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Spectroscopic and E-tongue evaluation of medicinal plants: A taste of how *rasa* can be studied

Rama Jayasundar*, Somenath Ghatak

Department of NMR, All India Institute of Medical Sciences, New Delhi, 110029, India

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ABSTRACT

Background: The use of medicinal plants in Ayurveda is based on *rasa*, generally taken to represent taste as a sensory perception. This chemosensory parameter plays an important role in Ayurvedic pharmacology.

Objective: The aim is to explore the use of structuro-functional information deduced from analytical techniques for the *rasa*-based classification of medicinal plants in Ayurveda.

Materials and methods: Methods of differential sensing and spectroscopic metabolomics have been used in select medicinal plants from three different taste categories (sweet, pungent and multiple taste): *Tribulus terrestris*, *Vitis vinifera* and *Glycyrrhiza glabra* from sweet category; *Piper longum*, *Cuminum cyminum* and *Capsicum annum* from pungent group; *Embolica officinalis* with five tastes. While Electronic tongue was used for evaluation of the sensorial property of taste, the chemical properties were studied with Nuclear Magnetic Resonance (NMR), Fourier Transform InfraRed (FTIR) and Laser Induced Break-down Spectroscopy (LIBS).

Results: In terms of taste and phytochemical profiles, all samples were unique but with similarities within each group. While the sensor response in E-tongue showed similarities within the sweet and pungent categories, NMR spectra in the aromatic region showed close similarities between the plants in the sweet category. The sensory, phytochemical and phytoelemental profiles of *E. officinalis* (with five *rasa*) in particular, were unique.

Conclusion: A combination of sensorial and chemical descriptors is a promising approach for a comprehensive evaluation and fingerprinting of the Ayurvedic pharmacological parameter *rasa*.

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1. Introduction

There has been a resurgence of interest in phytochemical research not only for identifying novel lead therapeutic compounds but also for understanding how traditional systems of medicine use plants [1–5]. Ayurveda has not only a long history of usage of plants for medicinal purposes but interestingly has its own pharmacological parameters to define the therapeutic attributes of plants. A major parameter in Ayurvedic pharmacology is *rasa*, which is generally understood and translated as taste. Taste is one of the organoleptic properties known to modern chemistry. Ayurveda has

classified plants under six types of *rasa*: *madhura* (sweet), *amla* (sour), *lavana* (salty), *katu* (hot/pungent), *tikta* (bitter) and *kashaya* (astringent) [6]. In this *rasa*-based classification of plants, each plant/plant part is grouped under one or more *rasa*. Having said this, it is not clear how *rasa* of plants was identified in days of yore. For instance, whether it was inferred from observations or experiments remains a moot point.

These questions apart, modern science has brought in its wake a number of analytical tools for research in various domains of knowledge, including that of medicinal plants. It is imperative for Ayurveda to take advantage of such tools to understand its knowledge base and add onto it. Much of the current research work on medicinal plants in Ayurveda however involves phytochemical and pharmacological analyses to identify and isolate active principles [7–10]. This is despite the fact that Ayurveda uses plant samples in entirety.

Considering that plants continue to be used in their native form in Ayurvedic medicines, there is an inevitable necessity to evaluate

* Corresponding author.

E-mail address: ramajayasundar@hotmail.com (R. Jayasundar).

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medicinal plants in terms of Ayurvedic pharmacological parameters, for instance, *rasa*. At the same time, there is demand for quality control and standardization of Ayurvedic plants and medicines [11,12], which has also created a pressing need for appropriate methodologies for comprehensive characterization of plants beyond molecules. In this context objective measurement of *rasa* assumes importance.

It is true that the analytical techniques used in phytochemical studies have conventionally focused on isolated single molecules. However, methodologies like differential sensing and spectroscopic techniques like Nuclear Magnetic Resonance (NMR) can be used to study plant materials as a whole and more importantly without subjecting them to any preparatory procedures [13,14]. This study is an exploratory effort, the first of its kind in literature, to study medicinal plants using Electronic tongue (E-tongue) and spectroscopic techniques like NMR, Fourier Transform InfraRed (FTIR) and Laser Induced Breakdown Spectroscopy (LIBS), but from the perspective of Ayurvedic pharmacology. The techniques used in this study are described in brief below.

1.1. Differential sensors for whole extracts

Sensors generally convert activity of a single or group of analytes dissolved in a solution into electrical potential. Conventional sensors are selective for specific target analytes – for example, hydrogen ions in selective electrodes. On the other hand, differential sensing inspired by sensory perceptions in mammalian gustatory and olfactory systems, uses an array of non-selective sensors, which are cross-reactive [13]. In other words, each sensor responds to a number of analytes but with a variance. The response from each of the sensors to an analyte is analysed using pattern recognition algorithms to obtain a fingerprint like response.

Differential sensors coupled with multivariate signal processing are increasingly used for gaining insight into complex processes like sensory functions. For instance, taste, the end result of complex functions from sensing of molecules by taste buds to sensory perception in brain [15] can be objectively assessed by Electronic tongue (E-tongue). This instrument employs electrochemical sensors with cross selectivity to study a wide spectrum of taste associated metabolites in a complex sample matrix, and uses multivariate analysis for data interpretation [16], [17]. This technique is used in food and pharmaceutical sectors for quality control and testing bitterness of drugs, respectively [17–19]. The non-specificity of the differential sensors used in E-tongue mimics taste perception in humans and make them ideal tools to evaluate *rasa*, important in Ayurvedic pharmacology.

1.2. Spectroscopic metabolomics

Spectroscopy plays a major role in phytochemical characterization of medicinal plants. Techniques such as NMR, Ultraviolet (UV) and FTIR are used widely in phytochemistry. Each technique uses different frequencies of the Electromagnetic spectrum to excite the samples and provide information on different aspects of their chemical nature [20]. Metabolomics is an approach to study complex systems [21], where metabolome represents collection of all metabolites in a sample. Spectroscopic metabolomics involve use of spectroscopic techniques to study complex sample matrix like crude extracts and analysing the results using multivariate analysis.

1.2.1. Nuclear Magnetic Resonance

NMR is a versatile analytical technique which uses magnetic properties associated with certain nuclei such as proton [22]. When a sample is placed in an external magnetic field, energy levels are

created in the protons. Applying appropriate radiofrequency (RF) pulse causes absorption of energy by the excited protons. NMR signal is produced as the excited protons relax back to their equilibrium position on the removal of the RF pulse. The NMR signal is dependent on the chemical environment of the protons in the sample. NMR, although traditionally used for studying single molecules, is ideally suited for metabolomics and has been applied in various studies of plants and whole extracts [23–25].

1.2.2. Fourier transform infrared spectroscopy

Chemical bonds vibrate and rotate at characteristic frequencies and when exposed to infrared, they absorb IR at frequencies that match their vibrational and rotational modes [20]. The IR frequency absorbed, depends on the chemical nature of the sample and helps identify functional groups (eg. hydroxyl, amines, alkenes, carbonyl, etc.) and compounds.

1.2.3. Laser-induced breakdown spectroscopy

This technique uses high energy laser pulse to excite elements in a sample [26]. Neutral, single, and double ionised atoms of elements are created in their excited states. The excited atoms and ions relax back to the lower energy states by emitting characteristic photons (frequency) providing information on trace elements present in the sample. The major advantage of this technique is minimal sample material and preparation.

1.2.4. Focus of the study

Taste has both subjective and objective components. While the former relates to sensory perception, the latter relates to chemical sense of taste, i.e. chemical constituents of the sample. For example, sucrose, fructose or glucose contribute to sweet taste [27,28], and acids to sour taste [29]. In this exploratory study, sensorial (taste) and phytochemical descriptors of select medicinal plants have been comprehensively evaluated. The latter have been studied using NMR, FTIR and LIBS, and the sensorial parameters of taste using E-tongue. This preliminary study has explored possible use of these techniques in medicinal plants from an Ayurvedic perspective.

2. Materials and methods

2.1. Plant materials

A total of seven plant samples from three taste categories were selected for the study – *Tribulus terrestris* (aerial part), *Vitis vinifera* (fruit) and *Glycyrrhiza glabra* (root) from sweet category; *Piper longum* (fruit), *Cuminum cyminum* (seed) and *Capsicum annum* (fruit) from pungent group; *Emblica officinalis* (fruit) with five *rasa* except that of salt but with a predominance of sour taste [6]. All were authenticated samples obtained from Kottakkal Arya Vaidya Sala, Kerala.

2.2. E-tongue based evaluation of taste

Taste was evaluated with the potentiometry based α -Astree Electronic tongue (Alpha MOS, France) using Sensor Array # 5 and a reference electrode. The array had seven sensors with three of them tuned each to sour (S1), salty (S3) and umami (S4) tastes. The other four sensors gave an integrated response. The electrochemical signals from the sensors were stored as data matrix and used for multivariate analysis. The response of each sensor was assessed on a relative intensity scale of 1–10, from the least to the most intense taste perception. All samples except *E. officinalis* were dry and powdered. 50 ml of distilled water was added to 5 gm of each sample, placed in a 40 °C water bath for 1 h, gently stirred every 10 min and then macerated. *E. officinalis* was chopped to small pieces and blended with water for 1 min. All the prepared solutions

were filtered to remove any suspended particles, left at room temperature for 1 h and used for analysis. The following were the experimental conditions: sample volume – 20 ml; acquisition time – 120 s; time per analysis – 180 s; four replicates per sample.

2.3. Spectroscopic studies

T. terrestris, *G. glabra*, *P. longum*, *C. annum* and *E. officinalis* were studied using NMR, FTIR and LIBS.

2.3.1. Nuclear Magnetic Resonance

Water suppressed proton NMR spectra were obtained from a 700 MHz NMR spectrometer (Agilent, USA) with deuterated trimethylsilyl propionate (TSP) in a coaxial insert as an external reference. The following acquisition parameters were used: relaxation delay – 14 s, no. of scans – 32, spectral width – 12 ppm and data points – 32 K. Although the aim was to profile the spectra rather than detailed identification of resonances, major peaks have been assigned using 2-Dimensional NMR (data not shown), in-house spectral library of phytochemicals and spectral data libraries (NMRshiftDB and SDBS) [23,30,31]. The samples were prepared as 50% aqueous solution by cold maceration for this study.

2.3.2. Fourier transform infrared spectroscopy

FTIR spectra were acquired using a Cary 660 FTIR (Agilent, USA). Parameters used were: wavelength range – 750 to 10,000 nm; no. of scans – 4; acquisition mode – Attenuated Total Reflection. The aqueous samples prepared for NMR were used for FTIR.

2.3.3. Laser-induced breakdown spectroscopy

Elemental profiling was carried out using a 4-channel LIBS spectrometer (Ocean optics LIBS 2000+) on samples prepared as dry pellets. Pulsed laser beam from a Q-switched Nd: YAG laser (Continuum Surelite III-10) (energy – 175 mJ) was focused on the sample by a quartz lens with consequent formation of plasma on the sample's surface. The emitted light from plasma was collected by a lens positioned at the tip of the optical fibre and fed into the entrance slit of the multichannel spectrometer. LIBS spectra were recorded with an average of 10 laser shots for each spectrum in the wavelength range 200–1100 nm with a repetition time of 2 Hz. Percentage elemental concentration was calculated with spectral intensity ratios using the calibration free method [32].

2.4. Data analysis

E-tongue data was analysed with the Alphasoft V12.3 software and the differential response of the sensors (S1–S7) is presented as a radar graph with intensity grading. NMR data on the other hand, was first processed using standard procedures (Fourier transformation, baseline and phase corrections) and then subjected to multivariate Principal Component Analysis (PCA) using MestReC and Unscrambler X10. The spectra for this were binned and bucketed at intervals of 0.01 ppm.

3. Results

3.1. Taste evaluation

Fig. 1 shows the radar plot of the individual discriminative efficiency of the seven sensors (S1–S7) for five representative samples. The signals have been converted to taste scale for quantitative interpretation. The seven sensors responded differently to each sample. Each plant registered a different set of readings indicating differences in their taste profile although with some similarities within a group. For example, *T. terrestris*, *V. vinifera* and

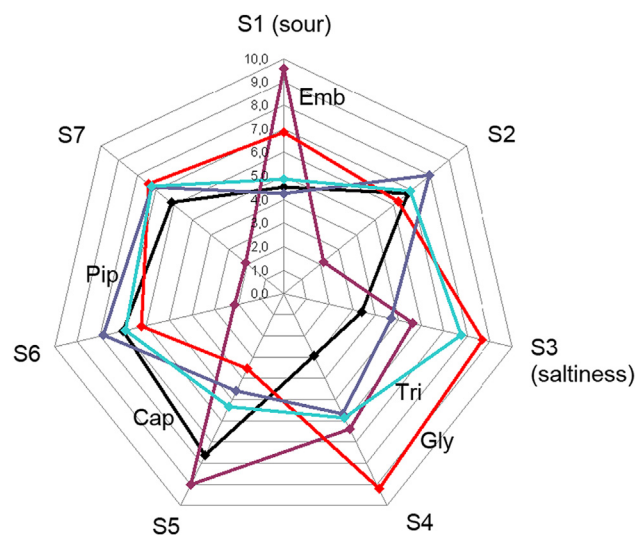


Fig. 1. Taste analysis of plant samples by E-tongue. The data from the seven sensors is represented as a radar map. Cap – *C. annum*; Emb – *E. officinalis*; Gly – *G. glabra*; Pip – *P. longum*; Tri – *T. terrestris*.

G. glabra from sweet category registered close ranking in S2, S3, S6 and S7. In the pungent group, *P. longum*, *C. cyminum* and *C. annum* registered similar readings in S1, S2, S6 and S7. When these two groups were compared for their taste rankings, differences were observed in the sensor readings of S1, S3 and S4. The averaged taste ranking for these sensors were 6 (S1), 8.5 (S3) and 7.5 (S4) for sweet group, and 4.2 (S1), 4.3 (S3) and 4.5 (S4) for the pungent group. This indicates the potential of sensors S1, S3 and S4 to differentiate between sweet and pungent group of plants. On the other hand, *E. officinalis* showed very different taste profile. It registered the maximum (9.5) in S1 sensor, which is specific to sour taste. This corroborates well with the information in Ayurveda that *E. officinalis* (*amla*) has a predominance of sour taste along with sweet, pungent, bitter and astringent [6].

3.2. NMR

Fig. 2 shows proton NMR spectral profile of the plant samples. Resonances from both primary (eg. carbohydrates, amino and organic acids) and secondary (eg. flavonoids, flavonols, flavonol glycosides, etc.) plant metabolites were observed in all the spectra. Peaks identified in the spectra include primary phytochemicals like sugars (glucose, galactose and sucrose), amino acids (valine, isoleucine, alanine, arginine, proline, tryptophan, phenylalanine, tyrosine and lysine) and organic compounds/acids (citrulline, citric acid, lactate, β -OH butyrate, methylamine, glutamate, succinate, N-acetyl glutamate, inositol, myoinositol, formate, malic acid, aspartate, fumarate, phenol and ethanol). Secondary metabolites like gallic acid, trigonelline, flavonoids and flavonol glycosides were also identified in the spectra. There were noticeable spectral differences in the region of amino and organic acids (1–3 ppm), sugars (3.4–4.5 ppm), and aromatic compounds (beyond 5 ppm). The multivariate analysis of NMR spectra however, revealed distinct patterns for the samples in the aromatic region of the spectra (Fig. 3). The plants from the sweet category were clustering closer in both the principal components (PC1 and PC2), indicating similarity between their spectra. No such pattern was seen for the pungent group of plants. The spectral data of *E. officinalis* was positioned away from those of other plants, indicating its unique spectral profile.

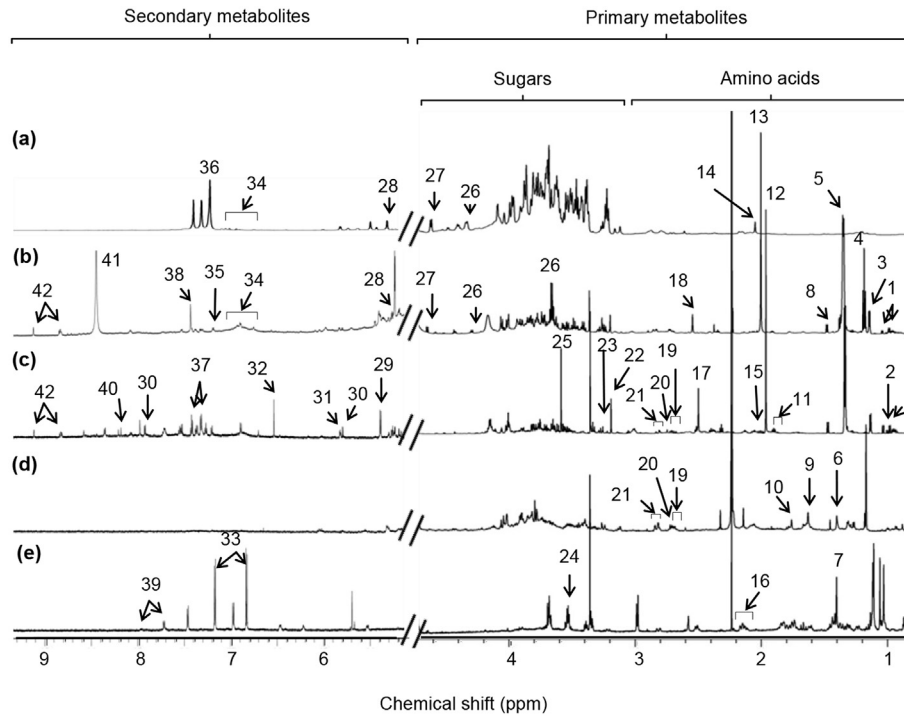


Fig. 2. Proton NMR spectra of (a) *Emblica officinalis*, (b) *Piper longum*, (c) *Tribulus terrestris*, (d) *Capsicum annum*, (e) *Glycyrrhiza glabra*. Peak assignments: 1-Valine, 2-Isoleucine, 3-Isobutyrate, 4-Ethanol, 5-Lactate, 6- β -OH butyrate, 7- λ -OH isobutyrate, 8-Alanine, 9-Arginine, 10-Lysine, 11-Citrulline, 12-Acetate, 13-N-acetylglycoprotein, 14-N-acetyl glutamate, 15-Proline, 16-Glutamate, 17-Succinate, 18-Methylamine, 19-Malic acid, 20-Citric acid, 21-Aspartate, 22-Cis-aconitate, 23-Myoinositol, 24-Inositol, 25-Glycine, 26-Galactose, 27- β -Glucose, 28- α -Glucose, 29-Sucrose, 30-Uridine, 31-Malic acid, 32-Fumarate, 33-Tyrosine, 34-Flavonol glycosides, 35-Flavonoids, 36-Gallic acid, 37-Phenylalanine, 38-Phenols, 39-Tryptophan, 40-ATP, 41-Formate and 42- Trigonelline.

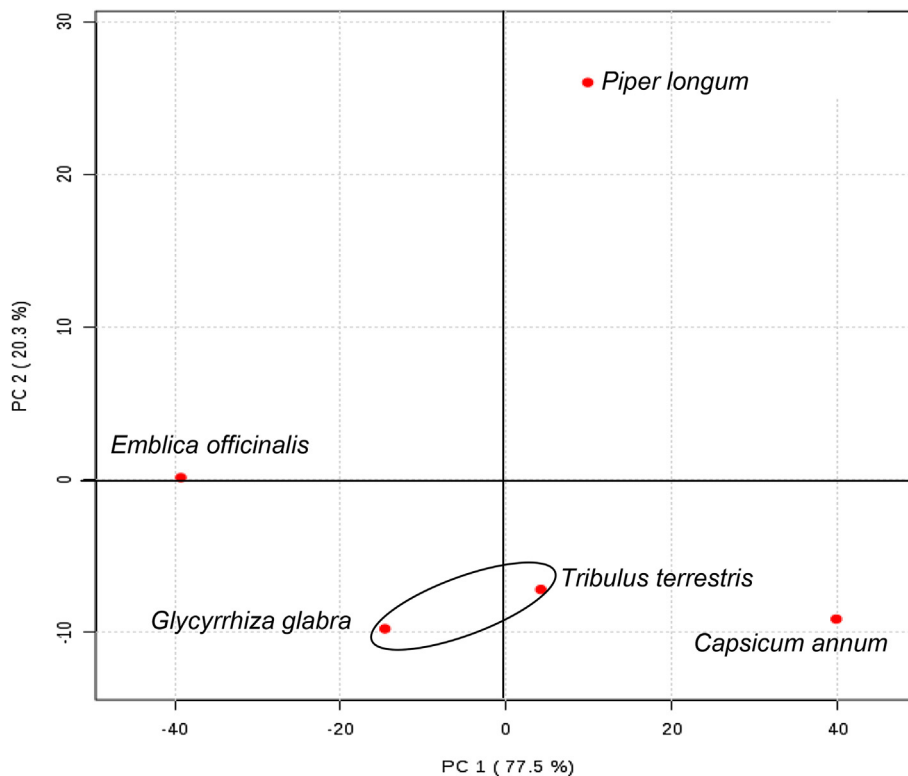


Fig. 3. Principle component analysis of NMR spectral data in the aromatic region of the spectra.

3.3. FTIR

While spectral similarities were observed, there were also differences in the 750–1850 cm^{-1} region (Table 1). Common functional groups (not shown in the Table) were observed in the stretching of C–O from ethers, N–H from protein amide groups, C=O from ester and aldehydes, and also from alkane C–H (2850 cm^{-1}) and carboxylic acid O–H (2931 cm^{-1}). Although the O–H stretching from carboxylic acid was observed in all plants, it was maximum in *E. officinalis*, which interestingly had also registered a maximum score for sour taste in E-tongue. It is pertinent to note that carboxylic acid is known to be associated with sour taste [29]. Table 1 highlights the spectral differences and similarities in the plant samples. Distinct peaks were observed for O–H at 1236 cm^{-1} (pungent group) and C–H at 932 cm^{-1} for the sweet group. Unique peaks were observed yet again for *E. officinalis* (O–H at 864 cm^{-1} , C–N at 1076 cm^{-1} and 1327 cm^{-1} , C=N at 1111 cm^{-1} , C–O–C at 1176 cm^{-1} , and an unidentified peak at 1400 cm^{-1}). The O–H and C–OH groups in *E. officinalis* are from gallic acid [10] [33], which has also been identified in the NMR spectrum (peak 36 in Fig. 2).

3.4. LIBS

All the plants showed presence of elements such as Mg, N, C, Ca, H, O and C in the spectral range 200–900 nm. Fig. 4 shows a representative spectrum. It is seen that each element displays several lines in the spectrum indicating multiple ionization states. For example, Ca displays Ca (I) at 422.6 nm, and Ca (II) at 393.3 and 396.8 nm. These represent the different ionization states created in the element due to the induced high plasma temperature of 10,000–15,000 K. The absence of toxic elements such as mercury, arsenic and lead in all the spectra confirms the purity of the samples.

Table 2 shows % concentration of elements which showed variations. Of these, Ca showed striking difference between groups: sweet (9.9, 8.7), pungent (4.1, 2.0) and *E. officinalis* (7.5). Another striking variation was in Mg between *E. officinalis* (16.7) and *C. annum* (5.0). Interestingly, a similar contrast was observed in the antioxidant data (not presented) of these two plants, with *E. officinalis* and *C. annum* registering respectively, maximum and minimum antioxidant activities. It is pertinent to note that high levels of Mg are reported to be associated with high antioxidant potential in plants [32].

4. Discussion

There is a revival of interest in traditional systems of medicine world over. This has prompted a growing interest in understanding Ayurveda and also its use of medicinal plants for therapeutic purposes. This work is in the context of Ayurvedic pharmacology, which uses parameters different to that used in modern medicine to understand and categorise medicinal plants. One such parameter is *rasa*, which is understood and translated as taste. This study is an attempt to explore the use of structuro-functional information

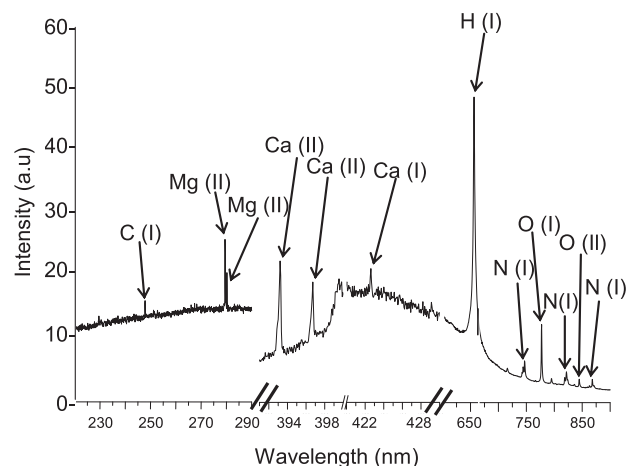


Fig. 4. Elemental profile of *Tribulus terrestris* using Laser-induced breakdown spectroscopy.

Table 2

Percentage concentration of elements assessed by LIBS.

Plants	% Concentration of elements				
	K	Na	Mg	Ca	Zn
<i>Glycyrrhiza glabra</i>	1.31	0.91	14.89	9.91	1.58
<i>Tribulus terrestris</i>	1.18	0.46	13.58	8.68	2.01
<i>Piper longum</i>	1.05	0.72	11.97	4.14	1.86
<i>Capsicum annum</i>	0.98	1.19	4.98	1.97	0.78
<i>Embolica officinalis</i>	1.13	0.62	16.69	7.52	1.21

deduced from spectroscopic techniques (NMR, FTIR and LIBS) and Electronic tongue to study the *rasa* of medicinal plants.

Taste being a chemosensory parameter, the sensory perception plays an equally important role as does the chemical composition. E-tongue closely mimics human tongue in perception and evaluation of taste, and provides an objective assessment of it. The preliminary data from this study indicates possible discrimination in the sensorial properties of medicinal plants in terms of taste. E-tongue could be a promising tool in assessment of the ayurvedic pharmacological parameter of *rasa*. Further in-depth studies are required and underway by this group.

Most of the analytical techniques used in phytochemical studies require sample preparation and have conventionally focused on isolated single molecules. However, NMR is an ideal tool to study plants in their native form without subjecting them to any preparatory procedures. Although the resonances observed from plants in NMR spectra essentially reflect common primary and secondary metabolites, the distinct differences observed in the spectra in this study suggest that taste phenotypes could be fingerprinted using NMR chemosensory markers [34]. NMR with its potential for simultaneous detection of all molecules is ideally suited for obtaining fingerprint of whole plant extracts. By identifying

Table 1

Functional groups unique to each plant identified from FTIR spectra

Plants	Functional groups and wavenumber (cm^{-1})									
	O–H	C–H	C–N	C=N	C–O–C	O–H	C–N	–NO ₂	Unassigned	C–OH
<i>G. glabra</i>	–	932	–	–	–	–	–	1387	–	1428
<i>E. officinalis</i>	864	–	1076	1111	1176	–	1327	1387	1400	1428
<i>T. terrestris</i>	–	932	–	–	–	–	–	–	–	–
<i>P. longum</i>	–	–	–	–	–	–	1236	–	–	–
<i>C. annum</i>	–	–	–	–	1149	–	1236	–	–	–

individual phytochemicals, NMR can also provide targeted information. Moreover, sample preparation in NMR is simple since separation of analytes is not required.

Comparing the NMR data with that of E-tongue in this study—*E. officinalis*, which registered a high score for sour taste, showed resonance from gallic acid (peak 36 in Fig. 2), known to be present in it. NMR identification of gallic acid is interesting and significant since acids such as ascorbic, citric, acetic, carboxylic and gallic are known to be associated with sourness [29,33]. The other plant where correlation between NMR and E-tongue data was observed was *G. glabra*. This plant not only displayed high score for sensor 4 in E-tongue (Fig. 1), but was also the only plant with signals from glutamate (peak 16 in Fig. 2e), the molecule associated with umami taste specific to S4 [35]. This is an interesting observation, despite the fact that Ayurveda does not mention this taste. The distinct peaks in the FTIR spectrum of *E. officinalis* support not only its NMR spectral profile but also its unique taste profile as mentioned in Ayurveda. Although many of the functional groups observed in the IR can also be seen in the corresponding NMR spectrum, FTIR is inexpensive and faster. It can be used for quick screening of functional groups and can be followed with in-depth analysis and confirmation using NMR.

Micro-minerals such as Na, K, Mg, P, and Ca are essential for functioning of both cells and the vital enzymes involved in major metabolic pathways [36]. Minerals are known to play a contributory role in enhancing the nutraceutical and medicinal properties of plants [37]. There is thus a growing interest in recent years in studying not only phytochemicals but also the biologically important elements in nutraceutical and medicinal plants. Interestingly, elements such as Ca and Mg may also play a role in taste [38,39]. LIBS has emerged as a sensitive and reliable analytical technique for rapid characterisation of phytoelements, as also seen in this study. A major advantage of this technique is its ability to provide quick and simultaneous information on all elements present in a sample with minimal sample preparation.

Plant chemistry has seen tremendous progress in recent years owing to the advancement in the field of natural product technologies and analytical techniques. Although whole extracts and complex mixtures do not fit the conventional model of single active molecule, the growing interest in multitargeting and synergism in phytomedicines have opened new vistas in phytochemistry research [40,41]. More importantly, the increasing attention on use of medicinal plants in Ayurveda has also necessitated identification of objective and reliable parameters for phytochemical evaluation of crude extracts. Plants have many properties ranging from sensorial to chemical and a comprehensive evaluation of these can also help in understanding their therapeutic properties from an Ayurvedic perspective. Such analyses can pave the way for novel parameters for quality control and standardisation from an Ayurvedic standpoint.

5. Conclusion

In this study, sensorial and chemical analyses that can comprehensively evaluate *rasa* of medicinal plants have been explored for the first time in literature. The multi-spectrometric analyses demonstrate their potential in providing phytochemical, phytoelemental and chemosensory information for plants. The advantages of the three techniques used (NMR, FTIR and LIBS) are minimum sample preparation, and simultaneous determination of functional groups, phytochemicals and phytoelements. E-tongue can play an important role in the sensorial evaluation of *rasa* of plants by providing a single signature for a broad group of metabolites associated with taste. The exploratory results indicate that a combination of sensorial and chemical descriptors can help in a

comprehensive study of *rasa* in Ayurveda. The novelty of this study is assessment of both the objective (chemical) and subjective (sensorial) aspects of the chemosensory property of taste using analytical techniques and more importantly, on whole extracts (as used in Ayurveda) rather than single molecules or fractionated extracts of plants. Further in-depth studies are underway.

Conflicts of interest

There are no conflicts of interest.

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