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Article

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Biochemical profile of two ethnically edible ectomycorrhizal mushrooms of southwest India

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Abstract

Edible mushrooms constitute the best natural source of bioactive compounds as well as antioxidants. Two edible ectomycorrhizal mushrooms of the Western Ghats of India (*Amanita* sp. and *Astraeus hygrometricus*) assessed for the biochemical profile. Aqueous extracts of dried powders of uncooked and cooked fruit bodies of mushrooms were evaluated for their soluble sugars and organic acids, while the methanol extract was used to detect polyphenols. Profiles of soluble sugars, organic acids, and polyphenols differed between the mushrooms as well as uncooked and cooked samples. Uncooked samples of both mushrooms possess higher quantities of sugars, organic acids, and polyphenols. Fructose is a common sugar in uncooked and cooked mushrooms, as well as tartaric acid, myricetin, and ethyl catechol. Trehalose was detectable only in *Amanita*, which has several industrial applications. Ascorbic acid found in both mushrooms and it is useful as a nutraceutical. Myricetin found in substantial quantities in both mushrooms, which serve as a nutraceutical owing to its antioxidant potential. The p-coumaric acid found in uncooked samples of both mushrooms. The novelty of these uncooked and cooked mushrooms in nutrition and bioactive potential will pave the way to adapt them for the human diet as well as in the production of health-promoting functional foods.

Keywords – *Amanita* – *Astraeus* – ectomycorrhizae – organic acids – polyphenols – sugars

Introduction

Mushrooms constitute a potential source of bioactive compounds of nutritional, health, and nutraceutical significance worldwide (Hobbs 2000, Cheung 2010, Chang & Wasser 2012). They became a popular dietary source due to their high levels of nutrients (carbohydrates, fiber, proteins, and vitamins) and low levels of fat and calories. In addition, mushrooms possess therapeutics like antioxidants, antimicrobials, antitumor, and immunomodulators substances that potentially useful in the production of value-added functional foods.

Edible ectomycorrhizal mushrooms have tripartite functions such as root symbionts with tree species facilitate drawing nutrients from the soil, and several of them are medicinally as well as nutritionally versatile. Many edible ectomycorrhizal mushrooms possess nutraceutical significance (e.g. Canterelles, milk caps, porcini, truffles, and *Russula*). There are up to 1,100 edible ectomycorrhizal mushrooms that possess medicinal, nutritional, and ethnic economic significance (Hall et al. 2011, Boa 2012, Zambonelli & Bonito 2012, Pérez-Moreno & Martínez-Reyes 2014,

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Hyde et al. 2019). Comparison of the number of species of edible ectomycorrhizal mushrooms in 15 genera performed with the WUFbase (Wild Useful Fungi database) (Boa 2004, Hall et al. 2011). Among them, *Russula* was first (72–110 species) followed by *Lactarius* (58–84 species). The maximum number of edible ectomycorrhizal mushrooms are sold in Mexico (77 species) followed by Nepal (13 species) (Boa 2004). Cultivation of ectomycorrhizal mushrooms is a challenge without the specific host tree (Hall et al. 2003). However, if it is possible to generate substantial biomass of mycelia of ectomycorrhizal fungi on agro-wastes, the possibilities are fair for fetching the health-promoting sugars, organic acids, and polyphenols.

The Western Ghats and west coast of India are endowed with several wild edible, medicinal and ectomycorrhizal mushrooms (Farook & Manimohan 2013, Karun & Sridhar 2013, Senthilarasu 2014, Senthilarasu & Kumaresan 2016, Pavithra et al. 2015, 2016a, 2016b, Karun & Sridhar 2014, 2016, 2017, Latha & Manimohan 2017, Sridhar & Karun 2019). The species richness of ectomycorrhizal species of *Inocybe* is the highest followed by *Amanita* and *Russula*. Interestingly, tree species belonging to the family *Dipterocarpaceae* also follow the same pattern of species richness. Although, *Amanita* generally regarded as poisonous, among the 15 edible ectomycorrhizal mushroom genera, *Amanita* positions in fourth place with 55 species (Hall et al. 2011).

While surveying the wild edible mushrooms of India's Western Ghats and southwest region, an *Amanita* sp. discovered in the lateritic scrub jungles and consumed by the local community at a tender stage (Karun & Sridhar 2014). Meticulous observations revealed that this *Amanita* sp. is ectomycorrhizal with many tree species in scrub jungles (e.g. *Acacia auriculiformis, A. mangium, Anacardium occidentale, Hopea ponga,* and *Terminalia paniculata*). *Astraeus hygrometricus* found in the foothills of the Western Ghats (Pavithra et al. 2015). It was associated with many tree species (e.g. *Artocarpus hirsutus, Holigarna arnottiana, Hopea parviflora, H. ponga, Phyllanthus emblica*, and *Syzygium cumini*). A series of studies have been carried out on the edibility and medicinal attributes of uncooked and cooked *Amanita* sp. (Greeshma et al. 2018a, 2018b, 2018c) as well as *Astraeus hygrometricus* (Pavithra et al. 2017, 2018). However, there is a knowledge gap on the biochemical profile and the pattern of ectomycorrhizal association of these mushrooms.

The organoleptic qualities (aroma, flavour, and taste) of mushrooms are dependent on the soluble sugars and organic acids. Soluble sugars are valued since they have therapeutic significance, while the organic acids possess radical scavenging, antimicrobial, anti-inflammatory, and neuroprotective attributes (Yoon et al. 2011, Barros et al. 2013, Leal et al. 2013). Similar to soluble sugars and organic acids, polyphenols present in mushrooms serve as potential antioxidants to quench free radicals (Cheung et al. 2003, Valentão et al. 2005a, Gąsecka et al. 2018). The current study envisaged assessing the soluble sugars, organic acids, and polyphenols of uncooked and cooked *Amanita* sp. and *Astraeus hygrometricus* obtained from the lateritic scrub jungles of southwest India and foothills of the Western Ghats of India, respectively.

Materials & Methods

Mushrooms and process

Tender sporocarps of ectomycorrhizal *Amanita* sp. were collected from the basins of *Anacardium occidentale* trees in the scrub jungles on the outskirts of Mangalore, India (12°48'N–74°55'E, 100–115 m asl). Tender sporocarps of another ectomycorrhizal mushroom, *Astraeus hygrometricus* (Pers.) Morgan, were collected from the Karkala forests of the foothills of the Western Ghats of India (13°12'N–74°58'E; 67–90 m asl). Mushrooms collected from three locations about 50–150 meters apart, rinsed separately in distilled water to eliminate soil, roots, and dirt. Cleaned sporocarps spread on a paper towel and blotted to remove the surface moisture. Divided each sample into two groups. The first group was oven-dried (55 ± 2°C), while the second was pressure-cooked with distilled water (1:0.25 v/v) followed by oven drying (55 ± 2°C). Continued drying of samples until the moisture content attain below 10%. Powdered dried sporocarps (in Wiley Mill, Thomas®, U.S.A.) transferred into airtight containers and refrigerated.

Extraction

Sonicated each sample of mushroom flour (1 g) in distilled water (5 ml) at an amplitude of 40% and centrifuged (10,000 rpm, 15 min). The supernatant concentrated in a vacuum concentrator before diluting to one ml with Millipore water. These aqueous extracts used for the determination of sugar and organic acid profiles. Followed the same procedure to determine the polyphenol profile with methanol for extraction instead of Millipore water.

Soluble sugars profile

Concentrated aqueous extract of each sample was filtered through a 0.45 µm PTEF (Polytetrafluoroethylene) membrane before assessing the soluble sugars using the HPLC (Shimadzu LC 10 A, Japan) coupled with a refractive index detector (Shimadzu RID-10A). Analysis carried out using the amino column (Kromasil NH_2, 250 mm × 4.6 mm, particle size 5 µm) using acetonitrile-water (75:25, v/v) as the mobile phase with a flow rate of 1 ml/min. Sugars identified based on a comparison of the retention time of the peak with the known concentration of standards.

Organic acids profile

The aqueous extract of samples was analysed for organic acids using the HPLC (Shimadzu LC 10 A, Japan) coupled with a UV detector. The analysis was performed using a reverse-phase C18 column (Intekchromasol RP-C18, 250 mm × 4.6 mm, particle size, 5 µm), injection volume was 5 µl, and detection at 210 nm. The mobile phase employed was 0.008 M sulphuric acid, at a flow rate of 1 ml/min in an isocratic mode of elution. Identified the organic compounds based on comparison of the retention time of the peak in comparison with known concentrations of standards.

Polyphenols profile

The methanol extract filtered through a 0.45 µm PTEF membrane onto the subject for polyphenol profile using the HPLC (Shimadzu LC 10 A, Japan). The analysis was performed by the RP C-18 column (Kinetex Reversed-Phase C18, 250 mm × 4.6 mm, particle size 5 µm) coupled with a PDA detector (Shimadzu SPD M20A). The injection volume was 5 µl, followed by the detection in the range of 280–320 nm. The mobile phases employed were A (100% acetonitrile) and B (0.1% o-phosphoric acid). The gradient programme equilibrated until 60 min, at a flow rate of 1 ml/min (Table 1). Polyphenols identified by comparing the retention time of the peak of samples examined with that of known concentrations of standards.

Table 1. The program followed for mushroom polyphenol determination using mobile phases A and B in HPLC.

Minutes	Mobile phase A (ml)	Mobile phase B (ml)
0	8	92
15	8	92
30	22	78
45	78	22
55	8	92
60	8	92

Statistical analysis

The *t*-test was followed to find the variation in sugars, organic acids and polyphenols between uncooked and cooked mushroom samples based on Statistica Version # 8.0 (StatSoft 2008).

Results

Soluble sugars

The soluble sugar profile revealed the occurrence of three and five sugars in *Amanita* sp. and

Astraeus hygrometricus, respectively (Table 2). The quantity of glucose, fructose, and trehalose was higher in uncooked than cooked *Amanita* sp. with a significant difference only in glucose content ($p<0.001$). In *Astraeus hygrometricus*, galactose, and maltose contents were significantly higher in uncooked samples ($p<1.001$), while it was the opposite for fructose content ($p<0.001$). The total soluble sugars were highest in uncooked samples of *Amanita* sp. (15.2 mg/g).

Table 2. Soluble sugar profile of aqueous extract of uncooked, and cooked *Amanita* sp. and *Astraeus hygrometricus* (mg/g) (n=3, mean±SD; *, $p<0.001$; BDL, below detectable limit).

Soluble sugar	***Amanita* sp.**		***Astraeus hygrometricus***	
	Uncooked	**Cooked**	**Uncooked**	**Cooked**
Glucose	5.47±0.25*	0.14±0.03	BDL	BDL
Fructose	0.51±0.03	0.48±0.02	0.15±0.002	0.44±0.01*
Galactose	BDL	BDL	9.47±0.16*	2.47±0.03
Rhamnose	BDL	BDL	0.49±0.01	0.35±0.004
Sucrose	BDL	BDL	0.38±0.01	0.19±0.002
Maltose	BDL	BDL	3.16±0.1*	0.45±0.01
Trehalose	9.2±0.26	8.83±0.4	BDL	BDL
Total	15.2	9.5	13.7	3.9

Organic acids

Altogether, six organic acids recovered from *Amanita* sp. and *Astraeus hygrometricus* (Table 3). In *Amanita* sp., uncooked samples consist of a higher number of organic acids than cooked samples (6 vs. 2), while in *Astraeus hygrometricus* three organic acids are common in uncooked and cooked samples. Tartaric acid was the highest in uncooked *Amanita* sp. followed by succinic and malic acids. Tartaric acid in cooked samples of *Amanita* sp. was significantly higher than in the cooked samples ($p<0.001$). In *Astraeus hygrometricus*, succinic acid was highest, followed by tartaric and ascorbic acids in uncooked as well as cooked samples. Tartaric ($p<0.01$) and ascorbic ($p<0.05$) acids were significantly higher in uncooked samples of *A. hygrometricus*, whereas succinic acid ($p<0.001$) was significantly higher in cooked than in uncooked samples. The total organic acids were highest in cooked samples of *A. hygrometricus* (25.9 mg/g).

Table 3. Organic acid profile of aqueous extract of uncooked, and cooked *Amanita* sp. and *Astraeus hygrometricus* (mg/g) (n=3, mean±SD; *, $p<0.05$; **, $p<0.01$, ***, $p<0.001$; BDL, below detectable limit).

Organic acid	***Amanita* sp.**		***Astraeus hygrometricus***	
	Uncooked	**Cooked**	**Uncooked**	**Cooked**
Tartaric acid	6.36±0.37***	0.65±0.1	2.87±0.2**	0.25±0.02
Succinic acid	4.73±0.15	BDL	5.00±0.34	25.45±2.04**
Ascorbic acid	0.05±0.002	BDL	0.21±0.01*	0.16±0.01
Malic acid	3.20±0.15	1.50±0.2	BDL	BDL
Pyruvic acid	0.42±0.03	BDL	BDL	BDL
Citric acid	1.46±0.04	BDL	BDL	BDL
Total	16.2	2.2	8.1	25.9

Polyphenols

Overall, 11 polyphenols were recovered from *Amanita* sp. and *Astraeus hygrometricus* (Table 4). The number of polyphenols was higher in uncooked than cooked samples of *Amanita* sp. (8 vs. 4) as well as *Astraeus hygrometricus* (7 vs. 6). In uncooked *Amanita* samples, the syringic acid content was highest, followed by gallic acid, myricetin, t-cinnamic acid, and epicatechin. Contents of gallic acid ($p<0.001$), ethyl catechol ($p<0.01$), and p-coumaric acid ($p<0.01$) were significantly higher in uncooked samples of *Amanita* sp. compared to cooked samples. In uncooked *Astraeus hygrometricus*, the vanillin content was highest followed by myricetin and ethyl catechol, while in

cooked samples, the epicatechin content was highest followed by myricetin and ethyl catechol. Contents of myricetin ($p<0.01$), ethyl catechol ($p<0.01$), and vanillin ($p<0.001$) were significantly higher in uncooked than cooked samples of *A. hygrometricus*.

Table 4. Polyphenols in methanol extract of uncooked, and cooked *Amanita* sp. and *Astraeus hygrometricus* (µg/g) (n=3, mean±SD; *, $p<0.01$; **, $p<0.001$; BDL, below detectable limit).

Polyphenols	***Amanita* sp.**		***Astraeus hygrometricus***	
	Uncooked	**Cooked**	**Uncooked**	**Cooked**
Gallic acid	10.30±0.11**	6.06±0.32	BDL	1.10±0.00
Kaempferol	BDL	BDL	4.00±0.0	BDL
Methyl catechol	2.10±0.05	BDL	BDL	0.02±0.00
Myricetin	9.36±0.25	6.66±0.2	100±0.01*	32.00±0.003
Ethyl catechol	6.70±0.36*	3.33±0.11	41.00±0.003*	8±0.001
Epicatechin	6.90±0.41	BDL	BDL	220±0.002
t-Cinnamic acid	8.28±0.16	BDL	1.30±0.0	BDL
Chlorogenic acid	BDL	BDL	1.30±0.0	BDL
p-Coumaric acid	2.05±0.14*	0.62±0.1	0.52±0.0	BDL
Vanillin	BDL	BDL	146±0.01**	3.04±0.0
Syringic acid	12.83±0.34	BDL	BDL	BDL
Total	58.5	16.7	294.1	264.2

Discussion

Soluble sugars

Accumulation of non-volatile compounds like soluble sugars in the fruit bodies of mushrooms and their organoleptic qualities (e.g. sweetness) are dependent on the soluble sugars (Litchfield 1967, Barros et al. 2007, Jedidi et al. 2016, Ravikrishnan et al. 2021). The type and quantity of soluble sugars varied greatly among the wild edible mushrooms of the Western Ghats (Ravikrishnan et al. 2021). Soluble sugars, as well as their quantities, differed among the uncooked and cooked mushrooms *Amanita* sp. as well as *Astraeus hygrometricus* in the present study, compared with another edible ectomycorrhizal mushroom *Amanita hemibapha* of the Western Ghats (Ravikrishnan et al. 2021). *Amanita* sp. was devoid of maltose and possibly broken into glucose during drying or processing (Barros et al. 2008). However, the maltose content was significantly higher in uncooked *A. hygrometricus* might have not been affected by the methods of processing followed. Galactose was below the detectable level in *Amanita* sp., while in *Astraeus hygrometricus*, it was as high as 2.5–9.5 mg/g. Similarly, *Astraeus hygrometricus* was devoid of trehalose, while it was as high as 8.8–9.2 mg/g in *Amanita* sp. Trehalose constitutes one of the principal carbohydrates in both European wild mushrooms as well as cultivated mushrooms in China (Kalač 2009, Li et al. 2014). Its content was drastically higher in *Amanita* sp. compared to *Amanita hemibapha* as well as *Amanita porphyria* (Reis et al. 2011, Ravikrishnan et al. 2021). Trehalose has several industrial applications, as it is useful in food industries as a food additive to increase sweetness and to promote freeze-dry preservation (Gibney et al. 2015). Sugar content in wild mushrooms seems to be dependent on various factors, like developmental stages, harvest conditions, and genetic makeup (Turfan et al. 2018). Since, the cultivated mushrooms have mostly uniform substrate as well as growth parameters (e.g. temperature, pH, conductivity, and O-R potential), the product composition may not vary too much. Hence, there is a scope to maneuver the substrates and conditions to fine-tune the soluble sugars in cultivated mushrooms favorably. However, such possibilities need further insight for those ectomycorrhizal mushrooms that produce mycelia or fruit bodies on composted agro-wastes.

Organic acids

Besides playing the main role in taste as well as flavour, they serve as antioxidants, anti-inflammatory, neuroprotective, and antimicrobial traits necessary for human health protection (Altmeyer et al. 1994, Valentão et al. 2005b, Seabra et al. 2006, Ribeiro et al. 2008, Brennan et al.

2000, Leal et al. 2013, Gąsecka et al. 2018). Gąsecka et al. (2018) reported a variety of organic acids in the wild as well as cultivated edible *Agaricus* spp. There is a drastic variation in the quantities of different organic acids between the mushrooms (*Amanita* sp. and *Astraeus hygrometricus*) as well as processing methods (uncooked and pressure-cooked) in our study. Such variations also reported in six wild edible mushrooms from the foothills of the Western Ghats (Ravikrishnan et al. 2021). According to Cámara et al. (1994), the organic acids not affected by the methods of processing and storage in foodstuffs like fruit juice and nectar. In our study, the succinic acid content in *Astraeus hygrometricus* was significantly increased on pressure-cooking, while it was the opposite for tartaric as well as ascorbic acids. However, pressure-cooking of *Amanita* sp. has drastically knocked off the organic acids (6 vs. 2) as well as their quantities (tartaric and malic acids). Uncooked *Amanita* sp. has a similar quantity of ascorbic acid, lower in citric acid, and higher succinic and malic acids compared to *Amanita rubescens* (Kouassi *et al.* 2016). Uncooked *Amanita* sp. has more quantity of organic acids compared with another edible ectomycorrhizal mushroom *Amanita hemibapha* of the Western Ghats (6 vs. 3) (Ravikrishnan et al. 2021). The *A. hemibapha* had lactic acid in addition to ascorbic and succinic acids. Similar to *Amanita caesarea* in Portugal, uncooked *Amanita* sp. in our study possesses citric as well as malic acids (Valentão et al. 2005b). Succinic acid serves as a precursor to many industrially important chemical compounds like adipic acid, 1,4-butanediol, tetrahydrofuran, N-methyl pyrrolidinone, 2-pyrrolidinone, succinate salts, and gamma-butyrolactone (Song and Lee, 2006). The mega doses of ascorbic acid (vitamin C) used to prevent many human ailments like diabetes, cataracts, glaucoma, macular degeneration, atherosclerosis, stroke, heart disease and cancer (Iqbal et al. 2004). In addition, the organic acids are capable of improving shelf life as well as preventing bacterial spoilage (Ouattara et al. 1997, Singla et al. 2012).

Polyphenols

In addition to fruits and vegetables, mushrooms possess several polyphenols (Barros et al. 2009). Interest in mushroom polyphenols is increasing recently owing to their therapeutic as well as nutraceutical potential (e.g. radical scavenging, metal chelation, and inhibition of lipid oxidation) (Cheung et al. 2003, Yoon et al. 2011). Gąsecka et al. (2016, 2018) have reported a variety of polyphenols in wild as well as cultivated edible *Agaricus* spp. as well as *Pleurotus* spp., and related their antioxidant potential to serve as nutraceuticals. Many studies have interrelated the antioxidant capacity of mushrooms with polyphenols (e.g. Ren et al. 2014, Lin et al. 2015, Smolskaité et al. 2015). In most instances, pressure-cooking of *Amanita* sp. and *Astraeus hygrometricus* reduced or knocked off the organic acids. However, in *A. hygrometricus*, gallic acid, methyl catechol, and epicatechin were below detectable limits in uncooked samples but showed up in cooked samples with an extremely high quantity of epicatechin (220 µg/g). When compared to *Amanita* sp., *Astraeus hygrometricus* had higher concentrations of myricetin and ethyl catechol. Myricetin has gained recognition for its nutraceutical value owing to its antioxidant, anticancer, antidiabetic, and anti-inflammatory activities (Semwal et al. 2016). The p-coumaric acid protects the liver and kidney against CIS-induced oxidative damage in experimental animal models, and it inhibits oxidative stress (Akdemir et al. 2017).

Conclusion

The composition of the edible mushrooms is dependent on the habitat, substrate, and growth stage. *Amanita* sp. as well as *Astraeus hygrometricus* assessed in this study are ectomycorrhizal and ethnically edible mushrooms in the early stage of development of the fruit body. They are easily available in the coastal belts of the southwest and the foothills of the Western Ghats of India, which provide food and economic security to the local people and tribal people during the rainy season. The profiles of soluble sugars, organic acids, and polyphenols shed light on the nutraceutical value of these mushrooms. Furthermore, studies on their proximal, mineral, amino acids, fatty acids, functional properties, and antioxidant potential show that they are superior in terms of the nutritional value as well as health-promoting potential. In the comparison of these mushrooms, the

total organic acids were highest in cooked *A. hygrometricus*, so also the total polyphenols in uncooked as well as cooked *A. hygrometricus*. Similarly, cooked *Amanita* sp. possesses a good quantity of trehalose, gallic acid, and ethyl catechol. The novelty of these mushrooms will allow them to employ in the production of attractive diets as well as health-promoting diets and value-added functional foods. There is a strong link between these mushrooms as ectomycorrhizas in many tree species, especially *Anacardium occidentale, Artocarpus hirsutus, Holigarna arnottiana, Hopea parviflora, H. ponga, Phyllanthus emblica, Syzygium cumini*, and *Terminalia paniculata*. The future concern is to protect these trees to conserve *Amanita* sp. as well as *Astraeus hygrometricus* for the benefit of human nutrition and health.

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