Introduction

Ganoderma species are regarded as higher fungi because the carbophores are visible enough to be seen with naked eye (Russell and Patterson, 2006; Happuarachchi et al., 2016). They are commonly known as “the king of mushrooms” or “the mushroom of immortality” (Uma Gowrie et al., 2014). The mushroom of immortality has been used for thousands of years by far eastern countries for its medicinal uses (Kenneth, 1990). The mushroom is one of the Chinese traditional mushrooms regarded as the world’s treasure of culture (Gao, 2011), and considered as therapeutic fungal biofactory (Russell and Paterson, 2006). Elemental analysis of extracts of wild Ganoderma spp. from Nigeria, in earlier studies, revealed important pharmacologically active elements (Ogbe et al., 2008; Ihayere et al., 2010, Shamaki et al., 2012). However, in other climatic conditions, Russell and Paterson (2006) reported the presence of proteins, peptides, amino acids, protein-peptide complexes and over 120 flavour compounds. Sun et al. (2004), Sun and Zhang (2011) and Lull et al. (2005) attributed the potent antioxidant activities and anti-inflammatory properties of this mushroom to peptides, polysaccharides and their complexes contained in this mushroom.

One important amino acid that has received less attention is arginine, which is reported to have ergogenic potentials and involved in protein synthesis (Campbell et al., 2004), in addition to its pharmacological potentials activities. It was equally reported that arginine possesses adjuvant properties that potentiates immune responses to an antigen, or moderate it towards a desired immune response (Delneste et al., 2004). These unidentified pharmacologically active compounds may be responsible for conferring hepatoprotective activities (Oluba et al., 2010), cardio-protective properties (Rajasekaran and Kalaimangal, 2012), anti-haemolytic activities against lead-exposed rats (Hossain et al., 2015), genito-protective effects in rats (Donmez and Yilmaz, 2014), anti-mutagenic effects (Lakshmi et al., 2006) and the suspected but unsubstantiated anti-cancer beneficial and immune-modulatory properties (Wasser, 2005; Happuarachchi et al., 2016; Wasser, 2017).

These postulations present the need to analyse for some important pharmacological elements and arginine contents in Ganoderma sp found in Nigeria, since this amino acid is reported to be involved in protein synthesis (Campbell et al., 2004), and that there may arise differences in chemical compositions of Ganoderma species. growing in different geographical zones (Liao et al., 2015).

ARGININE – an essential amino acid found in Ganoderma species from Northern Nigeria

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ABSTRACT

Introduction: The wild Ganoderma sp. is a mushroom found growing in most forest of Nigeria, and was analysed for elemental constituents and for the amino acid- arginine, using 0.5 g of the digested wild Ganoderma sp.

Method: The sample was analyzed against a blank using fluorescent atomic absorption spectrometry (FAAS) in triplicate. It was analyzed for concentration of elements such as iron (Fe), calcium (Ca) potassium (K), copper (Cu), zinc (Zn), manganese Mn), cobalt Co), magnesium (Mg), chromium (Cr), lead (Pb) and the essential amino acid-arginine (Ag).

Results: Findings revealed the presence of the analyzed selected elements and the arginine in various concentrations, thus explains the acronym “fungal biofactory” of this mushroom.

Significance: It was concluded that arginine content found in Ganoderma species is an important amino acid that provide the requirements needed for the synthesis of pharmacologically bioactive phytochemicals found in the Ganoderma species, and its medicinal usefulness should be explored.

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Materials and methods

Mushroom sample collection and preparation

The fruiting bodies of the Ganoderma species were collected in July and August 2016 from Lafia, Nassarawa State, during the rainy season. It was dried under room temperature for three weeks, grinded to coarse powder using grinding machine (Lister, China). This powder weighing 500g was carefully wrapped in a transparent polythene bag, inserted carefully in a plastic container, and transported to Maiduguri and kept at room temperature in the Pharmacology and Toxicology laboratory, Faculty of Veterinary Medicine, University of Maiduguri, until required for use.

Sample digestion

A (0.5g) Ganoderma sample was weighed and placed into a microwave tube. This was followed by 6 ml of concentrated nitric acid (64.3% HNO₃) was added followed by 3 ml of (20% v/v) hydrogen peroxide. The sample was then digested under microwave for 50 minutes and a temperature range of 130ºC - 180ºC was used in the digestion.

After digestion with microwave oven, the sample was transferred to a 25 ml volumetric flask and diluted with 10 ml distilled water, and then kept for further analysis. The sample was analyzed against a blank using fluorescent atomic absorption spectrometry (FAAS) at various wavelength in high performance lipid chromatography (HPLC), and the result was calculated using excel by extrapolating values derived with the standard calibration curve (Radojevic and Bashkin, 1999).

Statistical analysis

The data generated was statistical analyzed using one-way analysis of variance (ANOVA) and results were presented as mean ± standard deviation.

Results

The Ganoderma spp. sample was subjected to elemental analysis using high performance lipid chromatography (HPLC) at various wavelengths, and some selected elements as shown in Table 1, were found in different concentrations. Notably were iron (181.66±1.02), calcium 122.81±1.03, potassium (1210±2.83), copper (50.37±1.27), zinc (2.60±1.01), manganese (14.81±0.23) and chromium (0.21±0.01), lead (0.48±0.03). The result of the amino acid-arginine is presented in both Tables 1 and 2. It showed its concentration in the wild Ganoderma sp. to be 147.5 ± 0.13 ppm, which is equivalent to 0.0148 %. In figure 1, HPLC chromatogram reveals concentration of arginine spiking at 25 MaU*S when compared to the standard 30 MaU*S, thus showing some degree of significance.

| S/N | Wavelength (nm) | Concentrations (mg/l) | Mean ± SD |
|-----|-----------------|----------------------|-----------|
| 1   | 248.30          | 182.40               | 180.50    | 182.08    | 181.66±1.02 |
| 2   | 422.70          | 122.56               | 123.08    | 122.80    | 122.81±0.26 |
| 3   | 422.60          | 1207.20              | 1211.00   | 1212.81   | 1210.34±2.86 |
| 4   | 324.70          | 51.20                | 48.90     | 51.00     | 50.37±1.27  |
| 5   | 385.20          | 2.67                 | 2.50      | 2.62      | 2.60±0.09   |
| 6   | 398.70          | 0.32                 | 0.30      | 0.33      | 0.32±0.02   |
| 7   | 357.90          | 14.58                | 15.04     | 14.82     | 14.81±0.23  |
| 8   | 357.90          | 0.22                 | 0.20      | 0.20      | 0.21±0.01   |
| 9   | 283.00          | 0.48                 | 0.50      | 0.45      | 0.48±0.03   |
| 10  | 215.00          | 146.12               | 147.11    | 147.14    | 147.50±0.02 |

nm – nanometer, SD – standard deviation

Table 2. Arginine as determined by HPLC

| S/N | Wavelength (nm) | Retention time (mms) | Unspiked sample Signal peak area/MaU*S | Spiked sample Signal peak difference (Peak area/MaU*S) | Signal difference (Peak area/MaU*S) | Standard signal (Peak area/MaU*S) | Standard conc. | K standard conc. | Sample conc. (PPM) |
|-----|-----------------|----------------------|---------------------------------------|------------------------------------------------------|-------------------------------------|----------------------------------|----------------|-----------------|-------------------|
| 1   | 215.00          | 2412.00              | 209.20                                | 562.70                                               | 353.50                              | 28.37                            | 20.00          | 0.14            | 147.50            |
Discussion

The *Ganoderma* species in Nigeria is a fungus with great ecological role which depicts the Nigeria’s rich land flora, its features display an advanced heterotrophic property. This fungus was subjected to elemental and nutritional analysis. It showed an array of elements useful for supplementation and gave its handsome arginine constituents, an important amino acid involved in metabolism of amino acid and other important macromolecules for maintenance of homeostasis in a living organism.

The presence of pharmacologically active elements detected in wild *Ganoderma* species found in Nigeria, was earlier reported by Ogbe *et al.* (2008), Ihayere *et al.* (2010) and Shamaki *et al.* (2012). However, analyzed elements in this study are higher in concentration than the earlier reports of Shamaki *et al.* (2012), but similar to findings of Ihayere *et al.* (2010). This difference can be due to earlier reported factors which include; difference in methods used in the analysis and possibility of seasonal variations, geographical locations, variation in species, edaphic factors, anthropogenic factors, table water elemental constituents, nearby substances, and neighboring organic substances constituents while harvesting the mushroom, and also difference in soil types where tree trunk grew and upon which the *Ganoderma spp.* grows, as reported by Kamuya *et al.* (2010). The presence of pharmacologically active elements agrees with other findings by Mizuno (2011), thus accentuating the position of Russell and Peterson (2006) who reported that *Ganoderma* is a fungal biofactory. This finding supports the reported use of *Ganoderma lucidum* extracts as supplements in enhancing immune response in infectious bursa disease (IBD) in broilers (Ogbe *et al*., 2008).

Although, this mushroom was reported to contain large amount of amino acids by several authors, (Guzeldag *et al*., 2007; Dong and Han, 2015; Wasser, 2017), these amino acids are reported to be connected to the multiple medicinal properties of this mushroom by many researchers (Happuarachchi *et al*., 2016). Interestingly, those bioactive compounds, which amino acids form part of, are all linked to the amino acid-arginine, whose main function is its involvement in protein synthesis, in addition to other metabolic roles in the body (Wu and Morris, 1998; Tong and Barbul, 2004). This amino acid can also be catabolized in the
body to produce glucose for required energy supplies at gluconeogenesis (Campbell et al., 2004). Dietary supplementation of arginine and its anabolic effects has also been reported by Cremades et al. (2004). Thus, the immunological response observed by Ogbe et al. (2008) in IBD infected broilers, which are attributable to the presence of this precursor amino acid-arginine. The presence of arginine in Ganoderma can also be linked to its neutreutacetal properties (Boger, 2007). Arginine is also associated with nitric oxide synthesis that acts as a neurotransmitter and a vasodilator, which confers cytoprotective effect on the endothelium. This amino acid is also involved in the synthesis of Ganoderma derived sterols, that are reported to inhibit lanosterol 14α-demethylase activity in the biosynthesis of cholesterol (Hajjaj et al., 2005) supporting earlier findings (Stavinhoa, 2011; Shamaki et al., 2015; Wasser, 2002, 2017). Compounds from Ganoderma lucidum are equally reported to inhibit 5-alpha-reductase activity in the biosynthesis of dyhydrostestosterone (Liu et al., 2006) an enzyme involved in the production of testosterone, thus supporting the influence of dietary arginine on the anabolic effect of androgens (Cremades et al., 2004) by inhibiting cholesterol synthesis. This amino acid may be involved in the synthesis of triterpenoids found in Ganoderma lucidum, and these triterpenoids are reported to be responsible for neuraminidase inhibition in avian influenza H5N2 as reported by Shamaki et al. (2013) that was later revalidated by Zhu et al. (2015). High amount of arginine was found in this study, with the chromatogram of the sample 25 MaU*S spike level as compared to the standard 30 MaU*S. There are also reports of interaction between dietary arginine and hormones action (Ruzafa et al., 2003), since dietary restrictions of arginine resulted in abolished sexual dimorphism found in the levels of this amino acid. The involvements of arginine in various metabolic pathways have been reported by Wu and Morris (1998).

Conclusion

In conclusion, the pharmacological activities of extracts of wild Ganoderma spp. found in Nigeria, can be linked to the presence of this important amino acid –arginine which is involve in biochemical activities in the body.

Conflict of interest

There is no conflict of interest regarding the publication of this paper.

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