Raman Spectroscopy in Phytochemical Analysis

S. L. A. Gunawardana[1], T.D.C.P. Gunasekara[2,4], N.M.S. Sirimuthu[3,5], M.A. Siriwardhene[1,4] *
[1]Department of Pharmacy and Pharmaceutical Sciences, Faculty of Allied Health Sciences, University of Sri Jayewardenepura, Sri Lanka
[2]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka
[3]Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka
[4]Center for Plant Materials and Herbal Products Research, University of Sri Jayewardenepura, Sri Lanka
[5]Center for Nanocomposite Research, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka

*** Corresponding author email address**: siriwardhene@sjp.ac.lk

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Abstract

The importance of the use of Raman spectroscopy for the rapid identification and characterization of phytochemicals present in plants were investigated in the previous studies. Raman spectroscopy offers significant advantages for the analysis of complex chemical compounds because it does not require laborious sample preparation and it is a non-destructive method. Chemometric analysis of the Raman spectral data provides details for the detection of chemical structures which are responsible for a particular therapeutic activity. The details provided in this review article demonstrates the special potential of Raman spectroscopy for the study of plant metabolites.

Keywords: phytochemicals, Raman spectroscopy, analysis, chemometric, plants

1. Introduction

Medicinal plants are important sources for the treatment of various diseases of man and other animals. Various plant parts of the medicinal plants including leaves, roots, stems, barks, rhizomes, flowers, fruits, and grains are used for the treatment of disease conditions [1, 2]. These plants play a significant role as prototype drugs which have various therapeutic activities [3]. These therapeutic activities present in plants are provided by group of compounds which is commonly known as phytochemicals [4]. Generally, phytochemicals are biologically active and naturally occurring group of compounds belonging to plant secondary metabolites which protect the plant cells from environmental hazards [5]. When the dietary intake of the plant parts which contain these phytochemical compounds is significant, they have protective action on the human health [6]. Recently the attention is paid on the identification of the chemical nature of these phytochemicals in order to distinguish different functional groups which are responsible for their therapeutic activities [7]. Many researchers have carried out on the phytochemical analysis of the plants using the techniques like thin-layer chromatography [8, 9], high-performance liquid chromatography [10, 11], and liquid or gas chromatography [12], mass spectrometry etc. [13]. Although these methods are used in the phytochemical analysis, a significant problem the scientists encounter is that these methods are highly time consuming, and they need cumbersome pre-treatment procedures. Moreover, the experimental conditions are rigorous, the instruments are expensive and sample destruction takes place [5]. Therefore, the development of rapid, reliable, robust, and non-destructive spectroscopic analytical method is of great immediate interest. Thus, the attention is paid on the use of Raman spectroscopy which is a non-destructive, non-invasive and a rapid analysis method for the analysis of phytochemicals present in plants [14, 15].

The phytochemicals present in various plant parts such as leaves, flowers, bark, roots may differ from each other. Also, the content of a specific phytochemical may differ depending on the type of plant species and plant parts with the changes in the environment they grow [16]. These phytochemicals are reported to be used in ancient medicine to gain various therapeutic effects such as anti-inflammation, anti-oxidation, anti-cancer, diuretic and hepatoprotective effects with fewer side effects [17]. Analysing various plant parts provide a complete description of the distribution of various chemical compounds in different parts of the plant. This information obtained by analysing various plant parts can be used to isolate the chemical compounds with therapeutic effects and to enhance the possible therapeutic effects given by those phytochemicals. Also, the safety and efficacy of the phytochemicals can be improved concerning their chemical structure [18].

2. Raman spectroscopy

In 1928 an Indian scientist Sir C.V. Raman and his student Sir K.S. Krishnan illuminated a beam of sunlight by a telescope and invented that the light gets inelastically

collided through the medium [19]. This phenomenon of light scattering was named as "Raman effect" [20, 21]. The irradiation of monochromatic light gives rise to two types of light scattering known as elastic and inelastic light scattering. The elastic scattering takes place at the same wavelength and inelastic scattering takes place when shift in a photon frequency is resulting in a change in wavelength. The Raman spectroscopy is based on inelastic scattering [22]. This is commonly known as Raman scattering. In Raman scattering incident photon excites from ground state to virtual energy state. The excited molecule immediately relaxes into ground state or a vibrational state. This results in photons to undergo a wavelength/frequency shift. These frequency changes are recoded as Raman shifts. Raman shifts that gain energy are known as anti-Stokes scattering while the shifts that loose energy are known as Stokes scattering.

The Raman scattering provides information about molecular structure, symmetry, electronic environment, and binding. Therefore, Raman spectroscopy is a promising source for the investigation of molecular structure [23, 24]. Not only that, but Raman spectroscopy also enables the identification of even dark, fluorescing, and weak scattering samples within seconds [25]. This is a proven technology for the quantitative and qualitative analysis of the plant research and other areas like biological systems, biomedical diagnostics, pharmaceutics etc. [26].

Today the technique of Raman spectroscopy is developed into advanced analytical techniques like Surface-Enhanced Raman Spectroscopy (SERS), Fourier-Transform-Raman Spectroscopy (FT-RS) etc. Also, Raman spectroscopic technique is combined with other spectroscopic techniques such as Raman-Infrared (IR), Raman fluorescence, and Raman -Near Infrared (NIR) which enables the advanced analysis [23, 27].

SERS is a highly sensitive technique due to its excellent enhancement effect which reach up to several borders of magnitude with respect to Raman signals [28]. In this technique the laser beams are used to excite the vibrational transitions of the molecules adsorbed onto the nanostructures. This uses two mechanisms known as Electromagnetic field enhancement (EM) and Chemical Enhancement (CE)/ Charge Transfer (CT). The collective excitation of the free electrons in the metal surface gives rise to EM mechanism while CE mechanism arises from the overlap of wave functions between molecules and metals. This includes chemical binding interactions, resonance and photon driven energy transfer [29]. As a result of these two mechanisms the Raman scattering is increased up to 108 or 1015 [30]. SERS is capable of generating information rich data from extremely low concentrations of the analytes such as microfluid systems and also in biomedical assays like DNA detection, cancer tumour detection [31].

FT-RS uses IR excitation wave lengths in 1064 nm excitation range. IR radiations are obtained using Nd:YAG (Neodymium doped Yttrium Aluminum Garnet) lasers. These reduce the fluorescence issues that occur during the analysis of some samples. This is usually obtained within 15-30 minutes. FT-RS also provides high spectral resolution and proper wavelength accuracy. This provides high quality structural information and molecular level information [32].

Raman spectroscopic imaging is another significant advancement in the analysis techniques. This contain a huge amount of data since thousands of images are recorded across numerous wave lengths in this technology. The obtained hyper spectral images contain spatial and spectral information about a sample [25]. The Raman imaging enables to reveal the information regarding the distribution of chemical components at the molecular level, and extract tissue features depending on the chemical composition. Raman imaging is also a useful technique in the fields of biological and medical research, pharmaceutical research, plant and biomass research, art and product identification, and graphene research [26, 33].

The results obtained from vibrational spectroscopies are analysed using computational quantum chemical methods. These methods provide reliable interpretations of the results and prevent leading to erroneous conclusions. One such theoretical analysis method is Density Functional Theory (DFT) calculations at the B3LYP hybrid level. This theory is used to compute molecular structures, vibrational frequencies, and the energies of chemical reactions [34].

3. Raman spectroscopy in phytochemical analysis

3.1 Raman spectroscopy in glycosides

A study has been done by Ilze Vermaak et al (2010) to quantify and map steroidal glycoside named P57 (Figure 1) in the plant Hoodia gordonii using FT-Raman spectroscopy. Generally, this is done by liquid chromatography combined with mass spectrometry (LC-MS) which is expensive. In this experiment 145 natural and cultivated plant samples were analysed using FT-Raman spectroscopy and the concentration of P57 was determined. A calibration model was developed using the Partial Least Squares projections to latent structures (PLS) algorithm. This calibration model was evaluated according to the Root Mean Square Error of Prediction (RMSEP) and correlation coefficient (R2). Orthogonal Signal Correction (OSC) pre-processed model obtained a model which predicted P57 content based on the FT-Raman spectra with a correlation coefficient (R2) value of 0.9986 and an RMSEP of 0.004%. The results show that FT-Raman spectroscopy is a more accurate and a rapid way of quantifying the P57 in the H. gordonii raw materials [35].

Harpagophytum procumbens which belongs to family Pedaliaceae is a plant that can be found in Kalahari Desert region of southwestern Africa. This plant is commonly known as "Devil's claw" or "Grapple plant". The secondary roots or tubers of Devil's claw is famous for its use for the

treatment of pain and complications of pregnancy, skin diseases [36].

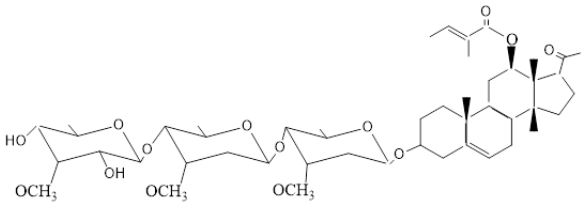

Figure. 1. Chemical structure of P57 (12-O-tigloyl-3β,12β,14β-pregn-5-en-20-one-3-O-β-D-thevetopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside)

The phytochemical studies have revealed the presence of chemicals such as harpagoside, harpagide and procumbide in this plant. A Raman quantification method of harpagoside in the plant and pharmaceuticals was developed using 150 samples of secondary roots of *H. procumbens* and 33 samples of ethanolic extract of the roots. According to the spectral analysis, the strongest band was observed at 1634 cm^{-1}. The bands at 1687, 1206 and 1001 cm^{-1} also conform the presence of harpagoside. These spectral bands can be used to detect the change in polymorphic form during the drug manufacturing process by identifying the changes in these weak bands. The characteristic bands identified in the spectral data were used to develop PLS models to determine the harpagoside content in the plant material. Using this PLS model, plant material can be classified according to the various contents of the harpagoside. Also, Raman mapping was performed on the root and the tablet samples. The results showed that the highest concentration of harpagoside was concentrated in the outer part of the secondary root and the tablet samples had a homogeneous distribution of harpagoside throughout the sample. This study confirms the use of Raman spectroscopy in the selection of high-quality plant material and detection of the change in the polymorphic form during the drug manufacturing process [37].

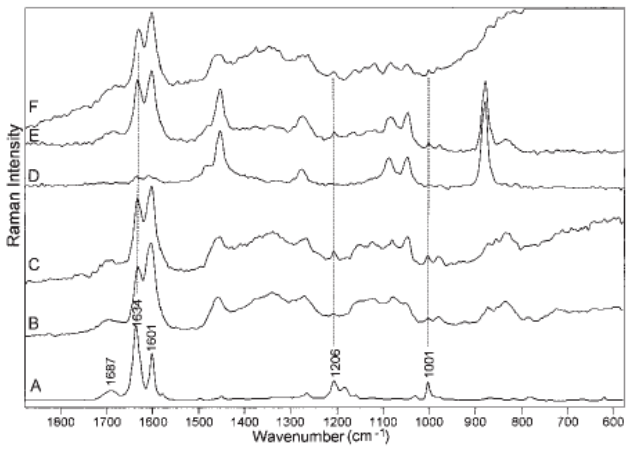

Figure. 2. FT-Raman spectra of pure harpagoside (A); freeze-dried devil's claw root with low (B) and high (C) harpagoside content; ethanolic extract with low (D) and high (E) harpagosid content; and tablet prepared from devil's claw roots (F).

3.2 Raman spectroscopy in alkaloids

Baranska and Proniewicz in 2007 investigated the use of Raman spectroscopy to directly analyse the caffeine from the plant tissue and in pharmaceuticals. The green tea leaves and guarana seeds were used as the plant sources whereas several painkillers and cordials were used as pharmaceuticals. Caffeine has its anhydrous form and hydrous form. These two forms have their characteristic peaks in the Raman spectroscopy. Anhydrous caffeine presents two intense bands at 1656 and 1698 cm^{-1} while only one peak at 1698 cm-1 is observed for the hydrated form. These characteristic peaks of caffeine in Raman spectroscopy can be used for the identification of the presence of caffeine in the plant materials and pharmaceuticals directly. The Figure 3 shows the FT-Raman spectra for the caffeine and plant drug samples. The bottom line of this figure shows the Raman spectrum of commercially bought caffeine. According to the spectrum the commercially bought caffeine is in its anhydrous form. In the spectrum, of guarana seed caffeine can be identified by the signal at 555 cm^{-1}. The occurrence of the band at 1658 cm-1 indicates that seeds contain anhydrous caffeine too. The occurrence of caffeine in the Raman spectrum of green tea leaf can be recognized by the signals at about 555, 738, 1333, 1656, 1698 cm^{-1}. Figure 4 contains the Raman analysis of two drugs, which composition differs only in the content of caffeine. APAP (acetaminophen) does not contain caffeine and APAP-Extra contain caffeine. The two drugs show several significant key bands due to the presence of main component Paracetamol. But the presence of significant band at 555 cm^{-1} in the spectrum of APAP-Extra can be assigned due to the presence of caffeine. This study demonstrated the use of Raman spectroscopy is an important tool for the rapid analysis of the plant materials as well as for the analysis of pharmaceuticals [38].

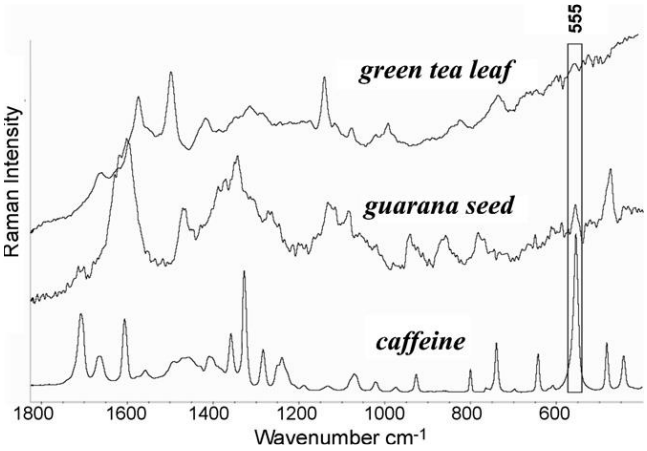

Figure. 3. FT–Raman spectra obtained from caffeine standard, guarana seed and green tea leaf

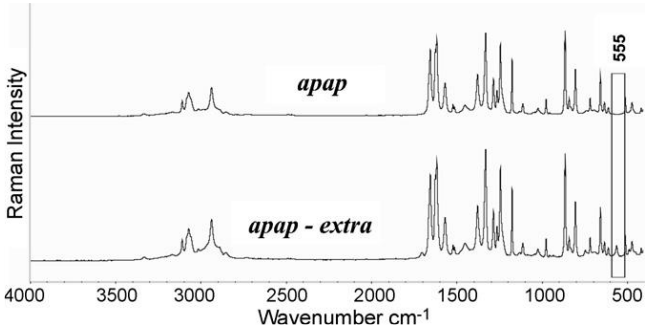

Figure. 4. FT- Raman spectra obtained from pharmaceutical drug containing paracetamol (APAP) and additionally, caffeine ('APAP-EXTRA').

Ancistrocladus heyneanus contain naphthylisoquinoline alkaloids which are synthesized as secondary metabolites in the plant. A study was done to analyze the distribution of naphthylisoquinoline alkaloids within the plant using micro-FT-Raman spectroscopy. Fresh plant materials obtained from the Botanical Garden, University of Wurzburg were used as the samples directly. The Raman spectra obtained in the range of 200–4000 cm-1 showed that the alkaloid ancistrocladine A, was present in the tip of the shoot and in the leaf midrib while ancistrocladine was found in the branch root of the plant. Raman spectra can be used to identify the distribution of the chemical compounds within the plant [39].

Hydrastis canadensis L. which is commonly known as Goldenseal is used to treat mild pathological conditions such as gastritis, colitis, loss of appetite, duodenal ulcers, and liver diseases [40]. Canadine is one of the main alkaloids present in this plant. The study was conducted to analyze alkaloid present in Goldenseal using structural and spectroscopic analysis. In the study *ab initio* Hartree-Fock (HF) and DFT employing B3LYP using 6-311++G (d,p) basis set were used for the calculations. The calculated vibrational frequencies were scaled and compared with the experimental infrared and Raman spectra. This has proved that experimental data complies with the calculated data. The structure activity relationship was mapped using the electrostatic potential surface and evaluating the reactivity descriptors. The evaluated structure was used for geometry optimization. The results have shown that the optimized structure is similar to the experimental structure. The Molecular Electrostatic Potential (MEP) Surface method was used in this study to identify the sites of relative charge distribution. This has shown that the nitrogen and oxygen atoms are the most active sites of this molecule. Natural bond orbital analysis has performed to detect the stability and hyper conjugative interactions of the molecule [41].

3.3 Raman spectroscopy in flavonoids

Flavonoids are a group of phytochemicals which are known to possess significant pharmacological activities. They mainly possess antioxidant activity [42]. A study was carried out to analyse the genistein (97%), daidzein (97%), and formononetin (98%) using FTIR and Raman spectroscopy. A full conformational analysis was performed using the spectroscopic data and density functional theory calculations (Figure 5). According to the calculations, genistein has shown a distinct chemical structure than daidzein and formononetin. The C5-OH group present in genistein was responsible for the formation of strong intramolecular interactions which leads to the formation of 6-membered intramolecular ring. The C7-OH group within the catechol moiety was necessary for the determination of the conformation of these three isoflavones. The hydroxylated isoflavone derivatives are responsible for their antioxidant properties. Detection of the structural conformation provides essential details of its biological activity [43].

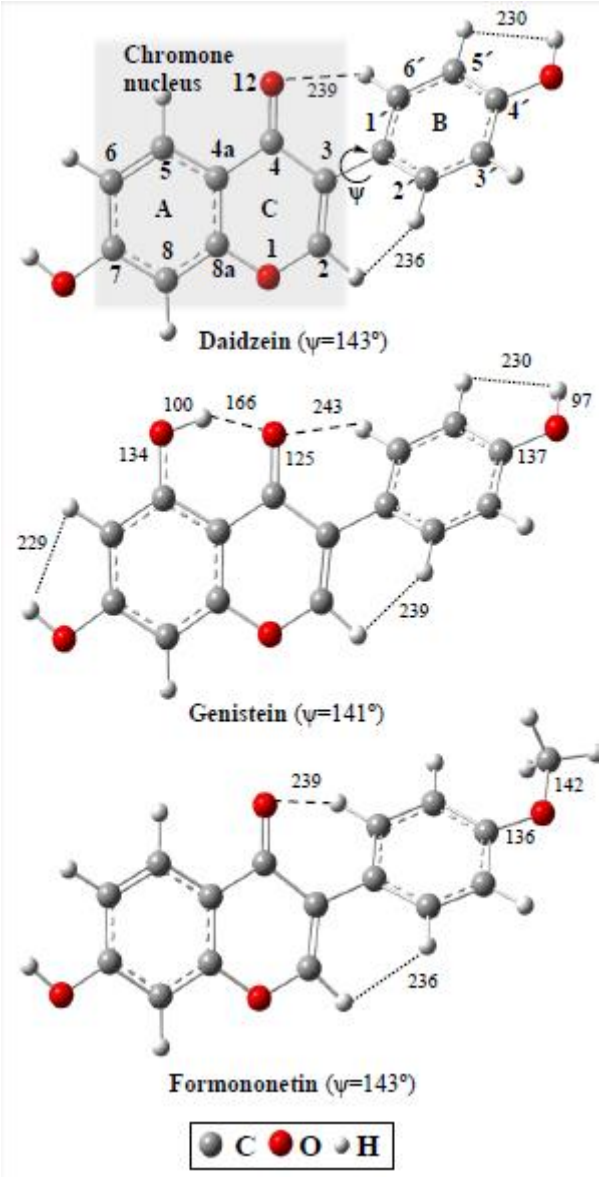

Figure. 5. Structures of Daidzein, Genistein and Formononetin

Aspalathus linearis which is commonly known as rooibos was analysed to identify and quantify the flavonoids present in the plant and to identify the distribution of the chemicals

within the plant. The leaves and the stem of the plant were collected from different areas within different years and analysed using FT-Raman spectroscopic method. The study demonstrated the presence of aspalathin, carotenoids and nothofagin. Aspalathin and nothofagin were reported to have anti-inflammatory activity [44] while carotenoids have antioxidant activity [45]. The Raman spectroscopic data was used to develop a calibration model to quantify the chemical compounds dihydrochalcone, aspalathin and nothofagin [46].

A study done in Japan has used Raman spectroscopy for the quantitative analysis of quercetin. Quercetin is a compound with many pharmacological benefits such as antioxidant, antiviral, antimicrobial, anticancer, anti-inflammatory and hepatoprotective activity [47, 48]. It was extracted from the onion peels discarded from a food factory. Raman spectrum showed characteristic peaks around 600 and 1600 cm^{-1}. An intensity ratio was calculated using the standard quercetin and the solvent using the Raman spectral data and the quantity of quercetin extracted from onion peels were determined. 73 mg/100 g and 70 mg/100 g masses of quercetins were extracted from dried onion peels by using hot ethanol and hot methanol for three hours. This conforms that the use of Raman spectroscopy for the quantitative analysis of phytochemicals provides accurate results [49].

3.4 Raman spectroscopy in terpenoids

Actea racemosa L (Black Cohosh) is used to relieve the symptoms such as hot flashes, mood disturbances and night sweats occur in menopause, and this is commonly used medicine in Western countries [50]. A new triterpene present in this plant was elucidated using 1H, 13C NMR, IR and Raman spectroscopy. The NMR studies detected that the structure of cimipodocarpaside is composed of four condensed ring skeletons in which rings A and C are 6-membered, whereas rings B and D are 7 and 5-membered. The Raman spectra was used to detect the complementary information such as C=C bonds and other fragments of the molecule. The vibrational spectroscopic data was interpreted using PED analysis of 273 fundamentals. The analysis of the chemical structure using these methods detected the structure as (24S)-3 β-hydroxy-24,25-oxiirane- 16,23-dione-9,10-seco-9,19-cyclolanost-7(8),9(11),10(19)-trien-3-O-β-D-xylopyranoside (Figure 6) [51].

The vibrational spectroscopic methods such as ATR/FT-IR spectroscopy, NIR spectroscopy and FT-Raman spectroscopy were used in a study to determine the monoterpenes and phenylpropanoids in the basil drug. The Raman spectrum showed characteristic bands at 740 cm^{-1} for thymol type 650 cm^{-1} for camphor type. The linalool type bands could be seen in 1643 and 1675 cm^{-1}. The Raman spectroscopic bands for methyl cinnamate were observed in 1717, 1638 and 1602 cm^{-1}. The data obtained from NIR spectroscopy was interpreted using chemometric methods and a calibration was developed. The study provides sufficient details for the efficient selection of basil plants and the purifying, blending and redistilling processes can be easily controlled using these details [52].

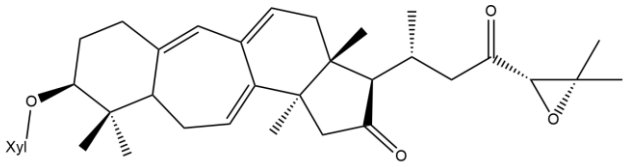

Figure. 6. Cimipodocarpaside: 3β-hydroxy-24,25-oxiirane-16,23-dione-9,10-seco-9,19-cyclolanost-7(8),9(11),10(19)-trien-3-O-β-D-xylopyranosid

3.5 Raman spectroscopy in coumestan

The leaf, stem, and root parts of the two medicinal plants *Eclipta alba* Hassk and *Eclipta prostrata* Linn were analysed in a study. FT-Raman spectroscopy at the range of 400-4000 cm^{-1} was used to analyse the plant samples. According to the results the main functional group present in both plants is wedelolactone. The presence of characteristic peaks confirmed the presence of characteristic functional groups such as carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, nitrates, chlorates, and carbohydrates. These are responsible for the specific medicinal properties given out by these herbal plants [53]

3.6 Raman spectroscopy in essential oils

In 2005 essential oils obtained from the plants belonging to Origanum, Satureja, Salvia, Sideritis, Thymus, Ziziphora, Calamintha, Lavandula and Thymbra genera were analysed using IR and Raman spectroscopy. The mid IR spectra were recorded in the range between 650 cm^{-1} and 4000 cm^{-1} with a ATR (Attenuated Total Reflection)/FT-IR spectrometer and the Raman spectra were recorded on a Nd:YAG laser at 1064 nm. The results were subjected to hierarchical cluster analysis in the range from 500 to 1800 cm^{-1} using OPUS programme (Bruker, Germany). According to the data Thymus contain mainly carvacrol, thymol, p-cymene and γ-terpinene. The spectral analysis of the data showed that *O.majorana* oil is mainly composed of carvacrol where the bands could be seen at 760 and 1623 cm^{-1} and *O.spyleum* oil composed of 41% of thymol and 35% of carvacrol. The Raman spectrum of *S. fructicosa* essential oil indicated 1,8-cineole and camphor as its main components. The bands of 645 cm^{-1} and 666 cm^{-1} indicated the presence of β-pinene and α-pinene respectively in *S. libanotica*. ATR-IR spectrum demonstrated the corresponding bands to Raman spectrum confirming the presence of the above compounds [54].

The Raman spectroscopy analysis of essential oils extracted from different citrus species demonstrated characteristic peaks. Myrcene showed a strong symmetric vibration at 1634 cm^{-1} and asymmetric vibration at 1594 cm^{-1}. The α-pinene, β-pinene and sabinene demonstrated characteristic bands at 1643-1657 cm^{-1} range. Also,

characteristic bands for limonene were detected in the peaks 758, 1435, 1644 and 1676 cm^{-1} range. A characteristic band at 1698 cm^{-1} was observed for γ-terpinene. The study confirmed that the both ATR/FT-IR spectroscopy and NIR-FT Raman spectroscopy could be used as a rapid analysis method of the citrus oils [55].

The plants oregano, thyme, dictamnus, marjoram, sage and pennyroyal of *Lamiaceae* species were collected, and the essential oils were extracted from hydrodistillation. The components of these extracts were determined by FT Raman spectrum and GC-MS (Gas Chromatography–Mass Chromatography). Characteristic peak for α-terpinene at 1611 cm^{-1}, for γ-terpinene at 1701 cm^{-1}, for thymol at 740 cm^{-1} and for carvacrol appeared at 759 cm^{-1}. The FT-Raman spectrum results showed characteristic peaks for the essential oils obtained from different sources. The essential oil obtained from sage oil showed characteristic peaks between 1500 and 1400 cm^{-1} and a strong peak at 652 cm^{-1}, while peaks at 1714 cm^{-1}, 1614 cm^{-1} and 647 cm^{-1}. According to this study it is proven that Raman spectrum of the essential oils is a fingerprint which exhibits a characteristic profile of the constituents. Therefore, the Raman spectroscopy could be used as a rapid non-destructive method for the quality control of the essential oils [56].

3.7 Raman spectroscopy in essential resins

'Dragon blood' is a natural resin used in ancient times for medicinal purposes. A study was done to analyse the resin obtained from the plants *Dracaena cinnabari L*, *Daemonorops draco*, *Eucalyptus terminalis* and sample obtained from dragon tress of Royal Botanic Garden, Edinburgh. A FT-Raman spectroscopic analysis was done to these samples and the characteristic biomarkers of dragon blood were observed in the region of 1400-1700 cm^{-1}. Also, characteristic peaks that differentiate samples obtained from each plant sample were identified in this study. This study of Edwards *et al* provide a complete FT-Raman spectral guide which can be used to differentiate the various sources of dragon's blood [57].

3.8 Raman spectroscopy in analysis of chemical composition of plants

The dried roots of *Puerariae Lobatae* Radix; (PLR) and *Puerariae Thomsonii* Radix; (PTR) are used in traditional Chinese medicine for the treatment of diarrhoea, fever, diabetes and cardiovascular and cerebrovascular diseases. A study was done to develop a method to analyse the plant samples using Raman spectroscopy. The spectral characteristics of PTR in the region of 400 to 1500 cm^{-1} are higher than PRL. Also, a significant peak was observed in 1626 cm^{-1}, which is present only in PRL. The data obtained from Rama spectroscopy was further analysed using Principal Component Analysis (PCA). Partial Least Squares-Discriminant Analysis (PLS-DA) was used to differentiate PLR from PTR from the Raman spectroscopic characteristics. PLS-DA 100% accuracy in the classification. This conforms the ability of PLS-DA to differentiate PLR from PTR using Raman spectra [58].

A study done in China has analysed several kinds of ginseng including Radix Ginseng (RG), Radix Ginseng Rubra (RGR) and Radix Panacis Quinquefolii (RPQ) and two samples of pseudo-ginsengs, Radix Codonopsis (RC) and Radix Platycodi (RP). FT-Raman spectrum was recorded at a resolution of 8 cm^{-1} in the range from 100 to 3700 cm^{-1}. From the spectral analysis the pseudo ginsengs could be differentiated correctly from the other ginsengs since their spectra were different from the others. But other ginsengs could not be analysed clearly from the spectral data since there were no characteristic bands. The cluster analysis was performed, and all the samples were clustered into four groups; white ginseng, red ginseng, American ginseng and pseudo ginseng correctly [59].

FT-Raman spectroscopic analysis of various *Panax ginseng* samples obtained from different origins have shown various similarities and differences. The ginseng obtained from China had a characteristic peak at 980 cm^{-1} was not present in the Korean and American ginseng spectra. Another peak at 1600 cm^{-1} responsible for C=C stretch was present in the American and Korean ginseng spectrum, whereas this peak was not present in the Chinese ginseng spectrum. American ginseng sample does not show any unique peaks or missing peaks. The peak of 1600 cm^{-1} can be seen in both American ginseng and Korean ginseng, while a peak at 1003 cm^{-1} can be seen in both American and Chinese ginseng. Therefore, American ginseng can be identified if both the peaks at 1600 cm^{-1} and 1003 cm^{-1} are present in the spectrum [60].

Yam is a food that is used as an important ingredient in Chinese traditional medicine. A study was conducted to determine the structures of Yam proteins obtained from *Dioscorea alata* L., *D. alata* L. var. *purpurea*, and *D japonica.* FT-Raman spectral results obtained were subjected to statistical analysis. According to Raman spectroscopic results the *D. alata* L., has shown amide I at 1662 cm^{-1} and amide III at 1263 cm^{-1} which confirms the presence of α-helix in the yam proteins. The presence of significance bands of the amide I at 1668 cm^{-1} and amide III at 1241 cm^{-1} confirmed the presence of β-sheet in the *D. alata* L. var *purpurea proteins*. Presence of amide I at 1667 cm^{-1} and amide III at 1257 cm^{-1} indicate that the *D. japonica* are in a mixed form of an α-helix and antiparallel β-sheet. According to low frequency Raman profile, different peaks have shown the presence of different amino acids. Peak of 621 cm^{-1} for phenylalanine; 643 cm^{-1}, 828 cm^{-1}, and 853 cm^{-1} for tyrosine and 759 cm^{-1} for tryptophan. The Raman ratios 0.46 for *D. alata* L., 0.79 for *D. alata* L. var. *purpurea*, and 1.03 for *D. japonica* have shown the different properties of these amino acids [61]

Near Infrared Fourier transform Raman spectroscopy was used in a study to determine the chemical components of samples of flax (*Linum usitatissimum* L.) stem and its anatomical parts. According to the results the characteristic

peaks for cellulose were observed to be prevalent in the fibers while hemi cellulosic polysaccharides in bast tissue and fibers. Peaks for waxes/fatty acid esters were mostly present in the cuticle/epidermal tissue. This provides a method of analysis for the detection of the relative amounts and location of the chemical compounds within the tissues of the flax plant [62]

A study done in Quedlinburg, Germany has analyzed pigments on differently colored flower petals of pansy cultivars (*Viola x wittrockiana* belongs to *Violaceae* family) using Fourier Transform Raman spectroscopy. For the further clarification pigment extracts obtained from the petals were separated by Thin Layer Chromatography (TLC) and analyzed using Raman spectroscopy. The results obtained were subjected to Hierarchical Cluster Analysis (HCA) using the Raman spectra of refence pigments of carotenoids, anthocyanins and flavonoids. The two-dimension Raman mapping technique provided the chemical image of the samples while the distribution and the concentration of the carotenoids, anthocyanins and flavanols were also determined using the Raman data. A satisfactory correlation was observed between the patterns seen on the visible images and the patterns on the chemical images obtained by Raman mapping [63].

A study done in Northeast India has analysed the antimicrobial activity of crude rhizome oils obtained from the plants *Curcuma amada, C. longa, Zingiber moran*, and *Z. zerumbet*. Rhizome oil was extracted using six different polar and non-polar solvents. The antimicrobial property was analysed using disc-diffusion and viability assay. The rhizome oil of all the extracts exhibited potent antimicrobial activity against all pathogenic bacterial and fungal strains tested. Water extract of *Z. moran* was found to be the highest effective antimicrobial agent. Antibacterial effect of the hydrodistilled fractions was also characterized against Gram-positive and Gram-negative bacteria using micro-Raman spectroscopy. Bacterial culture obtained from a single colony was cultured for 12 hours in liquid nutrient broth media and it was subjected to one subculture. The 6 hour grown cultures were treated with oil fractions and incubated at 28^{o}C for 12 hours at 180 rpm in an orbital shaker. The Raman spectroscopic readings were taken at 488 nm. The changes in the Raman shift of both Gram negative and positive bacteria were observed. The four oil fractions have shown characteristic Raman shifts revealing the antibacterial effect. Therefore, Raman spectroscopy can be used for the analysis of biological materials combined with phytochemical analysis [64].

4. Conclusions

Raman spectroscopy is evolving into an increasingly powerful and useful tool that can analyse variety of phytochemicals. It also determines the stoichiometric and hydration states of natural compounds. HPLC, GC, MS and other rational spectroscopic methods used in the analysis of phytochemicals are highly time consuming and needed pre-treatment procedures prior to analysis. Whereas the Raman spectroscopy provides unprocessed raw analysis of the plant materials. Hence it reduces the structural modifications likely to be happened during pre-treatment. Furthermore, the Raman spectroscopy is recently available in numerous applications with various techniques to demonstrate the structural features of complex molecules in plant analysis and pharmaceutical analysis. It has shown that Raman spectroscopy is becoming a promising tool for the fast analysis of phytochemicals derived from plants and pharmaceuticals.

Conflicts of Interest

The authors declare no conflicts of interest.

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