REVIEW ARTICLE

Chemical Profile of Value-added Oyster Mushrooms

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ABSTRACT

Oyster mushrooms have significance in cosmetics and nutrition. Cinnamaldehyde, amino acids such as histidine, arginine, lysine, glycine, and quercetin, play a crucial role in acne tissue repair and show anti-inflammatory activity. In the current research, quantitative and qualitative chemical evaluation of value-added mushrooms and control oyster mushrooms was performed. Value addition to mushrooms was accomplished by growing them on paddy substrate substituted with a bark of cinnamon 10.0% w/w or Moringa dried leaf powder 5.0% w/w separately. The mushroom extracts were subjected to thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) to perceive the modifications in the chemical profile. The liquid chromatographic study of mushrooms grown on paddy straw replaced with pieces of cinnamon (10.0% w/w) bark indicated that value-added mushrooms exhibited the presence of cinnamaldehyde and Eugenol, which were absent in the control group of mushrooms. In the same manner, paddy straw replaced with Moringa leaf dried powder (5.0% w/w) showed an increase in Quercetin concentration compared to the control group of mushrooms. The amino acid profile of value-added mushrooms and control group mushrooms was determined using standard amino acid samples by TLC and HPLC. The amino acid concentration was found to be higher in cinnamon-substituted mushrooms, followed by Moringa-substituted mushrooms, compared to the control. The chemical profile of the mushrooms grown on paddy substituted with 10.0% w/w Cinnamon/ Moringa leaf dried powder (5.0% w/w) showed more concentration of arginine, histidine, glycine, and lysine compared to the control group of mushrooms, reflecting that value addition to Oyster mushrooms was successful.

Keywords: Amino acids, Cinnamaldehyde, Eugenol, High-performance liquid chromatography, Quercetin, Value-added mushrooms

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INTRODUCTION

In recent times, there has been a tremendous increase in use of plant extracts in the Pharmaceutical and Cosmetic industries.[1,2] Understanding the chemical profile of plants requires the isolation, identification, and quantification of the active constituents from plants having biological activity.[3,4]

TLC (Thin layer chromatography) is frequently utilized to isolate and identify plant amino acids.[5,6] The technique is appropriate for the simultaneous determination of multiple components in less time with low detection limits. Silica gel, and modified silica gel are used as stationary phases, and the furthermost versatile, movable phasic systems are acetic acid ± *n*-butanol ± water, water ± phenol, or acetone ± *n*-butanol ± water ± acetic acid.[7]

Few High-performance liquid chromatography (HPLC) procedures have been described for the quantitation of Cinnamaldehyde and Eugenol. Reversed Phase Liquid Chromatography using C18 stationary phase with buffer ± acetonitrile based on phosphate, borate, or citrate as the movable phase can also be utilized. After derivatization with dansyl chloride, phthalic anhydride detection can be made by fluorescent determination, or identification may be accomplished by UV. When some amino acids are converted into hydroxamic constituents, they may be found by misting with 0.1M $FeCl_3$ solution in 0.1M HCl.[8,9]

MATERIALS AND METHODS

Extraction of Mushrooms

The control group of mushrooms and the produced value-added mushrooms were subjected to shade drying and then triturated to get coarser powder, separately. The resultant dried powder components were extracted using a hydroalcoholic mixture containing Alcohol and distilled water (80:20). The extract was fractionated with various organic solvent systems like fraction-I (dichloromethane), fraction-II (ethyl acetate), to isolate dissimilar groups of polar components like saponin, flavonoids by the process of liquid-liquid separation. The ethyl acetate fraction was found to be rich in flavonoids and was subjected to HPLC analysis.[7]

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Estimation of Quercetin by HPLC

Processing of Standard Reference Solution

Quercetin standard reference was weighed and dissolved using a sonicator and nearly 75.0% methanolic solution. To achieve a 100 ppm final concentration, the resulting aliquots were subjected for dilution with 75% methanolic solution.

Sample Solution Processing

100 mg mushroom powder sample was weighed and dissolved using a sonicator and nearly 75.0% methanolic solution. To achieve a 100 ppm final concentration, the resulting aliquots were subjected for dilution with 75% methanolic solution.[10]

Mobile phasic pH composition, flow rate, the wavelength of detection, and column pack, were established with reverence to the shape and position of Quercetin peaks in the respective chromatogram. The final chromatographic technique utilized methanol–distilled water–trifluoroacetic acid (7: 3 :0.1% v/v/v) as mobile phase (Alliance Waters, Milford) and Agilent 1200 with the flowing rate at 0.80 mL/min. The chromatographic elution was achieved with a pH-stable C18-Inertsil, 150 mm, 4.60 mm, 5.0 µm stationary phase. Ultraviolet detection was processed at 254 nm wavelength maximum. The samples were injected using a Rheodyne injector, and the infused volume was 50.0 µL. The retention time of quercetin was 2.886 minutes.

Identification of Amino Acids by TLC

About 10gms of fresh value-added mushrooms were triturated with 95.0% ethanolic solvent system and kept for the process of maceration for 7 days. The alcoholic extract was subjected to filtration and drying.[11,12]

A micropipette was used to apply the solution of mushroom extract and standard solution of the 19 essential amino acid components (1-mg/mL each) onto a TLC plate. A combination of water, acetone, n-butanol and acetic acid (23:35:35:7 % v/v/v) was used as the mobile phase to produce the elution in a saturated chamber. The plates were dried in hot air at 105°C after the elution, and UV detection was used to determine the results. By contrasting the R_f values, the amino acids' identification was accomplished.

Extraction of Free Amino Acids

Peptides/proteins Hydrolysis into Amino Acid

The mushroom sample solutions were dried under a vacuum and hydrolyzed with 200 mL of boiling 6N HCl and 40.0mL of Phenol over phasic vapor hydrolysis. The sample solutions were subjected to drying in a hot air oven in between 112–116ºC for 24 hours. Excess HCl was removed once the hydrolysis process was finished, and the tubes underwent a 90 minutes vacuum drying process. 500 *µL* of 20 mM boiling HCl was used to rehydrate the mushroom samples.

Amino Acid Derivatization

The 20 L sample solutions were reconstituted, and the AccQ-Fluor reagent kit was used to derivatize them (WAT 052880, Corporation of Waters, USA). Using a micropipette, 60 *µL* of AccQ-Fluor borate buffer was added to the sample tube before being vortexed. After adding 20 *µL* of AccQ-Fluor reagent, the mixture was immediately vortexed for 30 seconds before being transferred to vials with the highest potential for recovery. Before employing HPLC to separate the amino acids, the vials were heated for 10 minutes on a water bath at 55°C.

Amino Acid Elution Process

The amino acid constituents of AccQ-Fluor were isolated using a PDA detection system of *Waters 2707* Module LC Unit. Utilizing an autosampler, a 10 µL mixture was infused to a Waters reverse-phased AccQTag bonded silica Amino Acid C18 column (150 mm x 3.9 mm). In a separation gradient with a 1.0 mL/min flow rate, the Waters AccQ Tag Eluent A Concentrate (WAT052890) was diluted to 10% in Milli-Q water and used as eluent A, and 60% Acetonitrile was used as eluent B. The isolation gradient utilised up to 2 minutes at 100.0% A, 2.0 minutes at 98.0% A, 15.0 minutes at 93.0% A, 19.0 minutes at 90.0% A, 32.0 minutes at 67.0 percent A, 38.0 minutes at 0.0% A, and 56.0 minutes at 56.0 minutes (100.0% A). PDA was utilized to identify the amino acids at a wavelength of 254 nm with a column condition at 37°C. Using the Empower Pro Software, a standard solution of 2.5 µm/mL amino acid responses was obtained.[13]

Quantification of Cinnamaldehyde by HPLC

Cinnamon Substituted Mushroom Extraction

About 10grams of freshly taken cinnamon replaced mushrooms were taken in a mortar and triturated with 95.0% ethyl alcohol and preserved for the process of maceration for 7 days. The extract was subjected to filtration, and marc was discarded. The extract was dried and utilized as an extract of mushrooms.

Processing of Cinnamaldehyde Stock Solution

Cinnamaldehyde standard reference (100 mg) was weighed and dissolved using a sonicator, 100 mL volumetric flask, and methyl alcohol to get 1000 µg/mL solution.[14-18]

Processing of Cinnamaldehyde Working Standard Solution

Stock solution (1000 µg/mL) was subjected for dilution at 0.1 to 20 mL aliquots to get the final cinnamaldehyde concentration levels in between 1 to 200 µg/mL.

Preparation of Sample Solution

A stoppered tube was loaded with 100 mg Cinnamon value-added dried mushroom extract, which was precisely weighed. 10 mL of methyl alcohol was then poured to it. The tube's contents were sonicated for 15 minutes at room temperature (28 ± 2ºC) in an ultrasonicator. Whatman filter paper no. 41 was utilized to filter the tube contents, and the filtrate was then utilised as the solution of sample.[19]

HPLC Chromatographic Conditions

A reverse phase stationary phase of ODS-Intersil C_{18}-3V (5 µm, 4.6 mm×150 mm) was utilized with movable solvent system containing a combination of acetonitrile: methanol: water (20:35:45), was conveyed at the flowing speed of 1.0-mL/min.

The UV response was measured at a maximum wavelength of 221.0 nm. 50 µg/mL standard cinnamaldehyde and sample mushroom solutions were injected and subjected to chromatographic analysis.

Identification of Eugenol in Value-added Mushrooms

Cinnamon Replaced Mushrooms Extraction

About 10 grams of freshly taken cinnamon replaced mushrooms were taken in a mortar and triturated with 95.0% ethyl alcohol and preserved for the process of maceration for 7 days and mark was separated. The dried filtrate was utilized as an extract for the mushrooms.[9] The obtained extract was analyzed for the presence of Volatile oils by TLC, UV, and HPLC methods.

TLC of Eugenol

Eugenol standard and extract samples were dotted on silica (GF254) gel TLC stationary phase and subjected to the elution with movable phase (ethyl acetate: n-hexane, 1:4 % v/v). The resolved dots were detected at UV wavelength of 254.0 nm. After the separation of eugenol from the mushroom extract, the R_f value was analyzed and equated with the R_f finding of the Eugenol standard sample.

Estimation of Eugenol by UV-Visible Spectroscopy

Calibration Curve of Eugenol

Weighed eugenol was solubilized in ethyl alcohol to attain a 1000 µg/mL standard stock solution. Eugenol solutions were processed further to get the concentration levels between 20–100µg/mL for rectilinear plot. The resulting solutions' absorbance findings were estimated at 282 nm.

Processing and Estimation of Eugenol in Extract

50 mg of Cinnamon extract (dried) was weighed accurately, relocated into a stoppered flask, and mixed with 10.0 mL of ethyl alcohol. The contents of the flask were placed in to 100mL beaker and vortex for 15 minutes at 25.0°C with the help of an electric vortexer. The mixed sample was subjected to filtration, and the absorbance of the filtrate was determined at 282 nm. Eugenol levels were determined using the calibration curve.[20]

Determination of Eugenol by HPLC

A precisely weighed 100 mg sample of the dried mushroom extract was mixed with 1-mL of acetone before being added to 5 mL of methanol to create the sample solution. The acetone was then extinguished. The sample was ultrasonically processed (Altrasonic, Hyderabad, India) over 20 minutes before being diluted with methanol to a volume of 10 mL and filtered using a 0.2-µm syringe filter.[21]

HPLC Chromatographic Conditions

Acetonitrile and water in the proportion of 10:10 was elected as the movable solvent system. HPLC technique for the Eugenol estimation was processed through the Waters AllianceE2695 elution model (Waters-MA, US) utilizing a PDA detector (Waters-298) consisting of a column oven and autosampler. Empower 2 software (Europ Sciences, Ltd., United Kingdom), which was installed alongside the equipment for data collection and acquisition, was used to operate the instrument. On a reverse-phase C18 column (4.6 ×250.0 mm, 5 µm particle size; Merck, Mumbai, India) kept at room temperature, compounds were separated.[21]

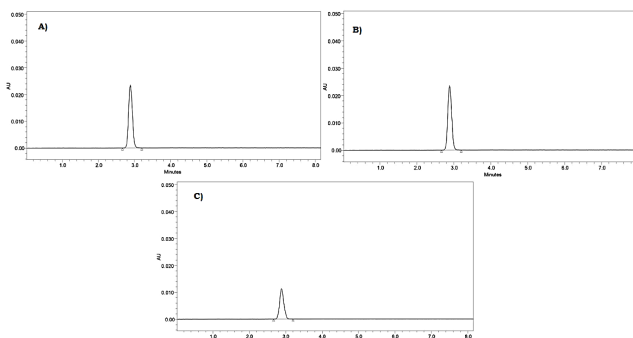

Figure 1: HPLC graph of Quercetin in A) Standard, B) Moringa substituted mushrooms, and C) Control mushrooms.

Table 1: Quercetin in Moringa mushrooms

Sample	Area	RT	Quercetin Concentration (µg/mL)
Standard	182469	2.886	100
Moringa substituted mushroom extract	141651	2.886	77.63
Control mushroom extract	99119	2.886	54.32

RESULTS AND DISCUSSION

Identification of Quercetin in Value-added Mushrooms

Standard Quercetin sample, control mushroom extract, and Moringa substituted mushroom extract solutions were processed and infused into a liquid chromatographic system. HPLC chromatogram of quercetin showed the RT at 2.886 with a standard concentration of 100 µg/mL. The Moringa mushroom extract showed the presence of quercetin compared to the control mushroom extract (Table 1 and Figure 1).

Identification of Amino Acids in Value-added Mushrooms

Identification of Free Amino Acids by TLC

Threonine, Valine, Methionine, I-Leucine, Leucine, Phenylalanine, Asparagine, Histidine, Alanine, Glycine, Arginine, Proline, Tyrosine, Cysteine, Hydroxyproline and Lysine were present in the *Moringa olifera* substituted mushroom extract and cinnamon substituted mushroom extract except Moringa substituted mushroom extract also contained glutamine and serine but lacked proline and Cystine. The R_f findings of amino acid compounds from mushroom extract (Table 2 and Figure 2).

Amino Acid Identification by HPLC

Histidine, Arginine, Glycine, and Lysine in the Control, *Moringa olifera*, and Cinnamon substituted mushroom extract was confirmed by HPLC analysis using standard amino acid samples (Figure 3 and Table 3).

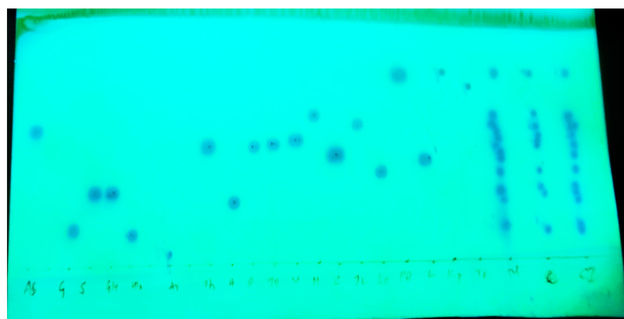

Figure 2: TLC of amino acids.

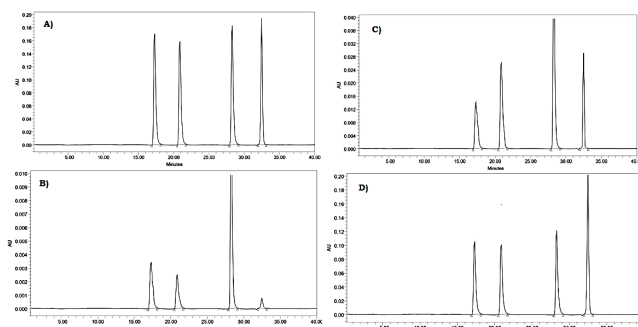

Figure 3: HPLC of amino acids A) Standard, B) Control extract, C) Moringa substituted mushroom extract D) Cinnamon substituted mushroom extract

Table 2: TLC of amino acids in control, moringa, and cinnamon mushrooms

S.no.	*Amino acid*	*Rf values*		
		Control mushrooms	*Moringa substituted mushroom extract*	*Cinnamon substituted mushroom extract*
1	Arginine	0.5	0.5	0.5
2	Glutamine	--	--	0.13
3	Serine	--	--	0.27
4	Glycine	0.26	0.26	0.26
5	Histidine	0.11	0.11	0.11
6	Asparagine	--	--	--
7	Threonine	0.44	0.44	0.44
8	Alanine	--	0.22	0.22
9	Proline	0.43	0.43	--
10	Tyrosine	--	0.45	0.44
11	Valine	0.46	0.46	0.46
12	Methionine	0.55	0.55	0.55
13	Cysteine	0.4	0.4	--
14	I-leucine	--	0.52	0.52
15	Leucine	0.33	0.33	0.33
16	Phenylalanine	0.72	0.72	0.72
17	Lysine	0.39	0.39	0.39
18	Hydroxy proline	--	0.7	0.7
19	Tryptophan	--	--	--

Table 3L: HPLC analysis of Amino acids in Control, Moringa, and Cinnamon mushrooms

Sample	*Amino acid*	*Area*	*RT*	*Concentration*
Standard	Histidine	850394	17.42	0.38789 µg/mL
	Arginine	790254	21`.21	0.4356 µg/mL
	Glycine	659794	28.61	33.3 µg/mL
	Lysine	800125	32.42	3.6549 µg/mL
Control	Histidine	15832	17.42	0.007 µg/mL
	Arginine	11308	21`.21	0.006 µg/mL
	Glycine	88206	28.61	4.451 µg/mL
	Lysine	2261	32.42	0.010 µg/mL
Moringa substituted mushroom extract	Histidine	67851	17.42	0.205 µg/mL
	Arginine	122131	21`.21	0.067 µg/mL
	Glycine	194505	28.61	9.816 µg/mL
	Lysine	126654	32.42	0.578 µg/mL
Cinnamon substituted mushroom extract	Histidine	461383	17.42	0.2104 µg/mL
	Arginine	447813	21`.21	0.246 µg/mL
	Glycine	773496	28.61	39.03 µg/mL
	Lysine	850394	32.42	3.88 µg/mL

Estimation of Cinnamaldehyde in Value-added Mushrooms by HPLC

In the current study, using a mobile phase made up of acetonitrile, water, and methanol allows for a more excellent resolution of the various components found in Cinnamomum zeylanicum Blume. In the mobile phase, acetonitrile and methanol were chosen because they are widely accessible in pure form, water-miscible, have low viscosities, and have low surface tension. 210 nm was chosen as the analysis wavelength because it increases the sensitivity of the standard (Figure 4 and Table 4).

TLC Analysis of Eugenol in Value-added Mushrooms

Cinnamon mushroom extract and Eugenol standard were dotted on silica (GF254) gel TLC plate and eluted with mobile phase (ethyl acetate: n-hexane, 1:4 % v/v). The separated dots were detected under UV at 254 nm. The R_f value of separated eugenol from the extract was calculated and compared with the standard Eugenol sample R_f value (Figure 5 and Table 5).

UV Analysis of Eugenol

Eugenol content was estimated by processing the standard and sample solutions and measuring the absorbance at 282 nm (Table 6 and Figure 6).

Quantification of Eugenol in Value-added Mushrooms by HPLC

In the present research work, using acetonitrile and water (1:1) as mobile phase showed better resolution of different

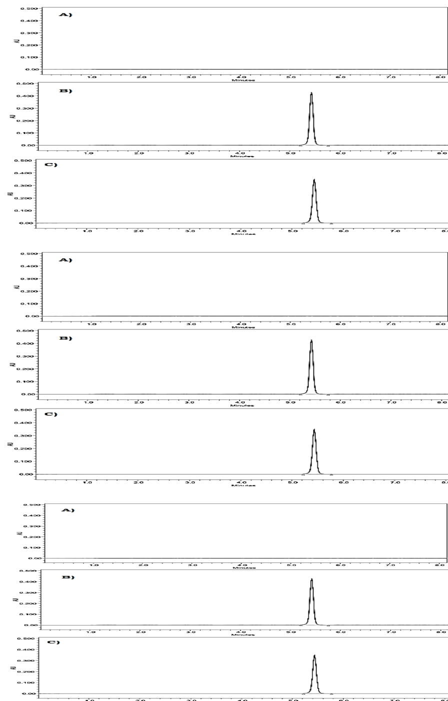

Figure 4: HPLC of Cinnamaldehyde A) Control, B) Standard C) Cinnamon value-added mushroom extract

Table 4: HPLC analysis of cinnamaldehyde in cinnamon mushrooms

Sample	*Area*	*RT*	*Concentration (µg/mL)*
Standard	245987	5.42	50
Cinnamon substituted mushroom extract	196582	5.42	39.95
Control mushroom extract	--	--	--

Figure 5: TLC of eugenol from cinnamon

Table 5: TLC findings of eugenol from cinnamon mushrooms

Eugenol	
Solvent system (n-hexane:ethyl acetate =4:1)	R_f value
Cinnamon Mushroom Sample	0.66
Standard Eugenol	0.66
Control Mushroom sample	No spot

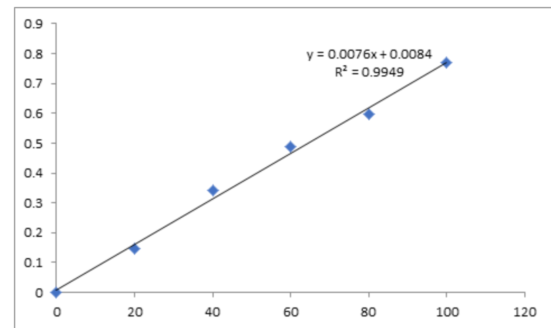

Figure 6: Calibration curve of eugenol

Table 6: UV-analysis of cinnamon mushrooms

Drug	*Concentration (µg/mL)*	*Absorbance*
Cinnamon oil (standard)	20	0.145 ± 0.0003
	40	0.342 ± 0.0005
	60	0.487 ± 0.0002
	80	0.596 ± 0.0001
	100	0.768 ± 0.0005
Cinnamon substituted mushroom sample	1mg/mL	0.148 ± 0.0003
Control	1mg/mL	Zero absorbance

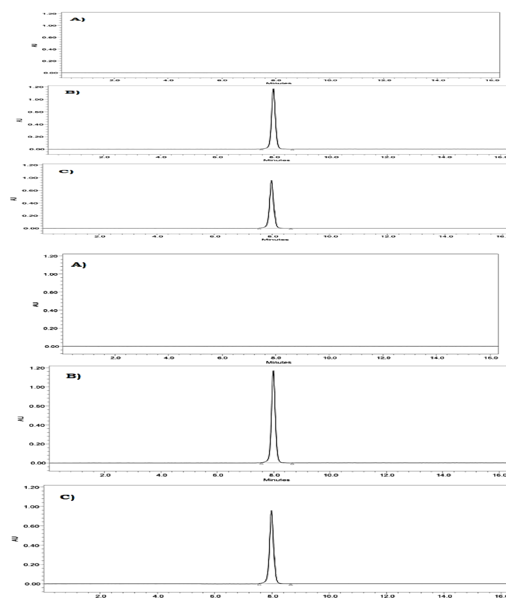

Figure 7: HPLC chromatograms A) Control B) Standard eugenol C) cinnamon substituted mushroom extract

Table 7: HPLC estimation of eugenol in cinnamon substituted mushrooms

Sample type	*Area*	*RT*	*Concentration(µg/mL)*
Standard	365478	8.02	150
Cinnamon substituted mushroom extract	256327	8.02	105.20
Control mushroom extract	--	--	--

components present in mushrooms grown on paddy substituted with cinnamon. Acetonitrile and water used as mobile phases in the ratio of 1:1 (v/v), low surface tension and viscosity, and are readily obtainable in pure form. The selected wavelength for analysis was 280 nm, which yielded better sensitivity for both the standard and the sample. Standard, control and sample solutions were subjected for the HPLC analysis (Table 7 and Figure 7).

The present study identified and quantitatively determined amino acids, quercetin, eugenol, and cinnamaldehyde in value-added mushroom extracts using TLC and HPLC analysis. The value addition of mushrooms grown on Cinnamon bark substituted paddy was also confirmed by analysis of their amino acid profile by TLC and HPLC. Amino acids, glycine, histidine, arginine, and lysine were observed in the extracts of mushrooms grown on *M. oleifera* leaf powder substituted and cinnamon-substituted mushroom extracts. But in cinnamon-substituted mushroom extracts, glutamine and serine were present, and proline and cystine were absent.

The mushrooms grown on paddy substituted with dried Moringa leaf powder were rich in quercetin and amino acids compared to the control group of mushrooms indicating that value addition to oyster mushrooms grown on Moringa leaf powder substituted paddy. The mushrooms grown on paddy substituted with cinnamon bark were found to contain cinnamaldehyde and eugenol and also contained more amino acid concentration than the control group of mushrooms, reflecting that value addition to oyster mushrooms grown on cinnamon bark substituted paddy. Maybe the phytochemicals present in Moringa leaves and cinnamon bark was responsible for triggering the production of new compounds which were absent in the control mushrooms and also for enhanced amino acid concentration in mushrooms grown on paddy substituted with dried Moringa leaf powder/cinnamon bark.

CONCLUSION

The value-added mushrooms grown on Moringa leaves were found to be a good source of quercetin and amino acids such as glycine, histidine, arginine, and lysine. The mushrooms grown on cinnamon bark were found to contain cinnamaldehyde and eugenol, which were absent in control mushrooms. These mushrooms were also a good source of glycine, histidine, arginine, and lysine amino acids compared to control mushrooms. Therefore, growing value-added oyster mushrooms is beneficial in producing mushrooms with desired chemical profile and biological activities.

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