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## ***Ganoderma* research activities and development in Namibia: A review**

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### **Abstract**

Medicinal mushrooms have provided a natural source of bioactive compounds since ancient times. *Ganoderma* is such a mushroom, which has worldwide recognition as a medicinally important mushroom. In this review, the authors provide a detailed summary of indigenous *Ganoderma* research in Namibia, looking at the ethnomycology, biological activity, physicochemical properties, food quality and safety, as well as cultivation of this mushroom. In this paper, all available records of *Ganoderma* research in Namibia were retrieved from the University of Namibia Institutional Repository with the keyword “*Ganoderma*”. Ten theses and four peer-reviewed articles with a total of 10 authors and 12 subjects, including ethnomedicinal plants, domestication, active compounds, medicinal and AIDS were reviewed. The main objective of this review is to guide researchers on the direction for future research and product development of *Ganoderma* in Namibia. The literature review highlights the potential to establish research and development activities of indigenous Namibian *Ganoderma* species. The study identifies an important knowledge gap on *Ganoderma* research, such as the complete morphological and molecular description of species, regulation and standardization of metabolites and characterization of novel compounds identified in indigenous Namibian *Ganoderma*. The domestication and cultivation of medicinal and edible mushrooms provide an excellent opportunity to contribute to the United Nations Sustainable Development Goals of promoting good health and well-being (SDG 3), nutrition (SDG 2: Zero Hunger), and income generation (SDG 1: no poverty).

**Key words** – biologically active – ethnomycology – indigenous – mushroom product

### **Introduction**

*Ganoderma* P. Karst. is a genus of the Ganodermataceae, with *Ganoderma lucidum* (Curtis) P. Karst as the type species (Gilbertson & Ryvarden 1986, Paterson 2006, Dai et al. 2009, Ryvarden & Melo 2014). It is characterized by distinct laccate and non-laccate basidiocarp frequently branched at the apex and truncated double-walled basidiospores (Karsten 1881, Moncalvo & Ryvarden 1997). Modern pharmacological research is ongoing to validate the immunotherapeutic benefits of this fungus by demonstrating the antitumor, antihypertensive, antiviral and immuno-modulating activities (Wasser 2011, De Silva et al. 2012) and affirmed the safe use of nutraceutical applications (Weng & Yen 2010). An array of *Ganoderma* nutraceuticals in the form of capsules, teas, coffees and other dietary supplements and health foods (Chang & Buswell 1999, Lai et al. 2004). Apart from their medicinal value, *Ganoderma* also contains important phytopathogenic species (Adaskaveg et al. 1990, Old et al. 2000, Flood et al. 2001).

*Ganoderma lucidum* is a species complex with 13 species from China, Europe and North America (Cao et al. 2012, Li et al. 2014, Zhou et al. 2015). *G. lucidum* is a world famous medicinal mushroom that has prominence in Traditional Chinese Medicine (TCM) since immemorial time (Jin et al. 2016). The fungus symbolized happiness, good fortune and good health, as well as immortality (Pegler 2002).

*Ganoderma* research in Africa is advancing, with twenty morphological species of *Ganoderma* reported being originated from southern Africa (Baxter & Eicker 1995). Application of the biological species concept and molecular tools has provided an appropriate means to resolve the taxonomical questions pertaining to *Ganoderma*, which has been in taxonomical chaos for many years (Adaskaveg & Gilbertson 1986, Ryvardeen 1995). The application of molecular tools has the advantage that species with similar basidiome morphology can be recognized and described (Smith & Sivasithamparam 2003, Cao et al. 2012, Kinge et al. 2012). The macroscopic features of indigenous *Ganoderma* having a woody, shiny red to brown or yellow/golden ear-shaped bracket in a wide range of sizes were consistent with the morphological description in the literature (Van der Westhuizen & Eicker 1994, Bhosle et al. 2010). Although a concerted effort has been made in many regions of the world to elucidate the taxonomy of *Ganoderma* species, little effort has been made to identify the *Ganoderma* species diversity in Africa (Ryvardeen & Johansens 1980, Douanla-Meli & Langer 2009, Kinge et al. 2012, Kinge & Mih 2014). *Ganoderma* has been described in South Africa (Coetzee et al. 2015), Ghana (Obodai et al. 2017) and Namibia (Kadhila-Muandingi 2010, Ekandjo 2012, Shikongo 2012).

In Namibia, the Zero Emissions Research Initiative (ZERI) at the University of Namibia hosts the NEPAD/SANBio Mushroom Node, with the mandate to promote research on edible and medicinal mushrooms, cultivation, and value addition of indigenous mushrooms. The Mushroom Node strives to create a better understanding of indigenous mushrooms with a potential for domestication and commercialization. They also conserve genetic resources of cultivated mushroom species for public distribution and research within southern Africa. Apart from the stated uses of indigenous *Ganoderma* in Namibia, it has not been exploited commercially (Hamwenye 2020).

To the best of the author's knowledge, all the available records of *Ganoderma* research in Namibia were retrieved for the writing of this review. This includes records on the University of Namibia Institutional Repository as well as materials that were not available on the repository at the time of writing. The objective of this review is to provide a comprehensive summary of the research work that was recorded for *Ganoderma* in Namibia. The second objective is to guide researchers on the direction for future research and product development of *Ganoderma*.

### **Host species of indigenous *Ganoderma* in Namibia**

Indigenous *Ganoderma* in Namibia was first reported by Van der Westhuizen and Eicker in 1994. *Ganoderma* has thick and woody basidiocarp, which are tough and hard to break (Van der Westhuizen & Eicker 1994). This fungus, which resembles elephant ears, is known locally as "omapakululu" in the Oshiwambo language or "omagege", a term used to describe all non-edible mushrooms (Kadhila-Muandingi 2010). To date, six *Ganoderma* species are confirmed to grow in Namibia, including *G. applanatum*, *G. enigmaticum*, *G. lucidum*, *G. neojaponicum*, *G. tsugae*, and *G. wiireonse* (Kadhila-Muandingi 2010, Hamwenye 2020). *Ganoderma lucidum* has been confirmed to grow in the north and north-eastern regions of Oshana, Ohangwena, Kavango and Caprivi (Kadhila-Muandingi 2010, Shikongo et al. 2013). In Southern Africa, fresh *Ganoderma* basidiocarps appeared in the summer and autumn seasons after rains (Van der Westhuizen & Eicker 1994, Shikongo 2012). Common *Ganoderma* host tree species have been summarized in Table 1. This fungus is not rare, as it could be found on dead trees, living or dead wood stumps and dead roots and tree barks buried underground.

### **Domestication**

Kamukwanyama (2009) reported the domestication of indigenous *Ganoderma* in Namibia.

The Kamukwanyama (2009) cultivation protocol for *Ganoderma* has been adopted by other researchers to improve yields and shorten the lengthy cropping cycle experienced during cultivation. In one such study, Ueitele et al. (2014a) used artificial wood logs prepared from hardwood sawdust and supplemented with pearl millet husks, which significantly shortened ( $p < 0.05$ ) the spawn run of the substrate. The vegetative phase recorded for this study was 40 days. In this study, pearl millet husk was considered a suitable supplement for sawdust substrate because it is soft and fine enough to absorb excess moisture and because of its fine hair-like structures to which the mycelia can attach and be propagated to the sawdust (Ueitele et al. 2014a). In a different study by Ueitele et al. (2014b), the experimental growing of *Ganoderma* on corn cobs produced a biological efficiency of 5.32%, which is extremely low compared to other studies. Similarly, according to Hamwenye (2020), the yield (0.762 g/kg) and biological efficiency (0.08%) obtained during cultivation of *Ganoderma* species in their study was very low compared to the yields (210.9–235.2 g/kg) and biological efficiencies (6.8–7.6%) reported by Roy et al. (2015).

**Table 1** Plant host species of indigenous *Ganoderma* in Namibia

Host tree species	Tree family	Common name	References
<i>Acacia erioloba</i>	Fabaceae	Camelthorn	Seymour (2006)
<i>A. sieberana</i>	Fabaceae	White thorn	Ajewole & Garba (2010)
<i>Baikiaea plurijuga</i>	Fabaceae	Zambezi teak	Nellis & Bussing (1990)
<i>Colophospermum mopane</i>	Fabaceae	Mopane	Kasanda & Kapenda (2015)
<i>Combretum collinum</i>	Combretaceae	Bushwillow	Setshogo & Venter (2003)
<i>Combretum frarans</i>	Combretaceae	Bushwillow	Setshogo & Venter (2003)
<i>Combretum zeyheri</i>	Combretaceae	Large-fruited bushwillow	Setshogo & Venter (2003)
<i>Croton gratissimus</i>	Euphorbiaceae	Lavendar croton	Setshogo & Venter (2003)
<i>Grewia retinervis</i>	Tiliaceae	Rough-leaved raisin bush	Setshogo & Venter (2003)
<i>Mundulea sericea</i>	Fabaceae	Cork-bush	Setshogo & Venter (2003)
<i>Pechuel-Loeschea leubuitziae</i>	Asteraceae	Wild sage/bitterbush	Tedder et al. (2012)
<i>Sclerocarya birrea</i>	Anacardiaceae	Marula	Kasanda & Kapenda (2015)
<i>S. caffra</i>	Anacardiaceae	Marula	Lewis (1987)
<i>Senegaria erioloba</i>	Fabaceae	-	
<i>Terminalia prunioides</i>	Combretaceae	Purple pod Terminalia	Setshogo & Venter (2003)
<i>Terminalia sericea</i>	Combretaceae	Silver cluster leaf	Setshogo & Venter (2003)
<i>Acacia</i> spp.			

Ueitele et al. (2014a) recommended that there is still a need to develop methods that can considerably reduce the cropping cycle and improve the yield of *Ganoderma lucidum* in Namibia. Another requirement is to analyse the nutrient composition of the substrates used for *Ganoderma* cultivation in Namibia. Low yields could be attributed to the inadequate nutrient composition of the substrates used (Bonatti et al. 2004). For this reason, different combinations and mixing ratios of agricultural wastes and supplements should be investigated in more detail to improve the fruiting of *G. lucidum* (Ueitele et al. 2014b). Further recommendations state that substrates such as corn cobs need to be supplemented before use as a substrate for *Ganoderma*. Ueitele et al. (2014b) have also identified the need for continuous effort to find suitable substrate combinations and supplements, which can increase *Ganoderma* yields from local agricultural material.

### Antimicrobial activity for health benefits

Traditionally, the local people at north-central and eastern Namibia use *Ganoderma* for a variety of health enhancement to treating specific ailments. These include strengthening the skull bone of infants, boosting the immune system of pregnant women and relieving nose bleeds (Ekandjo & Chimwamurombe 2012), stress relief, relaxant, treat colds and flu, treat wounds on children's heads as well as veterinary applications to treat various illnesses in cattle, goats and

chickens (Kadhila-Muandingi & Chimwamurombe 2012) and lastly, to treat skin and wound infections (Shikongo et al. 2013).

Ethnomycology investigations revealed that there are no standardized methods for the use of *Ganoderma* in Namibia. Mostly, preparations depend on the illness being treated; for example, in some cases, it was found that *Ganoderma* is first ground to a powder before use while in other cases *Ganoderma* tea is brewed by steeping the basidiocarp in boiled water for a couple of minutes until the water turns red from the mushroom extracts. In addition, the smoke of a burning *Ganoderma* or the ash obtained can be inhaled or mixed with tobacco for smoking (Ekandjo & Chimwamurombe 2012, Kadhila-Muandingi & Chimwamurombe 2012). The literature reviewed had no indication as to how long these treatments should last. Kadhila-Muandingi et al. (2013) illustrated that the *in-vitro* test by antiplasmodial properties of *G. lucidum* on *Plasmodium falciparum* 3D7, which grows in human red blood cells at 37°C. The anti-malarial activity of the *Ganoderma* was determined by the ability of the mushroom extract to reduce the number of parasites in the test cultures at 5, 10 and 50 µg/ml concentration (Kadhila-Muandingi et al. 2013). The activity was observed from the mushroom extract at all three concentrations, but only for the first 24 hours. The antimalarial activity was directly proportional to concentration, with the highest concentration of extract showing the highest reduction in parasitaemia. The IC<sub>50</sub> for the *G. lucidum* aqueous extract was 3.66.

Organic extracts prepared from indigenous *Ganoderma* showed biological activity similar to *G. lucidum* extracts tested elsewhere (Roberts 2004, Ofofodile et al. 2005, Kadhila-Muandingi et al. 2013, Shikongo et al. 2013). Shikongo (2012) reported that indigenous *Ganoderma* from Namibia may contain novel compounds which might be produced in response to the extreme environmental conditions in Namibia such as the harsh dry and hot conditions, different host species and many other factors. Mycochemical profiling of the indigenous *Ganoderma* showed compound classes of anthraquinones, anthrones, coumarins, arbutin drug compounds, cardiac glycoside drugs, alkaloids, heterocyclic nitrogen compounds, conjugated double bonds compounds, triterpenes, steroids (saponins and bitter principles) and sterols (cholesterol and esters), essential oil, bitter and pungent principles, saponins drugs, flavonoids, terpenes and essential oils (Shikongo 2012). These results have all been reported by previous writers (Eo et al. 1999, Boh et al. 2000, Kadhila-Muandingi et al. 2013). Researchers need to identify the exact types of compounds, complete purification of the components and structure elucidation using chromatographic and spectroscopic techniques (Shikongo 2012).

Hoases-Gorases & Goraseb (2013) conducted a study to determine the effects of *G. lucidum* as a potential health-promoting agent for people living with HIV/AIDS (PLWHA) in Namibia. The study also assessed the awareness of *Ganoderma* capsules by PLWHA as well as observing the effects of *Ganoderma* mushroom capsules on PLWHA, and finally, to make the recommendations for future expansions on the use of *Ganoderma* nutraceuticals in Namibia. For that study, *Ganoderma* capsules were obtained from Malaysia and were administered to PLWHA who were on antiretroviral (ARV) therapy as well as those who were not. It was observed that PLWHA gained an average of 4–18 kg in body mass after taking the *Ganoderma* capsules ( $n=30$ ). Other benefits recorded were regained strength, improved appetite and physical appearance, improved sleep and confidence. The participants did not report any side effects during the study (Hoases-Gorases & Goraseb 2013).

Although not providing direct evidence, the results obtained by Hoases-Gorases and Goraseb (2013) suggest that *Ganoderma* has an indirect effect on patients health by increasing their appetite, which in turn leads to health improved. Furthermore, the high protein content of *Ganoderma* might also be associated with the weight gain experienced by the participants. Despite the encouraging results, *Ganoderma* is under cultivated in Namibia, which causes a shortage of this powerful mushroom and points to a need for increased production in Namibia. Finally, the authors pointed to the need for a more detailed and experimental study design for future studies of this nature, where a control group is included for reliable results (Hoases-Gorases & Goraseb 2013).

## Food quality and safety of *Ganoderma*

Hainghumbi (2020) provides a baseline for safety standards by providing data on the microbiological and mycotoxin quality, safety and heavy metal content of wild-harvested indigenous mushrooms. Hamwenye (2020) assessed how the origin (wild or cultivated) of *Ganoderma* influenced the physicochemical properties, phenolics composition and *in vitro* antioxidant activity. This important study gives insight into the quality and purity of teas and tea infusions prepared from *Ganoderma* mushrooms (WHO 1998).

According to Hainghumbi (2020), wild *Ganoderma* mushrooms may need further processing before they are of suitable microbial quality and safe to consume. In their study, Hainghumbi (2020) reported that the total aerobic count and the total coliform counts were above the acceptable limits of <1 cfu/g set by the European Union Commission Recommendation (Directive 2004/24/EC). Mycotoxins were detected in the wild-harvested *Ganoderma* basidiocarps, with the most prevalent mycotoxins being Doxynivalenol, fumonisin B1, ochratoxin A, total aflatoxin and zearalenone (Hainghumbi 2020). Hainghumbi (2020) reported that they were not able to compare the microbial profiles of their *Ganoderma* since none were recorded in the literature that could be used for the comparison.

Furthermore, *Ganoderma* did not show susceptibility to yeast spoilage and may therefore have a better shelf life compared to other mushrooms (Hainghumbi 2020). *Ganoderma* mushrooms tested in this study had an acceptable limit for yeasts < log 3.7 cfu/g, which is in compliance with the European Union Commission Recommendation (Directive 2004/24/EC). A notable observation was that maximum mould count in *Ganoderma* differed significantly ( $p < 0.05$ ) between samples and ranged between 7.3 log cfu/g to 0 mould counts detected. Based on the European Union Commission Recommendation (Directive 2004/24/EC), the acceptable mould counts limit is <3.7 log cfu/g. These results by Hainghumbi (2020) suggest that further preservation may be required to reduce yeast spoilage and extend the shelf life of *Ganoderma* mushrooms.

The studies by Hamwenye (2020) and Hainghumbi (2020) demonstrate the importance of moisture in food quality, preservation and resistance to deterioration (Nielsen 2010). High moisture content in mushrooms can lead to mold formation. The results by Hamwenye (2020) showed that cultivated *Ganoderma* species had high moisture content (>10%) compared to the wild *Ganoderma* species (<10%). The moisture contents of Namibian wild and cultivated *Ganoderma* were comparable to 7.5% moisture contents (Ogbe et al. 2009), 8.10% (Abdalla et al. 2016), 10.78% and 11.47% (Slynko et al. 2017) reported elsewhere for wild *G. lucidum*. Ash contents of wild *Ganoderma* (1.91–5.32%) were within the range of ash contents (0.88–9.70%) reported elsewhere on wild *Ganoderma* species (Ogbe et al. 2009, Rawat et al. 2012, Takshak et al. 2014, Obodai et al. 2017). According to Hamwenye (2020), there was no significant difference ( $p > 0.05$ ) between the ash contents of wild *Ganoderma* versus cultivated *Ganoderma* and both were within the expected ranges (Hung & Nhi 2012, Wandati et al. 2013, Zhou et al. 2014).

Therefore, cultivated and wild species that presented a low water absorption index can be suitable for the formulation of nutraceuticals such as hot water extracts (infusions, tea). Infusions prepared from cultivated *Ganoderma* species had higher levels of total phenolics, condensed tannins and antioxidant activity except for total flavonoids than those prepared from wild *Ganoderma* species. Wild *Ganoderma* species still showed comparable levels to those reported in the literature, indicating that the wild species investigated in their study still had the potential for use as nutraceuticals and sources of medicinal compounds (Hamwenye 2020). The results show that all the infusions from wild and cultivated *Ganoderma* species had antioxidant activities higher than that of Quercetin (0.2 mg/ml) (Hamwenye 2020).

According to Hamwenye (2020), the wild *Ganoderma* basidiocarps used in their study appeared physically matured compared to the cultivated *Ganoderma*. This could explain the lower total phenolic contents of wild *Ganoderma* infusions compared to the cultivated *Ganoderma* infusions (Hamwenye 2020). This can be expected since the total phenolic content of a mushroom is influenced not only by its species and substrate but also by the maturity of the basidiocarp (Wandati et al. 2013).

## Discussion

This review highlights the potential to establish research and development activities of indigenous Namibian *Ganoderma* species which may encourage utilization of local *Ganoderma* species for medicinal purposes and commercialization as well as to lay a foundation for further research on Namibian *Ganoderma*.

Six species of *Ganoderma* have been identified in Namibia by sequencing the ITS region of basidiocarps that matched the general morphologic description of *Ganoderma* (Kadhila-Muandingi 2010, Hamwenye 2020). However, morphological descriptions of the different *Ganoderma* species are not recorded. It is preferable for the available gene sequences to be accompanied by detailed information about the site of collection and morphological descriptions of the specimen. Such information includes soil type, any host species in addition to species names in order to form the end product of any indigenous mushroom characterization (Nilsson et al. 2011, Bucar et al. 2013). Furthermore, molecular sequences need to be submitted to international databases such as UNITE and the National Centre for Biotechnology Information (NCBI) for inclusion, comparison and conservation. This is necessary for the phylogenetic position of a sequence to be known and for contradicting or competing for species names to be avoided (Nilsson et al. 2011, Coetzee et al. 2015).

Preservation of investigated *Ganoderma* species and strains in the form of a thoroughly annotated voucher specimen residing in a public herbarium or culture collection is highly recommended (Nilsson et al. 2011). This is necessary to continue work on the same species and for the conservation of genetic resources. Conservation of genetic resources is important because the recollection of wild species may not always be possible. Furthermore, the natural habitat of wild mushrooms is constantly changing under anthropogenic and environmental pressure (David et al. 2015) and the chemical composition of mushrooms influenced by various factors, including seasons, host species and geographic locations (Thomas et al. 2004, Maclean & Wilson 2011, Atanasov et al. 2015).

The gap in policy and quality assurance of mushrooms and mushroom products in Namibia was evident in the studies reviewed (Hainghumbi 2020, Hamwenye 2020). The quality and chemical composition of mushroom preparations, and therefore their biological activity, are affected by a number of factors such as time of harvest, storage and processing conditions which can all lead to the degradation of compounds (Bucar et al. 2013, Singh et al. 2014). The need to establish standardization for the identity, quality, purity and chemical composition of mushroom samples to be used for medicinal purposes cannot be overemphasized (Singh et al. 2014). In addition, traditional methods of preparing mushroom extracts for medicinal use may involve mixed formulations that exert synergetic effects on the patient and make it difficult to identify active compounds (Atanasov et al. 2015).

Unsustainable harvesting techniques and anthropogenic practices such as clearing land for cultivation and settling may reduce *Ganoderma* mushroom populations as well as host species, which may pose a threat to the sustainability of *Ganoderma* mushrooms in Namibia (Vines 2004, Canter et al. 2005, Cordell 2011). Therefore, the domestication and cultivation of *Ganoderma* are necessary to meet the increasing demand in the Namibian market. Added motivation for cultivated *Ganoderma* species is that wild-harvested *Ganoderma* mushrooms showed high levels of mycotoxin contamination (Hainghumbi 2020). This can be expected from mushrooms growing in outdoors conditions, with dust, feeding insects and animals prone to transfer more contaminants (Venturini et al. 2011). The quality of *Ganoderma* and *Ganoderma* products can also be influenced by changes in season, variations in soil type, stage of fruiting body development and maturity, thus making it difficult to maintain and control its quality (Chang & Miles 2004). Unless cultivated, the age or maturity of mushrooms is unknown. Generally, it was found that cultivated *Ganoderma* infusions showed higher levels of total phenolics, condensed tannins and antioxidant activity compared to infusions from wild *Ganoderma* species (Hamwenye 2020). However, wild *Ganoderma* infusions still showed comparable levels to those reported in the literature, indicating that these wild species do have the potential for use as nutraceuticals and sources of medicinal

compounds (Hamwenye 2020). All of these can result in the variable quality of the *Ganoderma*. Therefore, the cultivation of wild *Ganoderma* can help sustain and maintain the quality of *Ganoderma* in terms of monitoring the growth parameters and minimize pathogenic bacteria, insects' infestations, soil, and dust contaminations (Hainghumbi 2020). It is recommended to apply suitable decontamination processes before ingestion or application of these mushrooms and their products in order to reduce their microbial load (Hainghumbi 2020).

Furthermore, the cultivation of medicinal and edible mushrooms is an excellent opportunity to promote health (Sustainable Development Goal (SDG) 3; Good health and Well-being), nutrition (SDG 2: Zero Hunger), and income generation (SDG 1: no poverty), which are important sustainable goals of the United Nations (United Nations 2015). Reducing the cropping cycle and improving yields of mushrooms by developing strains that are better suited to the Namibian climate is another research opportunity that was identified. It is equally important to study available and potential substrates and substrate combinations and supplementations to improve mushroom yields. China is an excellent example of a country that has developed its mushroom industry to lift its people out of poverty. *Ganoderma* contributed the U.S. \$ 1628 million to the world market in 1995 (Hapuarachchi et al. 2018). China, Korea and Japan are the most producer and suppliers of medicinal *Ganoderma* and *Ganoderma*-based products worldwide (Chang & Miles 2004). The annual production of *Ganoderma* in China was estimated at 36700 MT in 2002 and 49200 MT in 2003 (Hapuarachchi et al. 2018). Product development and value addition of mushrooms promise to be effective in addressing not only Namibia but also Africa's challenges with poverty, hunger and diseases due to their nutritional and medicinal properties.

Lastly, it was found that indigenous Namibian *Ganoderma* may contain novel compounds with the potential for biological applications, due to the unique and extreme environmental conditions in the country (Nilsson et al. 2011, Shikongo 2012). This presents a research opportunity to identify novel compounds, purification and structure elucidation for in vitro and in vivo studies for drug exploration and discovery.

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