Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60277-9

Phytochemical properties of *Ganoderma applanatum* as potential agents in the application of nanotechnology in modern day medical practice

Akpera T. MANASSEH (FIMLS)[1], Jombo T.A. Godwin (FMCPath)[2],

EMANGHE E Ubleni (FMCPath)[3], Oguntayo O. Borisde (MSc)[4]

[1] *Department of Haematology and Blood Transfusion, College of Health Sciences, Benue State University, PMB 102119 Makurdi, Nigeria.*
[2] *Department of Medical Microbiology and Parasitology, College of Health Sciences, Benue State University, PMB 102119 Makurdi, Nigeria.*
[3] *Department of Medical Microbiology and Parasitology, Colege of Medical Sciences, University of Calabar, PMB 1115 Calabar, Nigeria.*
[4] *Department of Haematology, Federal College of Veterinary and Medical Laboratory Technology, Vom Jos, Nigeria.*

ARTICLE INFO

Article history:
Received 15 April 2012
Received in revised form 27 April 2012
Accepted 8 August 2012
Available online 28 August 2012

Keywords:
African Trypanosomiasis
Ganoderma applanatum
Nanotechnology
Phytochemistry

ABSTRACT

Objective: This study was set up to review the phytochemistry of *Ganoderma applanatum*, its potentiality in nanotechological engineering for clinical use as well as impact of aqueous extracts of *Ganoderma applanatum* on laboratoery rats infected with Trypanosoma brucei brucei. **Methods:** Aqueous extracts of *Ganoderma applanatum* were obtained using hot sterile distilled water and whatmann filter paper. The presence of saponins, alkaloids, tannins, anthraquinones, flavonoids, cardiac glycosides and steroids were tested using standard procedures. Acqueous extracts were also inoculated into laboratory rats infected with *Trypanosoma brucei brucei* along with both positive and negative controls. Blood samples were collected daily, stained with Giemsa's stain and examined for the presence of parasites. **Results:** *Ganoderma applanatum* aqueous extracts contained detectable levels of saponins, flavonoids, cardiac glycosides and steroids but undetectable levels of alkaloids, tannins and anthraquinone. All the infected rats died by day 12 from overwhelming trypanosomal infections. **Conclusion:** The biochemical constituents of *Ganoderma species* should be subjected to nanotechnological engineering in order to probably discover more of its wider therapeutic benefits, and to further disprove its suitability or otherwise in the treatment of African sleeping sickness.

1. Introduction

Nanotechnology is a process whereby engineered nanopeptides (particles) are normally embedded in the matrix of other composites to enhance certain characteristics. [1-3] The ability of engineered nanoparticles to interact with other particles, cells and tissues at a molecular level provides them with a distinct advantage over other polymeric or macromolecular substances. [4-6]

This engineering technique is fast gaining grounds with important applications in computer science, physical and organic chemistry, physics, science engineering and technology. Nanotechnology has similarly found wide applications in biology and medicine which usually employ dispersed nanoparticles for instance as fluorescent biological labels, tumour detection and destruction, contrast enhancement, bio-detection of pathogens, detection of proteins, probing of DNA structure, tissue engineering, separation and purification of biological molecules among others. This ranges from diagnostic, therapeutic as well as prognostic management of various human ailments. [7-9]

Treatment of infections and infestations, and related ailments which still constitute at least 70% of hospital attendees and admissions in sub-saharan Africa is recording great successes with the application of nanotechnology. [10-12] This has been observed in the management of viral infections such as HIV-AIDS; treatment of bacterial infections such as typhoid fever, Escherichia coli and Klebsiella pneumoniaeinfections, and tuberculosis; and parasitic infections such as cutaneous and muco-cutaneous leishmaniasis. [13-15]

Ganoderma species, a Basidiomycete fungus has been found to possess several medicinal properties useful in the treatment of diverse human infections. [16-18] It is in this regard that the phytochemical properties of the extracts of *Ganoderma applanatum* which could be potential raw materials for nanotechnological engineering in clinical

*Corresponding author: JOMBO G T A Department of Medical Microbiology & Parasitology, College of Health Sciences, Benue State University, P M B 102119 Makurdi, Nigeria.
Tel- +2348039726398
E-mail: jombogodwin@yahoo.com

practice were reviewed as well as its benefits or otherwise in the treatment of African trypanosomiasis. [19-21]

2. Materials and methods

Study Area and SettingThe study was carried out in Vom about 25 kilometres south-east of Jos, the Plateau state capital in north-central Nigeria. In Vom is sited the Federal College of Veterinary and Medical Laboratory Technology, and National Veterinary Research Institute where the study was carried out. Experimental rats were obtained from Nigerian Institute for Trypanosomiasis Research (NITR), Vom. The rats were kept in laboratory cages, fed with commercially prepared feeds (Vital feed) and allowed to acclamatise for four weeks. Blood samples were then collected from the tail vein on a microscope slide and examined under the microscope to exclude the presence of trypanosomes. Also Trypanosoma brucei brucei infected laboratory rats were obtained from NITR, Vom which supplied Trypanosoma species for the study.

2.1. *Ganoderma applanatum* extraction

One kilogram of the powder of *G. applanatum* was dissolved in three litres of distilled water. The sample was boiled for three hours, stirring every thirty minutes. It was then allowed to stand for 24 hours and then filtered using whatmann number 1 paper. The filtrate was evaporated to dryness in hot air oven set at 45oC, the extract obtained was reconstituted using sterile distilled water to obtain concentrations 500mg/ml and further diluted to obtain 250mg/ml. [22]

2.2. Phytochemical Screening

The crude extracts of *G. applanatum* were subjected to the following biochemical and applied molecular biological tests as follow:

Test for Saponins- To 0.5g of the extract was added few mls of distilled water to cover the extract and the mixture was shaken thoroughly. Frothing which persisted on warming indicated the presence of saponin. [23]

Test for Alkaloids- To 0.5g of the *G. applanatum* extract, 5 ml of 1% aqueous Hcl and few drops of Draggendanff's reagent were added on a steam bath and then filtered. To 1 ml of the filtrate, a few drops of Wagner's reagent were added. The non-formation of precipitate indicated absence of alkaloids. [23]

Test for Tannins- To 0.5g of the *G. applanatum* extract, 10 ml of distilled water was added and stirred and filtered. About 2 mls of 5% ferric chloride were added to the filtrate. The expected deep green colouration, which indicates the presence of tannins did not appear, showing non-availability of tannins. [23]

Test for Anthraquinone- To 0.5g of the *G. applanatum* extract was added 5 ml of chloroform and shaken thoroughly for 5 minutes. This was filtered and the filtrate shaken with an equal volume of 100% ammonia solution. No pink violet or red colour in lower layer was seen, indicating the absence of anthraquinones. [23]

Test for Flavonoids- A 0.5g of *G. applanatum* extract was dissolved in 2 ml of dilute NaOH solution. A few drops of concentrated H2So4 were added. The solution became colourless, indicating the presence of flavonoids. [23]

Test for Cardiac glycosides- A 0.5g of the *G. applanatum* extract was dissolved in 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was underlayered with 1 ml of concentrated H2So4. A brown ring at the interface indicated the presence of cardiac glycosides. [23]

Test for Steroids- A 0.5g of the *G. applanatum* extract was dissolved in 2 ml of chloroform H2So4 was added carefully from the side of the tube to form a lower layer. A reddish brown colour at the interface indicated the presence of steroids. [23]

Microscopy-*Ganoderma applanatum* extracts in various concentrations were injected into laboratory rats infected with Trypanosoma brucei brucei; positive and negative control rats were also set up concurrently. Blood samples were collected on daily basis, stained with Giemsa's stain and examined using X100 oil immersion objective lens for presence of blood parasites to assess the effect of the drug on Trypanosomes and its potential benefits in the treatment of African Trypanosomiasis. [24]

3. Results

Phytochemical screening of *Ganoderma applanatum* showed that aqueous extracts of the mushroom contained saponins, flavonoids, cardiac glycosides and steroids but did not contain detectable levels of alkaloids, tannins and anthraquinone.

All the laboratory rats infected with Trypanosoma brucei brucei and treated with or without aqueous extracts of *Ganoderma applanatum* died by day 12 of the experiment. Rats uninfected but treated with the aqueous fungal extracts remained alive and healthy at the time of rounding up of the experiment.

4. Discussion

Analysis of the phytochemistry of *Ganoderma applanatum* showed that it contains saponins, flavonoids, cardiac glycosides and steroids but undetectable levels of alkaloids, tannins and anthraquinone based on the procedure used. Also the fungal extracts had no cidal nor static effect on Trypanosoma brucei brucei.The scope and spectrum of antimicrobial activity of each or all of the metabolites recovered from aqueous *Ganoderma species* is yet to be clearly defined at the moment. [25-27] These biochemical findings and patterns from the present study are well in line with findings from several other studies in China, Poland and south Africa. [28-30]

In the wake of present trials and experimentation in nanotechnology for treatment of various infections and infestations, and against the backdrop of targeted drug

delivery, these biochemical substances form important engineering materials for that purpose. [31-33] This would probably contribute to addressing the major challenge of present day wide-spread antimicrobial resistance. [34,35] The fact that aqueous extracts of *Ganoderma species* have already been found to have potent antibacterial, antiviral, antiparasitic and anticancer properties readily make it a central tool for nanotechnology research in order to enhance its potency and activity along those lines. [36-38]

Contrary to the widespread antimicrobial properties of *Ganoderma species* from other studies, the drug failed to have any significant activity against Trypanosoma brucei brucei in the present study. [39,40] Utilization of targeted drug delivery using materials from Ganoderma extracts may need to be tried against Trypanosomes to ascertain the true state of activity against these parasites; also much higher doses of the extracts may similarly need to be tried for probable changes in activity profile. [41] Since treatment and control of African trypanosomiasis is increasingly becoming difficult due to the high resistance of available antitrypanosomal drugs, preliminary trials of drugs as well as application of relevant technologies to throw up newer and more potent drugs formulations should be a continuous process. [42,43]

The detection of phenols in China, glycopeptides in USA, and sterols in South Korea from*Ganoderma species* contrary to the present findings may be attributed to the differences in screening procedures used in those studies such as diffraction and electrophoresis. [44-46] It also demonstrates the heterogeneity of the biochemical composition of the fungus and also points to the fact that the complete phytochemical composition of Ganoderma is probably more diverse than the present available literature and hence as well, its therapeutic potentials. [47]

In conclusion, the present study has also added credence to the fact that aqueous extracts of *Ganoderma applanatum* contains saponins, flavonoids, cardiac glycosides and steroids. In its present for form is of no use in the treatment of African trypanosomiasis. Application of nanotechnological engineering on this fungus' biochemical constituents in drug carriage and targeted drug delivery across compact membranes may be required to really ascertain its usefulness or otherwise in the management of sleeping sickness.

Support/Grants

This paper is a product of a thesis by the first author for the award of Fellow of Institute of Medical Laboratory Sciences (FIMLS) of Nigeria and was supported in kind and logistics by the Benue State University, Makurdi, Nigeria.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Cui D, Tian F, Coyer S, Wang J, Pan B, Gao F, He R, Zhang Y. Effects of antisense-myc-conjugated single-walled carbon nanotubes on HL-60 cells. *J NeurosciTechnol* 2007; **7**: 1639-1641.

[2] Pantarotto D, Partidos CD, Hoebeke J, Brown F, Kramer E, Briand JP, Muller S, Prato M, Bianco A. Immunization with peptide-functionalized carbon nanotubes enhances virus-specific neutralizing antibody responses. *Chemistry and Biology* 2003; **10**(10): 961-966.

[3] Bhaskar S, Tian F, Stoeger T, Kreyling W, De la Fuente JM, Grazu V, Borm P, Estrada G, Ntiziachristos V, Razansky D. Multifunctional nanocarriers for diagnostics, drug delivery and targeted treatment across blood-brain barrier perspectives on tracking and neuroimaging. *Part Fibre Toxicol* 2010; **7**: e3. PMCID: PMC 2847536.

[4] Gabor F. "Characterization of Nanoparticles Intended for Drug Delivery". Sci Pharm 2011; 79(3): S701-S702. doi: 10.3797/scipharm.br-11-01.

[5] Saraiva J, Marotta-Oliveira SS, Cicillini SA, Eloy JO, Marchetti JM. Nanocarriers for Nitric Oxide Delivery. *J Drug Deliv* 2011; 2011: 936438. doi: 10.1155/2011/936438.

[6] Gandapu U, Chaitanya RK, Kishore G, Reddy RC, Kondapi AK. Curcumin-Loaded Apotransferrin Nanoparticles Provide Efficient Cellular Uptake and EffectivelyInhibit HIV-1 Replication In Vitro. PLoSOne 2011; 6(8): e23388. doi: 10.1371/journal.pone.0023388.

[7] Gyenge EB, Darphin X, Wirth A, Pieles U, Walt H, Bredell M, Maake C. Uptake and fate of surface modified silica nanoparticles in head and neck squamous cell carcinoma. *J Nanobiotechnology* 2011; **9**: 32. doi: 10.1186/1477-3155-9-32.

[8] Zhao W, Lu X, Yuan Y, Liu C, Yang B, Hong H, Wang G, Zeng F. Effect of size and processing method on the cytotoxicity of realgar nanoparticles in cancer cell lines. *Int J Nanomedicine* 2011; **6**: 1569-1577. doi: 10.2147/IJN.S21373.

[9] Vazquez E, Corchero JL, Villaverde A. Post-production protein stability: trouble beyond the cell factory. *Microb Cell Fact* 2011; **10**: e60. doi: 10.1186/1475-2859-10-60.

[10] Jombo GTA, Damen JG, Amechi I, Etukudo NS, Dabit O. Intestinal parasitosis among undernourished children of an urban settlement in West Africa: pattern and types. Asian J Pharmaceutical & Health Sci 2011: In Press.

[11] Tran PA, Webster TJ. Selenium nanoparticles inhibit Staphylococcus aureus growth. Int J Nanomedicine 2011; 6: 1553-1558. doi: 10.2147/IJN.S21729.

[12] Fang Q, Kani K, Faca VM, Zhang W, Zhang Q, Jain A, Hanash S, Agus DB, McIntosh MW, Mallick P. Impact of Protein Stability, Cellular Localization, and Abundance on Proteomic Detection of Tumor-Derived Proteins in Plasma. *PLoS One.* 2011; **6(7)**: e23090. doi: 10.1371/journal.pone.0023090.

[13] Taylor E, Webster TJ. Reducing infections through nanotechnology and nanoparticles. *Int J Nanomedicine.* 2011; **6**: 1463-1473.doi: 10.2147/IJN.S22021.

[14] Hsu CK, Liao MH, Tai YT, Liu SH, Ou KL, Fang S W, Lee IJ, Chen RM. Nanoparticles prepared from the water extract of Gusuibu (Drynariafortunei J. Sm.) protects osteoblasts against insults and promotes cell maturation. *Int J Nanomedicine.* 2011; **6**: 1405-1413. doi: 10.2147/IJN.S20473.

[15] Vivero-Escoto JL, Huang YT. Inorganic-Organic Hybrid Nanomaterials for Therapeutic and Diagnostic Imaging Applications. I*nt J Mol Sci.* 2011; **12**(6): 3888-3927. doi: 10.3390/ijms12063888.

[16] Wasser SP. Current findings, future trends and unsolved problems in studies of medicinal mushrooms. *ApplMicrobiolBiotechnol.*

2011; **89**(5): 1323-1332.
[17] Chai S, To KKW, Lin G. Circumvention of multiply drug resistance of cancer cells by Chinese herbal medicines. *Clin Med.* 2010; **5**: e26. doi: 10. 1186/1749-8548-5-26.
[18] Lindequist U, Niedermeyer THJ, Julich WD. The pharmacological properties of mushrooms. *Evidence Based Complement Alternat Med*. 2005; **2**(3): 285-299.
[19] Cavallaro AS, Mahony D, Commins M, Mahony TJ, Mitter N. Endotoxin-free purification for the isolation of Bovine Viral Diarrhoea Virus E2 protein from insoluble inclusion body aggregates. *Microb Cell Fact*. 2011; **10**: e57. doi: 10.1186/1475-2859-10-57.
[20] Ai J, Biazar E, Jafarpour M, Montazeri M, Majdi A, Aminifard S, Zafari M, Akbari HR, Rad HG. Nanotoxicology and nanoparticle safety in biomedical designs. *Int J Nanomedicine*. 2011; **6**: 1117-1127. doi: 10.2147/IJN.S16603.
[21] Torreela E, Kaiser M, Brun R, Mazue G, Bray MA, Pecoul B. Fexinidazole- A new oral Nitronidazole drug candidate entering clinical development for the treatment of sleeping sickness. *PLoSNegl Trop Dis*. 2010; **4**(12): e923. doi: 10. 1371/journal. pntd.0000923.
[22] Rodrigues ML, Nimrichter L, Cordero RJB, Casadevall A. Fungal Polysaccharides: Biological Activity Beyond the Usual Structural Properties. *Front Microbiol*. 2011; **2**: e171. : 10.3389/ fmicb.2011.00171.
[23] Umar IA, Maryoms NG, Daikwo E, Gidado A, Buratai LB, Igbokwe IO, Ibrahim MA. The Effect of Aqueous Extracts of Hibiscus Sabdariffa (Sorrel) Calyces on Heamatological Profile and Organ Pathological Changes in TrypanasomaCongolense - Infected Rats. *Afr J Tradit Complement Altern Med*. 2009; **6**(4): 585-591.
[24] Anere JI, Fajinmi AO, Lawani FAG. An analysis of humam African trypanosomiasis (HAT) in Nigeria. *Science World J* 2006; **1**(1): e. http://www.scienceworldjournal.org/article/view/644/510.
[25] Mazzio EA, Soliman KF. In vitro screening for the tumoricidal properties of international medicinal herbs. *Phytother Res* 2009; **23**(3): 385-398.
[26] Fouche G, Cragg GM, Pillay P, Kolesnikova N, Maharay VJ, Senabe JJ. In vitro anticancer screening of South African plants. *Ethnopharmacol* 2008; **119**(3): 455-461.
[27] Cai Y, Luo Q, Sun M, Corke H. Antioxidant effect and phenolic compounds of the traditional Chinese medicinal plants associated with anticancer. *Life science* 2004; **74**(17): 2157-2184.
[28] Romesh CH, Patter MG. Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of western ghats of Karnataka, India. *Pharmacognosy Res* 2010; **2**(2): 107-112.
[29] Getha K, Hatsu M, Wong HJ, Lee SS. Submerged cultivation of Basidiomycete fungi associated with root diseases for production of valuable bioactive metabolites. *J Trop Forest Sci* 2009; **21**: 1-7.
[30] Chunchao H, Guo TY. A hypothesis: supplementation with mushroom-derived active compound modulates immunity and increases survival in response to Influenza virus (H1N1) infection. *Evid-Based Complement Alternat Med* 2011; **2011**: e252501. Doi: 10.1093/ecam/neq037.
[31] McDowell G, Slevin M, Krupinski J. Nanotechnology for the treatment of coronary in stent restenosis: a clinical perspective. *Vasc Cell* 2011; **3**: e8. Doi: 10.1186/2045-824X-3-8.
[32] Ghanberi H, de Mel A, Seifalian AM. Cardiovascular application of polyhedral oligomericsilsesquiovanenanomaterials: a glimpse into perspective horizon. *Int J Nanomedicine* 2011; **6**: 775-786.
[33] Kovochich M, Marsden MD, Zack JA. Activation of latent HIV using drug-loaded nanoparticles. *PLoS One* 2011; **6**(4): e18270. Doi: 10.1371/journalpone.0018270.
[34] Lejon V, Robays J, N'siesi FX, Mumba D, Hoogstoel A, Bisser S, Reiber H, Boelaert M, Buscher P. Treatment failure related to intrathecal immunoglobulin M (IgM) synthesis, cerebrospinal fluid IgM, and interleukin-10 in patients with haemolymphatic stage sleeping sickness. *Clin Vaccine Immunol* 2007; **14**(6): 732-737.
[35] Bisser S, N'Siesi FX, Lejon V, Preus PM, Nicuwenhove SV, Bilengue MMC, Buscher P. Equivalence trial of melarsoprol and nifurtimoxmonotherapy and combination therapy for the treatment of second-stage Trypanosoma brucei gambiense sleeping sickness. *J Infect Dis* 2007; **195**: 322-329.
[36] Ofodile LN, Uma UN, Kokubun T, Grayer RJ, Ogundipe OT, Simmonds MSJ. Antimicrobial activity of some *Ganoderma speciesfrom* Nigeria. *Pharmacol Pharmaceutical Med* 2005; **19**(4): 310-313.
[37] Jonathan GS, Awotona FE. Studies on antimicrobial potentials of three Ganoderma species collected from University of Ibadan (Nigeria) botanical gardens. *Afri J Biomed Res* 2010; **13**(2): e. http:// www.ajbrui.net/ojs/index.php/ajbr/article/view/72/0.
[38] Liandris E, Gazoule M, Andreadou M, Sechi LA, Rosu V, Ikonomopoulos J. Detection pathogenic Mycobacteria based on functionalized quantum dots coupled with immunomagnetic separation. *PLoS One* 2011; **6**(5): e20026. Doi: 10.1371/journal. pone.0020026.
[39] Bhasker S, Tian F, Stoeger T, Kreyling W, de la Fuente JM, Grazu V, Borm P, Estrada G, Ntziachristos V, Razansky D. Multifunctional nanocarriers for diagnostics, drug delivery and targeted treatment across blood-brain barrier: perspectives on tracking and neuroimaging. *Part Fibre Toxicol* 2010; 7: e3. PMID: 20199661. PMCID: PMC2847536.
[40] Danesh-Bahreini MA, Shokri J, Samiei A, Kamali-Sarvestani EK, Barzegar-Jalali M, Mohammadi-Samani S. Nanovaccine for leishmaniasis: preparation of chitosan nanoparticles containing Leishmaniasuperoxide dismutase and evaluation of its immunogenicity in BALB/c mice. *Int J Nanomedicine* 2011; **6**: 835-842.
[41] Tran PA, Webster TJ. Selenium nanoparticles inhibit Staphylococcus aureus growth. *Int J Nanomedicine* 2011; **6**: 1553-1558.
[42] Sokolova AY, Wyllie S, Patterson S, Oza SL, Read KD, Fairlamb AH. Cross-resistance of nitro-drugs and implications for treatment of human African trypanosomiasis. *Antimicrob Agents Chemother* 2010; **54**(7): 2893-2900.
[43] Likeufack ACL, Brun R, Fomena A, Truc P. Comparison of the in vitro drug sensitivity of Trypanosoma brucei gambiense strains from West and Central Africa isolated in the periods 1960-1995 and 1999-2004. *Acta Trop* 2006; **100**: 11-16.
[44] Wu Y, Wang D. A new class of natural glycopeptides with sugar moiety-dependent activities derived from Ganoderma lucidum fruiting bodies. *J Proteome Res* 2009; **8**(2): 436-442.
[45] Mazzio EA, Soliman KFA. In vitro screening of tumoricidal properties of international medicinal herba. Part II. *Phytother Res* 2010; **24**(12): 1813-1824.
[46] Kim MY, Seguin P, Ahn JK, Kim JJ, Chun S, Kim EH. Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Kores. *J Agric Food Chem* 2008; **56**: 7265-7270.
[47] Amori A, Vaidya JG, Deokule SS. In vitro evaluation of anti-staphylococcal activity of Ganoderma lucidum, Ganoderma praelonggum and Ganoderma resinaceumfrom Pune, India. *Afric J Microbiol Res* 2011; **5**(3): 328-333.