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Evaluation of phansomba samples (*Phellinus* spp.) for amino acid composition

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Abstract: This study has been undertaken to investigate the amino acid contents in phansomba samples, a traditional medicinal mushroom used by the tribals of western Maharashtra. A total of 48 samples belonging to 16 *Phellinus* species were analyzed for the presence of amino acid qualitatively and quantitatively. The results of quantitative analysis indicated that the amino acid contents ranged from 0.56 to 3.38%. While qualitative analysis revealed presence of Alanine, Arginine, Cysteine, Cystin, Glutamic Acid, Glycine, Histidine, Leucine, Methionine, Ornithine, Proline, Tyrosine while Phenyl alanine was not detected in any of the samples.

Key Words- Amino acids, phansomba, *Phellinus*.

I. INTRODUCTION

Phellinus is one of the important genera in the family Hymenochaetaceae of order Aphyllophorales and decay heartwood and cause heart rot (white rot disease) and cankers of living trees, It degrades lignin, while cellulose is partly degraded. Most bracket forming species growing on *Atrocarpus integrifolia*, *Acacia nilotica*, *Mangifera indica* that are medicinally important and used as folk medicine in the Western Ghats of Maharashtra. The heavy rainfall, high humidity and hot temperature, in semi evergreen moist deciduous forest with few-isolated patches of evergreen vegetation in deep ravines favor the growth of *Phellinus* (Rabba, 1994; Lamrood, 2004).

Besides having economic importance being pathogenic fungus, Phellinus is also known for its medicinal used. Amongst the several species of *Phellinus*, only *Phellinus linteus* has received much attention. The mushroom is popularly known as **Sang-Hwang** in Korea; **Song Gen** in China, **Meshimakobu** in Japan (Mizuno, 2000) and **Phansomba** in India and have historically been recorded as traditional mushroom used to treat various ailments (Vaidya and Rabba, 1993, Lamrood, 2004, Vaidya et al., 2005, Zhu et.al., 2008; Sliva, 2010; Hua et al., 2016, He et al., 2021). Whereas, the medicinal use of this mushrooms, both Ayurvedic and traditional has been well documented in several ayurvedic books and research papers (Vaidya and Bhor, 1991, Vaidya and Lamrood, 2000, Lamrood and Ralegankar, 2021).

Unfortunately, there is no previous systematic study carried out to investigate the biochemical composition of this mushroom. Hence, the present study was undertaken with an aim to study the amino acids composition of phamsomba samples

II. Materials and Methods

II.1 Sample Collection

Samples of phansomba (*Phellinus* spp.) were collected in sexual stages from various regions of Western Maharashtra (i.e., Konkan region and Pune) from the forest trees and from the markets. Field collections were made from places like Alibaugh, Dapoli, Karnala, Kolhapur, Mumbai, Adali, Sawantwadi, Kankavali, Nardave, Ratnagiri, Harihareshwar, Deorukh of Konkan region and Pune University Campus, Amba valley, and Simhagarh fort for Pune region, by frequent visits and with the assistance of a Vaidu (local learned herbalist) or a tribal person. Market collections were made from cities like Pune, Mumbai, Alibaugh, and Kolhapur. Both, field and market samples were kept in a clean polythene bag with a label indicating the date of collection, place of the collection, name of the collector, name of the host (in case of field collection), name of the shop and shop owner (in case of the market collection), type of fruiting body and brief description of the specimen etc. (Lamrood et al., 2012)

After the collection, samples were dried at 40^0 – 45^0C and stored in cardboard boxes and deposited to Herbaria Poonensis, Department of Botany, University of Pune (now Savitribai Phule Pune University) under the accession number **'PH'**. The spore prints were obtained from fresh basidiocarps on cellophane paper and maintained in airtight plastic bags.

II.2. Identification

The specimens were identified by studying external and internal morphology. In external morphological study, characters like colour, texture, type of attachment of the fruit body with wood, hymenial and pileal surface of the basidiocarp, margin, pore morphology, dissepiment characters were observed. Measurements or range of measurements of the fruitbody were taken as per Lamrood (2004).

Detailed microscopic examinations were carried out by cutting free hand thin sections of fruit body passing through hymenium. The sections were first treated with absolute alcohol for few seconds and the transferred to a solution of 10% KOH for allowing the swelling of different hymenial structures.

The sections the were washed with distilled water **(D/W)** for two to three times allowed 1 minute for each washing and then stained with 1% phloxin (for basidia) and teased in lactoglycerine with 1% cotton blue (for other structures like setae, hyphal tips, hyphal type etc.). Semipermanent slides were prepared in lactoglycerine. Permanent slides were prepared in polyvinyl alcohol (PVA) medium as per Omar *et.al*. (1979) and observed under Olympus BX 40 microscope attached with HAMAMATSU 3CCD color camera C6157 and UVP. The photographs of powder were taken using Olympus 328 zoom trinocular microscope.

The Camera Lucida sketches were made by Ernet Leitz Wetzlar mirror type of camera lucida fitted to Bausch and Lomb compound microscope. Micrometric observations were carried out by using calibrated ocular micrometer of Erma Tokyo (Japan), Calibrated with Erma Tokyo (Japan) 1 mm stage micrometer.

The identification of specimens was done using a key suggested by Larsen and Cobb-Poulle (1990). Colour scheme of Jordan *et.al*. (1995) was used to describe colour of pileal, and hymenial surface, margin etc. Photographs of the specimens were done using Nikon Fm 2 Camera AF Nikkor 28-105 lens.

II.3 Estimation of total Amino acids.

Estimation of total Amino acids was done as per the method given in Sadasivam and Manikam (1992) with slight modification: 100 mg of sample was first grind with acid washed sand in mortar and pestle and extracted twice with 10 ml 80% (v/v) ethanol. The supernatant was collected and used as test sample. Lucine (1 mg ml^{-1}) served as standard. Reaction mixture contained 0.2 - 1.0 ml standard amino acid, 0.1 sample solution. Volume of each test tube was adjusted to 1 ml and 1 ml Ninhydrine reagent was added to each tube by mixed well. Later, 5 ml of diluent solution was added to each tube after keeping the tubes in boiling water for 15 min. Purple color developed after 15 min. was read at 570 nm. Distilled water served as blank. The standard curve was prepared and amount of amino acid content was calculated for each sample.

II.4 Qualitative estimation of amino acids

The estimation was carried out using the procedure of Rao *et. al.* (1974). Briefly, 0.5 gm of the sample was hydrolyzed under reflux with 10 ml of 6N HCl for 6h at 120 – 125^0C and then filtered. The excess of HCl was removed from the by filtrate by evaporating to dryness on water bath. To this dried residue, a small amount of warm water was added and was again re-evaporated to dryness. This procedure was repeated until the pH of the residue becomes around 5. The residue was then collected in minimum amount of 10 % isopropyl alcohol and the volume of the solution was made to 5 ml. This solution was served as test solution.

The test solution (*ca*. 1 ml) was loaded on to Whatman's filter paper no 1. The chromatogram was developed first in n-butanol:acetic acid : water (4 : 1: 5) and then in phenol : water (8 : 2). The chromatograms were developed by spraying 0.1% (w/v) ninhydrine in 95% alcohol, dried and developed in hot air oven at 100^0C for about 2-4 min. or till the colored spots appeared. The presence or absence of a particular amino acid was recorded as '+' or '-'respectively. The data was subjected to cluster analysis after coding and transforming the absence as 0 and presence as 1 of particular amino acid. This binary matrix was analyzed by generating distance matrix using Bray Curtis cluster analysis and tree was constructed using Biodiversity Professional program Version 2.0 software (http://www.sams.ac.uk, developed by Neil McAleece).

III. Results

Quantitative analysis of total amino acid contents of phansomba samples was carried out and represented in the following table

Table 1: Total amino acid content of different *Phellinus* species.

Species of *Phellinus*	Sample Code	Total Amino acid contents (%)
Phellinus adamantinus	PH – 3	2.94 ± 0.000
	PH - 5A	2.39 ± 0.006
	PH - 20	2.08 ± 0.011
Phellinus aureobrunneus	PH - 4	3.38 ± 0.006
Phellinus badius	PH - 12	0.56 ± 0.011
Phellinus coffeatoporus	PH - 13	1.12 ± 0.017
Phellinus crocatus	PH - 7	3.00 ± 0.011
Phellinus fastuosus	PH-A -20	2.95 ± 0.006
	PH-34 S_1	0.51 ± 0.000
Phellinus griseoporus	PH - 5	1.45 ± 0.006
	PH - 6	2.15 ± 0.011
Phellinus linteus	PH - 18	2.45 ± 0.011
	PH - 24	1.96 ± 0.000
	PH - 29	1.26 ± 0.011
	PH - 30	0.70 ± 0.011
Phellinus lloydii	PH - 9	2.72 ± 0.011
	PH - 9A	1.36 ± 0.006
	PH-33 L_2	0.56 ± 0.006
Phellinus melanodermus	PH - 38	1.20 ± 0.022
Phellinus merrillii	PH - 2	3.02 ± 0.006
	PH - 8	2.06 ± 0.006
	PH - 10	2.19 ± 0.017
	PH - 15	3.53 ± 0.006

	PH -15A	2.05 ± 0.011
	PH - 16	4.85 ± 0.006
	PH - 17	3.58 ± 0.011
	PH - 21	4.01 ± 0.017
	PH-32 L_1	0.58 ± 0.006
	PH-35 S_2	0.49 ± 0.006
	PH-36B_1	2.95 ± 0.000
	PH-37B_2	1.23 ± 0.017
	PH - 43	3.53 ± 0.006
	PH - 47A	2.85 ± 0.000
	PH - 47B	1.41 ± 0.017
	PH - 47C	1.52 ± 0.006
	PH - 47D	1.89 ± 0.011
	PH-A -21	2.89 ± 0.011
	M2	3.12 ± 0.006
	M4	3.22 ± 0.011
Phellinus minutiporus	PH - 31	1.50 ± 0.006
Phellinus orientalis	PH - 1	3.54 ± 0.000
Phellinus pappinus	PH - 22	3.28 ± 0.028
Phellinus sublinteus	PH - 19	2.50 ± 0.011
	PH - 23	3.00 ± 0.028
	PH - 25	2.32 ± 0.006
	PH - 26	0.64 ± 0.011
	PH - 42	0.97 ± 0.006
Phellinus torulosus	PH - 27	1.09 ± 0.011

Comparatively less variation was observed in total amino acid contents in the samples of respective species. However, the samples that are aged e.g. PH-A-20, PH-A –21, PH-34 S_1, PH-35 S_2, PH-33 L_2, PH-32 L_1 and PH-33 L_2 showed less amino acid contents as compared to other samples except PH-A-20 and PH-A-21 (see table 1)

The average amino acid contents were calculated and are given in following table 2.

Table 2: Average total amino acid contents in different *Phellinus* samples.

Name of the species	Sample Code	Mean
P. adamantinus	PH-5A	2.47 ± 0.006
P. aureobrunneus	PH - 4	3.38 ± 0.006
P. badius	PH- 12	0.56 ± 0.011
P. coffeatoporus	PH - 13	1.12 ± 0.017
P. crocatus	PH - 7	3.00 ± 0.011
P. fastuosus	PH-A-20	1.73 ± 0.003
P. griseoporus	PH-5	1.80 ± 0.008
P. linteus	PH - 18	1.58 ± 0.008
P. lloydii	PH-9	1.55 ± 0.007
P. melanodermus	PH - 38	1.20 ± 0.022
P. merrillii	PH-A-21	2.51 ± 0.008
P. minutiporus	PH - 31	0.70 ± 0.011
P. oriantalis	PH - 1	3.54 ± 0.000
P. pappinus	PH - 22	3.28 ± 0.028
P. sublinteus	PH-19	1.89 ± 0.012
P. torulosus	PH - 27	1.09 ± 0.011

It is observed for the above table that the total amino acid contents are less on *P. badius* and *P. minutiporus* viz. 0.56% and 0.76% respectively. The species like *P. aureobrunneus, P. oriantalis* and *P. pappinus* show relatively more or less similar amino acid contents i.e. 3.38%, 3.54% and 3.28% respectively. Similarly, the contents are more or less similar in *P. adamantinus* and *P. merrillii* viz. 2.47% and 2.51% respectively while rest of the species show the contents in the range of 1.09 – 1.80%

Table 3: Qualitative estimation of amino acids. (Note: + = present, – = absent)

Sample Code	Name of Amino acid																						
	Alanine	Amino n-butyric acid	Isolucine	Aspartic acid	L - cysteine	L - cystin	Dihydroxy Phenyl alanine	Glutamic Acid	Glycine	Histidine	L-Hydroxy Proline	Arginine	DL-nor Leucine	Valine	Tyrosine	Methionine	Ornithine	Lysine	L-Proline	L-Leucine	Threonine	Tryptophan	L- Phenylv alanine	Serine
12	+	–	–	+	–	–	–	–	+	+	–	+	+	–	–	–	+	–	+	–	+	+	–	–
9	+	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	+	+	+	–	–	–	–	–
1	+	–	–	–	+	+	–	–	+	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–
19	+	–	–	–	–	+	–	–	+	–	–	–	+	–	+	–	+	–	–	–	–	+	–	+
5A	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	–
31	+	+	+	–	–	–	–	+	–	+	–	–	+	–	+	+	+	–	–	–	+	+	–	–
22	–	–	–	–	–	+	–	–	+	+	+	–	+	–	+	+	+	–	–	–	–	–	–	–
5	+	+	–	+	–	+	+	+	+	–	+	–	+	–	–	+	–	–	–	–	–	–	–	–
13	+	+	–	+	–	+	–	–	+	–	–	–	–	–	–	–	+	+	–	–	+	–	–	+
4	–	+	–	+	–	+	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
38	–	–	–	–	–	+	–	–	+	–	+	–	+	+	+	–	+	–	+	–	–	–	–	–
18	–	–	–	–	+	+	–	+	+	+	+	–	+	–	+	–	+	–	–	–	–	–	–	–
7	+	–	–	–	+	–	+	–	+	–	+	–	+	+	–	+	–	+	+	–	–	+	–	–
27	+	–	–	–	–	+	–	–	–	+	–	+	+	–	–	–	–	–	+	–	–	–	–	–
A-20	+	–	–	–	–	+	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–
A-21	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–

samples were also screened qualitatively for the presence of amino acids. The spots were observed by spraying the chromatograms with a solution of ninhydrine and developing in hot air oven for about 2-4 min.

The R*f* values of each spot were then compared with the standard R*f* values and the results are given in above table 3. It was revealed from the above table that amino acids like L-cystin and Glycine were present in most of the samples while L-Phenyl alanine was not detected in any samples. Except *P. orientalis*, L-Leucine was also not detected in any samples similarly only *P.minutiporus* showed presence of Isolucine. Two sample viz. *P. griseoporus* and *P. crocatus* showed presence of Dihydroxy Phenyl alanine and samples like *P. melanodermus* and *P. crocatus* showed presence of Valine.

The total no. of amino acids per samples was calculated and is represented in the following table 4.

Table 4: Total no. of amino acids observed per species of *Phellinus*.

Name of the sample	Total no. of amino acids presents in sample	Percentage
P. adamantinus	11	45.83
P. aureobrunneus	5	20.83
P. badius	10	41.67
P. coffeatoporus	9	37.50
P. crocatus	11	45.83
P. fastuosus	4	16.67
P. griseoporus	10	41.67
P. linteus	9	37.50
P. lloydii	7	29.17
P. melanodermus	8	33.33
P. merrillii	4	16.67
P. minutiporus	11	45.83
P. orientalis	6	25.00
P. pappinus	8	33.33
P. sublinteus	8	33.33
P. torulosus	6	25.00

It can be observed from the above table that maximum number of amino acids was detected in *P. adamantinus*, *P. crocatus* and *P. minutiporus* followed by *P. badius* and *P. griseoporus* while a smaller number of amino acids was observed in *P. aureobrunneus*, *P. fastuosus* and *P. merrillii*. The total number of samples showing presence of a particular amino acid was also calculated and is represented in following table 5.

Table 5: Total no. of *Phellinus* species showing presence of a particular amino acid.

Name of the Amino acid	Total No. of samples showing presence of this amino acid	Percentage
Alanine	10	62.5
Amino n-butyric acid	4	25
Isolucine	1	6.25
Aspartic acid	5	31.25
L – cysteine	4	25
L - cystin	12	75
Dihydroxy Phenyl alanine	2	12.5
Glutamic Acid	4	25
Glycine	12	75
Histidine	6	37.5
L-HydroxyProline	7	43.75
Arginine	6	37.5
DL-nor Leucine	10	62.5
Valine	2	12.5
Tyrosine	6	37.5
Methionine	6	37.5
Ornithine	10	62.5
Lysine	5	31.25
L-Proline	5	31.25
L-Leucine	1	6.25
Threonine	3	18.75

Tryptophan	4	25
L- Phenyl alanine	0	0
Serine	2	12.5

It can be seen from above table that out of 16 samples, 12 (75%) samples show presence of L – cystin and Glycine. Similarly amino acids like Alanine, DL-nor Leucine and Ornithine were present in 10 samples out of 16 (see table 4 and 5). On the other hand L- Phenyl alanine was not detected in any of the sample. Also, amino acids like Isolucine and L-Leucine were present in only one sample while Dihydroxy Phenyl alanine and Valine were present in two samples (see table 4).

The data of presence and absence of was transformed in to binary matrix as shown in following table 6 and were subjected to cluster analysis.

Table 6: 1 / 0 matrix of chromatogram of Amino acids of different *Phellinus* species.

Sample Code	Name of Amino acid																							
	Alanine	Amino n-butyric acid	Isolucine	Aspartic acid	L – cystine	L - cystin	Dihydroxy Phenyl	Glutamic Acid	Glycine	Histidine	L-Hydroxy	Arginine	DL-nor Leucine	Valine	Tyrosine	Methioni	Ornithine	Lysine	L-Proline	L-	Threonin	Tryptoph	L- Phenyl alaline	Serine
12	1	0	0	1	0	0	0	0	1	1	0	1	1	0	0	0	1	0	1	0	1	1	0	1
9	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	1	1	0	0	0	0	1
1	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1
19	1	0	0	0	0	1	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0	1	1	1
5A	0	0	0	1	1	1	0	0	1	1	1	1	1	0	1	0	1	1	0	0	0	0	0	0
31	1	1	1	0	0	0	0	1	0	1	0	0	1	0	1	1	1	0	0	0	1	1	0	1
22	0	0	0	0	0	1	0	0	1	1	1	0	1	0	1	1	1	0	0	0	0	0	0	0
5	1	1	0	1	0	1	1	1	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1
13	1	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	0	1	1
4	0	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
38	0	0	0	0	0	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	0	0	0	0
18	0	0	0	0	1	1	0	1	1	1	1	0	1	0	1	0	1	0	0	0	0	0	0	0
7	1	0	0	0	1	0	1	0	1	0	1	0	1	1	0	1	0	1	1	0	0	1	0	1
27	1	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	1
A-20	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1
A-21	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0

Table 7: Similarity matrix.

Step	Clusters	Distance	Similarity	Joined 1	Joined 2
1	15	17.6470585	82.35294151	7	12
2	14	25	75	15	16
3	13	26.3157902	73.68420982	5	7
4	12	28.5714283	71.4285717	9	10
5	11	36.8421059	63.15789413	5	11
6	10	37.5	62.5	1	14
7	9	42.8571434	57.1428566	3	4
8	8	42.8571434	57.1428566	8	13
9	7	53.8461533	46.15384674	1	2
10	6	57.1428566	42.8571434	3	5
11	5	63.636364	36.36363602	6	8
12	4	66.6666641	33.33333588	1	3
13	3	77.7777786	22.22222137	1	6
14	2	84.615387	15.38461304	9	15
15	1	87.5	12.5	1	9

Table 8: Distance matrix.

	P.badius	*P. lloydii*	*P. orientalis*	*P. sublinteus*	*P.adamantinus*	*P.minutiporus*	P. pappinus	*P.griseoporus*	*P.coffeatoporus*	*P.aureobrunneus*	*P.melanodermus*	*P.linteus*	*P.crocatus*	*P.torulosus*	*P. fastuosus*	*P.merrillii*
P.badius	*	58.8235	37.5	55.5556	57.1429	57.1429	44.4444	40	52.6316	26.6667	44.4444	42.1053	47.619	62.5	28.5714	14.2857
P. lloydii	*	*	46.1538	40	55.5556	22.2222	40	35.2941	50	33.3333	53.3333	37.5	55.5556	46.1538	36.3636	18.1818
P. orientalis	*	*	*	57.1429	47.0588	23.5294	42.8571	37.5	53.3333	36.3636	42.8571	53.3333	35.2941	33.3333	40	20
P. sublinteus	*	*	*	*	52.6316	52.6316	62.5	44.4444	58.8235	30.7692	62.5	58.8235	42.1053	42.8571	33.3333	16.6667
P. adamantinus	*	*	*	*	*	36.3636	73.6842	47.619	50	50	63.1579	80	45.4545	47.0588	26.6667	26.6667
P. minutiporus	*	*	*	*	*	*	52.6316	47.619	40	12.5	31.5789	50	36.3636	35.2941	26.6667	26.6667
P. pappinus	*	*	*	*	*	*	*	55.5556	35.2941	30.7692	75	82.3529	42.1053	42.8571	33.3333	33.3333
P. griseoporus	*	*	*	*	*	*	*	*	52.6316	53.3333	44.4444	52.6316	57.1429	37.5	42.8571	42.8571
P. coffeatoporus	*	*	*	*	*	*	*	*	*	71.4286	35.2941	33.3333	30	26.6667	30.7692	15.3846
P.aureobrunneus	*	*	*	*	*	*	*	*	*	*	30.7692	28.5714	25	18.1818	22.2222	22.2222
P.melanodermus	*	*	*	*	*	*	*	*	*	*	*	70.5882	52.6316	42.8571	16.6667	16.6667
P. linteus	*	*	*	*	*	*	*	*	*	*	*	*	40	40	15.3846	30.7692
P. crocatus	*	*	*	*	*	*	*	*	*	*	*	*	*	35.2941	26.6667	13.3333
P. torulosus	*	*	*	*	*	*	*	*	*	*	*	*	*	*	60	40
P. fastuosus	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	75
P. merrillii	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

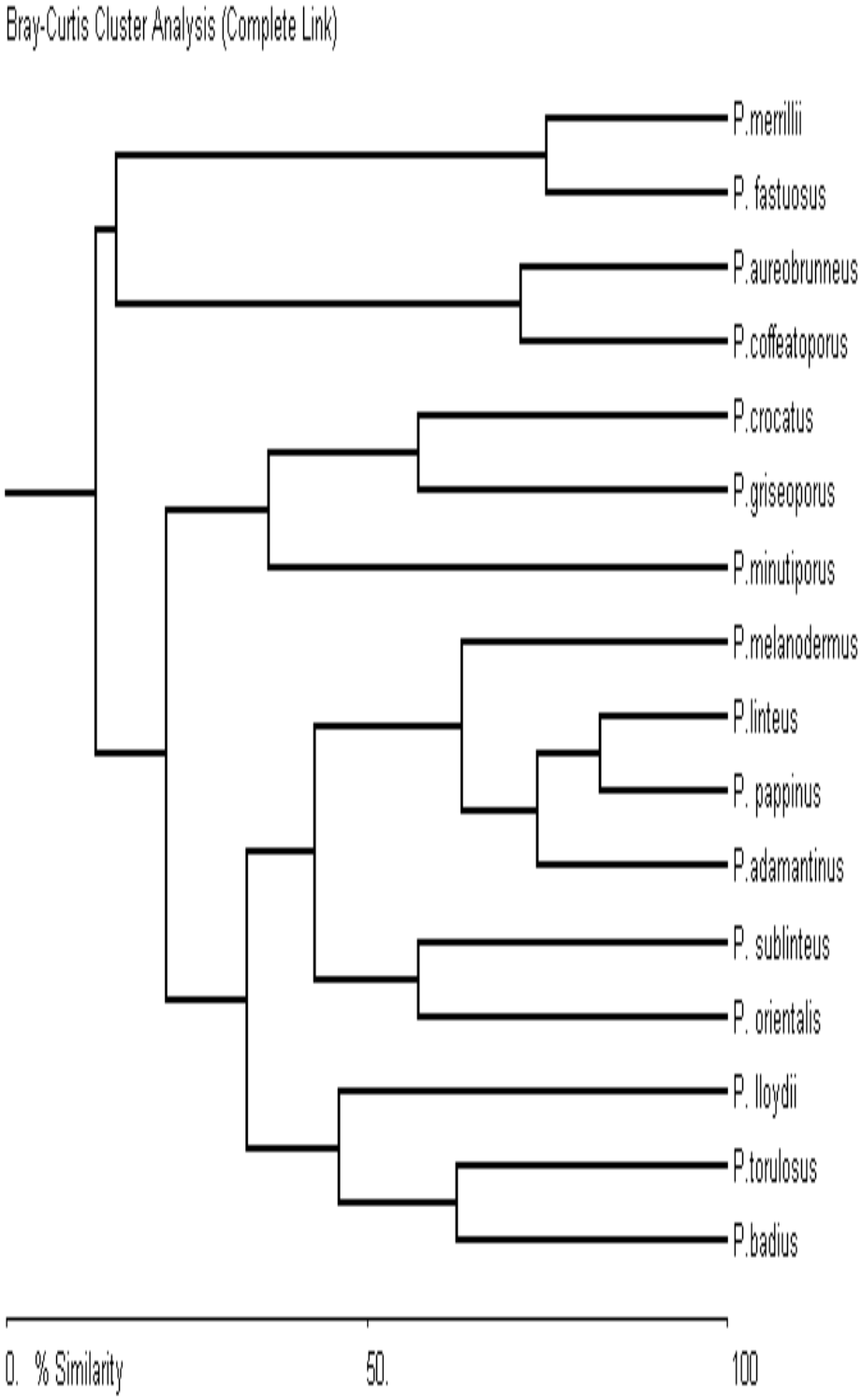

Fig. 1: Dendrogram generated of amino acids

The similarity and distance matrix generated are given in table 7 and 8 while the tree generated is given in figure1.

Three major clusters were observed as follows:

Cluster 1: Supported by *P. merrillii, P. fastuosus, P. aureobrunneus* and *P. coffeatoporus*.

The first half of the cluster was formed by *P. merrillii* and *P. fastuosus* while another half by *P. aureobrunneus* and *P. coffeatoporus*. This cluster is characterized by presence of amino acids like Alanine, Amino n-butyric acid, Aspartic acid, L – cystin, Glutamic Acid, Glycine, Arginine, Methionine, Ornithine, Lysine, Threonine and Serine.

L – cystin was found to be common for all these four species. Amino acids like Arginine, Methionine were present only in *P. merrillii* and *P. fastuosus*. Besides these amino acids, *P. fastuosus* also showed presence of Alanine while Glutamic Acid was present in *P. merrillii*.

On the other hand, both *P. aureobrunneus* and *P. coffeatoporus* showed presence of Amino n-butyric acid, Aspartic acid, Glycine and Lysine. Additionally, Alanine, Ornithine, Threonine and Serine were observed in *P. coffeatoporus*.

Cluster 2: Is supported by *P. crocatus, P. griseoporus* and *P. minutiporus*. *P. crocatus* and *P. griseoporus* were observed to be more apomorphic with respect to amino acid contents while *P. minutiporus* formed residual taxa in this cluster. The cluster is characterized by presence of all amino acids except Arginine, L-Leucine and Serine. Amino acids like Alanine, Dihydroxy Phenyl alanine, Glycine, L-Hydroxy Proline, DL-nor Leucine and Methionine were common in *P. crocatus* and *P. griseoporus* while Alanine, Amino n-butyric acid, DL-nor Leucine and Methionine were common for *P. griseoporus* and *P. minutiporus*. Amino acid Tryptophan was present in *P. crocatus* and *P. minutiporus* and was absent in *P. griseoporus*.

Besides these amino acids L – cysteine, Valine, L-Leucine and L-Proline were present only in *P. crocatus*. Aspartic acid and L – cystin were present in *P. griseoporus* while Isolucine, Tyrosine, Ornithine and Threonine were present only in *P. minutiporus*.

Cluster 3: This cluster was supported by species like *P. melanodermus, P. linteus, P. pappinus, P. adamantinus, P. sublinteus, P. orientalis, P. lloydii, P. torulosus, P. badius*. Three sub-clusters were observed in this mail cluster. The first cub-cluster includes species like *P. melanodermus, P. linteus, P. pappinus* and *P. adamantinus*. *P. melanodermus* and *P. adamantinus* forms residual taxa while *P. linteus* and *P. pappinus* show apomorphy.

Amino n-butyric acid, Isolucine and Dihydroxy Phenyl alanine were not detected in any of the species of this main cluster whereas L – cystin was observed in all species except *P. lloydii* and *P. badius*. Glycine and Ornithine was absent only in *P. torulosus* whereas DL-nor Leucine was absent in *P. orientalis* and *P. lloydii*.

Alanine was present only in last five species of this cluster i.e. *P. sublinteus, P. orientalis, P. lloydii, P. torulosus, P. badius* while Tyrosine was present in first five species viz. *P. melanodermus, P. linteus, P. pappinus, P. adamantinus, P. sublinteus*.

P. linteus and *P. pappinus* showed similarity with respect to amino acids like L – cystin, Glycine, Histidine, L-Hydroxy Proline, DL-nor Leucine, Tyrosine and Ornithine. *P. linteus* also showed presence of L – cysteine and Glutamic Acid while Methionine was observed only in *P. pappinus*.

On the other hand, in case of *P. sublinteus* and *P. orientalis* amino acids like Alanine, L – cystin, Glycine and Ornithine were common. Moreover, *P. sublinteus* additionally showed presence of DL-nor Leucine, Tyrosine, Tryptophan and Serine while L – cysteine and L-Leucine were present in *P. orientalis*.

The third sub-cluster is comprised of *P. lloydii, P. torulosus* and *P. badius* wherein *P. torulosus* and *P. badius* showed similarity while *P. lloydii* formed the residual taxa. Bothe *P. torulosus* and *P. badius* showed presence of Alanine, Histidine, Arginine, DL-nor Leucine, and L-Proline. *P. torulosus* also showed presence only of L – cystin whereas in *P. badius* Aspartic acid, Glycine, Ornithine, Threonine and Tryptophan were present.

The tree was constructed with sequential addition and weighing of each amino acid. It was observed during the character weighing that the topography of terminal taxa like *P. merrillii, P. fastuosus,P. lloydii, P. melanodermus, P. linteus, P. pappinus* and *P. adamantinus* were not disturbed. Furthermore, amino acids like Glycine, DL-nor Leucine followed by Ornithine contributed mainly in the construction of the tree.

IV. Discussion

The quantitative and Qualitative estimation of the amino acids showed that the contents in the studied *Phellinus* samples are very mush compatible to that of other mushrooms (see table 1)

Table 9: Amino acid contents in different medicinal mushrooms.

Name of the Mushroom	Amino Acid	Reference
Agaricus bisporus	Alanine, Arginine, Asparagine,Aspartic acid, Cysteine, Cystine, Glutamic acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine, Valine	Bernaś and Grażyna (2010), Bakowaski and Kosson (1985), AL-Hussainy and AL-Fadhly (2019), Rana et al. (2015), Mattila et al. (2002)
Pleurotus sajor-caju	Alanine, Arginine, Aspartate, Aspartic acid, Cysteine, Cystine, Glutamate, Glutamic acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine	Bisaria et al. (1987), Oyetayo et al. (2007), Kayoden et al. (2015), Pornariya and Kanok-Orn (2009)
Pleurotus ostreatus	Alanine, Arginine, Asparagine, Aspartic acid, Cysteine, Cystine, Gaba, Glutamic acid, Glutamine, Glycine, Histidine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, Norvaline, Ornithine, Phenylalanine, Proline, Serine, Thioproline, Threonine, Tryptophan, Tyrosine, Valine	Bernaś and Grażyna (2010), Mattila et al. (2002), Tagkouli et al. (2020), Pornariya and Kanok-Orn (2009)

Lentinus sajor-caju, L. connatus, L. torulosus, L. cladopus , L. squarrosulus	aspartic acid, Arginine, Alanine, Proline, tyrosine	Sharma et al. (2012)
Crepidotus applanatus, Daldinia concentrica, Oxyporus populinus , Trametes versicolor	Glutamic acid, Arginine, and Aspartic acid, Cystine, Lysine, and Histidine	Oni et al, (2021)
Pleurotus citrinopileatus	Leucine, Valine, Threonine, Lysine, Phenylalaline, Isoleucine, Methionine, Tryptophan	Musieba et al. (2013)
Pleurotus sp.	Arginine, Histidine, Lysine, Tryptophan, Phenylalanine, Methionine, Threonine, Leucine, Isoleucine, Valine	Bano et al. (1963)
Phellinus linteus	Aspartic acid, Serine, Glutamic acid, Glycine, Histidine, Arginine, Threonine, Alanine, Proline, Tyrosine, Valine, Methionine, Lysine, Isoleucine, Leucine, Phenylalanine	Jin et al. (2017)
Phellinus baumii	Aspartic acid, Threonine, Serine, Glutamic acid, Proline, Glycine, Alanine, Cystine, Valine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Histidine, Lysine, Arginine	Shon et al. (2006)
Pleurotus pulmonarius	Tyrosine, Histidine, Tryptophan, Arginine, Cysteine, methionine	Rana et al. (2015)
Lentinula edodes	Cystine, methionine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, histidine, lysine, arginine, tyrosine, phenylalanine	Mattila et al. (2002), Lasota and Sylwestrzak, 1989; Fasidi and Kadri, 1990; Kiribuchi, 1991
P. eryngii, P. nebrodensis	Alanine, Glycine, Valine, Leucine, Isoleucine, Threonine, Serine, Proline, Asparagine, Thioproline, Aspartic acid, Methionine, Hydroxyproline, Glutamic acid, Phenylalanine, Glutamine, Ornithine, Lysine, Histidine, Tyrosine, Tryptophan, GABA	Tagkouli et al. (2020)
Ganoderma lucidum	Tryptophan, Alanine, Serine, Cysteine, threonine, Asparagine, Glutamine, lysine, Glycine, Histidine, Proline, Isoleucine, Tryptophan, phenylalanine, arginine, methionine, leucine, Valine	Zhang et al. (2018)
Schizophyllum commune	Alanine, Arginine, Aspartate, Aspartic acid, Cystine, Glutamate, Glutamic Acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine	Al Azad and Ping (2021), Ivanova et al. (URL: https://www.researchgate.net (accessed 25/10/2021)
Phansomba samples (*Phellinus* spp.)	**Except Phenyl alanine all essential and non-essential amino acids**	**Present Study**

The qualitative contents were compared with previous reports of Mizuno, 2000, Bhor, 1984. The data is represented in table 2.

Table 10: Comparative account of qualitative amino contents in *P. linteus*, *P. merrillii* and *P. fastuosus*

	P. linteus (Mizuno, 2000)	*P. linteus* Jin et al. (2017)	*P. linteus* (Present study)	*P. merrillii* (Bhor, 1984)	*P. merrillii* (Present study)	*P. fastuosus* (Bhor, 1984)	*P. fastuosus* (Present study)
Name of the Amino acid	Aspartic acid, Glycine	Aspartic acid, Serine, Glutamic acid, Glycine, Histidine, Arginine, Threonine, Alanine, Proline, Tyrosine, Valine, Methionine, Lysine, Isoleucine, Leucine, Phenylalanine	**Cysteine, Cystin, Glutamic Acid, Glycine, Histidine, Proline, Leucine, Tyrosine and Ornithine**	Aspartic acid, Arginine, Glutamic acid, Cystine, Leucine, Methionine	**Cystin, Glutamic Acid, Arginine, Methionine**	Alanine, Arginine, Aspartic acid, Cystine, Leucine, Methionine	**Alanine, Cystin, Arginine, Methionine**

It was observed that samples contain both essential and non-essential amino acids however, Aspartic acid and Leucine was not detected in *P. merrillii* and *P. fastuosus* but were reported by Bhor (1984) whereas, Aspartic acid was not detected in *P. linteus* that was reported by in the studies of Mizuno (2000) and Jin et al. (2017). Similarly, Phenylalanine was also not detected in any of the study sample.

V. Conclusions:

Except for *Phellinus merrillii* and *Phellinus fastuosus* the amino acid contents were not previously reported from other *Phellinus* species that were used as 'phansomba. All the study samples showed presence of both essential and non-essential amino acids, except Phenylalanine. Although, finding from present study is noble; however, variations may exist in the amino acids reported with similar species under different environmental conditions. This mushroom being non-edible, more often consumed in the form of tea or decoction and may serve as important source of essential amino acid.

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