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Novel Innovations in Herbal Wine and Pharmacological Potential

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ABSTRACT
In recent years, the wine industry has undertaken various innovations to support the development of functional foods, as evidenced by numerous wine products formulated with herbal plants. These combinations have resulted in a diversity of secondary metabolites, which are of significant interest to the pharmaceutical industry. Nevertheless, further breakthroughs are needed to introduce novelty in herbal wine products. One such innovation involves the utilization of *Cymbopogon citratus*, *Zingiber officinale*, *Citrus hystrix*, *Elettaria cardamomum*, *Cinnamomum verum*, *Syzygium aromaticum*, and *Caesalpinia sappan* L. This study identified 85 compounds, demonstrating distinct differences in the chemical of bioactive compounds between the raw herbal plants and the fermented product. These differences are attributed to substitution reactions occurring in the carbon framework, resulting in the formation of derivative compounds in the herbal wine. Additionally, enzymatic reactions mediated by *Saccharomyces cerevisiae* during fermentation led to the formation of biologically important precursors, such as N-Acetylmannosamine, which plays a role in the regulation of GNE gene mutations. Therefore, the herbal wine produced in this study presents potential as a functional food with promising pharmacological applications.
Keywords: *Bioactive compounds; Herbal; Pharmacological; Saccharomyces cerevisiae; Wine*

INTRODUCTION

Wine is generally an alcoholic beverage produced through the fermentation of fruits (substrates) that contain sugars (Bhise & Morya, 2021). Based on its classification, wine is divided into two main categories: grape wine and non-grape wine. The latter group is further subdivided into six types, namely fruit wine, table wine, fortified wine, medicinal wine, sparkling and still wine, and dessert wine (Morya et al., 2024). According to Soni et al. (2021), medicinal wine is a beverage with medicinal properties derived from herbal plants. In recent years, medicinal wine has been extensively studied, revealing a variety of interesting findings. Morya et al. (2024) reported that wines containing extracts from *Phyllanthus emblica*, *Aloe vera*, *Ocimum tenuiflorum* Linné, *Cymbopogon flexuosus*, *Mentha piperita* L., *Cinnamomum verum*, *Sambucus*, and *Zingiber officinale* exhibit antimicrobial and antioxidant effects. Similarly, Zhao et al. (2024) found that a combination of grapes and wolfberries with jujube produced both volatile and non-volatile components. Furthermore, the presence of leucine and phenylalanine was noted to enhance the nutritional value of the wine. The plant *Lycium ruthenicum* Murray has long been used in traditional Chinese medicine to treat heart-related and other ailments (Lee & Choi, 2023). When combined with grapes, this plant yields a fermented product that shows increased levels of chemical compounds with antioxidant and anti-inflammatory benefits (Lu et al., 2022). The use of herbal plants from 15 families—such as *Rutaceae*, *Lamiaceae*, and *Asteraceae*—in local wines from Eastern Spain and the Balearic Islands has also been documented. Some of these wines have been found to provide medical benefits, such as alleviating symptoms of diarrhea (Martínez-Francés et al., 2021).

Herbal wine is commonly made by adding medicinal plants during the fermentation process or by blending wine with plant extracts that contain bioactive compounds. Perestrelo et al. (2018) examined the combination of wine with *Musa sapientum* L., *Passiflora edulis* L., and *Malus domestica* Borkh. Kumaresan et al. (2024) studied wine combined with *Humulus lupulus*, *Mentha arvensis*, *Emblica officinalis*, and *Allium sativum*. Snopek et al. (2018) investigated wine with *Camellia sinensis*, while (Nguyen et al., 2019) studied a blend of red wine with *Ganoderma lucidum*. In addition, Liu et al. (2024) reported combinations of wine with *Cyclocarya paliurus*, and Chawafambira, (2021) explored wine with *Lippia javanica* and *Uapaca kirkiana*. These studies indicate that wine can be combined with medicinal herbs to enhance its value and function as a beverage that is not only rich in flavor but also possesses pharmacological properties. Herbal plants, according to Sujarwo et al. (2015) and Estiasih et al. (2025), are traditionally known in Indonesia as jamu or wedangan, which are commonly consumed by the local population. These traditional herbal drinks can be innovatively combined with wine-making (fermentation) techniques. In developing a new type of herbal wine, this study uses medicinal plants fermented through wine fermentation methods. This innovation differs in both methodology and the type of wine produced compared to previous studies.

This study aims to investigate the effect of wine fermentation techniques on herbal plants in relation to the composition of their bioactive compounds. This innovation in herbal wine production is expected to open new avenues for research in the fields of wine science and herbal beverage processing. In addition, it has the potential to be developed for fortification with additional nutrients or as a functional food.

METHODS

Fermentation Procedure

The ingredients used are divided into two groups: fresh and dried. The fresh ingredients include *Cymbopogon citratus* (16 g), *Zingiber officinale* (3 g), and *Citrus hystrix* (1 g). The dried ingredients consist of *Elettaria cardamomum* (4 g), *Cinnamomum verum* (1.5 g), *Syzygium aromaticum* (0.1 g), and *Caesalpinia sappan* L. (3.3 g). All ingredients are thoroughly cleaned to remove any foreign materials. Sugar (333 g/L) is then added, and the mixture is boiled for 10 minutes, followed by a cooling process. After cooling, the mixture is filtered, and *Saccharomyces cerevisiae* yeast is added. The solution is then fermented for one week at room temperature (approximately 28–30°C). Next, it is transferred to a new container for a second fermentation period lasting one month. Once the herbal wine reaches one month of age, it is transferred again to a clean container and stored in a dark room until the analysis is conducted.

GC-MS Analysis

The analysis of volatile components in the herbal wine was conducted using Gas Chromatography-Mass Spectrometry (GC-MS), specifically the Agilent 5977B Series GC/MSD system, USA. A sample of 3–5 mL was dissolved in methanol and injected into the GC-MS equipped with an HP-5 MS column, using helium as the carrier gas at a flow rate of 1 mL/min. The injector temperature was set at 290°C

with a split ratio of 50:1. The temperature program started at 40°C, increased at a rate of 15°C/min up to 290°C, and was held for 10 minutes. The MSD detector had a solvent delay of 2.6 minutes, and the auxiliary temperature was set at 290°C.

RESULTS AND DISCUSSION

The results of the investigation on the bioactive compound components of the herbal wine are shown by the GC-MS spectrum peaks (Figure 1). Based on the spectrum, a total of 44 peaks were detected, each with varying peak areas. Within these peak areas, more than one compound was identified.

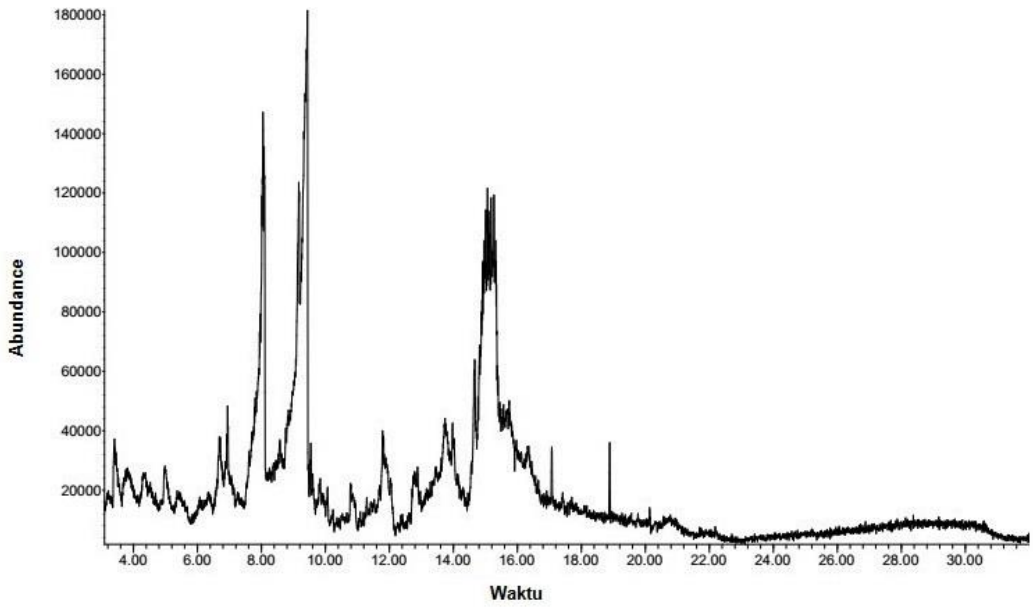

Figure 1. GC-MS spectrum of herbal wine

Referring to Figure 1, the chemical composition of herbal wine identified 85 compounds and summarized in Table 1.

Table 1. Bioactive compound of herbal wine

RT	Area%	Compounds
3.408	1.07	2-Furanmethanol 3-Furanmethanol
4.985	1.08	Propane, 1-isothiocyanato- 3H-1,2,4-Triazole-3-thione, 1,2-dihydro-
6.688	1.2	2,5-Dimethylfuran-3,4(2H,5H)-dione Furaneol Hydrouracil, 1-methyl-
6.946	1.26	D-Alanine, N-propargyloxycarbonyl-, dodecyl ester 1,3,5-Triazine-2,4,6-triamine 4(1H)-Pyrimidinone, 2,6-diamino-
7.62	1.05	Isosorbide Dinitrate 2,3-Dimethyldecane

RT	Area%	Compounds
		L-Mannose
7.731	1.33	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
		Acetamide, N-(2-acetyl-3-oxo-4-isoxazolidinyl)-
		N-propyl-butyramide
7.809	1.54	1-[3-Hydroxypropyl]-aziridine
		cis-3-Methyl-2-n-propylthiophane
7.832	0.53	Formamide, N,N-diethyl-
7.854	0.64	Hexanoic acid, 2-acetyl-, ethyl ester
7.965	3.68	1,3-Dioxolane, 2,4,5-trimethyl-
8.053	6.93	N-Acetylmannosamine
8.851	1.13	Glycerin
9.172	8.31	5-Hydroxymethylfurfural
		4-Fluorobenzyl alcohol
9.242	2.7	Furan, 2,3-dihydro-4-(1-methylpropyl)-, (S)-
		1,2-Benzenediol, 3-methyl-
9.547	0.55	1,2-Benzenediol, 4-methyl-
		Benzene, (methylthio)-
		Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis
10.789	0.48	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-trans)-
		Cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans-
		Thiophene, 2-ethyl-5-(2-methylpropyl)-
11.79	0.98	4,5,6-Pyrimidinetriamine
		Benzene, (ethenylsulfonyl)-
		4-Isopropoxy-2-butanone
11.89	0.09	Hexanoic acid, 3-oxo-, methyl este
		Ethanol, 2-(pentyloxy)-, acetate
		2-Thiophenecarboxylic acid, 3-methyl-, methyl ester
12.743	0.66	Methyl 4-methylthiophene-2-carboxylate
		N-Methoxycarbonylmaleimide
12.789	0.78	N-Methylpyrrole-2-carboxylic acid
		Benzenethiol, 2-amino-
		Bicyclo[3.1.1]heptan-3-one, 6,6-dimethyl-2-(2-oxopropyl)-, isomer 1
12.88	0.73	Pyrimidine, 2,4,5-triamino-
		Octan-6-one-2-ol, 3-ethyl-, acetat
		Butanoic acid, 2-methyl-, propyl ester
13.741	1.06	Butanoic acid, 2-methyl-, pentyl ester
		Butanoic acid, 2-methyl-, 1-methylpropyl ester
		N-Acetylpyrrolidone
13.764	0.41	2H-Pyran-2-methanol, 6-ethoxy-3,6-dihydro-3-hydroxy-
		9-methylheptadecane
13.976	0.15	4-Methyl-2,6,7-trioxa-1-phosphabicyclo(2.2.2)octane-1-sulfide
		4-Cyano-2-phenylpyridine

RT	Area%	Compounds
14.652	4.33	6H-Purine-6-thione, 9-ethyl-1,9-dihydro- 7-Phosphabicyclo[2.2.1]hept-2-ene, 5-isopropyl-2,7-dimethylBenzene, 1,2,3-trimethoxy-5-methyl 2H-imidazole-2-thione, 1,3-dihydro-5-(1-methylethyl)-1-(2-propen-1-yl)-
14.773	0.92	Sorbic acid, TBDMS derivative Dimethyl 2,5-thiophenedicarboxylat
14.817	1.64	4-(Dimethylamino)-6-(methylamino)-1,3,5-triazin-2-ol
14.876	2.2	4-Amino-3-mercaptobenzoic acid 2-Chlorophenyl isothiocyanate
14.899	2.26	2-Hydroxy-5,6-dihydro-4H-benzothiazol-7-one 6-Fluorovanillin Benzaldehyde, 2-fluoro-5-hydroxy-4-methoxy-
14.938	2.04	4H-Cyclopenta-1,2-dithiol-1-ium, 3-ethyl-5,6-dihydro-, bromide [1,1'-Biphenyl]-3-amine
14.974	2.49	Demeton-S-methyl sulfone
15.011	2.3	Fumaric acid, 2,4-dimethylpent-3-yl pentyl ester Cyclohexanebutanal, 2-methyl-3-oxo-, cis-
15.069	4.39	Cyclohexene, 3-ethyl-1-trimethylsilyloxy-lyloxy- 1,3-Cyclohexanedicarbohydrazide
15.107	1.94	Phenol, 4-methoxy-2-nitro- Fumaric acid, 4-methylpent-2-yl pentyl ester
15.136	2.08	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-1,3-dimethyl-5-(1-methylethyl)- Phenol, 3,4,5-trimethoxy-
15.182	3.57	[1,2,4]Triazolo[4,3-a]quinoline Pyrimidine-2,4(1H,3H)-dione, 6-hyd roxy-5-methyliminomethylDimethyl 2,5-thiophenedicarboxylat
17.078	0.51	Dodecanoic acid n-Hexadecanoic acid
18.888	0.59	.alpha.-Methyl-2-naphthalenemethanol Octadecanoic acid 7-Chloro-2,3-dihydro-3-methyl-5-phenyl-1H-1,4-benzodiazepin-2-one

The findings in Table 1, when compared to the bioactive compound reported in previous studies on the chemical composition of herbal plants (referring to the types of plants used in the method), show several similarities in compounds. Only two identical chemical components were found, namely n-Hexadecanoic acid and Octadecanoic acid, in *E. cardamomum* and *S. aromaticum* (Benmakhlouf et al., 2022; Castillo et al., 2023). The remaining compounds are clearly different, which is likely due to various chemical factors. The compound 1,2-Benzenediol, 3-methyl- (Table 1) is not the same as 1,3-Benzenediol found in *C. sappan* L (Adnan

et al., 2022), although both belong to the same chemical class, namely phenols, specifically the benzenediol subclass. The chemical component 6-Fluorovanillin (Table 1), which was detected in the herbal wine, differs from vanillin found in extracts of *Z. officinale* and *S. aromaticum*, although they belong to the same class, phenols (El-Saber Batiha et al., 2020; Schaller & Schieberle, 2020) (Figure 2).

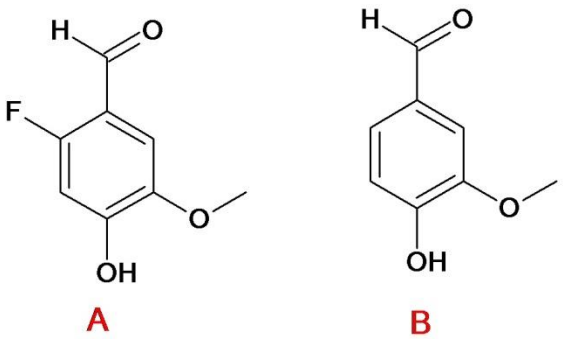

Figure 2. Structure of bioactive components (A) 6-Fluorovanillin and (B) vanillin

The structural difference between the two bioactive compounds presented in Fig. 2 lies in the substitution at the C6 carbon atom, where a fluorine (F) atom is attached; 6-Fluorovanillin is a known derivative of vanillin (Creveling et al., 1981; Nie & Kirk, 1991). In addition, all the identified compounds listed in Table 1 are classified as derivative compounds. N-Acetylmannosamine (Table 1), detected in the herbal wine analyzed in this study, is a product of sugar degradation by *S. cerevisiae*. This degradation pathway is consistent with previous isotopic studies tracing the biosynthesis of N-Acetylmannosamine in bacteria, which confirmed its classification as a sugar derivative (Combt & Roseman, 1960). N-Acetylmannosamine is pharmacologically significant as a key precursor for the synthesis of N-acetylneuraminic acid, which is utilized in enzyme replacement therapy for GNE myopathy (Nonaka distal myopathy) (Carrillo et al., 2021; Van Wart et al., 2021; Mullen et al., 2022). Another compound identified, 3H-1,2,4-Triazole-3-thione, 1,2-dihydro- (Table 1), is considered an intermediate due to the presence of a 1,2,4-triazole ring. This heterocyclic structure exhibits a wide range of biological activities, including antibacterial, antifungal, antioxidant, antitubercular, antitumor, analgesic, anti-inflammatory, and pesticidal properties (Aggarwal & Sumran, 2020; Abdelli et al., 2021; Strzelecka & Świątek, 2021)

The investigation of herbal wine and its bioactive compound in this study reveals two aspects of novelty. First, the chemical composition of the herbal plants differs almost entirely from the components identified in the fermented herbal wine. This variation is attributed to the addition of bonds in the carbon chain, resulting in the formation of derivative compounds. Second, the bioactive compounds present in the herbal wine exhibit potential pharmacological properties, as supported by previous research findings. Based on these two observations, further studies are warranted to confirm the pharmacological benefits of herbal wine.

CONCLUSION

During the fermentation process, electrophilic substitution reactions occur on the benzene ring, along with other chemical and enzymatic reactions mediated by *S. cerevisiae*. These reactions lead to the formation of several derivative compounds that are highly relevant in pharmacology. The herbal wine investigated in this study demonstrates potential as a health-oriented functional food.

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