Abstract

Dental treatment has focused on assessment of treatment outcomes rather than patients experience of their disease. A better understanding from individual's point of view is needed for planning and evaluation of health interventions and allocation of resources. Patient assessments are important to understand the effects of the treatment. This assessment can be used to demonstrate the burden of periodontal disease on wellbeing of population and use resources to improve access to oral health care services. Over the last decade, patient centered evaluation tools have been developed to assess patient's subjective oral health in terms of how it affects patient’s daily activities, psychological well being and impact on individual’s quality of life. Patient-centered outcome measures provide feasible and appropriate methods for addressing patients' concerns. This review discusses the various aspects of patient centred outcome assessment for periodontal therapy.

Keywords:
Phytochemical
Antimicrobial
HR-LCMS

Introduction

Zingiberaceae is the largest monocotyledons family in India. Zingiberals group has 52 genera and 1400 species concentrated in Indo-Malaysian region of Asia. Out of these 22 genera and 121 species reported from tropical and sub tropical regions of Asia, India represents 11 species [1], distributed in south east India, North east India, Andaman and Nicobar [2]. In Maharashtra 5 species are reported along Western Ghats in Konkan, Kolhapur, Satara, Ahemadnagar, in semi evergreen forest [3]. It gets the name Zingiber from Sanskrit, meaning 'Bull’s Horn’ [4-5], not only because of their unique flavour but also because of their medicinal properties. Some of the dietary ingredients have been identified and their biological activities elucidated [6-7].

Z. zerumbet (L.) Smith, is used as an anti-inflammatory adjuvant for stomach ache, sprain, and fever. Z. zerumbet (L.) Smith has important economic properties, as the rhizome can be used as both a spice and a traditional medicine [8]. Z. zerumbet (L.) Smith is a vigorous ginger with leafy stems growing to about 1.2 m tall. It is a perennial herb with tuberous root that is normally found in the damp, shaded region of low land and hill slopes. In the midst of summer, a separate stalk starts growing out of the ground with green cone shaped bracts, which resembles that of pinecones. Within a couple of weeks, the green cone turns red and then small creamy yellow flowers appear on the cone. So it is known as 'pinecone ginger' in some region. However, it is most widely known as the "shampoo ginger" because of the creamy liquid substance produced in the cones [9-10].

Mass spectrometry (MS) imaging combines spatial information and molecular information for a wide range of compounds. This method can, therefore, complement the classical approaches of metabolomics studies and microscopic methhods. MS imaging is the method of scanning a sample of interest and generating images of the intensity distribution of analyte ions. In contrast to classical histochemical methods, MS imaging is a label free technique and thus, can be used without prior knowledge of the analyte. Due to this untargeted nature, hundreds of compounds can be detected simultaneously. While numerous studies have been published in the field of clinical research, plant research is a relatively new application of MS imaging which, however, offers important advantages for the detailed investigation of metabolites from complex plant tissues [11-12]. LC/TOF (time of flight)-MS analysis gives accurate mass measurements, high resolution and provides the elemental compositions of unknown peaks with more accuracy in complex matrices [13-14].

The objective of the current study was focused to investigate the antibacterial potential of crude rhizome extract and its minimum inhibitory concentration against pathogenic bacterial strains and identification of metabolites by ESI-Q-TOF-MS i.e. high resolution mass spectrometry.

Original article

Profiling volatile compounds from crude rhizome extract of Zingiber Zerumbet (L.) Smith by solid phase micro extraction and liquid chromatography-high resolution time of flight mass spectrometry

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2. Material and Method

2.1. Plant collection and preparation

Fresh rhizomes of Z. zerumbet were collected from areas of Southern Western Ghats of Maharashtra like Kolhapur and Ratnagiri districts in monsoon (September to October). Collections were identified taxonomically using Flora of Kolhapur District, Flora of British India*. Rhizomes were thoroughly washed with distilled water and cut into small pieces, shade dried at room temperature. Completely dried rhizomes were crushed into fine powder by grinder mixer.

2.2. Extraction and fractionation

Fine dried powder 25 gm of the ground rhizome material were extracted with methanol 300 ml using Soxhlet apparatus for 18 hrs and Soxhlet was evaporated to dryness at constant temperature of 72 0C at reduce pressure. The extracts were filtered through Whatman filter paper. Resulting extracts were n-hexane, chloroform, ethyl acetate and methanol. The methanol extract exhibited showed the highest activity. Hence methanol from extract was evaporated to dryness and the resulting weight of extract was 10 g. (stick and brown color) store at 4 0C for further use.

2.3. Bacterial strains selected for susceptibility

A total number of 5 bacteria (human pathogens) were selected namely Staphylococcus aureus (NCIM-5021), Salmonella typhimurium (NCIM-2501), Pseudomonas aeruginosa (NCIM-5029), Shigella flexneri (NCIM-2931), Escherichia coli (NCIM-2931). All the above mentioned bacterial strains were collected from Department of Microbiology, Vivekanand Arts Sardar Dalipisingh Commerce and Science college, Samarth Nagar, Aurangabad. These bacterial cultures were maintained in nutrient agar slants at 37 0C. Each of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37 0C.

2.4 Antibacterial assay

Antibacterial activities of methanolic rhizome extract of Z. zerumbet were determined by standard 96 well plate methods. For this method extracts of rhizomes of 2,4,6,8 and 10 l concentration were used. The efficiency of rhizomes extract was tested against five human pathogenic bacteria as Staphylococcus aureus (NCIM-502), Salmonella typhimurium (NCIM-2501), Pseudomonas aeruginosa (NCIM-5029), Shigella flexneri (NCIM-5265), and Escherichia coli (NCIM-2931). All these bacteria were maintained in nutrient agar slants at 37 0C. The MIC was calculated as the lowest concentration of the extract inhibiting the growth of bacterial strength.

2.5. Equipment and conditions

Identification of metabolites from an active sub-fraction of methanol extract was carried out at SAIF, IIT, Bombay. Samples were analyzed on a LC-ESI-Q-TOF-MS (Agilent Technologies 6550 i-Funnel) system equipped with a G4220B pump, G4226A auto sampler and G1316C, and a diode array detector (DAD). The elution solvent consisted of a gradient system of 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.3 ml/min. The gradient system started with 95% A: 5% B reaching 5% A: 95% B in 50 min, then back to initial composition 95% A: 5% B in 10 min which was held at same composition for 5 min. The MS analysis was carried out by ESI positive ionization mode. MS source conditions were as follows: capillary voltage 3500 V, Gas temperature 250 0C, drying gas flow 13 L/min, sheath gas temp 300, sheath gas Flow 11, nebulizing gas pressure 35 (psig), fragmentor 175 V, Skimmer 65 V, Octopole RF Peak 750 V, and mass range m/z 50–1000. The resolution was 40,000 FWHM. Metlin database was used to structure confirmation.

3. Result discussion

Table 1 shows the antibacterial activity of methanolic crude extract from rhizome of Z. zerumbet. All antibacterial activities observed varied with the type of test organism. The result indicated that extract were more effective in inhibiting Gram positive bacteria than Gram negative bacteria. The bacterium Staphylococcus aureus shows maximum inhibition at Salmonella typhimurium, Pseudomonas aeruginosa, Escherichia coli and Shigella flexneri. Thus result indicated that methanolic extract was found significant inhibitor for bacterial strains and inhibited Staphylococcus aureus at lower concentration while Escherichia coli and Shigella flexneri at higher concentration. It means that Gram positive bacteria are more sensitive as compared to Gram negative bacteria. Thus methanolic extract was found to be potential antibacterial agent against human pathogenic bacteria. The earlier findings also supports this view that organic extracts are more susceptible than aqueous extract. The methanolic extract was screened for phytochemical analysis by the standard methodology given by Harborne. It revealed the presence of carbohydrates, glycoside, saponin, phenols, terpenoids and absence of tanins and alkaloids.

<table>
<thead>
<tr>
<th>Z. zerumbet</th>
<th>P. aeruginosa</th>
<th>S. typhimurium</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>S. flexneri</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.58</td>
<td>1.61</td>
<td>2.00</td>
<td>1.18</td>
<td>2.00</td>
</tr>
<tr>
<td>4</td>
<td>2.00</td>
<td>1.92</td>
<td>1.92</td>
<td>2.66</td>
<td>2.90</td>
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<tr>
<td>6</td>
<td>1.02</td>
<td>0.96</td>
<td>1.69</td>
<td>1.68</td>
<td>1.64</td>
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<tr>
<td>8</td>
<td>1.68</td>
<td>1.72</td>
<td>1.02</td>
<td>1.68</td>
<td>0.82</td>
</tr>
<tr>
<td>10</td>
<td>1.18</td>
<td>1.15</td>
<td>0.98</td>
<td>1.68</td>
<td>0.82</td>
</tr>
<tr>
<td>MIC</td>
<td>6.00</td>
<td>6.00</td>
<td>10.00</td>
<td>6.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 1- Antibacterial activity of Z. zerumbet against human pathogens

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Phytochemical constituents</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizome</td>
<td>Carbohydrate</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Starch</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Saponin</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Glycoside</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Flavonoid</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Terpenoid</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Alkaloid</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Tannin</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Saponin</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Phenol</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

Grades of development: +: present; - - : absent
2.1. Identification of metabolites by ESI-Q-TOF-MS

Metabolites analysis by ESI-Q-TOF-MS revealed the presence of fatty acids, organic compounds, phenolics, alkaloids, aminopyrimidines, dipeptide and tripeptides like important metabolites (Tables 4). The major abundant metabolites identified in the Z. zerumbet ethanol extract by ESI-QTOF-MS analysis were Tamoxifen, Lecanoric acid, Oxyphencyclimine, 4-Nonylphenol, Estradiol valerate, Cedrol, Phylloquinone (Vitamin K1) of mass 371.23, 318.07, 344.20, 220.18, 356.23, 222.19 and 450.35 respectively (Fig. 1). The retention time, mass, molecular formula and the DB difference (ppm) of the major seven abundant metabolites are shown in (Table 4). The chromatogram spectra showed counts versus retention time (Fig. 2).

<table>
<thead>
<tr>
<th>Name</th>
<th>RT</th>
<th>Mass</th>
<th>Formula</th>
<th>M/Z</th>
<th>Ion</th>
<th>DB diff. (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoxifen</td>
<td>8.53</td>
<td>371.23</td>
<td>C$<em>{23}$H$</em>{20}$NO</td>
<td>354.22</td>
<td>(M+H)$^+$</td>
<td>14.7</td>
</tr>
<tr>
<td>Lecanoric acid</td>
<td>8.73</td>
<td>318.07</td>
<td>C$<em>{22}$H$</em>{20}$O$_4$</td>
<td>307.06</td>
<td>(M+H)$^+$</td>
<td>14.7</td>
</tr>
<tr>
<td>Oxyphencyclimine</td>
<td>10.53</td>
<td>344.20</td>
<td>C$<em>{22}$H$</em>{22}$NO$_2$</td>
<td>357.25</td>
<td>(M+H)$^+$</td>
<td>2.92</td>
</tr>
<tr>
<td>4-Nonylphenol</td>
<td>12.36</td>
<td>220.18</td>
<td>C$<em>{13}$H$</em>{14}$O</td>
<td>213.17</td>
<td>(M+H)$^+$</td>
<td>0.99</td>
</tr>
<tr>
<td>Estradiol valerate</td>
<td>15.56</td>
<td>356.23</td>
<td>C$<em>{23}$H$</em>{22}$O$_4$</td>
<td>361.23</td>
<td>(M+H)$^+$</td>
<td>5.27</td>
</tr>
<tr>
<td>Cedrol</td>
<td>17.49</td>
<td>222.19</td>
<td>C$<em>{13}$H$</em>{20}$O$_2$</td>
<td>225.19</td>
<td>(M+H)$^+$</td>
<td>5.27</td>
</tr>
<tr>
<td>Phylloquinone (Vitamin K$_1$)</td>
<td>21.24</td>
<td>450.35</td>
<td>C$<em>{44}$H$</em>{50}$O$_4$</td>
<td>443.33</td>
<td>(M+H)$^+$</td>
<td>14.48</td>
</tr>
</tbody>
</table>

![Fig. 2. Counts versus mass to charge (m/z) ratio](image)

**Table 4:** Major abundant metabolites from Z. zerumbet

Tamoxifen, sold under the brand name Nolvadex among others, is a medication that is used to prevent breast cancer in women and treat breast cancer in women and men [16]. It has been used for Albright syndrome [17]. Tamoxifen is used to treat infertility in women with an ovulatory disorders. It is given at days 3–7 of a woman’s cycle [18]. Tamoxifen is used to prevent or treat gynecomastia [19]. (PubchemCID: 2733526).

Lecanoric acid is a common medullary lichen depside. Lecanoric acid and its derivatives had been found to have a lot of bioactivities in previous studies [20]. Demonstrated that lecanoric acid is an inhibitor of histidine decarboxylase with extremely low toxicity; and another research indicated that a lecanoric acid analogue, exhibited the inhibitory activity on skin tumor promotion [21-22]. The potent fungitoxic activity of lecanoric derivatives. (PubchemCID: 99613).
Oxyphencyclimine is an antimuscarinic. Oxyphencyclimine is only found in individuals that have used or taken this drug. It is an anticholinergic drug (trade name Daricon) used in treating peptic ulcers. (Pubchem CID: 4642).

4-Nonylphenols, from the Latin nonus (number 9) and phenol, are a family of closely related organic compounds composed of phenol bearing a 9 carbon-tail. Nonyl phenols can come in numerous structures, all of which may be considered alkyl phenols. They are used in manufacturing antioxidants, lubricating oil additives, laundry and dish detergents, emulsifiers, and solubilizers [23]. These compounds are also precursors to the commercially important non-ionic surfactants alkylphenol ethoxylates and nonylphenol ethoxylates, which are used in detergents, paints, pesticides, personal care products, and plastics. Nonylphenol has attracted attention due to its prevalence in the environment and its potential role as an endocrine disruptor and xenoestrogen, due to its ability to act with estrogen-like activity [24]. (Pubchem CID: 67296).

Estradiol valerate, sold under the brand names Progynova, Progynon Depot, and Delestragen among others, is a medication which is used in hormone therapy such as for menopausal symptoms and in hormonal birth control [25-26]. It is also used in the treatment of prostate cancer in men [8]. It is taken by mouth or by injection into muscle. Estradiol valerate was introduced for medical use in 1954 [27-28]. Along with estradiol cypionate, it is one of the most widely used esters of estradiol [29]. (Pubchem CID: 13791).

Cedrol is a sesquiterpene alcohol found in the essential oil of conifers (cedar oil). It has also been identified in Origanum monites, a plant related to oregano [30]. Its main uses are in the chemistry of aroma compounds [31]. It makes up about 19% of cedar wood oil Texas and 15.8% of cedarwood oil Virginia [32]. (Pubchem CID: 65575).

Vitamin K is a group of structurally similar, fat-soluble vitamins that the human body requires for complete synthesis of certain proteins that are prerequisites for blood coagulation (K from Koagulation, Danish for "coagulation") and which the body also needs for controlling binding of calcium in bones and other tissues [33]. The vitamin K-related modification of the proteins allows them to bind calcium ions, which they cannot do otherwise. Without vitamin K, blood coagulation is seriously impaired, and uncontrolled bleeding occurs. Preliminary clinical research indicates that deficiency of vitamin K may weaken bones, potentially leading to osteoporosis, and may promote calcification of arteries and other soft tissues. (Pubchem CID: 65575).

Conclusions

The methanolic rhizome extract showed maximum inhibition and MIC Pseudomonas aeroginosa (NCIM-5029), Salmonella typhae (NCIM-2501), Staphalococcus aureus (NCIM-5021), Escherichia coli (NCIM-2931) and Shigella flexneri(NCIM-5265). Phytochemical screening shows the presence of carbohydrate, glycoside, saponin, phenols, terpanoids and absence of tannins and alkaloids. Therefore the active plant extract is subjected to further HRLC-MS analysis to investigate various chemical components for isolation of the therapeutic antimicrobials for detailed pharmacognosy and phytochemistry.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgment:

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[32] Susan Barclay-Nichols. swiftcraftymonkey.blogspot.com


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