

Fermentation of Polyherbal Preparations as in Ayurveda: a Novel Protocol for Drug-Lead Discovery

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Abstract

Background: Alcoholic fermentation of polyherbal preparations to yield medicinal wines has been in vogue since the dawn of Ayurveda. It was not explicitly stated in the classics of Ayurveda, why to ferment medicines? However, this unique class of medicines was considered to be providing better/higher active medicinal principles than their unfermented forms. There are only a few reports expressing evidences from investigations driven by this hypothesis. Berberine and its derivatives form such a model for assessing the positive changes brought about by Ayurvedic fermentation, as reported earlier.

Materials and methods: Plants were collected and polyherbal formulations were prepared as per the classic Ayurvedic texts and the formulation was compared with whole plant extract through LC/MS-MS for the production of their derivatives.

Results: We were austerely analysing for the derivatives of piperine during the formulation production. In our analysis it is proven that piperine and its derivatives were found in the herbal formulation which leads to another model for assessing the positive changes due to Ayurvedic fermentation.

Conclusion: The significance of looking at Ayurvedic fermentations with novel interests as a drug discovery protocol is suggested, since it might provide new, more potent, water soluble and biocompatible drug-leads. It may be used in conjunction with high throughput systems and would deliver drug-leads present naturally.

Keywords: Ayurvedic Fermentation; Medicinal Wines; Biotransformation of Piperine; Biocompatible; Enhancement of Medicinal Value

Introduction

Black pepper has been in use for millennia in continents where it would not grow normally. Hence, it has been a commodity to trade since the dawn of

civilization. Piperine is a major compound present in black pepper as well as in certain other related plants [1]. Piperine and its derivatives, both natural and synthetic, are of great significance in food and medicine [2]. It has been a topic for intensive and extensive research for

decades to get better forms of it with higher activity [3]. Attempts to synthesize piperine and its derivatives have yielded great success [4-9]. The enquiries to find active principles of food and medicine paved the way of identifying the significance of piperine and its natural analogues [3]. The synthetic forms for naturally occurring piperine derivatives and novel derivatives derived from the chemical and structural knowledge of compounds too have been investigated for their properties [2]. The novel, synthetic derivatives often have been looked upon for enhanced properties than the natural forms [10,11].

Natural products have been excellent sources of therapeutically effective medicines, though they may be the oldest form of medication. Among them, plants are the most important sources of medicines. In any part of world, traditional medicines were in vogue where plants were the most important and available materials [12-15]. At present, outstanding developments in synthetic drug designing have been of great success for delivering more powerful drugs than their natural cousins. However, plant-based compounds have retained their place, probably due to safety reasons. Therefore, research on plant products with their alleged traditional uses as drugs and compounds present in them is viewed as important as modern methods of synthesizing compounds [16,17].

The compounds present in *Piper* sp. have shown *in vitro* inhibitory activity against the therapeutically important enzymes such as COX 1 and LOX. These enzymes are responsible for prostaglandin and leukotriene biosynthesis respectively [18], hence critical in managing inflammation. Since inflammation is a common process observed in all pathological encounters of biosystems, the bioactive compounds of *Piper* sp. are of great importance. Piperine, an active phenolic component extracted from black pepper is quite useful in stimulating the pancreatic enzymes, protecting against oxidative damage, lowering of lipid peroxidation, and enhancing the bioavailability of many other therapeutic compounds as well [19]. Black pepper may also contain many derivatives of piperine such as piperidine, piperetine, piperaninein etc. in very small quantities [20]. The anti-inflammatory activities of piperine and its derivatives have been demonstrated in different rat models [21]. The effect of piperine seems to be beneficial for severely painful inflammatory diseases such as rheumatoid arthritis. In olden days, pepper had been used against many diseases such as cancer, malaria, cholera etc. [22]. Although, activities of piperine have gained wide recognition, the activities of its derivatives have not received any specific, noticeable acceptance. There are reports of piperine biotransformation, although it is considered to be difficult to get metabolized [23,24].

Detectable quantities of piperine metabolic derivatives such as piperonylic acid, piperic acid, and piperonal are reported [19].

As lipoxygenase (LOX) metabolizes arachidonic acid to leukotrienes, it is regarded as a potent target for drugs for treating many diseases [25]. We have reported the comparative LOX inhibitory activity of piperine, piperonylic acid, piperic acid, and piperonal within themselves and with a standard [26]. There are several other reports showing higher desired activity of piperine derivatives than piperine [9,5]. Since many fermented Ayurvedic medicines (asava, arishta) contain black pepper and certain other peppers, it is natural to expect piperine derivatives in such preparations. The presented work, driven by the above mentioned hypothesis, focused on the fermentative experiment in search of piperine derivatives, with the Ayurvedic protocol for a medicinal wine pippalyasava, containing three peppers [26]. The pippalyasava prepared in the laboratory as per the ancient protocol and the samples of pippalyasava procured from market did contain piperine together with its derivatives, such as piperonylic acid, piperic acid, and piperonal. Piperine was considered as a model for a medicinal compound only. There may be many such medicinal compounds getting transformed into more potent forms by fermentation in Ayurvedic medicinal wines.

Materials and Methods

All the materials for fermenting pippalyasava at the authors' institution were a general gift of the Vaidyaratnam P. S. Varrier's AryaVaidyaSala, Kottakkal, Kerala, India. It was made according to the Ayurvedic Pharmacopoeia of India, Part II, Vol. II [26]. The pippalyasava fermented in the laboratory and the samples procured from marketing outlets of different Ayurvedic Pharmaceutical companies (AryaVaidyaSala, Santhigiri, Kerala, India) were clarified by centrifugation to remove debris left out by fermentation and the clear liquid was dried under vacuum, to vaporize water, self-generated the ethyl alcohol and other volatile solvents present in them. The residue was re-dissolved in mass spectra grade water and methanol: water (1:1). The samples were filtered through appropriate filters for application on to the Acquity UPLC system fitted with C18 column and connected to Xevo G2-S Q-ToF MS/MS system (Waters). The UPLC run conditions were standardized for a 15 min run with mobile phase A (0.1% Formic Acid in Water) and B (Acetonitrile). The gradient elution starting from 95(A):5(B) to 5(A):95(B) in the end. For the better separation of the peaks, particular gradients were kept in hold at specific time points. The masses and

corresponding Retention Time were compared with the standards. The MS analyses were performed with a capillary voltage of 3 kV. The desired masses were then to undergo collision induced dissociation in the same instrument. The CID was also standardized by applying varying collision energies (25 V to 50 V). The fragmentation patterns were then compared with the standard compounds using Massfrag software (Waters). Samples of piperine, piperonylic acid, piperic acid, and piperonal, procured from Sigma were used as standards.

Results and Discussion

Piperine, piperonylic acid, piperic acid, and piperonal were identified and confirmed by the MS and MS/MS data given below:

1. Piperine (MW 285.343 g/mol)- UPLC: Rt = 8.716, ESIMS (positive): MS, m/z 286.1395 (M+H)⁺, Tandem mass spectrometry (MS/MS) (Precursor ion at m/z 286.1395 [C₁₇H₁₉NO₃]), m/z 201.05 [C₁₂H₁₁NO₂]⁺, m/z 135.036 [C₈H₇O₂]⁺, m/z 112.0691 [C₆H₁₀NO]⁺.

2. Piperonylic Acid (MW 166.0266) UPLC: Rt = 14.421, ESIMS (Positive): MS, m/z 167.0845 (M+H)⁺, Tandem Mass Spectrometry (MS/MS). (Precursor ion at m/z 167.0845 [C₈H₆O₄]), m/z 99.96 [C₇O]⁺, m/z 129.97 [C₈H₂O₂]⁺.

3. Piperic Acid (MW 218.208 g/mol)(C₁₂H₁₀O₄)- UPLC: Rt = 6.20, ESIMS (Positive): MS, m/z 219.08 [M+H]⁺, Tandem Mass Spectrometry (MS/MS) (Precursor ion at m/z 219.07,[C₁₂H₁₀O₄]), m/z 201.0582 [C₁₂H₉O₃]⁺, m/z 173.0662 [C₁₁H₉O₂]⁺, m/z 159.0457 [C₁₁H₁₁O]⁺, m/z 143.0475 [C₁₀H₇O]⁺, m/z 135.0437 [C₈H₇O₂]⁺, m/z 115.0518 [C₈H₃O]⁺

4. Piperonal (150.03)- UPLC: Rt = 13.304, ESIMS (Positive): MS, m/z 151.1356 (M+H)⁺, Tandem Mass Spectrometry (MS/MS) (Precursor ion at m/z 151.1356[C₈H₆O₃]), m/z 133.99 [C₇H₂O₃]⁺, m/z 91.51 [C₇H₇]⁺

Piperine and its derivatives as obtained in laboratory sample, commercial sample 1 and commercial sample 2 are given as Figures 1-3 respectively (supplementary data).

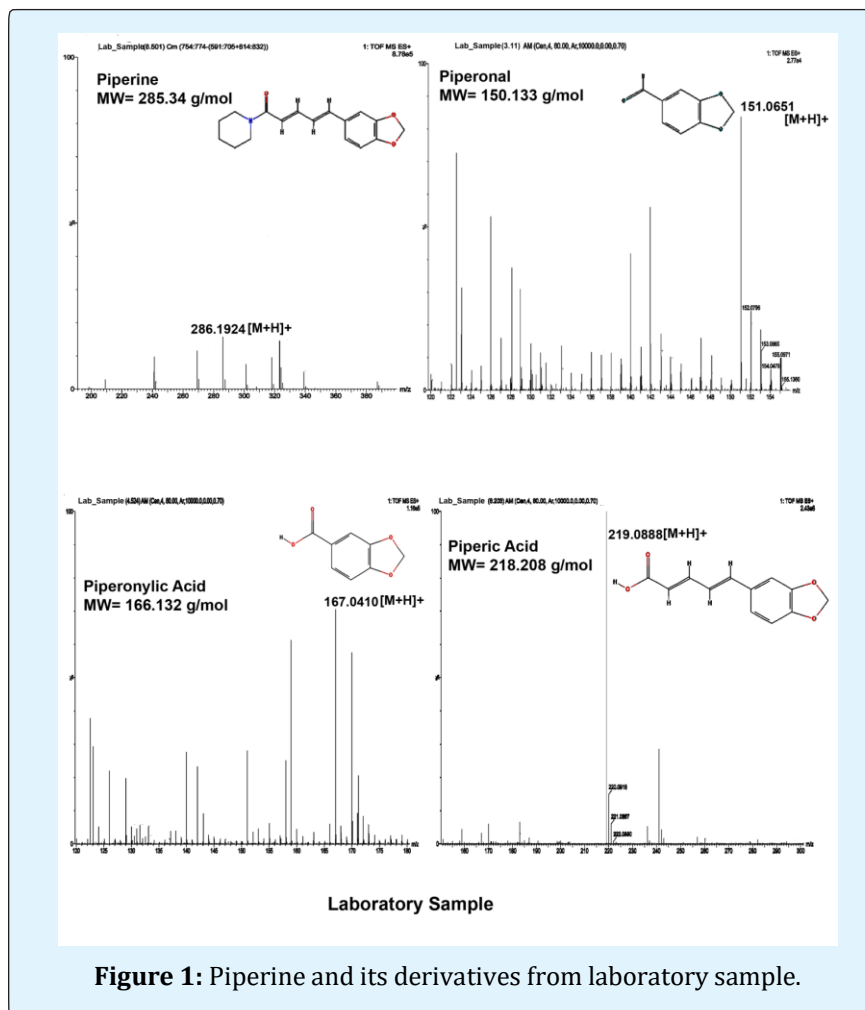
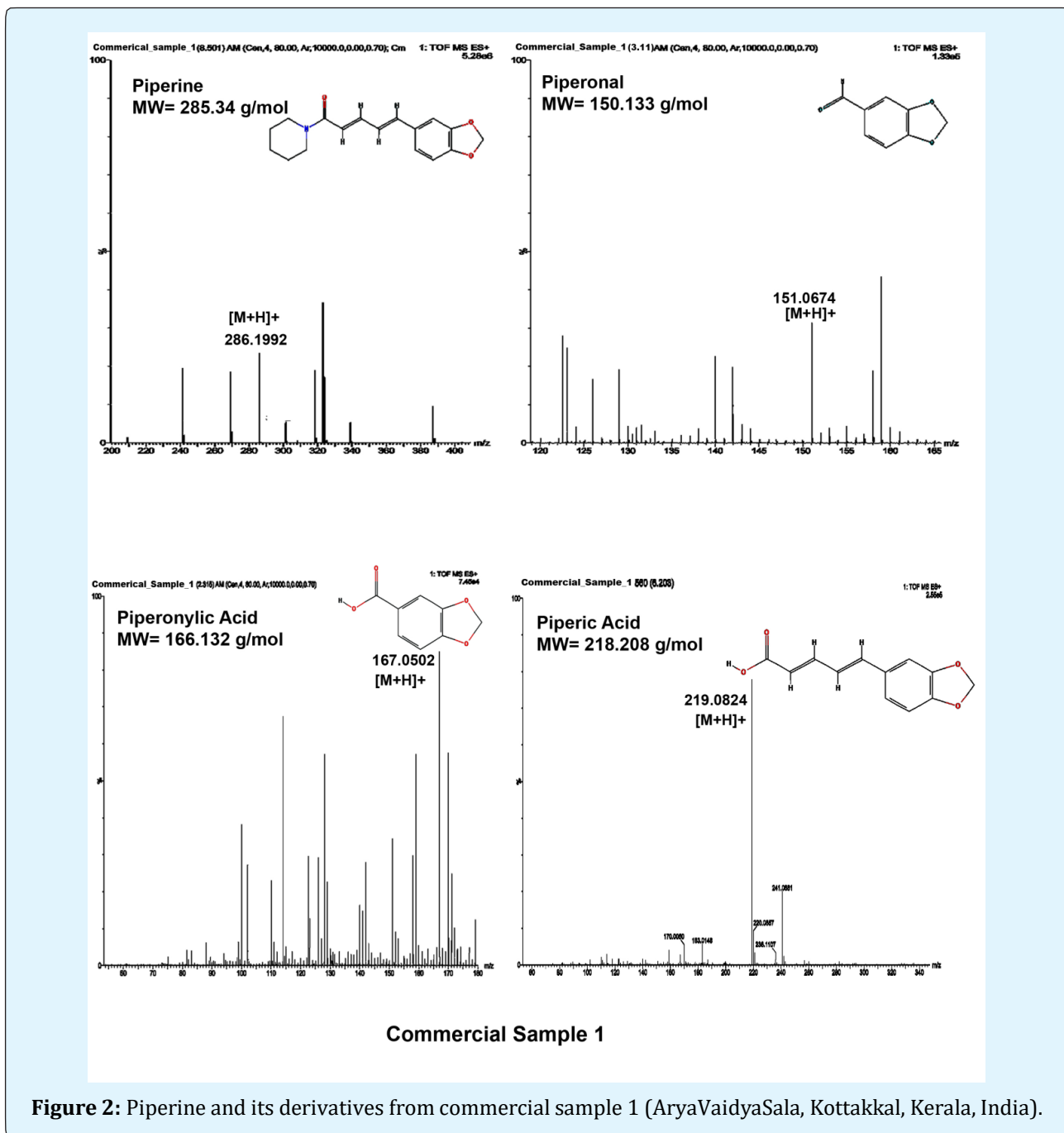
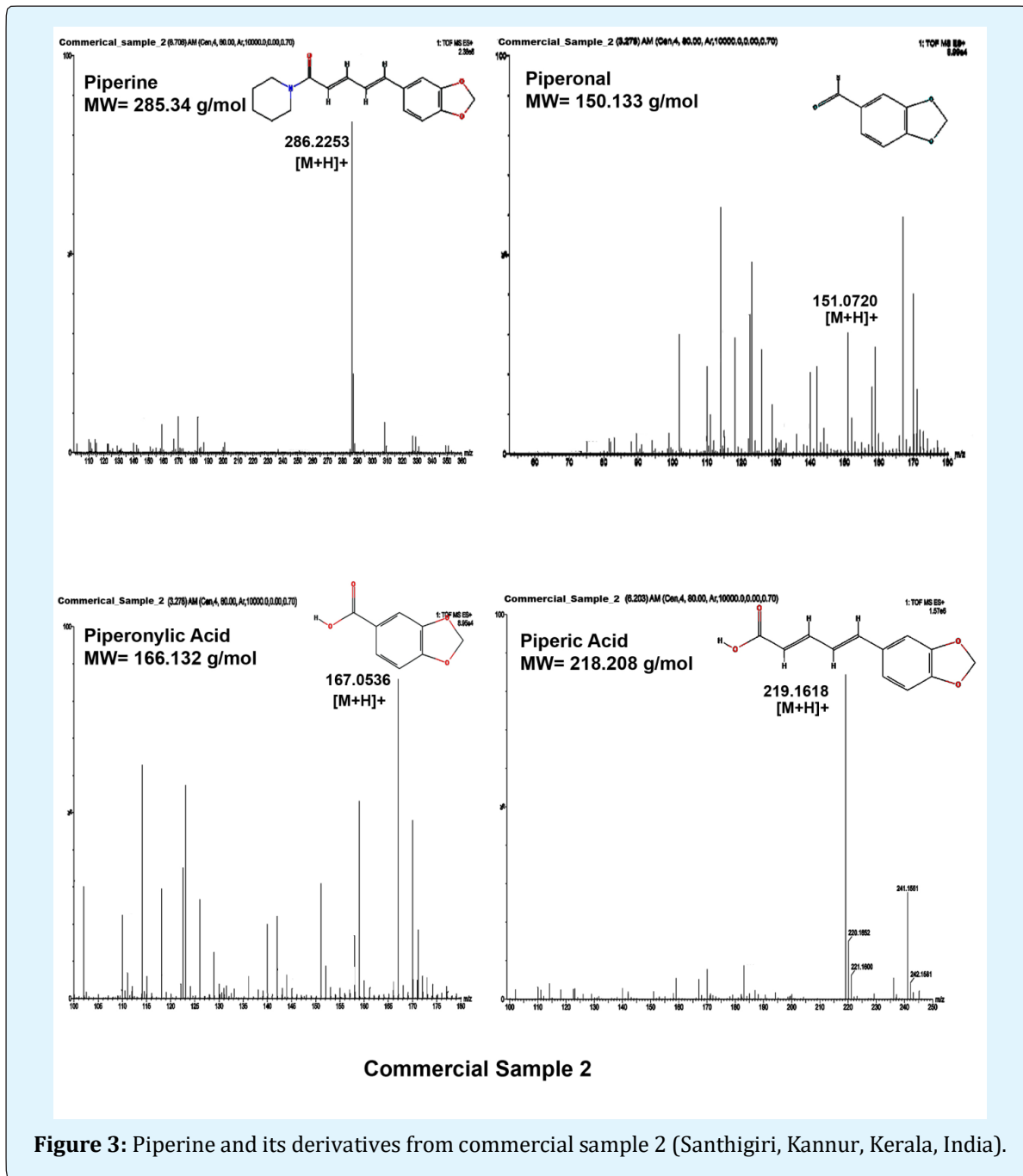


Figure 1: Piperine and its derivatives from laboratory sample.

The pippalyasava made in the laboratory of the authors as per the Ayurvedic Pharmacopoeia of India, Part II, Vol. II [26] and the samples procured from market agreed to each other as far as the presence of piperine, piperonylic acid, piperic acid, and piperonal are concerned. Though quantitative estimation was not undertaken, a picture of equivalence in quantity of the

different derivatives of piperine present in the different samples analyzed was gained by assessing of the dilution factors of the assayed samples. If the preparation protocol of pippalyasava was practiced rigorously, this could be expected from the fermentation by the wild yeast consortium, with a self-regulating population structure.





Derivatives of piperine, piperonylic acid, piperic acid, and piperonal, demonstrated superiority over piperine in inhibiting the therapeutically significant enzyme, LOX as expressed in the previous report by the authors [27]. The derivatives of piperine are expressively superior to piperine. Piperine derivatives have been found to have more potential in Alzheimer's disease treatment since Piperine, piperic acid, and piperic ester exhibit AChE *in*

vitro inhibitory activity of 46.5, 50.6, and 63.6%, respectively [11]. Piperine derivatives, piperonylic acid, piperic acid, and piperonal have been found to be better LOX inhibitors than piperine which are implicated in inflammation [27]. Several such instances are reported elsewhere [10]. Similarly, berberine derivatives inhibit phospholipase A₂, another enzyme implicated in anti-inflammatory therapy, than the parent compound

berberine. Berberine will get bio transformed into such higher active derivatives by fermentation [28-30]. Biotransformation of berberine has been considered as a model for assessing the positive changes possibly brought about by Ayurvedic fermentation [30].

The biotransformation of piperine delivers compounds which are superior in water solubility and bioavailability implicating as potential better therapeutic leads in specified instances, as expressed by the results of present work. Hence, the protocol adopted in Ayurvedic medicine fermentation may provide piperine a boost to its special status of 'enhancers of bioavailability', by diversifying and strengthening the repertoire of active molecules [31-34]. Piperine forms metabolic derivatives such as piperonylic acid, piperic acid and piepronal in rat [19]. However, such higher active metabolic derivatives would be available in the body for lesser time compared to their delivery through fermented samples. It may be considered logically and hopefully that their possibility to be developed to a drug may be high because of better pharmacological activity, favorable pharmacokinetic and pharmacodynamic behaviors or less toxicity This is an obvious advantage of fermenting poly herbal preparations into medical wines: to enhance their medicinal value. Hence, the expressed work would qualify to be another model for assessing the positive changes possibly brought about by Ayurvedic fermentation in the case of berberine as a model [30].

Conclusion

Since a reverse pharmacological approach would be fruitful to identify drug-leads for targeted diseases, Ayurvedic fermentation would prove to be better than a blind approach with random source materials. Hence, apart from providing popular dosage forms of medicinal wines, fermenting of Ayurvedic medicines would be able to provide better drug leads for any system of medicine. Since the products are out of fermentation by organisms including probiotic systems, they may be assumed to be more biocompatible that suit to the human system than anything produced by synthetic means. It may be used in conjunction with high throughput systems and would deliver drug-leads present naturally. The work suggests the need for looking at the Ayurvedic fermentations with relevant and novel interests as observed in a recent review [35].

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Disclosures

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