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Challenges in integrating component level technology and system level information from Ayurveda: Insights from NMR phytometabolomics and anti-HIV potential of select Ayurvedic medicinal plants

Rama Jayasundar [a, *], Somenath Ghatak [a], Muzamil Ashraf Makhdoomi [b], Kalpana Luthra [b], Aruna Singh [a], Thirumurthy Velpandian [c]

[a] Department of NMR, All India Institute of Medical Sciences, New Delhi, India
[b] Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India
[c] Department of Ocular Pharmacology and Pharmacy, All India Institute of Medical Sciences, New Delhi, India

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ABSTRACT

Background: Information from Ayurveda meeting the analytical challenges of modern technology is an area of immense relevance. Apart from the cerebral task of bringing together two different viewpoints, the question at the pragmatic level remains 'who benefits whom'.
Objective: The aim is to highlight the challenges in integration of information (Ayurvedic) and technology using test examples of Nuclear Magnetic Resonance (NMR) metabolomics and anti-HIV-1 potential of select Ayurvedic medicinal plants. The other value added objective is implications and relevance of such work for Ayurveda.
Materials and methods: Six medicinal plants (*Azadirachta indica*, *Tinospora cordifolia*, *Swertia chirata*, *Terminalia bellerica*, *Zingiber officinale* and *Symplocos racemosa*) were studied using high resolution proton NMR spectroscopy based metabolomics and also evaluated for anti-HIV-1 activity on three pseudoviruses (ZM53 M.PB12, ZM109F.PB4, RHPA 4259.7).
Results: Of the six plants, *T. bellerica* and *Z. officinale* showed minimum cell cytotoxicity and maximum anti-HIV-1 potential. *T. bellerica* was effective against all the three HIV-1 pseudoviruses. Untargeted NMR profiling and multivariate analyses demonstrated that the six plants, all of which had different Ayurvedic pharmacological properties, showed maximum differences in the aromatic region of the spectra.
Conclusion: The work adds onto the list of potential plants for anti-HIV-1 drug molecules. At the same time, it has drawn attention to the different perspectives of Ayurveda and Western medicine underscoring the inherent limitations of conceptual bilinguism between the two systems, especially in the context of medicinal plants. The study has also highlighted the potential of NMR metabolomics in study of plant extracts as used in Ayurveda.

1. Introduction

Of the knowledge assets India holds, Ayurveda not only occupies a prime position but is also among the few Indian Knowledge Systems that has kept alive its vibrant tradition till date. Despite Western medicine being the frontline medical system in India, Ayurveda continues to address many health issues [1–5] thereby contributing to the healthcare, independent of the Allopathic system of medicine [6]. At the same time, Ayurveda has also allowed its vast documented knowledge of clinical practices, formulations and medicinal plants to be used by Western medicine [7–13]. For instance, drug molecules such as curcumin [14], guggulsterone [15,16] and reserpine [17] have been identified with lead information from Ayurvedic pharmacopoeia. Increasing interest in Western medicine in the use of *kshārasutra* in fistula-in-ano [9], and leech to

* Corresponding author.
E-mail: ramajayasundar@hotmail.com.
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relieve pain [7] and venous congestion [8] are other examples. Ayurveda thus remains extremely resourceful and of interest to the Allopathic system of medicine. At the same time, how such work will benefit Ayurveda is a moot point. This article highlights this issue via case in point studies using Nuclear Magnetic Resonance (NMR) phytometabolomics and anti-HIV-1 activity of select medicinal plants used in Ayurveda. It also underscores the challenges in using information from Ayurveda for reductionism based studies.

Although conventional spectroscopic studies such as NMR, Mass Spectroscopy (MS) and Fourier Transform Infrared (FTIR) spectroscopy study single isolated molecules, techniques such as metabolomics is fast becoming the method of choice for study of complex mixtures [18]. Metabolome represents collection of all metabolites in a sample, in particular, the small metabolites and phytometabolome denotes compounds in plants. In phytometabolomics, chemical profiling of plants is carried out [19]. Untargeted metabolomics provides in general, a broad overview of the chemical composition of the sample without requiring prior knowledge of the metabolites. The experimental data is usually subjected to multivariate analysis for data interpretation. The aim in general is to look for fingerprints rather than specific metabolites. NMR is an ideal technique for phytometabolomics when plants are used in their native form (as in Ayurveda) since separation of analytes is not a pre-requisite for this technique [20].

Many diseases have now become an enormous human and economic burden, and Acquired Immunodeficiency Syndrome (AIDS) caused by Human Immunodeficiency Virus (HIV) is one such disease [21]. Due to the extensive diversity in the virus, there is no effective vaccine till date against HIV-1. Anti-retroviral therapy (ART) is successful in reducing the viremia, but the development of ART resistance mutations in the viral strains in the infected individuals is a major impeding challenge. This underscores the need to discover new effective drugs, which are also less expensive and less toxic, to be used alone or in combination with ART in order to suppress the viremia at early stages of the infection [22–24]. Medicinal plants, which have always contributed to drug development in modern medicine [25], continue to evoke interest in exploring their potential and in the context of AIDS, to identify anti-HIV-1 compounds [26–32].

This article has three focus points: (i) untargeted NMR metabolomics for study of plant extracts in their native form, as used in Ayurveda (ii) attempting for the first time in literature, correlation between NMR phytometabolomics data and Ayurvedic pharmacological parameters (iii) using information from Ayurveda to screen select medicinal plants for anti-HIV activities and highlighting the key challenges in such information exchanges between Ayurveda and modern pharmacological studies. The article also draws attention to the fundamentally different approaches to use of medicinal plants in Ayurveda and modern medicine.

2. Materials and methods

2.1. Sample selection and preparation

Six plants, namely *Azadirachta indica* (AI) (bark), *Tinospora cordifolia* (TC) (stem), *Swertia chirata* (SC) (whole plant), *Terminalia bellerica* (TB) (fruit rind), *Zingiber officinale* (ZO) (rhizome) and *Symplocos racemosa* (SR) (stem bark) were selected on the basis of their therapeutic use in Ayurveda for treatment of *visarpa*, which is usually correlated with herpes, a viral disease [33]. Authenticated samples were obtained from Kottakkal Arya Vaidya Sala, Kerala. Five gm of the raw material was soaked in 50 ml of water and ethanol in 9:1 ratio for 24 h and cold macerated. This solution was then centrifuged twice at 5000 rpm for 10 min and the supernatant filtered through Whatmann Paper No.1 to remove the very fine suspended particles. The supernatant was collected and lyophilized to get a dry powder. Polar metabolites from the plant samples are extracted by this method. It is pointed out that the process of boiling plant samples in water involved in the preparation of *kvatha* (decoction) in Ayurveda also essentially extracts the polar metabolites. The removal of water content in the sample by lyophilization involves no chemical reactions and hence no changes in its properties.

2.2. Nuclear magnetic resonance spectroscopy

The NMR studies were carried out on a 700 MHz high resolution NMR spectrometer (Agilent, USA). The lyophilized sample (25 mg) was dissolved in 1 mL of deuterium oxide (D_2O) and taken in a 5 mm diameter NMR tube with a coaxial insert containing deuterated trimethylsilylpropionate (TSP). The latter was used as an external reference. All the samples were maintained at pH 7. Water suppressed proton NMR spectra were obtained with the following parameters: relaxation delay – 14 s, spectral width – 12 ppm, scans – 64, data points – 32 K, flip angle – 90°. The Free Induction Decay was Fourier-transformed and phased (zero and first order). A line-broadening factor of 0.3 Hz was then applied to each spectrum and the baseline corrected. All the peaks were referenced to TSP.

Although untargeted metabolomics, the strategy followed in this study, does not require detailed identification of phytometabolites and an attempt has been made to assign peaks. It was pointed out that since the plant parts were used in their native form, the spectra had a number of peaks making spectral assignments a challenge. Still, effort has been made to assign the peaks using in-house and NMR data libraries such as NMRshiftDB, SDBS (Integrated Spectral Data Base System for Organic Compounds) and HMDB (Human Metabolome Database) [20,34,35].

Principle Component Analysis (PCA) was carried out on the NMR spectral data using Metaboanalyst 3.0. In addition to evaluation of the entire spectral region (0–10 ppm), the spectra were also divided into 3 major regions for analysis: 0–4.5 ppm (predominantly primary metabolites), 0–3 ppm (predominantly amino acids), and 5–10 ppm (aromatic compounds). The spectra were binned and bucketed at an interval of 0.04 ppm using MestReC. $p < 0.05$ and 95% Confidence Interval (CI) were considered statistically significant.

2.3. In vitro MTT assay for cytotoxicity

2.3.1. Sample preparation

The lyophilized plant sample was dissolved in autoclaved milliQ water and filter purified using 0.4 μm. The reconstituted plant extracts were stored in dark brown microcentrifuge tubes at 4 °C until when required for use.

2.3.2. Cell lines

TZM-bl cells, also known as JC 53-bl cells, is a HeLa cell clone that expresses CD4, CXCR4, CCR5 and contains *tat* responsive reporter genes for firefly luciferase and *Escherichia coli* β-galactosidase under the regulatory control of the HIV-1 long terminal repeat [36]. These were obtained from NIH AIDS Research and Reference Reagent Program (catalog no. 8129). The cells were maintained in minimal essential media supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml) in a humidified atmosphere of 5% CO_2 at 37 °C.

2.3.3. MTT assay

The assay was performed using Alkan et al. method with some modifications [37]. Briefly, TZM-bl cells (7000/well) were plated in

100 μl of medium/well in 96-well plates (Costar Corning, Rochester, NY). After 48 h of incubation, the cells reached the desired confluence. The cells were then incubated with different concentrations (1000 μg/ml, 500 μg/ml, 250 μg/ml, 125 μg/ml, 62.5 μg/ml, 31.2 μg/ml, 15.6 μg/ml and 7.8 μg/ml) of each of the plant extract for 48 h at 37 °C. After removal of the plant extracts, the cells were washed with phosphate-buffered saline (pH 7.4), and 100 μl/well (10 mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) and phosphate-buffered saline solution was added. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 μl of DMSO. Viable cells were determined by absorbance at 570 nm.

$$\%\ \text{cell viability} = 100 - \left[\frac{\text{Absorbance (sample)}}{\text{Absorbance (control)}}\right] \times 100$$

Untreated TZM-bl cells were used as control. The study was carried out in triplicate. The LD_{50} values calculated for each plant extract from this data were used in the anti-HIV-1 activity assays against the three pseudoviruses.

2.4. Anti-HIV-1 activity assay

Pseudoviruses are infectious molecular clones of HIV-1 envelope which are produced by co-transfection of the envelope clone of interest and another clone containing the whole HIV-1 genome except the envelope, in mammalian cells [38]. The pseudoviruses thus produced are capable of single round of infection into the target cells. They can be easily produced in large amounts and are less biohazardous than HIV-1 primary isolates. A standard panel of HIV-1 pseudoviruses belonging to clade C and clade B were procured from NIH AIDS Research and Reference Reagent Program. In order to determine the cross clade anti-HIV-1 activity of plant extracts, each of them were tested against representative clade B and C pseudoviruses. Each plant extract was tested for its anti-HIV-1 activity against two clade C and one clade B pseudoviruses belonging to tier 2 from the panel (Table 1).

For determining $TCID_{50}$, serial five-fold dilutions of pseudovirus were made in 96-well culture plate in a total volume of 100 μl of growth medium for a total of 11 dilution steps. Then TZM-bl cells (10,000 cells in 100 μl of growth medium containing 75 μg/ml DEAE-dextran) were added to each well, and the plates were incubated at 37 °C in a humidified 5% CO_2–95% air environment. After a 48 h incubation, 130 μl of culture medium was removed from each well and 50 μl of Bright Glo reagent (Promega Corp., Madison, WI) added to the cells. After a 2-min incubation at room temperature to allow cell lysis, 90 μl of cell lysate was transferred to 96-well white solid plates (Corning-Costar) for measurements of luminescence using a luminometer. The $TCID_{50}$ was calculated as per procedure given elsewhere [39].

Inhibition of viral infectivity by plant extracts was measured as a reduction in luciferase gene expression after a single round of infection of TZM-bl cells with virus. Pseudovirus (200 $TCID_{50}$) was pre-incubated for 1 h at 37 °C, 5% CO_2 in 96-well flat-bottom culture plates with the plant extracts with six serial dilutions (dilution range = 4.1–1000 μg/ml). For the TB extract, eight serial dilutions were taken (dilution range = 2–1000 μg/ml). Freshly trypsinised cells [10,000 cells in 100 μl of growth medium containing DEAE Dextran (25 μg/ml)] were added to each well of the pre-incubated virus. One set of control wells received cells plus virus (virus control) and another set received cells only (background control). After 48 h of incubation at 37 °C, 5% CO_2, luciferase activity was measured by using the Bright-Glo Luciferase Assay System (Promega Inc.). VRC01, broadly known as monoclonal antibody that targets the CD binding site, was taken as the positive control. A ten-fold serial dilution of VRC01 (0.0001–10 μg/ml) was used in the experiment.

For all dilutions of plant extracts, the percent anti-HIV-1 activity was calculated based on the relative luminescence units (RLU) of the test wells containing plant extract divided by the virus control. Cell control value was subtracted from the plant extract RLU value as background cut off. The 50% inhibitory concentration (IC_{50}) for each plant extract was determined. At this dose, relative luminescence units were reduced by 50% compared to virus control wells. The 50% inhibitory titer was determined from the linear portion of the titration curve using the method of least square.

Table 1
Pseudovirus panel used in HIV-1 neutralization assay.

Pseudovirus	Country of origin	Subtype	Tier	GenBank accession number
ZM53 M.PB12	Zambia	C	2	AY423984
ZM109F.PB4	Zambia	C	2	AY424138
RHPA 4259.7	USA	B	2	AY835447

3. Results

3.1. Proton NMR spectroscopy

Fig. 1 shows a representative proton NMR spectrum from one of the plant (*T. cordifolia*). A number of resonances are seen in the spectrum, as expected of a plant sample used in its native form. Signals in the 0–4.6 ppm spectral region are predominantly from primary metabolites (eg. carbohydrates, amino and organic acids) and those in the 5–11 ppm region are primarily from secondary plant metabolites (eg. flavonoids, flavonols and flavonol glycosides). Both primary and secondary metabolites are seen not only in this spectrum but in others as well, although with variations. Many common resonances were observed in the primary metabolites – amino acids (isoleucine, valine, lactate, alanine and arginine), sugars (α-glucose, β-glucose) and organic acids (formate).

Fig. 2 shows 3D plots of the principle component analysis of the NMR data from the following spectral regions: entire spectrum (0–11 ppm) (Fig. 2a); primary metabolites (0–4.5 ppm: sugars and amino acids) (Fig. 2b); predominantly amino acids (0–3.5 ppm) (Fig. 2c); aromatic compounds (5–11 ppm) (Fig. 2d). The dispersion of data in the plots show that each plant has its unique phytochemical profile, be it primary or secondary group of metabolites. Maximum differences were observed in the aromatic region of the spectra (Fig. 2d). The score plot shows 65.3% variance in the first axis (PC1) and 21.5% in PC2 indicating significant differences in the spectral profile in the aromatic region of the spectra. It is pertinent to note that each of these six plants have different Ayurvedic pharmacological properties (Table 2). Even AI and SC, which have similar *rasa*, *guna*, *virya* and *vipaka* differ in their *karma* [40,41].

3.2. MTT assay

The percentage cell viability was calculated for eight different concentrations (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.2 μg/mL, 15.6 μg/mL and 7.8 μg/mL) of the plant extract (Table 3). The LD_{50} concentration for ZO, TB, TC, SR, AI and SC were 846 μg/mL, 334 μg/mL, 582 μg/mL, 94 μg/mL, 282 μg/mL and 172 μg/mL, respectively. While ZO, AI and TB showed minimum cytotoxicity, SR displayed the maximum.

3.3. Anti-HIV-1 activity assay

Table 4 shows the anti-HIV-1 activity of the plant extracts against the three pseudoviruses presented as the reciprocal value of the IC_{50}, which is the dilution at which virus infectivity is inhibited

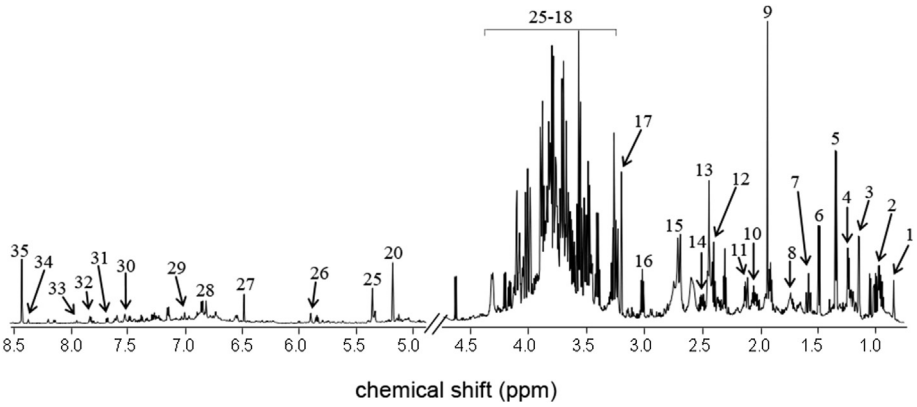

Fig. 1. One dimensional proton NMR spectrum from *T. cordifolia*. The assigned resonances are: 1 – isoleucine, 2 – valine, 3 – isobutyrate, 4 – β-hydroxyisobutyrate, 5 – lactate, 6 – alanine, 7 – citrulline, 8 – arginine, 9 – acetate, 10 – proline, 11 – glutamate, 12 – pyruvate, 13 – oxalacetate, 14 – succinate, 15 – glutamine, 16 – malate, 17 – choline, 18 – glucose, 19 – inositol, 20 – galactose, 21 – cellobiose, 22 – fructose, 23 – sucrose, 24 – proline, 25 – sucrose, 26 – NAD/NADP, 27 – fumarate, 28 – flavonoids/glucosinolates, 29 – tyrosine, 30 – phenylalanine, 31 – chlorogenic acid, 32 – tryptophan, 33 – uridine, 34 – ATP, 35 – formate. ATP – Adenosine triphosphate; NAD – Nicotinamide adenine dinucleotide; NADP – Nicotinamide adenine dinucleotide phosphate.

to 50%. Two plant extracts ZO and TB exhibited inhibition of viral activity against all the three pseudoviruses. While TB showed maximum anti-HIV-1 activity (IC_{50} < 2 μg/ml) against all the pseudoviruses, ZO showed maximum activity against ZM53 and RHPA (IC_{50} < 4.1 μg/mL) and moderate efficacy against ZM109 (IC_{50} of 98 μg/mL). The other plant extracts did not reach IC_{50} against the above panel of pseudoviruses. For VRC01, the IC_{50} was reached at 0.10 μg/mL, 1.40 μg/mL and 0.09 μg/mL respectively, against ZM109, ZM53 and RHPA (Fig. 3).

3.4. NMR spectrum of T. bellerica

Fig. 4 shows the NMR spectrum of TB, which had demonstrated maximum entry level inhibition of the pseudo HIV-1 viruses. It is interesting to note the presence of ferulic and caffeic acids (highlighted in the figure), both of which are reported to have anti-HIV activities [42,43]. Although further in-depth studies with NMR and other analytical techniques are required to assign these peaks with certainty, presence of these two peaks in the NMR spectra of the inhibitory (Fig. 4) but not the non-inhibitory (Fig. 1) extracts is nevertheless interesting. Further in-depth studies are underway.

4. Discussion

Medicinal plants continue to contribute enormously to drug development in modern medicine. In the context of the current trend considering Ayurveda as a data bank for discovery of new drug molecules, this work adds onto the list of potential plants with anti-HIV-1 activity. Acquired Immunodeficiency Syndrome is a major health problem worldwide [21]. Notwithstanding availability of vaccines for HIV, there is a need to discover effective medicines, which are also less expensive and less toxic.

Although a number of plants have been studied and reported for their anti-HIV-1 activities [26–32], there are very few reports on virus infected cells for the plants used in this study, namely *A. indica*, *T. cordifolia*, *S. chirata*, *T. bellerica*, *Z. officinale* and *S. racemosa*. Sabde et al. (2011), in their studies on human CD4+ T cell line (CEM-GFP cells infected with HIV-INL4.3 virus), have reported significant anti-HIV-1 activity for *Aegle marmelos*, *Argemone mexicana*, *Asparagus racemosus*, *Coleus forskohli* and *Rubia cordifolia* but not *T. cordifolia* [30]. Absence of anti-HIV activity in *T. cordifolia* reported by them is in agreement with that observed in the present study.

A. indica, however, was found to inhibit HIV-1 reverse transcriptase in HIV-1 infected CD166 CD4 cell lines [44]. Valsaraj et al. have reported weak anti-HIV-1 activity of termilignan, thannilignan, 7-hydroxy-3′,4′-(methylenedioxy) flavan and anolignan B (non-polar compounds) isolated from *T. bellerica* on MT-4 cells [45]. It is interesting to note that in the present study, the polar compounds of TB have shown strong anti-HIV-1 activity, although on a different cell line. There are no reports however, on anti-HIV-1 activity of ZO. To the best of our knowledge, the present study may be the first report on anti-HIV-1 potential of ZO.

Spectroscopic analysis of medicinal plants provides an important platform to understand their phytochemical constituents thereby leading to studies on drug development. In this context, NMR is an ideal analytical technique for phytochemical analysis since it allows simultaneous detection of diverse groups of primary and secondary metabolites without the need for fractionating the extract. Metabolomics is a new approach to study the phytochemical complexity of plants [18,19] and NMR, conventionally used for structure elucidation of phytomolecules is best suited for such studies on whole extracts [20]. The NMR spectroscopy data from this study has also demonstrated the usefulness of metabolomics approach for studying whole extracts, as used in Ayurveda.

Plants produce a range of metabolites classified as primary and secondary, both of which are seen in the NMR spectra from whole extracts – primary metabolites predominantly in the 0–4.6 ppm (aliphatic region) and secondary in the 5–11 ppm (aromatic region) of the spectra. The multivariate analysis has brought out the subtle but striking differences between the NMR phytochemical profile of the six plants. The spectral data from the primary metabolite region (Fig. 2b) showed clustering of TB and ZO, the two plants with maximum anti-HIV-1 activities. This not only indicates spectral similarity in the primary metabolites between the two plants, but also a possible correlation between their bioactivities. At the same time, the marked differences between the plants in the aromatic region of the spectra (Fig. 2d) can be a reflection of the plants' different Ayurvedic pharmacological properties. Although in-depth studies are required to make affirmative conclusions, this study has shown the possibility of using modern technology and statistical analysis from an Ayurvedic point of interest.

4.1. What does the anti-HIV-1 potential findings mean for Ayurveda?

A basic appreciation of how Ayurveda understands, selects and uses plants for treating diseases is imperative, prior to addressing the above-mentioned issue.

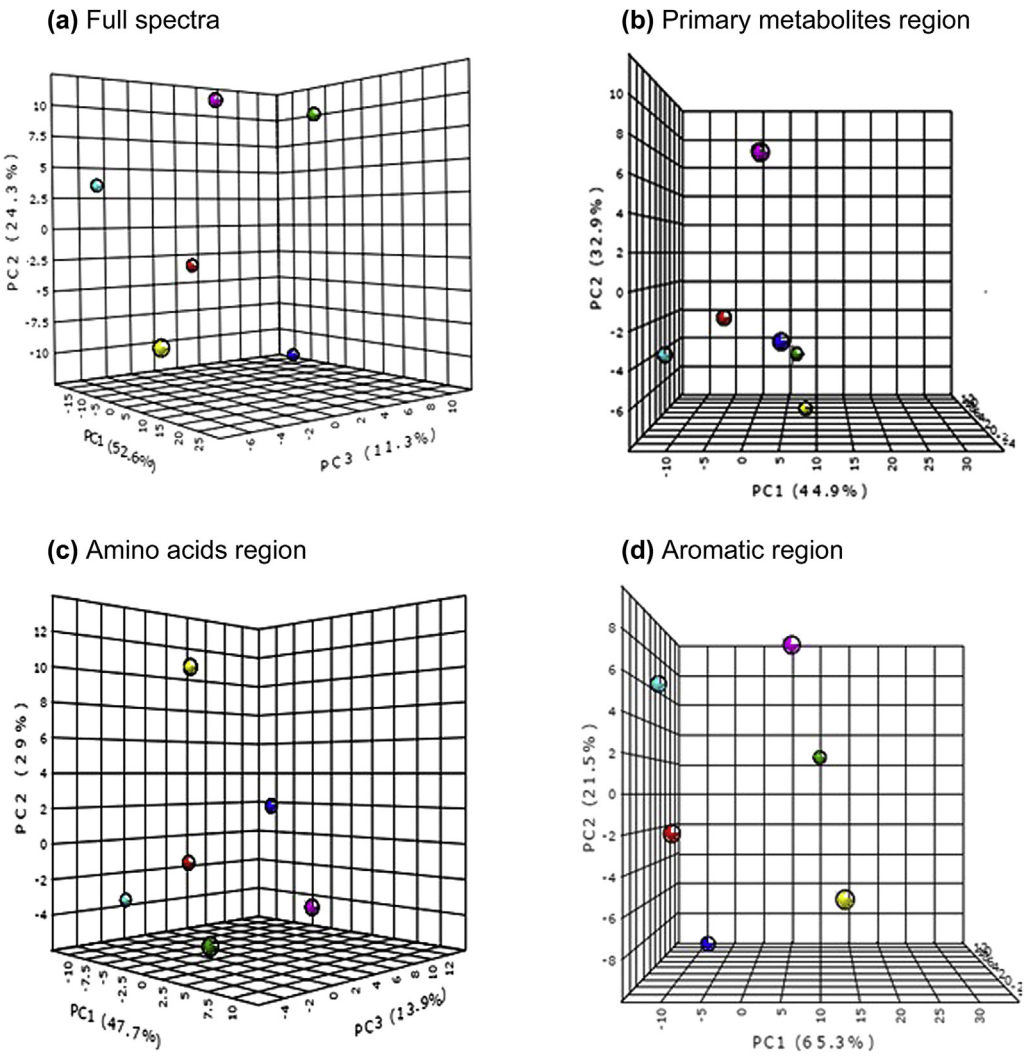

Fig. 2. Three dimensional PCA plots of the proton NMR data from different spectral regions for *A. indica* (AI), *S. chirata* (SC), *S. racemosa* (SR), *T. bellerica* (TB), *T. cordifolia* (TC) and *Z. officinale* (ZO).

4.1.1. Ayurveda's understanding and use of medicinal plants

The use of medicinal plants in Ayurveda is dictated by their Ayurvedic pharmacological properties (*rasa*, *guna*, *virya*, *vipaka* and *karma*) and their effects on *dosha* (*vata*, *pitta* and *kapha*). The latter act as a common denominator to understand and translate all factors impacting health and disease, from lifestyle regimens and environment to plants, which are used as both food and medicine [46,47].

Ayurveda has a functional and inclusive perspective of the system (defined by *vata*, *pitta* and *kapha*) whereas Western medicine and the allied areas like drug research, has a predominantly structural and reductionistic viewpoint. Understood as inter-dependent domains (physical, physiological, psychological, consciousness, and biotic and abiotic environmental factors) of different dimensions, each influencing the other, Ayurveda perceives the human body as a holistic and complex entity [48]. This inclusive vision forming the fundamental basis of ayurveda is well integrated into its clinical and pharmacological practices.

Health in Ayurveda is seen as a reflection of the ability of the system to adapt to stress (environmental and endogenous) [48]. Disease (except traumatic injuries) results when this adaptive mechanism fails. On the other hand, addressing disease at the molecular and cellular levels is a central feature of Western

Table 2
Ayurvedic pharmacological parameters for the plants studied. AI – *A. indica*, TC – *T. cordifolia*, SC – *S. chirata*, TB – *T. bellerica*, ZO – *Z. officinale*, SR – *S. racemosa*.

Ayurvedic parameters	Plants					
	TC	AI	TB	SC	ZO	SR
rasa	*tikta, kashaya*	*tikta*	*kashaya*	*tikta*	*katu*	*kashaya*
guna	*laghu*	*laghu, ruksha*	*ruksha, laghu*	*laghu, ruksha*	*laghu, snigda*	*laghu*
virya	*ushna*	*sheeta*	*ushna*	*sheeta*	*ushna*	*sheeta*
vipaka	*madhura*	*katu*	*madhura*	*katu*	*madhura*	*katu*

Table 3
MTT assay of hydroalocoholic plant extracts on TZM-bl cell line. ZO – *Z. officinale*, TB – *T. bellerica*, TC – *T. cordifolia*, SR – *S. racemosa*, AI – *A. indica*, SC – *S. chirata*.

Concentration (μg/mL)	Dilution	Cell viability (%)					
		ZO	TB	TC	SR	AI	SC
1000	Neat	41.26 ± 1.89	35.27 ± 2.25	21.44 ± 1.40	32.33 ± 2.13	34.75 ± 3.50	29.51 ± 2.81
500	1:1	73.65 ± 3.14	44.99 ± 1.55	54.47 ± 2.21	33.79 ± 3.84	44.62 ± 2.07	33.17 ± 2.21
250	1:2	77.62 ± 2.06	53.08 ± 2.15	61.75 ± 2.73	42.03 ± 1.52	48.23 ± 2.97	44.73 ± 3.01
125	1:4	90.03 ± 4.82	68.61 ± 1.35	67.68 ± 1.58	49.31 ± 0.91	57.51 ± 1.48	53.33 ± 1.99
62.5	1:8	92.52 ± 2.28	68.61 ± 1.44	73.53 ± 3.31	54.56 ± 2.14	79.98 ± 1.73	55.99 ± 3.18
31.2	1:16	92.10 ± 1.11	75.59 ± 2.02	77.21 ± 2.90	57.26 ± 1.04	85.54 ± 4.03	66.09 ± 2.49
15.6	1:32	96.47 ± 0.79	78.39 ± 4.73	81.71 ± 1.82	67.75 ± 3.07	87.97 ± 2.71	73.33 ± 1.87
7.8	1:64	97.51 ± 1.78	90.43 ± 2.08	82.96 ± 2.11	81.76 ± 1.30	97.79 ± 3.13	84.69 ± 3.52
Cell control	–	100.00	100.00	100.00	100.00	100.00	100.00

medicine. These unique perceptions form not only the conceptual frameworks of each system but also highlights the basic differences in the way therapies and medicines are strategised. For the same reason, the selection and use of plants differ significantly between Ayurveda and Western medicine.

Since Ayurveda views disease as a system perturbation (imbalance in *vata*, *pitta* and *kapha*), a number of factors are considered to play a role in adaptation and hence the Ayurvedic treatment is always multimodal [33,49]. This also indicates why Ayurvedic medicines are not single molecule-single target as in Western medicine. Moreover, the plant parts identified with medicinal properties are used in entirety resulting in the Ayurvedic medicines having multiple molecules and targets. It is critical to know that Ayurveda has its own pharmacological parameters such as *rasa* to discern the therapeutic use of medicinal plants [40,41]. For this reason, work on medicinal plants should not be simplistically classified as Ayurvedic research unless based on Ayurvedic pharmacological parameters.

4.1.2. Selection criteria for medicinal plants: Ayurveda to lead drug molecules

Going by the conceptual differences in the standpoints of Ayurveda and modern medicine, Ayurvedic knowledge based selection of right candidate plants for modern drug discovery is not simple and straight forward. The lead information can rely either on similarities based on clinical symptoms or causative factors. In the context of the former, *rajayakshma* and *ojo kshaya* are considered to mimick HIV [50,51]. The causative factor based approach with regard to HIV, points to identifying diseases caused by virus. In this study, the six plants chosen are used in Ayurveda for treatment of *visarpa*, correlated to the viral disease herpes. However, it is also pointed out that Ayurveda does not consider *visarpa* as viral in origin (*krimi*) but regards it as a perturbation of *dosha* [33].

Selection of plants for evaluation of anti-HIV potential need not be only disease- (*visarpa*) or plant category (*krimighna*)-based. In fact for treating *visarpa*, *balya* and *rasayana* drugs can also be used to improve the *bala*/*oja* of the patients, thus targeting the immune system to efficiently resist the *krimi*. The rationale would be that herpes zoster infections occur in immuno-compromised hosts.

When the experimental results of bioassays presented in this study are viewed against this background and the many nuances in Ayurvedic approach to treatment in general, their inconsequentiality to Ayurveda becomes apparent. For instance, of the six plants studied, Ayurveda indicates anti-microbial (*krimighna*) activities only for *A. indica* (*nimba* in Ayurveda) – *krimighna* plants are generally considered to have anti-microbial, anti-bacterial, anti-fungal and/or anti-viral activities. The two plants [*T. bellerica* (*vibhītakī*) and *Z. officinale* (*ārdraka*)] which exhibited anti-HIV-1 potential in this study are not *krimighna*. This underlines the inherent limitations of conceptual bilinguism between Allopathy and Ayurveda, especially in the context of therapeutic attributes and use of medicinal plants.

There is another potential inference from this study. While the experimental data provides preliminary leads for anti-HIV-1 drugs, pharmacological effectiveness of plant extracts rather than single molecules have also been demonstrated. Although conventional pharmacological standpoint is single molecule-single target, there is now growing consensus that multi-targeting multi-molecular drugs might be more effective [52–54]. Plant extracts with multi-molecules are likely to have multiple targets. In this context, it is pertinent to note clinical trials on HIV/AIDS patients reporting the effectiveness of Ayurvedic polyherbal formulations and single herbal products (plants used in their native form) [51,55,56]. The present study also underlines the interesting possibility of using whole extracts

Table 4
Anti-HIV-1 activity of six plant hydroalcoholic extracts against three pseudoviruses.

Plants	IC_{50} (μg/mL)		
	Clade C		Clade B
	ZM109	ZM53	RHPA
T. bellerica	< 2	< 2	< 2
Z. officinale	98	< 4.1	< 4.1
S. racemosa	> 100	> 100	> 100
S. chirata	> 200	> 200	> 200
A. indica	> 300	> 300	> 300
T. cordifolia	> 600	> 600	> 600

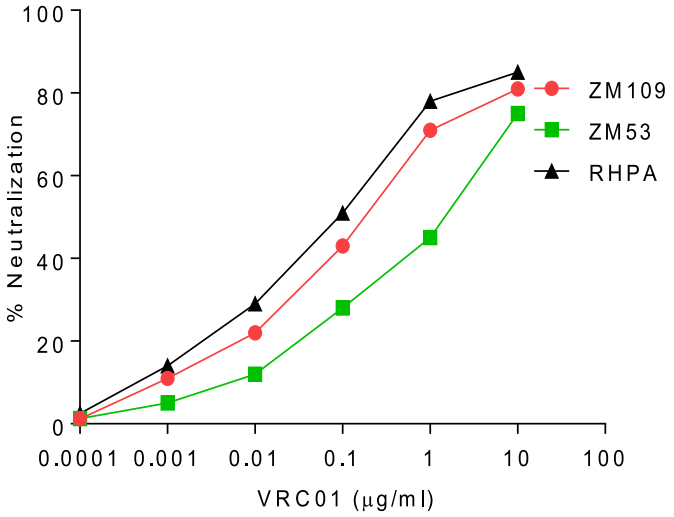

Fig. 3. Neutralization of pseudoviruses by VRC01: serial dilutions of antibody were pre-incubated with pseudovirus for 1 h and then added to TZM-bl cells. Two days after infection, luciferase values were measured and percent neutralization calculated.

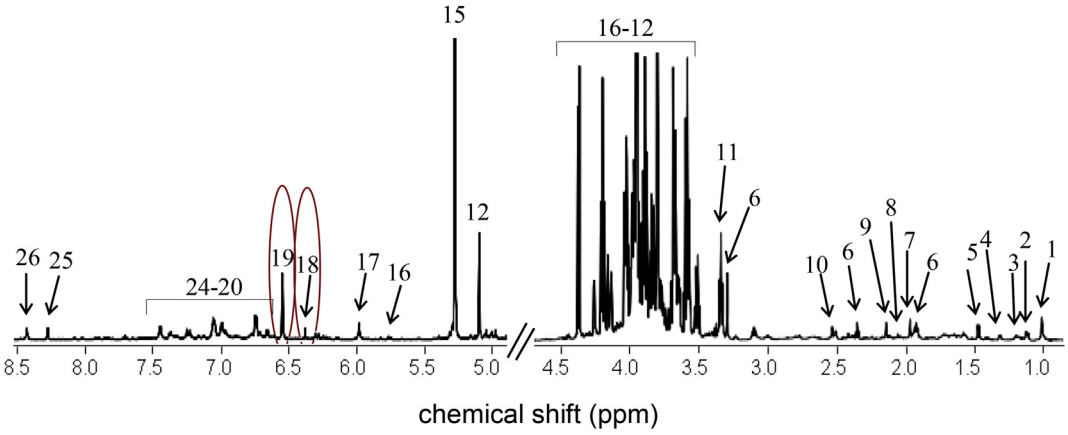

Fig. 4. One dimensional proton NMR spectrum of *T. bellerica*, which showed anti-HIV-1 activity. The resonances are: 1 – valine, 2 – rhamnose, 3 – lactate, 4 – alanine, 5 – arginine, 6 – γ-amino n-butyrate, 7 – acetate, 8 – N acetyl aspartate, 9 – methionine, 10 – succinate, 11 – choline, 12 – glucose, 13 – fructose, 14 – glycerol, 15 – sucrose, 16 – galactose, 17 – fumarate, 18 – ferulic acid, 19 – caffeic acid, 20 – phenylalanine, 21 – transaconitate, 22 – flavonoids/glucosinolates, 23 – tyrosine, 24 – tryptophan, 25 – formate, 26 – ATP (Adenosine triphosphate).

rather than single molecules to address multifactorial diseases like AIDS, which would have several therapeutic targets.

5. Conclusion

Of the six plants studied in unfractionated form, *T. bellerica* and *Z. officinale* showed minimum cell cytotoxicity and maximum anti-HIV-1 potential. By correlating Ayurvedic pharmacological properties with NMR metabolomics data, the study has also shown that the latter can be a useful technique to study plant extracts as used in Ayurveda. At the same time, the article has also drawn attention to the fundamental differences in the conceptual frameworks of Ayurveda and Western medicine, especially in the context of medicinal plants, and the relevance of work such as this for Ayurveda.

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Conflict of interest

None.

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