Aesculin | C ₁₅ H ₁₆ O ₉ | 340.0804 | 345.0591 | - | + | - |
| 67 | 8.155 | Chlorogenic acid | C ₁₆ H ₁₈ O ₉ | 354.0957 | 377.085 | - | + | - |
| 68 | 8.723 | Acetylaminodantrolene | C ₁₆ H ₁₄ N ₄ O ₄ | 326.1008 | 331.0794 | - | + | - |

| | | | | | | | | |
|-----|--------|--|--|----------|----------|---|---|---|
| 69 | 8.895 | Rhamnetin | C ₁₆ H ₁₂ O ₇ | 316.0565 | 299.0531 | - | + | + |
| 70 | 9.049 | Dihydrodeoxy streptomycin | C ₂₁ H ₄₁ N ₇ O ₁₁ | 567.2869 | 568.294 | - | + | - |
| 71 | 9.893 | 2,3,4-Trihydroxy-4-Methoxy benzophenone | C ₁₄ H ₁₂ O ₅ | 260.0693 | 283.0582 | - | + | - |
| 72 | 13.677 | 2,4-dimethyl-tetradecanoic acid | C ₁₆ H ₃₂ O ₂ | 256.2412 | 279.2304 | - | + | + |
| 73 | 14.52 | GPEtn(10:0/11:0)[U] | C ₂₆ H ₅₂ NO ₈ P | 537.3404 | 279.2304 | - | + | - |
| 74 | 15.039 | 9,13-dihydroxy-11 octadecenoic acid | C ₁₈ H ₃₄ O ₄ | 314.2441 | 297.2408 | - | + | - |
| 75 | 17.698 | Khayanthone | C ₃₂ H ₄₂ O ₉ | 570.2837 | 593.2728 | - | + | - |
| 76 | 19.523 | 23-methyl 5Z,9Z tetraacosadienoic acid | C ₂₅ H ₄₆ O ₂ | 378.3504 | 401.3397 | - | + | - |
| 77 | 3.231 | 3-Indolylactic acid | C ₁₁ H ₁₁ NO ₃ | 205.0727 | 188.0693 | - | - | + |
| 78 | 4.032 | p-Hydroxy phenyllactate | C ₉ H ₁₀ O ₄ | 182.057 | 165.0536 | - | - | + |
| 79 | 5.171 | Metrapone | C ₁₄ H ₁₄ N ₂ O | 226.1095 | 209.1063 | - | - | + |
| 80 | 5.63 | Fluvoxamine acid | C ₁₄ H ₁₇ F ₃ N ₂ O ₃ | 318.1214 | 341.1118 | - | - | + |
| 81 | 5.855 | 17-phenyl-trinor PGF2 alpha | C ₂₃ H ₃₂ O ₅ | 388.2215 | 463.0849 | - | - | + |
| 82 | 6.475 | Salsalate | C ₁₄ H ₁₀ O ₅ | 258.0518 | 241.0485 | - | - | + |
| 83 | 6.539 | Mefenamic acid Metabolite (b-D-Glucopyranuronic acid, 1-[2[[3(hydroxymethyl)-2 methylphenyl]amino] | C ₂₁ H ₂₃ NO ₉ | 433.1378 | 456.1269 | - | - | + |
| 84 | 6.922 | Rosmarinic acid | C ₁₈ H ₁₆ O ₈ | 360.0837 | 343.0802 | - | - | + |
| 85 | 7.022 | Acebutolol | C ₁₈ H ₂₄ N ₂ O ₄ | 336.2057 | 359.1948 | - | - | + |
| 86 | 7.14 | Normorphine 3-glucuronide | C ₂₂ H ₂₅ NO ₉ | 447.1533 | 470.1424 | - | - | + |
| 87 | 7.179 | Leukotriene E4 | C ₂₃ H ₃₇ NO ₅ S | 439.24 | 440.247 | - | - | + |
| 88 | 7.646 | 5-Nitro-2-Phenylpropyl amino benzoic acid [NPPB] | C ₁₆ H ₁₆ N ₂ O ₄ | 300.1122 | 305.091 | - | - | + |
| 89 | 7.841 | Dehydrorotenone | C ₂₃ H ₂₀ O ₆ | 392.1265 | 397.1041 | - | - | + |
| 90 | 7.859 | 4-Dedimethyl-6-dehydro anhydrotetracycline | C ₂₀ H ₁₈ N ₂ O ₇ | 398.1104 | 399.1178 | - | - | + |
| 91 | 9.085 | Oxfendazole | C ₁₅ H ₁₃ N ₃ O ₃ S | 315.0697 | 298.0696 | - | - | + |
| 92 | 9.495 | Epiafzelechin trimethyl Ether | C ₁₈ H ₂₀ O ₅ | 316.1319 | 339.1212 | - | - | + |
| 93 | 9.596 | Triptonide | C ₂₀ H ₂₂ O ₆ | 358.1404 | 341.1373 | - | - | + |
| 94 | 11.025 | Evoxine | C ₁₈ H ₂₁ NO ₆ | 347.1379 | 370.1271 | - | - | + |
| 95 | 11.132 | 13R-hydroxy 9E,11 Zoctadecadienoic acid | C ₁₈ H ₃₂ O ₃ | 296.2342 | 279.2308 | - | - | + |
| 96 | 11.185 | Deoxysappanone b 7,3'- dimethyl ether acetate | C ₂₀ H ₂₀ O ₆ | 356.1267 | 361.1054 | - | - | + |
| 97 | 11.619 | Mitoxantrone | C ₂₂ H ₂₈ N ₄ O ₆ | 444.2032 | 445.2102 | - | - | + |
| 98 | 11.77 | 18-Oxocortisol | C ₂₁ H ₂₈ O ₆ | 376.1898 | 381.1682 | - | - | + |
| 99 | 12.089 | Avocadene acetate | C ₁₉ H ₃₆ O ₄ | 328.2621 | 351.2516 | - | - | + |
| 100 | 12.397 | 18-Hydroxycortisol | C ₂₁ H ₃₀ O ₆ | 378.2053 | 383.1839 | - | - | + |
| 101 | 13.218 | Normeperidine | C ₁₄ H ₁₉ NO ₂ | 233.143 | 238.1217 | - | - | + |
| 102 | 15.371 | (Z)-2-tetracos-15- enamidoethanesulfonic acid | C ₂₆ H ₅₁ NO ₄ S | 473.349 | 496.3378 | - | - | + |
| 103 | 26.633 | 14-hydroxy-5Z-tetradecenoic acid | C ₁₄ H ₂₆ O ₃ | 242.1862 | 247.165 | - | - | + |

+ Presence of the compound, - Absence of compound, R-root, S-stem, L- leaf

Substitution of underground parts with aerial parts have been studied earlier in other medicinal root species like *Aegle marmelos* [12] and *Premna latifolia* [13]. From the present phytochemical study of various part of *O. indicum*, it was clear that a good extend of similarity exist for root and stem rather than leaves. The GC-MS and HR-LCMS chromatograms of root and young stem revealed similar peaks. The principal compounds oroxylin and chrysin were present both in root and stem, almost in similar quantity. This report is the first of its kind which reveals a GCMS and LCMS profile of root stem and leaf of *O. indicum*. LC-MS based phytochemical profiling can also be used for checking the authenticity of crude drugs available in market.

Conclusion

Majority of the bioactive compounds present in the root of *O. indicum* are present in the stem too. Thus it can be concluded that young stem of *O. indicum* can be effectively substituted for its roots in Ayurvedic formulations. In leaf the principle compound Oroxylin is present in small quantity, but Chrysin is below detectable level. So leaf do not form a suitable substitute for root.

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