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Biochemical Characterization and LC-MS/MS Analysis of Phenolic Compounds in Ginger Wine

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Abstract

The present investigation proposes a biochemical characterization of ginger wine by LC-MS/MS analysis for a variety of phenolic compounds. Seven biochemical parameters were characterized by using standard methodologies. External calibration standards were used to quantify 15 phenolic acids (5 points). The use of 0.1 percent formic acid improves sample stability throughout the analysis. Matrix interference peaks were observed in some cases, which were separated chromatographically using gradient mobile phase. The first transition was used for the quantification whereas the second transition was used for the confirmation. The biochemical parameters observed in ginger wine were within the range of the Bureau of Indian Standard for wine. Six phenolic acids were reported out of 15, with vanillic acid being the most abundant and resveratrol being the least abundant.

Keywords
LC-MS/MS, Ginger, Wine, Phenolics.

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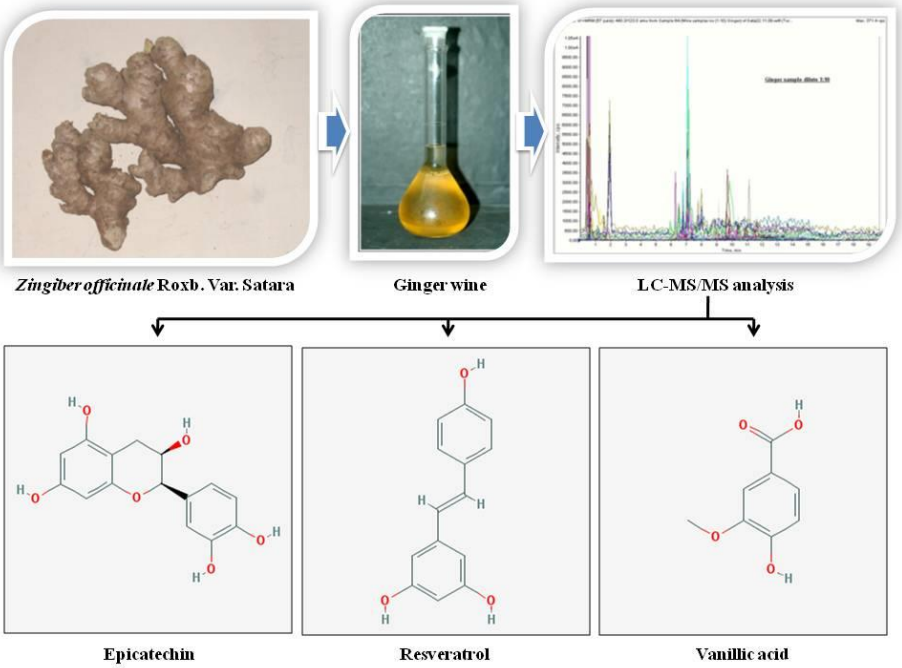


1. INTRODUCTION:

Wine is one of the most popular alcoholic beverages on the planet. The phenolic compounds of the wine, notably flavanols (i.e., catechins), have been the focus of current research since their relationship to the beneficial effects of moderate consumption of wine was observed; often known as the "French Paradox" [1]. Phenolic compounds present in red wine cause an increase in the total antioxidant capacity of serum thereby triggering low-density lipoprotein [2] and reducing the risk of cardiovascular diseases. The high-performance liquid chromatography (HPLC) technique has been widely used in order to determine the phenolic compounds in wine samples [3-5]. Simple phenolic acids (such as hydroxybenzoic acid and hydroxycinnamic acid) and sophisticated polyphenols (such as flavonols, anthocyanins and tannins) are mostly derived from grape skins and seeds during the vinification process in red wine [6] or from yeast metabolites and aging in oak barrels. Due to its great sensitivity and ease of use, reverse-phase, high-performance liquid chromatography (RP-HPLC) with a diode array detection (DAD) detector is extensively used to analyze these chemicals in wine [7-10]. However, the UV spectra of some phenolic compounds are quite similar, making their identification ambiguous. Analytical technology for phenolic structures in grapes and wines was thus developed using LC-MS and multiple MS/MS (MSn) stages with an ESI source operating in the negative mode [11-13]. With LC-MS, differences in phenolic compositions and structures could be identified, so nowadays LC-MS is considered the best analytical approach for studying phenolic compounds in grapes and wines [14,15].

2. METHODOLOGICAL DESIGN:

2.1. Preparation of Ginger Wine

Ginger wine was made from local ginger variety using Yeast strain – *Saccharomyces cerevisiae* MTCC – 463, in the laboratory at Shivaji University, Kolhapur, Maharashtra, India.

2.2. Biochemical Parameters

All the parameters excluding pH were characterized by using method [16] with some modifications and pH was determined by using method [17].

2.3. Standard Solutions

All the reference standards were purchased from Sigma Aldrich and stored in dark vials in a refrigerator at 4^0C until further use. Standard stock solution (10 mg/L) was prepared by mixing the standard compound in an appropriate solvent (methanol-water (1:1, v/v)). A working standard mixture of 1 mg/L was prepared from the dilution of the above stock. The calibration standards were made by serial dilution of 1 mg/L working standard with methanol-water (1:1, v/v) in the range of 10-500 ng/ml.

2.4. Sample Preparation

The ginger wine samples were injected with dilution up to 10 times by using 0.1% formic acid in water. Diluted samples were passed through 0.2µm nylon membrane filter paper and injected into LC-MS/MS for analysis.

2.4.1. LC-MS/MS Conditions

The LC-MS/MS analysis was done with Agilent Technologies 1200 series hyphenated to API 4000

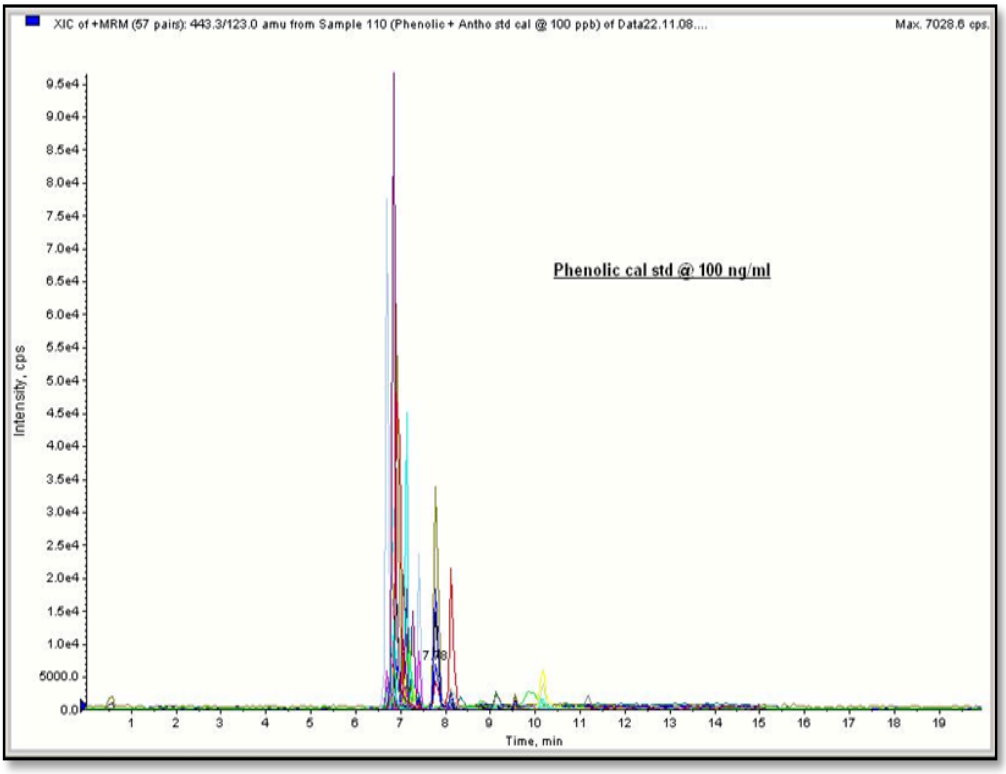
Qtrap (ABS Sciex) mass spectrometer equipped with electrospray ionization (ESI+). Pricenton SPHER-60 C-18 60A^{o} (150x2mmx5µm) with mobile phase A-0.1% formic acid in water: methanol (90:10), B-0.1% formic acid in water: methanol (10:90). The gradient 0-1min 98% A phase,1-12min 98-5% A phase,12-14min 5% A phase,14-15min 5-98% A phase and 15-22min 98% A phase. The flow rate was 0.400 ml/min. The oven temperature was 35^{o}C and the injection volume was 10µl. Mass parameters: curtain gas 20 psi, ion spray voltage 5500V, and temperature 500^{o}C. Mass parameters were given in table 1.

3. RESULTS AND DISCUSSION:

The biochemical parameters characterized from ginger wine were depicted in Table 1. The ethyl alcohol (8.12%) was marginal compared to other wines; pH (3.85) was in the range, while total sulphur and free sulphur were reported in very less amounts. Total acids and volatile acidity were both found to be within the BIS range. The phenolic compounds were analyzed from ginger wine by LC-MS/MS with electrospray ionization in positive polarity. Those compounds positively detected as per the above criteria were mentioned in Table 2. The chromatogram (Fig 1a) depicts standard phenolic compounds with retention time, while the chromatogram (Fig 1b) depicts a detailed account of phenolic compounds found in ginger wine with retention time. Significant changes in the composition and concentration of phenolic compounds occur during the winemaking process, because of the disintegration of the fruit, as well as wine fermentation and aging [18]. Ginger wine contained different polyphenolic compounds in different amounts. A detailed account of all 15 compounds quantified in ginger wine was depicted in table 3. Among the polyphenols analyzed, Vanillic acid – I (643 ng/ml) was found to be the most abundant followed by Vanillic acid – II (505 ng/ml), while Resveratrol – I (31.20 ng/ml) was found to be the least amount. Researchers conducted on the compositions of phenolics in wines are greatly focused on the concentrations of resveratrol and anthocyanidins [19-23].

Fig 1: a) Chromatogram of standard phenolics b) Chromatogram of ginger phenolics.

a)



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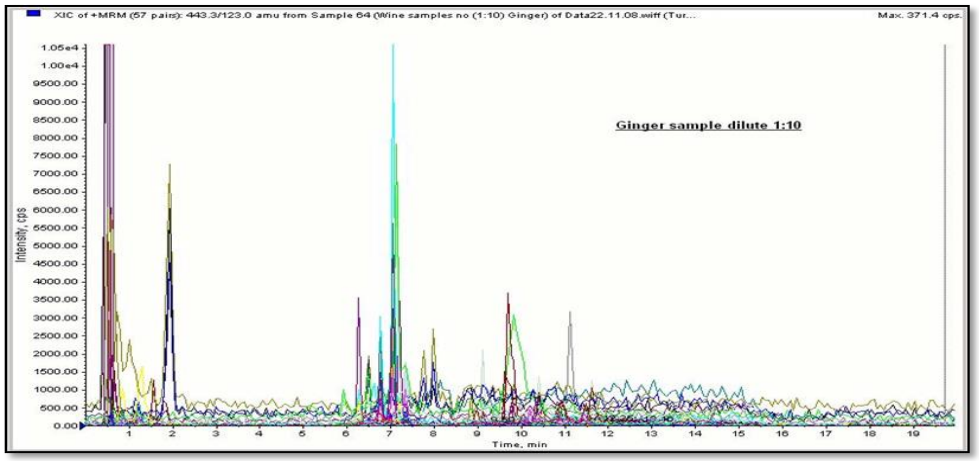

Table 1: Biochemical content of ginger and other wines

Sr. No.	Characteristic	Dry White/Red	Sweet White/Red	Sparkling Wine	Ginger wine
1	Ethyl Alcohol	8 to 15.5 (% by volume) ±5	8 to 15.5 (% by volume) ± 5	8 to 15.5 (% by volume) ±5	8.12%
2	Reducing residual sugar, g/l, Max	10	10 to 150	100	4.23
3	pH	3.0-4.0	3.0-4.0	3.0-4.0	3.85
4	Total Acids (as tartaric acid), g/l, Max	10.00	10.00	10.00	7.5
5	Volatile Acidity expressed as acetic acid, g/l, Max	1.0	1.0	1.0	0.57
6	Total sulphur dioxide, mg/l, Max	250	250	250	12.28
7	Free sulphur dioxide, mg/l, Max	100	100	100	1.53

Table 2: Different polyphenol compounds analyzed in ginger wine by LC-MS

Analyte Peak Name	Calc. Conc. (ng/mL)	Analyte RT
Epicatechin -I 291.0 /123.0	313.00	7.06
Rutin hydrate -I 611.0 /303.0	113.00	7.35
Syringic acid -I 199.0 /155.0	259.00	7.21
Vanillic acid -I 169.0 /125.0	643.00	7.13
Resveratrol -I 229.0 /135.0	31.20	9.22
Catechin -I 291.0 /165.0	176.00	6.78

Table 3: Mass spectrometer parameters

Sr. No.	Compound	ESI $(M+H)^+$	Transition-I					Transition-II		
			Q*	Q_1	DP	CE	CXP	Q_2	CE	CXP
1.	Epicatechin gallate	$(M+H)^+$	443	123	51	19	5	273	12	5
2.	Caffeic acid	$(M+H)^+$	181	89	26	43	6	135	27	8
3.	t-Piceatannol	$(M+H)^+$	245	199	65	25	5	135,107	32, 28	9, 5
4.	Epicatechin	$(M+H)^+$	291	123	50	23	5	139,165	20 ,18	6
5.	Chlorogenic acid	$(M+H)^+$	355	163	56	19	10	89, 135,145	81,53,43	4,8,6
6.	*p*-Coumaric acid	$(M+H)^+$	165	147	56	16	6	119, 91	25, 35	5,3
7.	Quicetrin hydrite	$(M+H)^+$	449	303	45	15	9	129, 85	22, 35	6
8.	Rutin hydrate	$(M+H)^+$	611	303	61	29	16	465, 85	19,59	18,4
9.	Syringic acid	$(M+H)^+$	199	155	56	14	7	123, 77	18,38	5,2
10.	Vanillic acid	$(M+H)^+$	169	125	55	14	5	93	21	3
11.	Quercetin	$(M+H)^+$	303	153	55	45	5	137, 69	45,80	5,1
12.	Ellagic acid	$(M+H)^+$	303	257	116	40	4	229, 201	45,50	4,3
13.	Resveratrol	$(M+H)^+$	229	135	29	22	6	107, 119	30,55	4,5

14.	Catechin	$(M+H)^+$	291	165	38	17	7	139,123	22	7,2
15.	Kaempferol	$(M+H)^+$	287	165	53	40	2	121	50	5

Note: Q*- parent ion, Q_1- quantitation ion, Q_2- confirmatory ions

4. CONCLUSION:

According to our knowledge, there are no detailed data regarding the biochemical characterization and composition of phenolic compounds in ginger wine was present. So, this preliminary study contributes new knowledge of the biochemical content and composition of phenolic compounds by LC-MS in the wine. The report of an anti-aging compound like resveratrol will lead to its commercialization. Further studies, with a larger number of samples, are necessary to confirm the differences observed.

CONFLICT OF INTEREST: None

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