Abstract
Xanthine oxidase is a highly versatile enzyme that is widely distributed among different Indigofera linnaei Linn. is a potential folklore medicinal plant (Fabaceae) used for Ayurveda and Siddha systems of medicine. In this study Alkaloids, Carbohydrate Glycoside, Saponin, Flavonoids, tannins and Phytosteroids were identified as the major phytochemical constituents in the methanol, acetone and toluene fractions of Indigofera linnaei Linn. leaf extract. Their structures were elucidated, on the basis of GC-MS data. 2,4,6-Octanerione (9.24%), 4, (methyl cyclopropyl)-1-butene(8.87%), non-ionic acid methyl ester(5.78%), trans-N-methyl-3-oxo-5,6-dimethoxy morphian (9.63%). (IRS, 2SR) 2-Dimethyl (Phenyl) silylpentane-3-ol (7.39%), 2, 2-bis (t-phenyl 3, 4" dimethyl phosphate) (5.84%), 2-cyclopropylenetic acid(6.49%) these different active phytochemicals have been found to possess a wide range of activities. In conclusion Indigofera linnaei Linn. contains biologically active compounds that may serve as candidate for the discovery of new drugs in the treatment of antimicrobial activities.

Keywords: GC-MS, Phytochemicals, Indigofera linnaei Linn., antimicrobial activities
ovate to orbicular, 3-4.5 × 2.5-4 mm, outside hairy; wings 3-4 × 1-1.5 mm, glabrous, margin shortly ciliate; keel 3-4 × ca. 1.5 mm, glabrous, margin shortly ciliate, lateral spur ca. 0.5 mm. Stamens 2.5-4 mm; anthers glabrous.

2. Materials and Methods

2.1 Collection of plant material: The leaves of *Indigofera linnaei* Linn. were collected from the Bharadhidasan university herbarium, Thiruchirappalli, Tamil Nadu, India. They were identified and authenticated by the Bharadhidasan university herbarium, Trichirappalli, Tamil Nadu, India.

2.2 Preparation of powder and extract: Leaves of *Indigofera linnaei* Linn. (500g) was shade dried, powdered and extracted with ethanol for 8 hours using soxhlet apparatus. The extract was then filtered through Whatmann filter paper No.41 along with 2g sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate is wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and reduce the volume to 1ml. The extract contains both polar and non-polar phytocomponents.

2.2 GC-MS Analysis: The GC-MS analysis of *Indigofera linnaei* Linn. powder leaves extract with absolute alcohol, was performed using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary column (5% phenyl 95% dimethyl polysiloxane) (30nm X 0.25mm ID X 0.25µmdf) and mass detector turbomass gold of the company which was operated in EI mode. Helium was the carriers gas at a flow rate of 1ml/min. and the injector was operated at 290ºC and the oven temperature was programmed as follows; 50ºC at 8ºC/min to 200ºC (5min) at 7ºC/min to 290ºC(10min).

2.3 Identification of components: Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST), WILEY8, FAME having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the (NIST), WILEY8, FAME library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. Results and Discussion

3.1 Ultra Violet - Visible Spectroscopy: The plant sample extracts of two solvents (methanol and toluene) has been taken for UV-vis study.

The plant extracts of methanol and toluene is been tested for UV-vis spectrum. The principle of UV-spectral analysis is for separation of functional group and electron transition compound respectively. The functional group of active compounds by UV-visible spectrum by position of peak values ranges from 404.17 to 666.27 in methanol extract and 412.12 to 669.96 in toluene extract.

| S. No. | Methanol  | Toluene  |
|--------|-----------|----------|
| 1.     | 404.17    | 412.12   |
| 2.     | 534.04    | 534.60   |
| 3.     | 607.76    | 609.31   |
| 4.     | 666.27    | 669.96   |

3.2 Fourier Transformed Infrared: Performing the next advanced phytochemical analysis technique of FTIR the presence of various functional groups of different compounds was found.

| S. No. | Methanol |
|--------|----------|
| 1.     | 3819.71  |
| 2.     | 3937.71  |
| 3.     | 3410.62  |
| 4.     | 2925.22  |
| 5.     | 2362.12  |
| 6.     | 2134.44  |
The FTIR method is the radiation passed through sample to be separated the functional group of compounds, the FTIR analysis is done in methanolic extract, the peak area ranges from 3819.17 to 621.03.

The FTIR and UV spectrum was used to identify the functional group of the active components based on the peak values in the infrared radiation. The methanol extract of *G. kollimalayanum* was passed through FTIR, the functional groups of the components were separated based on its peak ration and the same was passed into UV spectroscopy for electron transition of compounds.

The FTIR analysis confirmed the presence of the carboxylic acid, and Alkenes-CH₂, CH₃, Aromatic streching which shows major peaks at 1019.87 and 2922.33 etc. (Yuvarajan et al.).

### 3.3 Gas Chromatography Mass Spectrometry:

The plant sample taken on subjecting to the GC-MS provided the result of different peaks determining the presence of 19 compounds it’s found that most of compounds showed various therapeutic properties revealing its medicinal properties.

The GC-MS analyses of 19 bioactive compounds were identified in the methanolic extracts of *I. linnaei* Linn. they were 2,4,6-Octanerione, 4, (methyl cyclopropyl)-1-butene, non-ionc acid methyl ester, trans-N-methyl-3-oxo-5,6-dimethoxy morphian. (IRS, 2SR) 2-Dimethyl (Phenyl) silylpentane-3-ol, 2,2-bis (t-phenyl 3,4” dimethyl phosphate), 2-cyclopropylenetic acid etc. has the following peak areas.

#### Table 3: Phytocompounds identified in the *Indigofera linnaei* Linn. whole plant extract (GC-MS study).

| S.No. | RT  | Name of Compound | M     | MW     | Peak Area |
|-------|-----|------------------|-------|--------|-----------|
| 1.    | 5.33| 2,4,6-Octanetion-E | C₈H₁₂O₃ | 156    | 3.99      |
| 2.    | 18.18| 4-Methyl Cyclopropyl-1-butene | C₄H₁₄ | 110    | 2.62      |
| 3.    | 20.99| Nonanoic acid, methyl ester | C₁₀H₂₀O₂ | 172    | 7.86      |
| 4.    | 24.55| Trans-N-Methyl-3-Oxo,5,6-dimethoxy morphian | C₁₀H₁₅O₃ | 315    | 4.17      |
| 5.    | 27.08| (1RS, 2SR)-2-methyl (Phenyl) silylpentane-3-ol | C₁₃H₂₂O₅Si | 222    | 3.67      |
| 6.    | 27.51| 2,2.bis [t-phenyl-3”-4”-dimethyl phosphate) | C₂₃H₂₃N₄O₂ | 438    | 2.88      |
| 7.    | 27.90| 2-Cyclopropylacetic acid | C₆H₁₂O₂ | 100    | 6.67      |
| 8.    | 28.26| 5-a-androst-16-en-3-ol-[t-butyl]dimethylsily] ether | C₂₂H₄₄O₅ | 388    | 4.59      |
| 9.    | 28.90| t-Butyl [(4-methylprop)-2,5-dioximidazolidin-4-yl] methyl carbonate | C₁₃H₂₅N₃O₄ | 285    | 2.70      |
| 10.   | 32.04| 2,2,Dimethyl-3-hydroxy Propyl 2,2-dimethyl butonate | C₁₁H₂₂O₃ | 202    | 2.06      |
| 11.   | 32.83| Dichloroquinolin-8-olataluminium (3) | C₄H₆ACl₂NO | 241    | 2.97      |
| 12.   | 33.59| 2-[2-bromo-4-(1-methyl ethyl) phenyl]amino-5[6-(3-pyridinyl) hexyl] Pyridine | C₁₂H₉BrN₅ | 451    | 2.04      |
| 13.   | 35.24| 2-tert-butoxy-3-methyl-5-(trimethylsilyl) Cyclohexa-2,5-diene-1,4-dione | C₁₂H₂₂O₆Si | 266    | 2.19      |
| 14.   | 36.54| Diethyl-2,6-dimethyl-4-(3-Pyridazinyl) 1,4-dihydropyrindine-3,5-carboxylate | C₁₇H₂₁N₃O₄ | 331    | 2.33      |
| 15.   | 39.28| N-(tert-Butoxycarbonyl)-2-(4-methoxyphenyl)allylanine | C₁₅H₁₃NO₃ | 263    | 2.45      |
| 16.   | 41.88| (5a, 6a)4,5-Epoxy-6-acetoxy-17b hydroxyl-17-cyclopropymethyl-3a- pthalimidomorphinan | C₃₉H₅₀N₄O₆ | 514    | 4.00      |
S.No. | RT  | Name of Compound                                                                 | M                  | MW     | Peak Area |
|------|-----|----------------------------------------------------------------------------------|--------------------|--------|-----------|
| 17.  | 42.11 | 7,16-Dichloro-7,16-di(phenyl sulfinyl) diicosane                                      | C_{34}H_{52}Cl_{12}O_{2}S | 626   | 1.81      |
| 18.  | 43.05 | (2S, 3S) - 2, 3 - Epoxy-l-hexanol                                                   | C_{6}H_{12}O_{2}     | 116   | 2.80      |
| 19.  | 44.47 | (1R*, 2R*, 6S*)-2-(tert-Butyldimethysiloxy)-6,9,9-trimethyl bicycle [4.2.1] no, nun-8-one | C_{18}H_{34}O_{2}Si | 310   | 7.15      |

Table 4: Preliminary phytochemical activities of *Indigofera linnaei* Linn

| S. No. | Name of Compound                                                   | Nature of Compound Group | Activity                                                                 |
|--------|-------------------------------------------------------------------|--------------------------|-------------------------------------------------------------------------|
| 1.     | 2,4,6-Octanetrion-E                                               | Polyketone               | Antiinflammatory response, provides functional support against leukemia |
| 2.     | 4-Methyl Cyclopentyl-1-butene                                      | Alkane                   | Promotes growth reduction of mutation rate, antitoxicity against compounds. |
| 3.     | Nonanoic acid, methyl ester                                       | Ester                    | The antimethylthia properties                                           |
| 4.     | Trans-N-Methyl-3-Oxo-5,6-dimethoxy morphian                        | Alkene                   | Antitumour activity.                                                    |
| 5.     | (1RS, 2SR)-2-methyl (Phenyl) silylpentane-3-ol                     | Pentane                  | Cytotoxicity and efficacy of allergenic extracts                         |
| 6.     | 2,2, bis [1-phenyl-3"-4"-dimethyl phosphate)                       | Alkene                   | Antidiabetic activity.                                                  |
| 7.     | 2-Cyclopropylacetic acid                                          | Propionic acid           | Tumour and antiseptic activity on lesion of skin                         |
| 8.     | 5-a-androst-16-en-3-ol-[t-butyl]dimethylsily] ether                | Ether                    | Anti-leper against the skin                                             |
| 9.     | t-Butyl [(4-methylprop)-2,5-dioximidazolidin-4-yl] methyl carbonate | Propane                  | Provides control liver damage against antiseptic activities              |
| 10.    | 2,2,Dimethyl-3-hydroxy Propyl 2,2-dimethyl butonate                | Butane                   | Anticancerous activity shows presence of compounds.                      |
| 11.    | Dichloroquinolin-8-olatoaluminium (3)                             | Ketone                   | Anti-tumour activity                                                    |
| 12.    | 2-[2-bromo-4-(1-methyl ethyl phenyl]amino-5[6-(3-pyridinyl) hexyl] Pyridine | Aldehyde             | Antihyperplasmic activity of growth reduction in intestinal enzymes.    |
| 13.    | 2-tert-butoxy-3-methyl-5-(trimethylsilyl) Cyclohexa-2,5-diene-1,4-dione | Isohexobutane          | Antioheoplastic activity                                                 |
| 14.    | Diethyl-2,6-dimethyl-4-(3-Pyridazinyl) 1,4-dihydropyridine-3,5-carboxylate | Diethylyl butane       | Control hypersensitive reaction                                          |
| 15.    | N-(tert-Butoxycarbonyl)-2-(4-methoxypheny)allylamine                | Allyl amine butane       | Phytocompound having liver susceptibility of reactions                   |
| 16.    | Cyclopropane                                                       | Antiallergenic reactions. |                                                          |
3.4 Phytochemical Studies

3.4.1 Preliminary Phytochemical Analysis: Qualitative phytochemical studies of different extracts of leaves of *Indigofera linnaei Linn.* were performed on its alcoholic and water extracts to identify its Alkaloid, Carbohydrate and Glycoside, Saponin, Protein & Amino acid, Phenolic compounds & Flavonoids and Phytosterols by using suitable chemicals and reagents (Table 2). Alkaloid test results of leaf showed slightly positive in all four tested reagents. Qualitative phytochemical studies of Carbohydrate & Glycoside showed a good characteristic colour and precipitate in all five tested reagent. Slight presence of Saponin was confirmed by foam test in leaf in all extracted solvents. Protein and amino acid was found absent in all tests. However in Millon’s test alcoholic extract showed slight presence of protein. Phenolic compounds and Flavonoids were abundantly present in all the extracts. However alkaline test showed the moderate result in comparison to other two tests. Libermann-Burchards test showed slight presence of phytosterol in all the extracts. The above qualitative phytochemical screening showed that the whole plant is a rich source of Glycosides, Phenols & Flavonoids. However, presence of protein and alkaloids is limited in leaves.

| S. No. | Name of Compound | Nature of Compound Group | Activity |
|--------|-------------------|--------------------------|----------|
| 17.    | 7,16-Dichloro-7,16-di(phenyl sulfinyl) diocosane | Sulphohydroxydine | Antitumour activity |
| 18.    | (2S, 3S) - 2, 3 - Epoxy-1-hexanol | Epoxyhexane | Antiinflammatory response against skin lesions |
| 19.    | (1R*, 2R*, 6S*)-2-(tert-Butyldimethylsilox)-6,9,9-trimethyl bicycle [4.2.1] no, nun-8-one | Methyl butane | Anti anaesthetic properties. |

Table 5: Qualitative Phytochemical Screening of leaves of *Indigofera linnaei Linn.*

| Phytochemical test          | Cold Maceration | Sohxalation |
|-----------------------------|-----------------|-------------|
| **1. Alkaloids**            |                 |             |
| Mayer’s test                | +               | -           | +           |
| Wagner’s test               | +               | -           | +           |
| Hager’s test                | +               | +           | -           |
| Dragendorff’s test          | +               | +           | +           |
| **2. Carbohydrates & Glycosides** |             |             |
| Molish’s test               | +++             | +++         | +++         |
| Fehling’s test              | +++             | +++         | +++         |
| Barfoed’s test              | +++             | +++         | +++         |
| Benedict’s test             | +++             | +++         | +++         |
| Borntrager’s test           | +++             | +++         | +++         |
| **3. Saponins**             |                 |             |
| Foam test                   | +               | +           | +           |
| **4. Proteins & amino acid**|                 |             |
| Millon’s test               | -               | -           | -           |
| Biuret’s test               | -               | -           | -           |
| Ninhydrin test              | -               | -           | -           |
| **5. Phenolic compounds & flavonoids** |             |             |
| Ferric chloride test        | ++              | +++         | +           |
| Lead acetate test           | ++              | +++         | +           |
| Alkaline test               | ++              | ++          | +           |
| **6. Phytosterol**          |                 |             |
| Libermann-Burchard’s test   | +               | +           | +           |

- Negative; +, Slight; ++, Moderate; ++++, Frequent;

The phytochemical screening of whole plant extract *Indigofera linnaei Linn.* revealed the presence of alkaloids, flavonoids, Phytosteroids, glycosides, carbohydrates, saponins *etc.*
The phytocompounds which exhibits the properties of antitoxic and antibacterial activity, so the plant extracts are subjected to further studies. The phytochemical analysis of the passiflora incarnate leaf extract shows the presence of tannins, alkaloids, flavonoids and carbohydrates etc. Tannins have been found to form irreversible complexes with proline rich proteins resulting in the inhibition of the cell protein synthesis (Hagerman et al.).

Conclusion:
The several secondary metabolites were present in the plant extracts of solvents methanol, acetone and toluene. The phytocompounds were alkaloids, flavonoids, glycosides, Phytosteroids, carbohydrate, saponins, tannins etc. were found in plant extracts. The UV-visible spectrum which shows the peak area having functional groups in methanol, and toluene solvents. The FTIR analysis which shows distinct peak areas of functional group. This functional groups having N-acetyl, alkene, forming of groups etc. There are 19 compounds is separated through GC-MS analysis. The 19 compounds were listed and their compounds, their nature, biological functions of that particular compounds. This GC-MS analysis which exhibits certain new compounds also but their biological properties were not found. The phytocompounds of the plant Indigofera linnaei Linn. can be detected through the qualitative, UV-Vis spectrum, FTIR-analysis and GC-MS. This detection of compound and its structure and activities will lead to the number of new drugs invention for various incurable diseases.

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