TECHNICAL NOTE

Analysis of Secondary Metabolites from Fungus *Ganoderma lucidum*

Yordan Martinez Aguilar *, Román Rodríguez Bertot **, Gretel Borrero Pita **

*Department of Science and Agricultural Production, Pan-American Agricultural School, Zamorano, Honduras.
** Center for Animal Production Studies, Faculty of Agricultural Sciences, University of Granma, Granma, Cuba.
Corresponding author: rrodriguezb@udg.co.cu

Received: November, 2020; Accepted: December, 2020; Published: December 2020.

INTRODUCTION

Quite a few alternatives to diet antibiotics in animal production have been developed in the past decade as probiotics, prebiotics, and phytobiotics. In that sense, the scientific community has studied new natural products available, and show effectiveness under different *in vitro* and *in vivo* schemes (Molina *et al*., 2019). Fungus *Ganoderma lucidum*, order *Polyporales* and family *Ganodermataceae*, is originally from China. It is spread worldwide, mainly in tropical and subtropical areas, growing as a parasite or saprophyte, which feeds on dead organic matter from a large variety of trees, where it gets the organic compounds needed as nutrients (Ogbe *et al*., 2009).

Various studies have demonstrated that this toadstool promotes immune function due to an increase in the production and excretion of interferon (IFN) through host cells, which have anti-tumor and antiviral properties. Besides, it promotes the action of macrophages, T-lymphocytes, and K cells (natural killers) (Oluwafemi *et al*., 2020). Additionally, the utilization of *G. lucidum* as an additive in the diet of monogastric animals improved their productive performance, humoral response, the antioxidant capacity, and intestinal microflora (Molina *et al*., 2019; Oluwafemi *et al*., 2020). Despite the medicinal properties of *G. lucidum*, few studies have dealt with the phytochemical characterization of this natural product, according to a phytochemical screening of wild strains, for further research as functional additives in animal diets. The aim of

Citation (APA)
Martinez Aguilar, Y., Rodríguez Bertot, R., & Borrero Pita, G. (2021). Analysis of Secondary Metabolites from Fungus Ganoderma lucidum Journal of Animal Production, *33*(1). https://revistas.reduc.edu.cu/index.php/rpa/article/view/e3683

this study was to conduct quality identification of the presence of secondary metabolites of *Ganoderma lucidum.*

DEVELOPMENT

Sample collection and preparation

The experiment was performed at the Center of Applied Chemistry (CEQA), Faculty of Technical Sciences, University of Granma, Cuba. The fungal strains were collected from dry tree trunks in the experimental areas of the seed nursery at the University of Granma, located 17 km from the city of Bayamo, Granma, Cuba, with an average temperature of 25±2 °C.

The fungi were identified morphologically (size and color), and microscopically, at the Department of Microbiology of the University of Granma, Cuba. Then, they were dehydrated for three days in a stove (WSU 400, Germany), with air recirculation at 65 ºC. Later, the material was crushed, using a hammer mill with side blades (1 mm size particle). The samples were stored in sterile airtight plastic bags, at room temperature, in the dark, to prevent decomposition of active substances due to the action of light.

Phytochemical screening

A volume of 5 g of *Ganoderma lucidum* powder was weighed in an analytical balance (BS 2202S Sartorius, China), then 50 mL of 70% ethanol was added to make the alcohol extract, along with 50 mL of distilled water to make the aqueous extract in the same way through fluid percolation. The samples were left to rest for 3 days at 8 °C. Test tubes (10 cm long by 1 cm diameter), rack, flasks, pipettes (2 and 10 mL), beaker, Erlenmeyer flasks, funnel, filter paper, burner, and digital magnetic shaker (IKA RH Basic 1), were used.

The phytochemical screening was done according to the methodology suggested by Miranda and Cuellar (2000). The following assays were done: Mayer, Dragendorff, Wagner (alkaloids), Baljet (coumarines), Liebermann-Burchard (triterpenes or steroids), foam (saponins), ninhydrin (free amino acids), Fehling (carbohydrates as reducers), iron chloride (phenols or tannins), Borntrager (chinones), Shinoda, (flavonoids), Kedee (glycosides), Molish (sugars), catechins, triterpenes and steroids, resins, and anthocyanins. Cross-system analysis was used as a measure criterion for quantification of secondary metabolites.

Table 1 shows the phytochemical screening of *G. lucidum.* This study concluded that alkaloids (the two extracts) and flavonoids (the two extracts) were the only secondary metabolites detected in the aqueous extract. However, coumarins, phenols and tannins, amino acids and amines, anthocyanidines, sugars, catechins and triterpenes, and steroids, were quantified.

Table 1. Presence of metabolites according to the phytochemical screening of *G. lucidum*

Assays	Ethanol extract	Aqueous extract
Dragendorff assay (alkaloids)	+++	+
Mayer (alkaloids)	+	+
Wagner (alkaloids)	+++	+
Baljet (coumarins)	+	
Bontranger (chinones)	-	-
Fehling (carbohydrates as reducers)		-
Iron chloride (phenols and tannins)	+	-
Ninhydrin (amines and amino acids)	+	
Anthocyanins	+	
Shinoda (flavonoids)	+	+
Kedee (glycosides)	-	
Molish (sugars)	+	
Mucilages (polysaccharides)		-
Resins	-	
Foam (saponins)		-
Catechins	+	
Triterpenes and steroids	+	

Legend: (-) Absence (+) Presence (+++) Abundance

Although phytochemical screening does not provide secondary metabolite quantification of the samples, it is a starting point to know the presence of secondary metabolites, which can improve the nutritional and health status of animals, even in small concentrations. Overall, alkaloids are the most representative secondary metabolites in the extract of *Ganoderma lucidum.* The quantification of alkaloids in both extracts (ethanol and aqueous) shows the polarity of the secondary metabolites detected. According to Martínez *et al*. (2020), alkaloids are mostly found in the ethanol extract, and in smaller quantities in the aqueous extract, as shown in Table 1. It has been demonstrated that in small concentrations of alkaloids (particularly isochemical ones), they reduce oxidative stress and intestinal swelling, so its use in animals may contribute to a reduction of postprandial stress in intensive productions (Martínez *et al*., 2020). In that sense, Ogbe *et al.* (2009) demonstrated that the aqueous extract of *G. lucidum* reduced the oocysts of *Emeria tenella*, and the intestinal injuries, and it improved the productive performance of broilers. However, high concentrations of alkaloids caused irritation in the gastrointestinal tract, intestinal hemorrhage, and respiratory paralysis in animals (Martínez *et al*., 2020).

Moreover, the anthocyanidines detected in the ethanol extract proved therapeutic effects in relation to antioxidant properties, such as lipid control, secretion of insulin, and vasoprotective effects (Miranda and Cuellas, 2000). Likewise, the coumarins detected are powerful anticoagulants and bactericides (Martínez *et al*., 2020). The flavonoids in *Ganoderma ludicum* have a phytoestrogenic effect, and an antioxidant capacity (free radical catcher RH*). They can also mediate in the synthesis of eicosanoids, platelet aggregation, and low-density protein oxidation (Ogbe *et al.*, 2009). No reducing sugars, saponins, and mucilages were observed in the aqueous extract. These metabolites cause symptoms related to anti-nutritional factors, like other

secondary metabolites identified in *Ganoderma lucidum*, such as alkaloids and polyphenols, when found in high concentrations in the diet.

CONCLUSIONS

Alkaloids (in both extracts) and flavonoids (in both extracts) were quantified based on the preliminary analysis of secondary metabolites of wild *Ganoderma lucidum*. Meanwhile, coumarins, phenols and tannins, amines and amino acids, anthocyanidines, sugars, catechins and triterpenes, and steroids, were quantified in the ethanol extract. An *in vivo* study that evaluates the use of this natural product as a zootechnical additive in the diet of animals is recommended.

REFERENCES

Martínez, Y., Más, D., Betancur, C., Gebeyew, K., Adebowale, T., Hussain, T., Wensheng, L., & Ding, X. (2020). Role of the Phytochemical Compounds like Modulators in Gut Microbiota and Oxidative Stress. *Current Pharmaceutical Design*. DOI: 10.2174/1381612826666200515132218

Miranda, M., & Cuellar, A. (2000). Manual de prácticas de laboratorio: Farmacognosia y productos naturales. *Ciudad Habana: Universidad de la Habana*.

Molina, D. S., Bernardo, J., Machado, O. D., Más, D., & Martínez, Y. (2019). Nutraceutical effect of *Ganoderma lucidum* fungus on neonatal broilers diet. *International Journal of Poultry Science, 18*(12), 641-647. DOI: http://dx.doi.10.3923/ijps.2019.641.647

Ogbe, A. O., Atawodi, S. E., Abdu, P. A., Sannusi, A., & Itodo, A. E. (2009). Changes in weight gain, faecal oocyst count and packed cell volume of Eimeria tenella-infected broilers treated with a wild mushroom (*Ganoderma lucidum*) aqueous extract. *Journal of the South African Veterinary Association*, *80*(2), 97-102. DOI: https://jsava.co.za/index.php/jsava/article/view/179

Oluwafemi Adetuyi, B., Olamide Okeowo, T., Adefunke Adetuyi, O., Abraham Adebisi, O., Ogunlana, O. O., Janet Oretade, O., Najat, M., Magdy Beshbishy, A., Welson, N., & Batiha, G. E. S. (2020). *Ganoderma Lucidum* from red mushroom attenuates formaldehyde-induced liver damage in experimental male rat model. *Biology*, *9*(10), 313. DOI: 10.3390/biology9100313

AUTHOR CONTRIBUTION

Conception and design of research: YMA; data analysis and interpretation: YMA; RRB; GBP, redaction of the manuscript: YMA, RRB.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.