IDENTIFICATION AND CULTIVATION OF *GANODERMA* MUSHROOM SPECIES IN NAMIBIA AND THE PHYSICOCHEMICAL PROPERTIES, PHENOLICS COMPOSITION AND *IN VITRO* ANTIOXIDANT ACTIVITY OF THEIR INFUSIONS

A THESIS SUBMITTED IN THE FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN AGRICULTURE (FOOD SCIENCE AND TECHNOLOGY)

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Abstract

Ganoderma is a fungus that grows in natural habitants from different parts of the world. It is a prized mushroom in developed nations especially in Asian countries due to its health promoting properties such as anticancer, antibacterial, anti-inflammatory, antioxidant, anti-diabetic. These health promoting effects are attributed to bioactive compounds such as the phenolics. However, in developing countries such as Namibia, *Ganoderma* is still an untapped and barely identified resource. The objectives of this study was to identify and cultivate wild *Ganoderma* species harvested from different host trees in Namibia and to determine their physicochemical properties (moisture, ash, water absorption and water solubility indices). The phenolics composition (total phenolics (TP), total flavonoids (TF), condensed tannins (CT)) and *in vitro* antioxidant activity (AA) of hot water infusions of the wild and cultivated mushrooms were also determined. Folin-Ciocalteu, Aluminium Chloride, Vanillin-HCl, DPPH assay methods were used to determine TP, TF, CT and AA, respectively. *Ganoderma* species were identified using DNA based on the sequences in the GenBank with 98-100% similarity. Two new *Ganoderma* species: *Ganoderma enigmaticum* and *Ganoderma wiireonse* were identified for the first time in Namibia. Wild species had 6.12- 11.70% moisture, 1.91- 5.32% ash, 11.55- 24.40 (g of absorbed water/g of dry sample) water absorption index, 3.60- 24.10% water solubility index, 18.37- 44.78 (mg GAE/g of sample) TP, 0.09- 1.67 (mg QE/g of sample) TF, 2.97- 6.37 (mg CAE/g of sample) CT and 40.8- 49.3% AA. Cultivated species had 9.64- 13.45% moisture, 2.34- 6.20% ash, 13.55- 28.30 water absorption index, 6.40- 25.35% water solubility index, 36.70- 52.73 (mg GAE/g of sample) TP, 0.41- 0.86 (mg QE/g of sample) TF, 11.38- 15.29 (mg CAE/g of sample) CT and 53.6- 63.7% AA. Infusions prepared from cultivated

Ganoderma species had higher levels of TP, CT and AA than those prepared from wild *Ganoderma* species suggesting that they can be potential nutraceuticals and source of healthful antioxidants. Further studies should be done on *in vitro* and *in vivo* medicinal effects of *Ganoderma* species in Namibia.

List of publication(s)/Conference(s) proceedings

The following article has been presented in conference proceedings based on the work carried out in this thesis.

Conference Paper

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List of Abbreviations and Acronyms

AA	Antioxidant Activity
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BLAST	Basic Local Algorithm Search Tool
CAE	Catechin Equivalent
CC	*Combretum collinum*
CM	*Colophospermum mopane*
CT	Condensed Tannins
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picryl-hydrazyl
EDTA	Ethylenediaminetetraacetic acid
GAE	Gallic Acid Equivalent
GE	*Ganoderma enigmaticum*
GL	*Ganoderma lucidum*
GW	*Ganoderma wiiroense*
HCl	Hydrochloric

IC	Inhibition concentration
ITS	Internal Transcribed Spacer
MS	*Mundelea sericea*
NaCl	Sodium Chloride
NCBI	National Center for Biotechnological Information
NCRST	National Commission on Research Science and Technology
NSFAF	Namibia Students Financial Assitance Fund
PA	*Pterocarpus angolensis*
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PL	*Pechuel-Loeschea leubuitziae*
QE	Quercetin Equivalent
SB	*Sclerocarya birrea*
SE	*Senegaria erioloba*
SPSS	Statistical Package for Social Sciences
TBE	Tris Borate EDTA
TFC	Total Flavonoids Content

TPC	Total Phenolic Content
UV	Ultra-violet
WAI	Water Absorption Index
WSI	Water Solubility Index
ZERI	Zero Emissions Research Initiative

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Dedication

This Thesis is dedicated to my heavenly father, God the almighty and my father Mr Gotpen Hailonga Hamwenye, my mother Mrs Ester Haulofu, my grandmother meekulu Susan Wakambata and my siblings. I love you and highly appreciate you all for I could not have done this without your love and support that kept me going even during my downfalls.

Declarations

I, Karlin Kundeinge Naimbodi Hamwenye, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

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Name of Student Signature Date

Chapter 1: Introduction

1.1. Background of the study

About 1.5 million species of mushrooms are estimated to exist in nature (Dharmaraj, Kuberan & Sivasankari, 2015). These are regarded as edible, medicinal, or poisonous (Chang & Miles, 2004). *Ganoderma* species are one of the mushrooms that are considered to be edible (Hapuarachchi, Wen, Deng, Kang & Hyde, 2015; Wachtel-Galor, Yuen, Buswell & Benzie, 2011) and medicinal (Kozarski, Klaus, Niksic, Vrvic, Todorovi, Jakovljevic & Griensven, 2012; Obodai, Mensah, Fernades, Kortei, Dzomeku, Teegarden, Schwartz, Barroros, Prempeh, Takli & Ferreira, 2017, Wachtel-Galor *et al*., 2011).

Ganoderma is a fungus that grows naturally in woodlands (Figure 1) and forests from different parts of the world such as USA, United Kingdom, Asian countries, Australia (Hapuarachchi *et al*., 2015) and in Africa (Obodai *et al*., 2017; Coetzee, Marincowitz, Muthelo & Wingfield, 2015; Kadhila-Muandingi, 2010). More than 300 species of *Ganoderma* have been described globally (Bhosle, Ranadive, Bapat, Garad, Deshpande & Vaidya, 2010). A few of the identified *Ganoderma* species include *G. applanatum*, *G. lucidum* (Bhosle *et al*., 2010; Kadhila-Muandingi, 2010), *G. australe*, *G. annulare*, *G. flexipes*, *G. chalceum*, *G. colossum*, *G. multiplicatum*, *G. curtisii*, , *G. resinaceum*, *G. lipsiense*, *G. philippi*, *G. tsugae*, *G. destructans* (Bhosle *et al*., 2010*)*, *G. neo-japonicum* (Kadhila-Muandingi, 2010), *G. lucidum* (Shikongo, 2012), *G. enigmaticum* (Coetzee *et al*., 2015), *G. wiiroense* (Otto, Blanchette, Barnes & Held, 2015) with *G. lucidum* being the most studied because it is considered a leader of medicinal mushrooms (Chang and Miles, 2004).

Figure 1: Wild *Ganoderma* fruiting body (marked with letter a) on a tree stump.

Although edible, *Ganoderma* is not included directly or consumed as a meal due to its thick, corky and tough fruiting body as well as a non-fleshy texture (Chang & Miles, 2004). It is processed into consumable dietary supplements such as capsules, tablets and nutraceuticals (coffee, tea and hot water extracts). Different parts of the mushroom such as mycelia, spores or fruiting bodies are used (Hapuarachchi *et al*., 2015; Wachtel-Galor *et al*., 2011; Yang, Chen, Leong, Zhao, Duan, Tang & Li, 2012). Furthermore, *Ganoderma* powder has been investigated for incorporation in emulsion type sausages (Ghobadi, Mohammadi, Chabavizade & Sami, 2018) and in rabbit meat (Trebušak, Levart, Salobir & Pirman, 2014).

Asian countries such as Japan, China and Korea are the major producers of *Ganoderma* and suppliers of *Ganoderma* based products (Chang & Miles, 2004; Hapuarachchi, Elkhateeb, Karunarathna, Cheng, Bandara, Kakumyan, Hyde, Daba & Wen, 2018). The production of *Ganoderma* in Japan was estimated at 500 metric tons (MT) in the year 1995 (Hapuarachchi *et al*., 2018). In China, total *Ganoderma* production was estimated at 3000 MT in 1997 and increased to 49200 MT in 2003 (Hapuarachchi *et al*., 2018). The U.S. market price of *Ganoderma* based products was estimated at U$ 1628.4 million in 1995 (Chang & Miles, 2004; Hapuarachchi *et al*., 2018).

Although *Ganoderma* is present in Namibia the knowledge of this indigenous mushroom is limited to traditional medicinal use at household level in rural areas, where it is used for instance for stress and cold/flu relief by sniffing its ash and treating wounds by applying ground *Ganoderma* on infected skin area (Kadhila-Muandingi & Chimwamurombe, 2012). Apart from the stated uses of indigenous *Ganoderma* in Namibia, it has not been exploited commercially.

The medicinal effect of *Ganoderma* is attributed to its biologically active compounds such as polysaccharides, triterpenoids (Xu & Beelman, 2015) and phenolic compounds (Kozarski *et al*., 2012). These bioactive compounds have been reported to have health promoting activities such as antibacterial (Dharmaraj *et al*., 2015; Fathima & Reenaa, 2016; Shah, Modi, Shukla & Lahiri, 2014), anticancer (Fathima & Reena, 2016), antitumor and anti-inflammatory (Joseph, Sabulal, George, Antony & Jonardhanan, 2011), antidiabetic (Ma, Hseih & Chen, 2015) and antioxidant (Kozarski *et al*., 2012).

Ganoderma has been widely studied especially in Asia. These studies range from taxonomy investigations (Singh, Dhingra & Shri, 2014a; Hapuarachchi *et al*., 2015), physicochemical parameters and chemical constituents (Singh *et al*., 2014a; Singh, Singh, Dhingra & Shri, 2014b) to nutritional composition (Sharif, Shahid, Mushtaq, Akram & Rashid, 2017; Sharif, Mustafa, Munir, Weaver, Jami & Shahid, 2016 ; Hung & Nhi, 2012; Salamat, Shahid & Najeeb, 2017; Roy, Azad, Sultana, Anisuzzaman & Khondkar, 2015a; Abdalla, Ahmed, Abdalla, Abdelmaboud, Khiery, Elriah & Saeed, 2016) particularly those of *G. lucidum*. They also include antioxidant activity (Sharif *et al*., 2017; Hung & Nhi, 2012; Rawat, Mohsin, Negi, Sah & Singh, 2013; Rajoriya, Tripathy & Gupta, 2015; Nithya, Ambikapathy & Panneerselvam, 2015; Cör, Botić, ŽKnez, Gregori, & Pohleven, 2017 ; Kozarski *et al*., 2012), biochemical composition (Rawat *et al*., 2012; Takshak, Chaudhary, Sindhu & Singh, 2014; Sharif *et al*., 2016; Roy *et al*., 2015a; Abdalla *et al*., 2016; Salamat *et al*., 2017), antimicrobial activity (Dharmaraj *et al*., 2015; Fathima & Reena, 2016; Shah *et al*., 2014), polyphenolic contents (Rawat *et al*., 2013; Shah *et al*., 2014) and other bioactive compounds (Cör *et al*., 2017), anti-diabetic activity (Ma *et al*., 2015), anti-cancer effects (Fathima & Reena, 2016), anti-tumor and anti-inflamatory effects (Joseph *et al*., 2011) of *G. lucidum*.

The mycochemical components (Nagaraj, Mallikarjun, Naika & Venugopal, 2013; Manasseh & Godwin, 2012), *in vitro* antimicrobial potential (Nagaraj *et al*., 2013), antioxidant activity (Rajoriya, Tripathy & Gupta, 2015; Kozarski *et al*., 2012) of *G. applanatum* have also been studied. Dharmaraj *et al*., (2015) and Rajoriya *et al*., (2015) evaluated antimicrobial and antioxidant activities of *G. tsugae*, respectively. Liew, Khong & Kutoi (2015) evaluated qualitative profile, antioxidant activity and antimicrobial activity of *G. australe*. Laungharn, Karunarathna, Khan, Xu, Mortimer & Hyde (2017) also evaluated the antimicrobial activity of *G. australe*. Tan, Kuppusamy, Phan, Tan, Raman, Anuar & Sabaratnam (2015) evaluated antioxidant activity of *G. neo-japonicum*.

In Namibia, studies on *Ganoderma* species are limited and emphasised on the distribution, genetic diversity and on the community views on how they use *Ganoderma* mushrooms (Kadhila-Muandingi, 2010; Ekandjo, 2012) as well as the mycochemical screening and antibacterial activities of wild *G. lucidum* (Shikongo, 2012).

The chemical composition of mushrooms can be species specific and the host tree on which it is growing can affect it (Wandati, Kenji & Onguso, 2013; Singh, Harsh & Gupta, 2015; Zhu & Tan, 2015). In the north and north eastern parts of Namibia, wild *Ganoderma* species were observed on live and dead stumps of host tree species such as *Croton gratissimus*, *Colophospermum mopane*, *Sclerocarya caffra*, *Terminalia sericea*, *Mundulea sericea*, *Terminalia prunioides*, *Acacia erioloba* and *Combretum collinum* (Kadhila-Muandingi, 2010; Shikongo, 2012; Ekandjo, 2012).

1.2. Statement of the problem

There is limited scientific identification of *Ganoderma* species in Africa. Indigenous communities hardly differentiate between species possibly because they identify mushrooms primarily based on phenotypical features. However, *Ganoderma* species cannot be easily differentiated based on morphological characters alone due to variation in diverse geographical locations under dissimilar climatic conditions, cultivation and the natural genetic development (e.g. mutation, recombination) of specific species (Wachtel-Galor *et al*., 2011; Kim, Shim, Seo & Kim, 2002). Hapuarachchi *et al*., (2015) emphasised on the taxonomic confusion of *G. lucidum* species complex that was brought about through classification of *Ganoderma* species based on their morphology. Therefore, to avoid inconsistency in the naming of the *Ganoderma* species Hapuarachchi *et al*., (2015) suggested that molecular analyses such as informative DNA markers should be used. There is little information on the identity of the *Ganoderma* species found in Namibia. Three *Ganoderma* species namely *G. lucidum* (Kadhila-Muandingi, 2010; Shikongo, 2012), *G. applanatum* and *G. neo-japonicum* (Kadhila-Muandingi, 2010) have been identified in Namibia. Kadhila-Muandingi (2010) focused on the distribution, genetic diversity and on the use of *Ganoderma* mushrooms as narrated by indigenous users in Oshana and Ohangwena regions of northern Namibia.

The potential health benefits of wild *Ganoderma* species and their irregular distribution in the natural habitat promoted their commercial cultivation to sustain their increasing demand in different parts of the world, mostly in Asian countries (Wachtel-Galor *et al*., 2011; Zhu & Tan, 2015). For these reasons, it would be important to provide scientific knowledge on biochemical components and bioactivity of cultivated *Ganoderma* species.

This would allow for the comparison of the chemical composition between wild and cultivated *Ganoderma* species which is said to be influenced by geographic origin, the type of species and host tree or substrate on which the mushroom was growing (Wandati *et al*., 2013; Singh *et al*., 2015). *G. lucidum* has been cultivated by Ueitele, Chimwamurombe & Kadhila-Muandingi (2014) to optimise its cropping cycle in Namibia. Shikongo (2012) focused on the qualitative mycochemical analysis of the *Ganoderma* in Namibia. However, there is no quantitative data on the physico-chemical properties, quantitative phenolics composition and antioxidant activity of wild and cultivated indigenous *Ganoderma* mushrooms in Namibia. Proximate composition such as ash content, protein content, fiber content, fat content and total carbohydrates have not been investigated on both wild and cultivated *Ganoderma* species in Namibia. However, in this study only ash content was investigated on both wild and cultivated *Ganoderma* species. This was because the sample weight of most wild *Ganoderma* species and all cultivated species investigated in this study could not accommodated other above mentioned proximate composition parameters.

1.3. Objectives of the study

The overall objective of this study was to investigate the effect of cultivation on the physicochemical properties, phenolics composition, and *in vitro* antioxidant activity of infusions prepared from harvested wild and cultivated *Ganoderma* mushrooms. The specific objectives were:

1.3.1. To identify the harvested wild *Ganoderma* mushrooms.

1.3.2. To cultivate the harvested wild *Ganoderma* mushrooms.

1.3.3. To determine the physicochemical properties (moisture, ash, water absorption index, and water solubility index) of wild and cultivated *Ganoderma* mushrooms.

1.3.4. To determine the phenolics composition (total phenolics, total flavonoids and condensed tannins) of infusions prepared from harvested wild and cultivated *Ganoderma* mushrooms.

1.3.5. To determine the *in vitro* antioxidant activity of infusions prepared from harvested wild and cultivated *Ganoderma* mushrooms.

1.4. Hypotheses of the study

1.4.1. All collected wild *Ganoderma* mushrooms belong to the same species.

1.4.2. All collected wild *Ganoderma* species can be cultivated.

1.4.3. Wild and cultivated *Ganoderma* species have no significant difference in moisture content, ash content, water absorption index and water solubility index.

1.4.4. Infusions prepared from wild and cultivated *Ganoderma* species have no significant difference in total phenolics, total flavonoids and condensed tannins contents.

1.4.5. Infusions prepared from wild and cultivated *Ganoderma* species have no significant difference in *in vitro* antioxidant activities.

1.5. Significance of the study

Ganoderma mushrooms especially *G. lucidum* is highly prized and its worldwide sales have contributed U$1628.4 million to the U.S. market in 1995 (Hapuarachchi *et al*., 2018). Therefore, the information provided in this study on the physicochemical properties, phenolics composition and antioxidant activity of *Ganoderma* species could be used to create awareness on the health potential of these mushrooms as well as promote their consumption as a source of nutrients and nutraceuticals (Upadhaya, Raut & Koirala, 2017). This would in turn help create *Ganoderma* market through selling of *Ganoderma* and it's by products. Moreover, cultivation of *Ganoderma* species can help sustain and improve their availability since it is difficult to collect wild *Ganoderma* species due to their irregular distribution in nature. This study identified two new *Ganoderma* species (*G. enigmaticum* and *G. wiireonse*) for the first time in Namibia, hence providing awareness on the types of *Ganoderma* species that can be found in Namibia.

Furthermore, infusions prepared in this study provide insight on the alternative ways of preparing and consuming *Ganoderma* as a functional food and a nutraceutical. In addition, the knowledge on cultivation of *Ganoderma* mushrooms provided in this study could help sustain the existence of the irregularly distributed wild *Ganoderm*a species that are most likely to become extinct from overharvesting, if commercialised. This knowledge can be used as a basis to create awareness to community members on the potential of these mushrooms for cultivation, health benefits and income-generation (self-employment). The manufacturing of value-added *Ganoderma* products such as infusions can be promoted by the national ministries and agencies tasked with manufacturing, industrialisation and agro-processing for both local consumption and to earn foreign currency via export of these healthful products.

Chapter 2: Literature Review

2.1. *Ganoderma* species and their distribution

Ganoderma is a fungus that grows naturally in woodlands and forests from different parts of the world such as in USA, United Kingdom, Asia, Australia and Africa. More than 300 species of *Ganoderma* have been described globally (Bhosle *et al*., 2010). A few of the identified *Ganoderma* species include *G. applanatum*, *G. lucidum* (Bhosle *et al*., 2010; Kadhila-Muandingi, 2010), *G. australe*, *G. annulare*, *G. flexipes*, *G. chalceum*, *G. colossum*, *G. multiplicatum*, *G. curtisii*, , *G. resinaceum*, *G. lipsiense*, *G. philippi*, *G. tsugae*, *G. destructans* (Bhosle *et al*., 2010*)*, *G. neo-japonicum* (Kadhila-Muandingi, 2010), *G. lucidum* (Shikongo, 2012), *G. enigmaticum* (Coetzee *et al*., 2015), *G. wiiroense* (Otto *et al*., 2015).

2.1.1. Host species of wild *Ganoderma*

Wild *Ganoderma* has been reported growing on host tree species such as *Mangifera indica L*., *Ficus religiosa L*., *Cassia siamea Lam*., *Cassia fistula L*., *Albizia lebbek Benth*., *Terminelia bellerica Roxb*., *Acacia nilotica L*., *Dalbergia sissoo Roxb*., *Delonix regia Raf*., *Dalbergia sissoo Roxb* in India (Singh *et al*., 2015). Another host tree species on which wild *Ganoderma* species were found growing in India include *Tamarindus indica* and *Leucaena lastisiliqua* (Bhosle *et al*., 2010). In South Africa, wild *Ganoderma* species such as *G. destructans* and *G. enigmaticum* were reported growing on *Ceratonia silqua* and *Jacaranda mimosifolia* host trees (Coetzee *et al*., 2015).

Furthermore, wild *Ganoderma* species were also found growing on a *Delonix regia* tree in Ghana (Obodai *et al*., 2017). In the northern and north-eastern parts of Namibia wild

Ganoderma species were reported growing on stumps of trees such as *Croton gratissimus*, *Colophospermum mopane, Sclerocarya caffra*, *Terminalia sericea*, *Mundulea sericea*, *Terminalia prunioides*, *Acacia erioloba*, *Combretum collinum* as outlined in Table 1 (Kadhila-Muandingi, 2010; Shikongo, 2012; Ekandjo, 2012).

Table 1: The local names and scientific names of some *Ganoderma* tree hosting species found in north central and north-eastern parts of Namibia.

Local Name	Scientific Name	Region	Reference
Omupupa	*Baikiaea plurijuga*	Oshana, Ohangwena, Zambezi, Kavango	Kadhila-muandingi, 2010; Ekandjo, 2012
Omusaati	*Colophospermum mopane*	Oshana, Ohangwena, Zambezi, Kavango	Kadhila-muandingi, 2010; Shikongo, 2012; Ekandjo, 2012
Omugolo	*Terminalia sericea*	Oshana, Ohangwena, Zambezi, Kavango,	Kadhila-muandingi, 2010; Shikongo, 2012;
Omugongo	*Sclerocarya birrea (caffra)*	Oshana, Ohangwena	Kadhila-muandingi, 2010; Shikongo, 2012
Omumbaganyana	*Mundulea sericea*	Zambezi, Kavango	Shikongo, 2012
Omuhama	*Terminalia prunioides*	Ohangwena, Zambezi, Kavango	Shikongo, 2012
Omupupwaheke	*Combretum collinum*	Oshana, Ohangwena, Zambezi Kavango	Shikongo, 2012
Omumbango	*Croton gratissimus*	Oshana, Zambezi, Kavango	Shikongo, 2012
Unknown	*Grewia avellana*	Zambezi, Kavango	Ekandjo, 2012
Omwoonde/ Omuthiya	*Acacia erioloba*	Zambezi, Kavango	Ekandjo, 2012
Omukuku	*Combretum imberbe*	Zambezi, Kavango	Ekandjo, 2012

2.2. Taxonomy of *Ganoderma*

The genus *Ganoderma* describes a group of fungi with large fruiting bodies growing above ground on tree stumps. It is characterised into two different groups, the laccate (*G. lucidum* complex) and the non–laccate (*G. applanatum* complex) species, which denote the subgenera *Ganoderma* and *Elfvingia* respectively (Hapuarachchi *et al*., 2015). Laccate *Ganoderma* is characterised by a shiny pileus and non-laccate *Ganoderma* is characterised by a dull pileus surface. The hierarchy of *Ganoderma* classification is in the following descending order: kingdom fungi; phylum Basidiomycota; class Agaricomycetes; order polyporales; family Ganodomataceae and genus *Ganoderma* (Wachtel-Galor *et al*., 2011). *Ganoderma* is described as a fungus which is a multicellular eukaryotic heterotrophic organism. It is characterised as Basidiomycota because it consists of hyphae and reproduce sexually through the formation of basidia (club-shaped spore bearing structure). Agaricomycetes are earthbound fungi that have a symbiotic association with wood and mainly act as decomposers. They can, however, also be parasitic. The order polyporales refers to a group of fungi that have many pores underneath the fruiting body. The family Ganodomataceae characterise fungi with a double-walled basidiospores (Wachtel-Galor *et al*., 2011). The traditional taxonomy of *Ganoderma* is built on its morphological traits such as shape and colour of the fruit body, host specificity, and geographical origin that are used to classify individual members of the species (Wachtel-Galor *et al*., 2011; Hapuarachchi *et al*., 2015).

2.2.1. Identification of *Ganoderma* mushroom

2.2.1.1. Macro-morphological identification

Ganoderma mushrooms are characterised based on their basidiocarp which is a fruiting body (above ground) emerged from mycelium (underground) (Figure 2). The basidiocarp comprises of different features such as pileus (cap), context, tubes, pores, stipe (stem), hyphae, pileipellis, basidiospores (López-Peña, Samaniego-Rubiano, Morales-Estrada, Gutiérrez, Gaitán-Hernández & Esqueda, 2019). The basidiocarp is characterised according to its colour, length, width, shape, and presence of stipe (stipitate) or absence of stipe (sessile). The pileus is characterised according to its colour, texture and shape. The context is characterised according to its thickness, colour and whether it is fibrous or spongy. The tubes are characterised according to their length and colour. The pores are characterised by their size, colour and shape. The stipe is characterised according to length, width, colour and shape. The hypha is characterized according to its size, colour, wall and shape. The pileipellis is characterized according to its size, shape, colour and wall. Basidiospores are characterised according to size, shape, pillars and colour (López-Peña *et al*., 2019).

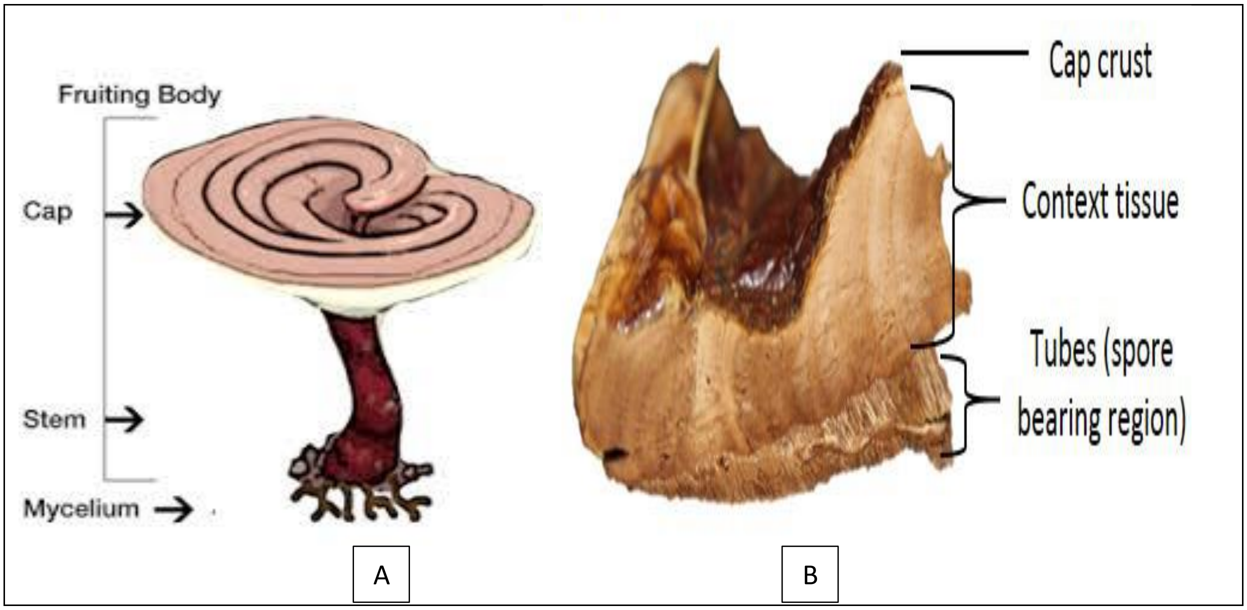

Figure 2: Structure of *Ganoderma*. A: Rough sketch of basidiocarp of *Ganoderma* mushroom. B: Inner parts of *Ganoderma* basidiocarp (Loyd, Smith, Richter, Blanchette & Smith, 2017).

The morphology of *Ganoderma* mushrooms varies within species depending on their geographic origins. Kim, Seo and Kim (2001) compared the morphological features of *G. lucidum* from Korea and Taiwan. Their study focused on basidiocarps hardness; context, tubes and pore colour; context and tubes thickness; pore size and shape of *G. lucidum*. *G. 0lucidum* from Korea had 15904 kg/cm^2 (pore) and 10617 kg/cm^2 (context) hardness of basidiocarp; 5.9 mm thick and whitish orange to brownish orange context; 6.5 mm thick and light brown tubes; 6-7 No/mm (number of pores per mm) and circular pores. *G. lucidum* from Taiwan had 7695 kg/cm^2 (pore) and 4722 kg/cm^2 (context) hardness of basidiocarp; 4.8 mm thick and whitish orange context; 7.9 mm thick and brown tubes; 4-5 No/mm and angular to round pores (Kim *et al*., 2001).

Coetzee *et al*., (2015) characterised morphology of *G. enigmaticum* from South Africa. They described this fungi as having perennial and stipitate basidiocarp; pores size of 3-5 No/mm that are round, uneven and extended; soft, corky and dark brown context; dark brown tubes that are 0.5-1.5 mm long and ellipsoid basidiospores. Otto *et al*., (2015) characterised the morphology of *G. wiiroense* from Ghana. They labelled this fungal species to have an annual and sessile basidiocarp which has yellowish brown to reddish brown pileus when dry; pores size of 3-5 No/mm that are round, uneven and slightly extended; brown tubes that are 0.1-1 mm long and ellipsoid to cylindrical-ellipsoid basidiospores.

2.2.1.2. Molecular identification

The morphological features differ due to variances in cultivation in diverse geographical locations under dissimilar climatic conditions and the natural genetic development (e.g. mutation, recombination) of specific species (Wachtel-Galor *et al*., 2011). Due to inconsistency in macro-morphological identification, biochemical, genetic, and molecular methods have also been applied in *Ganoderma* species taxonomy. Molecular-based methodologies such as genomic DNA extraction have been implemented (Wachtel-Galor *et al*., 2011). Cetyltrimethylammonium bromide (CTAB)-based is one of the commonly used method to extract DNA in *Ganoderma* (Chong, Abdullah & Tong-Leong, 2013). The method involves the use of CTAB buffer to digest cell matrix of the isolate at a certain temperature (60–65°C) in a water bath or oven (Chong *et al*., 2013).

2.3. Physicochemical properties of *Ganoderma*

Foreign matter, colour, bulk density, particle size, moisture content, water absorption index (WAI), water solubility index (WSI), pH, viscosity and total soluble solids (TSS) are used to determine quality and purity of teas and tea infusions (World Health Organization [WHO], 1998). WAI is used to indicate how much of the solvent will be absorbed by the solute (Rweyemamu, Yusuph, & Mrema, 2015). WSI is used as an indicator of the total soluble solids. Moisture is an important factor in food quality, preservation, and resistance to deterioration (Nielsen, 2010). Wild *G. lucidum* was found to have moisture contents of 7.5% (Ogbe, Ditse, Echeonwu, Ajodoh, Atawodi & Abdu, 2009), 8.10% (Abdalla *et al*., 2016), and 10.78%-11.47% (Slynko, Blinov, Babenko, Mihailova, Bannikova, Shekhovtsov, Nechiporenko, Goryachkovskaya, Veprev, & Peltek, (2017) on dry weight basis. Cultivated *G. lucidum* was reported to contain 10.61% moisture content (Upadhaya, Raut, & Koirala, 2017).

Singh *et al*., (2014a) evaluated the extractive values, absorption properties, foaming properties, dispersibility and bulk density of powders of two *Ganoderma* species (*G. lucidum* and *G. philippii*) collected from Uttarakhand, India to set standards for the quality and purity. The extractive values were found in the range of 3.80–4.17% in alcohol soluble extracts and 4.83–6.70% in water soluble extracts for the two *Ganoderma* species (Singh *et al*., 2014a). Singh *et al*., (2014b) reported that *G. applanatum* had 5.28% water soluble extractives and *G. brownii* had 8.35% water soluble extractives. The water absorption capacity of *G. lucidum* and *G. philippii* was found in the ranges of 11.67–12.33 mL/g (Singh *et al*., 2014a).

The foaming capacity was reported to range between 41.51–48.80% while the foaming stability ranged between 36.21–37.63%, for the two species of *Ganoderma* (Singh *et al*., 2014). The foaming ability is associated with the content of solubilized protein. Dispersibility of the two species was said to be ranging from 53.97–59.60% (Singh *et al*., 2014). The dispersibility of powder shows its ability to reform in water. Singh *et al*., (2014) also evaluated the bulk density *G. lucidum* and *G. philippi* and found that it was ranging between 0.457–0.498 g/L. Bulk density indicates the heaviness of the powder sample which determines the volume of the packaging material required (Singh *et al*., 2014).

2.4. Biochemical composition of *Ganoderma*

2.4.1. Proximate composition of *G. lucidum*

Proximate composition of a substance involves different classes of nutrients present in the sample such as protein, fat, carbohydrates, fiber and ash. Proteins are large molecules made up of amino acids that our body cells need to function well (Lykke & Padonou, 2019). A protein comprises of 20 amino acids and nine of these amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) are essential. Thus, the body can only obtain them through the diet (Lykke & Padonou, 2019). They play an important role in the body as they help cells maintain their structure, strengthen the immune system, metabolise body enzymes and regulate body functions by acting as chemical messengers to help cells, tissues and organs communicate (Lykke & Padonou, 2019). The daily protein intake recommended for both children and adults is 0.8-1.2 g/kg of body weight per day (Cardon-Thomas, Riviere, Tieges & Greig, 2017).

Several studies evaluated the proximate composition of wild and cultivated *Ganoderma* species specifically *G. lucidum*. Wild *G. lucidum* was reported to contain 15.04% protein (Sharif *et al*, 2016), 16.07% protein (Salamat *et al*., 2017), 17.92-21.97% protein (Takshak *et al*., 2014) and 17.55% protein (Usman, Kyari, Abdulrahman, Ogbe, Ahmad, Ibrahim & Sakuma, 2012). Cultivated *G. lucidum* was reported to contain 17.12-18.22% protein (Swati, Kumar, Bisht, Negi & Singh, 2016), 60.23% protein (Upadhyaya *et al*., 2017) and 13.3% protein (Hung & Nhi, 2012).

Moreover, fats are known to be a main source of energy and they provide essential energy for the brain and growing fetus (Lykke & Padonou, 2019). Fats also contain health boosting vital fatty acids that the body cannot produce by itself and they also improve the flavour and deliciousness of food (Lykke & Padonou, 2019). The daily recommended total fat intake for adults from 19 years of age and above is 29-70 g (15-30%) (Alasfoor, Rajab & Al-Rassasi, 2013). Wild *G. lucidum* was reported to contain 0.53% fat (Sharif *et al*, 2016), 0.64% fat (Salamat *et al*., 2017), 2.78-4.80% fat (Takshak *et al*., 2014) and 2.60% fat (Usman *et al*., 2012). Cultivated *G. lucidum* was reported to contain 3.20-3.48% fat (Swati *et al*., 2016) and 3.0% fat (Hung & Nhi, 2012).

Carbohydrates play a main role in the body as they provide the most energy and their daily recommended intake is 55-75% (Alasfoor *et al*., 2013). Wild *G. lucidum* was reported to contain total carbohydrates of 82.47% (Sharif *et al*, 2016; Salamat *et al*., 2017), 27.97-53.95% (Takshak *et al*., 2014) and 33.13% carbohydrates (Usman *et al*., 2012). Cultivated *G. lucidum* was reported to contain total carbohydrates of 38.04-40.04% (Upadhyaya *et al*., 2017) and 82.3% (Hung & Nhi, 2012).

Dietary fiber is important for the body as its deficiency leads to diseases such as constipation, diabetes, coronary heart diseases and obesity (Li & Komarek, 2017). Adequate intake of dietary fiber will prevent such diseases and it also helps lower blood lipid. The daily recommended intake of dietary fiber is 15 to 16 g per person (Li & Komarek, 2017). Wild *G. lucidum* was reported to contain 54.12% fiber (Sharif *et al*, 2016), 54.12% fiber (Salamat *et al*., 2017), 19.67-37.69% fiber (Takshak *et al*., 2014) and 30.25% fiber (Usman *et al*., 2012). Cultivated *G. lucidum* was reported to contain 30.55-32.41% fiber (Swati *et al*., 2016) and 14.21% fiber (Upadhyaya *et al*., 2017).

Ash content is obtained through burning of organic matters to give inorganic matters such as minerals. It indicates the amount of total mineral content in foods and it is also used as a parameter for nutritional labelling on food products (Nielsen, 2010). Wild *G. lucidum* was reported to contain 2.01% ash (Sharif *et al*, 2016), 2.10% ash (Salamat *et al*., 2017), 3.66-9.70% ash (Takshak *et al*., 2014) and 5.93% ash (Usman *et al*., 2012). Cultivated *G. lucidum* was reported to contain 3.27-3.85% ash (Swati *et al*., 2016), 1.4% ash (Hung & Nhi, 2012) and 6.3% ash (Roy *et al*., (2015a).

2.4.2. Mycochemical compounds

2.4.2.1. Classes of mycochemicals and their health benefits

Mycochemicals are biologically active chemicals produced by mushrooms. The term mycochemical is sometimes used in literature interchangeably with phytochemicals, which are biologically active chemicals produced by plants and they are secondary metabolites of plant (Saxena, Saxena, Nema, Singh & Gupta, 2013). These chemicals often include phenolic compounds, alkaloids, terpenoids and saponins. Phenolic compounds are the major class of phytochemicals and are comprised of three major groups namely the phenolic acids, flavonoids and polyphenols (Saxena *et al*., 2013). Another group of phenolics is called tannins, which are polymers of phenolics and are separated into two groups such as hydrolysable and condensed tannins (Saxena *et al*., 2013). Phenolics are not nutrients, however, they play major role on health by preventing chronic diseases such as cancer, cardiovascular and neurodegenerative diseases as well as inflammations, aging due to their pharmacological properties such as anti-carcinogenic, anti-mutagenic, antioxidant, antiviral and anti-inflammatory activities (Ozcan, Akpinar-Bayizit, Yilmaz-Ersan & Delikanli, 2014).

2.4.2.1.1. Phenolic acids

Phenolic acids are compounds with only one carboxylic acid functional group with seven carbon atoms and are divided into two groups namely the hydrocinnamic and hydroxybenzoic acids (Saxena *et al*., 2013) as depicted in Figure 3. Hydrocinnamic acids are formed as simple esters with glucose or hydro carboxylic acids (Saxena *et al*., 2013). Hydrocinnamic acids include cinnamic acid, ferulic acid, sinapic acid and caffeic acid while hydroxybenzoic acids include benzoic acid, salicylic acid, vailinilic acid and gallic acid.

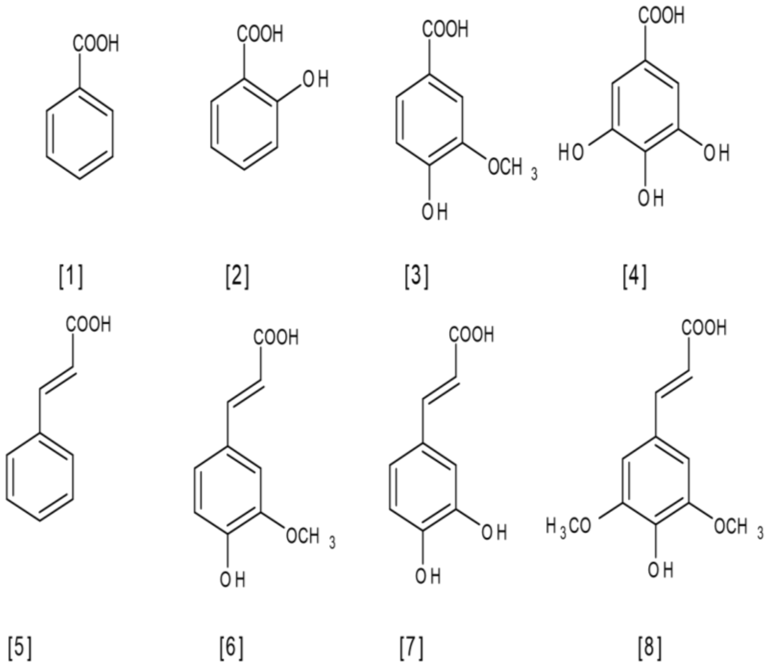

Figure 3: Structures of phenolic acids. Hyroxybenzoic acid are Benzoic acid (1), Salicylic acid (2), Vanilic acid (3), Gallic acid (4) and Hydroxycinnamic acid are Cinnamic acid (5), Ferulic acid (6), Sinapic acid (7) and Caffeic acid (8) (Saxena *et al*., 2013).

2.4.2.1.2. Flavonoids

Flavonoids are low molecular weight compounds that are made up of 15 carbon atoms (Saxena *et al*., 2013). They are separated into two groups namely: anthocyanins such as anthocyanidin and anthoxanthins that include flavones, flavans, flavonols, isoflavones (Ozcan *et al*., 2014). Some structures are depicted in Figure 4.

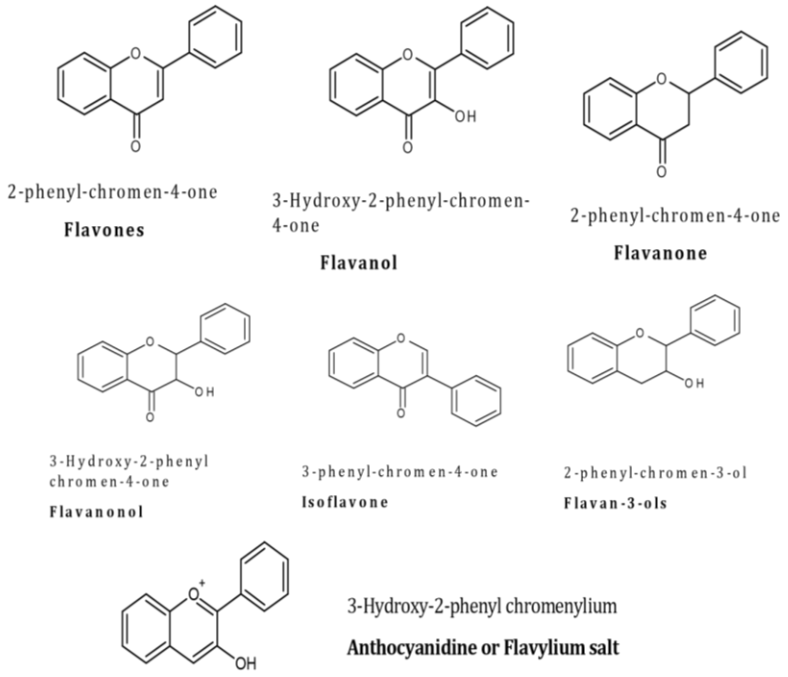

Figure 4: Structures of Flavonoids (Saxena *et al*., 2013)

2.4.2.1.3. Tannins

Tannins are compounds with high molecular weight and are divided into two groups namely: hydrolysable and condensed tannins (Saxena *et al*., 2013). Hydrolysable tannins are said to be esters of gallic acid such as gallo- and ellagi-tannins while condensed tannins are said to be polymers of polyhydroxyflavan-3-ol monomers (Ozcan *et al*., 2014; Saxena *et al*., 2013). The structures of hydrolysable and condensed tannins are demonstrated in Figure 5.

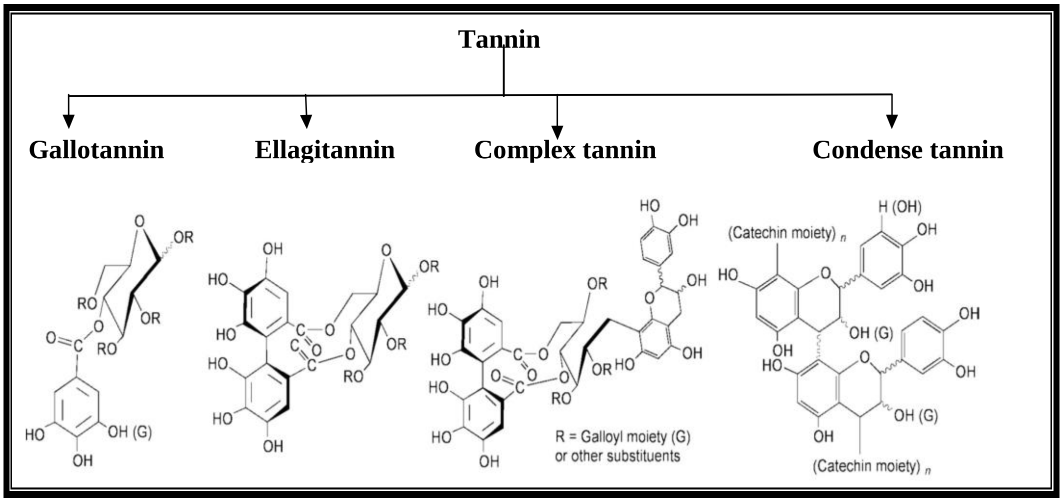

Figure 5: Structures of tannins (Saxena *et al*., 2013)

2.4.2.2. Phenolics composition of *Ganoderma*

Total phenolic content (TPC), total flavonoids content (TFC) and tannins in mushrooms depend on the type of species, growth type (wild or cultivated), extraction solvent and geographic origin (Wandati *et al*., 2013). Wild *G. lucidum*, wild *G. tsugae* and wild *G. applanatum* were reported to contain 9.00 mg GAE/g, 9.00 mg GAE/g and 11.60 mg GAE/g total phenolic content, respectively. The same species were reported to contain 0.63 mg QE/g, 0.84 mg QE/g and 0.62 mg QE/g total flavonoids, respectively as well as 2.29 mg CAE/g, 1.82 mg CAE/g and 2.43 mg CAE/g tannins, respectively (Rajoriya *et al*., 2015). Rajoriya *et al*., (2015) findings are comparable to those of Rawat *et al*., (2013) whose study reported that wild *G. lucidum* contain 8.44- 9.25 mg GAE/g total phenolic content and 1.84- 2.14 mg GAE/g total flavonoids, although the total flavonoids reported by Rawat *et al*., (2013) were a bit higher. Cultivated *G. lucidum* has been reported to contain TPC ranging between 21.06 mg GAE/g and 46.97 mg GAE/g (Cor *et al*., 2017) which is higher than that of wild one. The difference in levels TPC of the mushrooms is said to be influenced by extracting solvents used as well as by the extraction temperature used (Cor *et al*., 2017).

2.5. Edibility of *Ganoderma* and its uses

Palatable food (eaten as a meal) and edible food although both defines food quality and infer that food is fit for human consumption, the terms have distinctive meanings. Palatable focus on the taste and deliciousness of food and the food is rather eaten for enjoyment. On the other hand, edible focus on food safety and that the food is not poisonous and will do no harm on health when consumed (Hapuarachchi *et al*., 2015). *Ganoderma* mushrooms are therefore considered edible but not necessarily palatable because of their tough and non-fleshy texture (Chang & Miles, 2004). They have been regarded as edible mushrooms due to their medicinal uses in various forms (Arko, Marzuki & Kusmoro, 2017; Roy *et al*., 2015, Hapuarachchi *et al*., 2015).

To facilitate an easier consumption of *Ganoderma* mushrooms, they have been processed into various products such as tablets, capsules, tea, coffee and hot water extracts (Wachtel-Galor *et al*., 2011; Hapuarachchi *et al*., 2015). These mushrooms are also used in the formulations of other products such as soap, toothpaste and oil and are marketed as health promoting food and drug supplements (Hapuarachchi *et al*, 2015; Singh *et al*. 2013; Wachtel-Galor *et al*., 2011).

2.5.1. Processing of *Ganoderma* products as nutriceuticals and nutraceuticals

Nutriceuticals and nutraceuticals both offer similar health benefits however, they are presented differently. Nutriceuticals involves extraction of a specific compound or compounds (for example polysaccharides) from a food substance and are consumed in a form of capsules or tablets (Chang & Miles, 2004). On the other hand, nutraceuticals involves the consumption of the entire food portion as part of a normal diet. Encapsulated or tabulated *Ganoderma* nutriceuticals such as polysaccharides, triterpenes or phenolics are processed through extraction from fruiting bodies or mycelia grown through submerged liquid cultures and then dried via evaporation (Wachtel-Galor *et al*., 2011; Chang & Miles, 2004). In terms of *Ganoderma* mushrooms that are not eaten directly as a meal, their source of nutraceuticals would be hot water extraction of their entire fruiting bodies as tea infusion or ground fruiting bodies into powder and processing them into capsules or tablets as well as consuming food enriched with *Ganoderma* powders (Chang & Miles, 2004).

2.5.2. Hot water infusions

An infusion is a drink prepared by steeping herbs or mushrooms in boiled water for a specific amount of time (Herrera, Aguilera, Rebollo-Hernanz, Bravo, Benítez, Martínez-Sáez, Arribas, Castillo & Martín-Cabrejasa, 2018). An herb is any plant with leaves, seeds or flowers that can be used for flavouring, food, medicine or perfume. There are various kinds of infusions namely; herbal infusions, tea infusions and mushroom infusions as presented in table 2 (Pereira, Barros, Antonio, Verde, Buelga & Ferreira, 2016; Herrera *et al*., 2018; Paramasivam, Deepa, Selvi & Chandrasekaran, 2017; Hussein, Shedeed, Abdel-Kalek, & El-Din, 2011; Acharya, Ghosh & Biswas, 2018). Tea infusions have been prepared from tea plant (*Camellia sinensis*), Berry leaves, Carob and Doum (Paramasivam *et al*., 2018; Hussein *et al*., 2011). Herbal infusions have been prepared from Lemon balm, Olive tree, Rosemary and Thymus vulgaris (thyme) plant (Herrera *et al*., 2018; Pereira *et* *al*., 2016). Mushroom infusion has been prepared from *Lepista sordida* mushroom species through hot water extraction (Acharya *et al*., 2018).

Table 2: Different types of infusions and their preparation

Ingredients	Infusion type	Weight/volume infusion ratios	Brewing time	References
Thymus vulgaris powder	Herbal infusion	1 g of sample/ 200 mL of boiling water	5 minutes	Pereira *et al*., 2016
Camellia sinensis powdered leaves	Tea infusion	5 g of sample/ 150 mL of boiling water	5 minutes	Paramasivam *et al*., 2018
Lepista sordida fruiting body powder	Mushroom infusion	0.1 g of sample/ 20 mL of boiling water	5 minutes	Acharya *et al*., 2018
Lemon balm; Olive tree; Rosemary	Herbal infusions	1 g of sample/ 50 mL of boiling water	5 minutes	Herrera *et al*., 2018
Berry leaves; Carob; Doum	Tea infusions/ Herbal infusion	15 g of sample/ 100 mL of boiling water	5 minutes	Hussein *et al*, 2011

2.6. Biologically active compounds of *Ganoderma*

Bioactive compounds are non-nutritional food substances that when consumed causes a reaction in living tissues and may have positive or negative health effects based on the dosage (Guaadaoui, A., Benaicha, S., Elmajdoub, N., Bellaoui, M., & Hamal, 2014). There are several biologically active compounds namely, polysaccharides, triterpenoids, lectins, statins and phenolic compounds. *Ganoderma* species have been reported to have biologically active compounds such as polysaccharides, triterpenoids (Xu & Beelman, 2015) and phenolic compounds (Kozarski *et al*., 2012; Rajoriya *et al*., 2015; Sharif *et al*., 2017; Cor *et al*., 2017; Tan *et al*., 2015).These bioactive compounds have been reported to have health promoting activities such antibacterial (Dharmaraj *et al*., 2015; Fathima & Reena, 2016; Shah *et al*., 2014, Shikongo, 2012), anticancer (Fathima & Reena, 2016), antitumor and anti-inflammatory (Joseph *et al*., 2011), antidiabetic (Ma *et al*., 2015) and antioxidant (Kozarski *et al*., 2012). Table 3 shows the bioactive compounds of different *Ganoderma* species as well as their bioactivities. *Ganoderma* polysaccharides act as antitumor through activation of different immune systems in the host. They are also know to induce apoptosis, inhibit cell proliferation and suppress cell migration of highly invasive human prostate cancer cells (Joseph *et al*., 2011).

Different *Ganoderma* species have common bioactive compounds and similar bioactivities. *G. lucidum* and *G. applanatum* both have antioxidant properties due to the presences of flavonoids (Joseph *et al*., 2011; Manasseh *et al*., 2012). *G. tsugae* and *G. microsporum* have immunomodulatory activities due to the presences of proteins such as Ling Zhi-8 known as imm (Sánchez, 2017). *G. lucidum* and *G. tsugae* both have anti-inflammatory activities due to the presence of triterpenoids compounds (Joseph *et al*., 2011; Chen, Hsieh, Chiang, & Lin, 2014). *G. lucidum* has antibacterial and anti-cancer activities due to the presence of polysaccharides (Fathima & Reenaa, 2016).

Table 3: Bioactive compounds of *Ganoderma* species and their bioactivities

Species	Bioactive compounds	Bioactivity	References
G. lucidum	Polysaccharides	Antibacterial Anti-cancer	Fathima & Reenaa, 2016
	Triterpenoids	Anti-tumor Anti-inflammatory	Joseph *et al*., 2011
	Flavonoids	Antioxidant	Uma *et al*., 2014
G. tsugae	Triterpenoids	Anti-allergic Anti-inflammation	Chen *et al*., 2014
	Protein (Ling Zhi-8)	Immunomodulatory	Sánchez, 2017
G. applanatum	Flavonoids	Antioxidant	Manasseh *et al*., 2012
G. microsporum	Protein (Ling Zhi-8)	Immunomodulatory	Sánchez, 2017

2.6.1. Medicinal uses and side effects of *Ganoderma*

Ganoderma species have been said to play a major role in the prevention and treatment of immunological diseases namely; hypertension, tumorigenesis, insomnia, migraine, as well as arthritis, bronchitis, asthma, anorexia, gastritis, hemorrhoids, hypercholesterolemia, nephritis, dysmenorrhea, constipation, lupus erythematosis, hepatitis, and cardiovascular complications (Wachtel-Galor *et al*., 2011, Russell &Paterson, 2006; Wang, Xi, Li, Wang & Yao, 2012). Besides the health benefits of *Ganoderma, G. lucidum* has been said to have some side effects (Wasser, 2005). Intake of 1.5–9 g per day when taking a powder extract of *G. lucidum* for the first time may result in sleepiness, thirst, rashes, bloating, frequent urination, abnormal sweating, and loose stools (Wasser, 2005). Furthermore, non-nutrients phenolic compounds such as tannins found in *Ganoderma* mushrooms are said to be non-reducing agents of essential minerals such as calcium and iron causing their deficiency in the body (Ashok & Upadhyaya, 2012).

2.6.2. Antioxidant activity of *Ganoderma*

Antioxidants are compounds that scavenge free radicals from the body cells to lower or prevent oxidation damage of the body cells (Ozcan *et al*., 2014). Oxidation is a chemical reaction that can produce free radicals, thus leading to chain reactions that may damage the cells of organisms. *Ganoderma* species have been reported to contain compounds that have antioxidant properties such as phenolics that inhibit the initiation stage and interrupt the propagation stage by capturing radicals (Raseta, Vrbaski, Boskovic, Popovic, Mimica-Dukic, & Karaman, 2017; Rajoriya *et al*., 2015; Liew *et al*., 2015; Tan *et al*., 2015).

Furthermore, three antioxidant assays namely; DPPH (1,1-diphenyl-2-picryl hydrazyl radical), ABTS (3-ethylbenzothiazoline-6-sulphonic acid) and FRAP (Ferric Antioxidant Reducing Power) have been commonly used to demonstrate the scavenging ability of *Ganoderma* species as presented in table 4 (Raseta *et al*., 2017; Rajoriya *et al*., 2015; Liew *et al*., 2015; Tan *et al*., 2015; Cor *et al*., 2017). Wild *Ganoderma* species have been reported to inhibit free radicals at percentages of 0.0003% to 95.51% (Raseta *et al*., 2017; Rajoriya *et al.*, 2015; Liew *et al*., 2015). Cultivated *Ganoderma* species have scavenging ability ranging from of 0.13% to 17. 29% (Tan *et al*., 2015; Cor *et al*., 2017).

Table 4: Antioxidant activities of different *Ganoderma* species

Assay	Scavenging ability	Equivalents	Extracting solvents	Species	References
ABTS	23.30 (mg TE/g d.w)	2.33-10.24%	H_2O	Wild *G. lucidum*	Raseta *et al.*, (2017)
DPPH	2.75- 3.64 (µg/mL)	0.0003-0.004%	H_2O	Wild *G. lucidum*	Raseta *et al.*, (2017)
FRAP	127.00- 696.38 (mg AAE/g d.w)	12.70-69.63%	H_2O	Wild *G. lucidum*	Raseta *et al.*, (2017)
DPPH	93.19%	931.9 (mg/ mL)	MeOH	Wild *G. lucidum*	Rajoriya *et al.*, (2015)
DPPH	95.51%	955.10 (mg/ mL)	MeOH	Wild *G. tsugae*	Rajoriya *et al.*, (2015)
DPPH	91.64%	916.40 (mg/ mL)	MeOH	Wild *G. applanatum*	Rajoriya *et al.*, (2015)
DPPH	89.50%	895.00 (mg/ mL)	EtOH	Wild *G. australe*	Liew *et al.*, (2015)
DPPH	17.12%	171.20 (mg/ mL)	H_2O	Cultivated *G. lucidum*	Cor *et al.*, (2015)
DPPH	15.68%	156.80 (mg/ mL)	MeOH	Cultivated *G. lucidum*	Cor *et al.*, (2015)

2.7. Cultivation of *Ganoderma*

Artificial cultivation of *G. lucidum* was practiced in Bangladesh to assess the best cultivation media for achieving high yield, biological efficiency, and growth (mycelia, primordial and fruiting body) rate of *G. lucidum* using different sawdusts (Roy, Jahan, Das, Munshi & Noor, 2015b). Roy *et al*., (2015b) demonstrated that the yield of *Ganoderma* is dependent on the type of sawdust and supplement used. In Namibia, cultivation of *G. lucidum* was carried out by Ueitele *et al*., (2014) with the aim of shortening the cropping cycle of this particular mushroom.

2.7.1. Stages of cultivation

There are four stages/phases involved in the cultivation of *Ganoderma* species namely, (1) Selection of a suitable mushroom for pure culture; (2) Development of spawn; (3) Substrate preparation and (4) Fruiting phase (Ueitele *et al*., 2014). Stage 1 basically involves inoculating a piece of *Ganoderma* mushroom onto Potato Dextrose Agar in petri dishes to obtain mycelia growth that will be used to inoculate the spawn in stage two. Stage 2 involves inoculating the wheat grain with mycelia grown in stage one and incubating it until mycelia overcrowd the grains to produce spawn (Ueitele *et al*., 2014). Stage 3 is the vegetative phase which involves inoculation of substrate (woodchips) placed in polyethylene plastic bags with spawn and incubating them to allow mycelia to fully overcrowd the bags. Stage 4 is the fruiting phase in which the substrate bags from the vegetative phase are transferred to the fruiting room and slashed open to allow fruiting bodies to emerge (Ueitele *et al*., 2014).

2.7.2. Growth parameters for *Ganoderma* species

2.7.2.1. Temperature

Temperature is one of the crucial factors needed for the mycelium growth of fungi. Woo-Sik, Yun-Ju, Doo-Hyun, So-Deuk, Young-Bok & Soon-ja (2009) investigated culture conditions for the mycelial growth of *G. applanatum* from Korea, cultivated on Potato Dextrose Agar (PDA) for 6 days. Their study reported that mycelia of *G. applanatum* grow very well at optimal temperature of 25–30°C. Another study done in Korea on wild strains in *G. lucidum* evaluated the culture conditions for mycelia growth isolated using PDA and incubated for 10 days at 25°C (Chandana, Ahmed, Hyun, Geon, Tae & U, 2008). Their study indicated that suitable growth temperatures for *G. lucidum* ranges from 25–35°C of which the best mycelia growth was achieved at 30°C, followed by 35°C and the optimum growth was obtained at 25°C.

2.7.2.2. Potential of Hydrogen (pH)

Different species of *Ganoderma* grow best at different pH levels. *Ganoderma* species have been reported to grow at pH ranges of 4.0–9.0 (Magday, Bungihan & Dulay, 2014; Luangharn *et al*., 2017; Jo, Cho, Cho, Park, Yoo & Seok, 2018). *G. lucidum* has been reported to grow best at the pH range of 6.0–7.5 with an optimal growth at pH 8.0 (Magday, Bungihan & Dulay, 2014). *G. australe* has been reported to grow best at pH level of 7.0–8.0 with an optimal growth at pH 5.0–6.0 (Luangharn *et al*., 2017). *G. applanatum* has been reported to have a maximum growth at the pH range of 6.0–8.0 and an optimum growth at pH levels 4.0, 5.0 and 9.0 (Jo *et al*., 2018).

2.7.2.3. Moisture and Humidity

Moisture and humidity are two of the important growth parameters for *Ganoderma* species. Kamr and Bhatt (2013) reported that *Ganoderma* has an optimum growth at moisture content of 60–70% and at relative humidity (RH) of 85–95%. Bernabé-González, Cayetano-Catarino, Bernabé-Villanueva, Romero-Flores, Ángel-Ríos and Pérez-Salgado (2015) reported that *Ganoderma* can also grow at moisture content of 69% and relative humidity of 70%.

2.7.3. Duration of cultivation cycle and yield for *Ganoderma* species

The duration of the cropping cycle of *Ganoderma* depends on the species, substrate, geographic origin and growth parameters (Kamra & Bhatt, 2013; Roy *et al*., 2015b). Kamra and Bhatt (2013) study on first attempt of an organic cultivation of red *G. lucidum* under subtropical habitat and its economics found that spawn run incubated at 30°C took 51 days to be completely covered by mycelia growth. They further found that vegetative phase took 67 days, primordial initiation took 35 days at 90–95% RH, and fruiting phase took 92 days. Kamra and Bhatt (2013) also reported that the total duration of the cropping cycle of *G. lucidum* was 224 days. The yield obtained by Kamra and Bhatt (2013) was 570 g/100 kg. Furthermore, artificial cultivation of *G. lucidum* was done using different sawdusts as substrates (Roy *et al*., 2015b). Roy *et al*., (2015b) study found that the primordial initiation of *G. lucidum* grown on *Swietenia mahagoni* took 33-35 days and the fruiting phase took 60-71 days before harvest. The primordial initiation of *G. lucidum* grown on *Dipterocarpur turbinatus* took 36-40 days and the fruiting phase took 66-90 days before harvest (Roy *et al*., 2015b). The yield obtained by Roy *et al*., (2015b) was

132.9 g/kg with 4.3% biological efficiency and 110.4 g/kg with 3.6% biological efficiency for *S. mahagoni* and *D. turbinatus*, respectively.

2.8. Methods used for phenolics composition analyses

2.8.1. Determination of total phenolic content

Folin-Ciocalteu is a method commonly used for determination of total phenolic content and was outlined by Singleton and Rossi (1995). This method involves the reaction of Folin-Ciocalteu reagent with sample extract and sodium carbonate to extract phenols from the sample. A dark blue colour is observed after the reaction and absorbance is read at 750 – 760 nm. The results are expressed as Gallic acid equivalent. This method has been used by several researchers to determine total phenolic in different mushrooms (Orhan & Ustun, 2011; Islam, Yu, Xu, 2016; Rajoriya *et al*., 2015; Rawat *et al*., 2013; Sharif *et al*., 2017; Cor *et al*., 2017). Individual phenolic compounds are determined using a High Performance Liquid Chromatograph (HPLC) method (Obdai *et al*., 2017)

2.8.1.1 Sample extraction

Different extracting solvents such as methanol (MeOH), ethanol (EtOH), ethyl acetate (EtAC), water, and n-hexane can be used for sample extraction prior to total phenolic content determination (Sharif *et al*., 2017). Commonly used extracting solvents are water, methanol and ethanol. Extracting solvents are said to have different polarity which influences their extracting yield (Cor *et al*., 2017). Extracting temperature is also said to have an influence on extracting yield and it varies with the solvent used (Cor *et al*., 2017).

2.8.2. Determination of total flavonoids

A colorimetric Aluminium Chloride ($AlCl_3$) method has been used by several researchers to determine total flavonoids in mushroom (Islam *et al.*, 2016; Rajoriya *et al.*, 2015; Rawat *et al.*, 2013; Sharif *et al.*, 2017). This method involves the reaction of aluminium chloride and potassium acetate with the sample extract at room temperature for 30 minutes. Some researchers used reagents such as sodium nitrate ($NaNO_2$) and sodium hydroxide (NaOH) in place of potassium acetate (Sharif *et al.*, 2017; Gil-Ramírez, Pavo-Caballero, Baeza, Baenas, Garcia-Viguera, Marín & Soler-Rivas, 2016). The absorbance is read at 415 – 510 nm and the results are expressed as quercetin or catechin equivalent.

2.8.3. Determination of condensed tannins

There are no outlined methods for determination of condensed tannins in mushrooms. A Vanillin-HCl method is used to determine condensed tannins in plants and plant based products (Price, Van Scoyoc & Butler, 1978). It involves the reaction of sample extract with vanillin reagent (4% HCl (v/v) in methanol and 0.5% (w/v) vanillin in methanol) in a water bath. The absorbance is read at 500 nm and catechin is used as a standard.

Chapter 3: Research

3.1. Research Design

The study involved three experiments (Figure 6). The first experiment included extracting DNA of collected wild *Ganoderma* species from their natural habitats in northern Namibia. The second experiment involved cultivating the collected wild *Ganoderma* species. The third experiment was to perform physicochemical, phenolics composition and *in vitro* antioxidant analyses on the wild and cultivated *Ganoderma* species' crushed fruiting bodies and hot water extracts (Infusions).

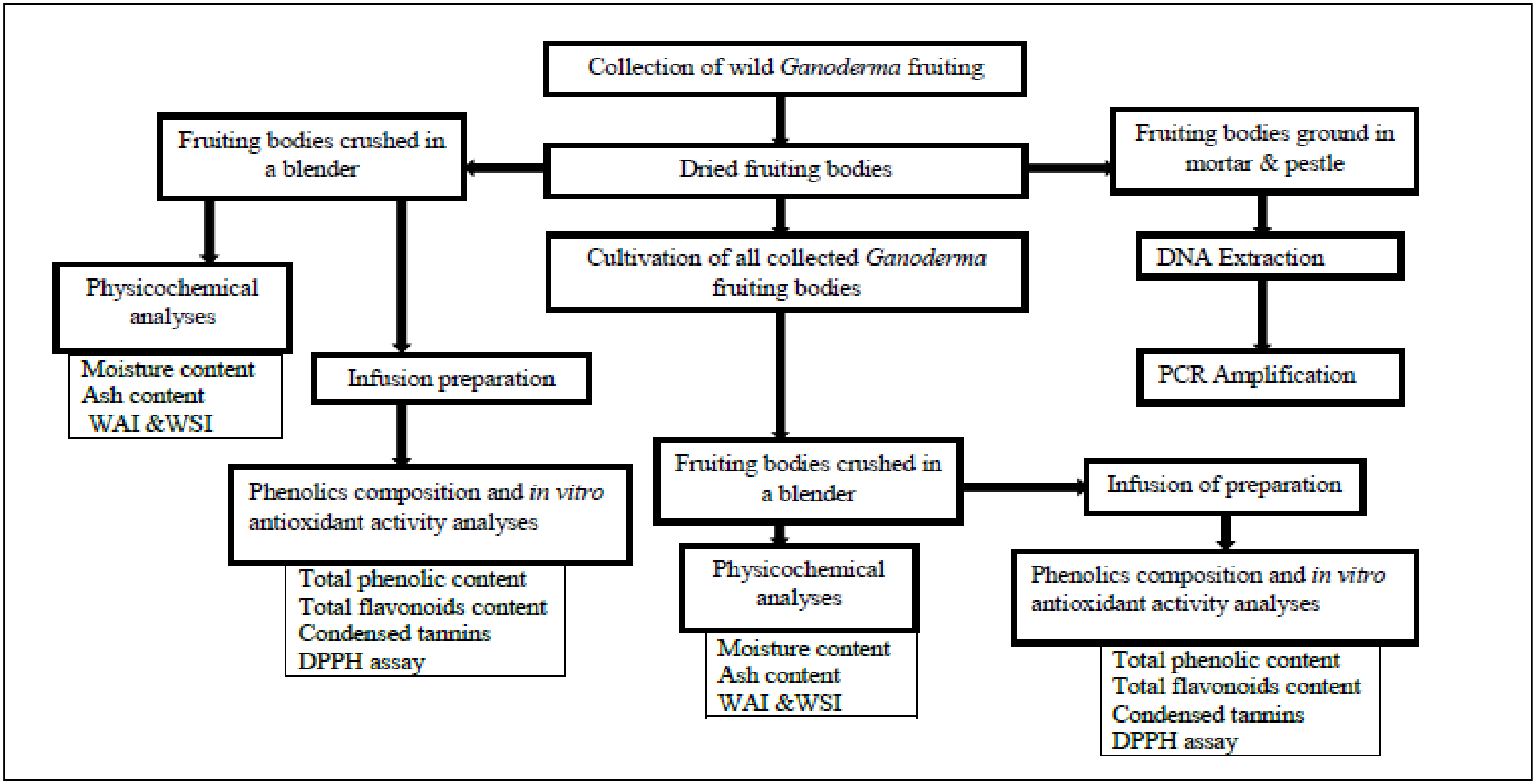

Figure 6: Experimental design for identification, cultivation and quantification of physicochemical properties, phenolics composition, antioxidant activities of wild and cultivated *Ganoderma* species. DNA: Deoxyribonucleic acid; PCR: Polymerase Chain Reaction; DPPH: 2,2-diphenyl-1-picryl-hydrazyl; WAI: Water Absorption Index; WSI: Water Solubility Index.

Identification, physicochemical properties, phenolics composition and *in vitro* antioxidant activity of wild and cultivated *Ganoderma* species in Namibia

Abstract

Ganoderma is one of the prized mushrooms in developed nations especially in Asian countries due to its health promoting properties such as anticancer, antibacterial, anti-inflammatory, antioxidant and anti-diabetic. These are attributed to bioactive compounds such as the phenolics. However, in developing countries such as Namibia, *Ganoderma* mushrooms are untapped and barely identified. This study identified *Ganoderma* species collected from different host trees in the wild, cultivated them on one substrate and determined their physicochemical properties. The total phenolics (TP), total flavonoids (TF), condensed tannins (CT) and *in vitro* antioxidant activity (AA) were determined in the hot water infusions made from the wild and cultivated *Ganoderma* mushrooms. Folin-Ciocalteu, Aluminium Chloride, Vanillin-HCl, DPPH assay methods were used to determine TP, TF, CT and AA, respectively. Two new *Ganoderma enigmaticum*, *Ganoderma wiireonse* species were identified for the first time in Namibia based on the DNA sequences in the GenBank with 98-100% similarity. Wild species had 6.12-11.70% moisture, 1.91-5.32% ash, 11.55-24.40 (g of absorbed water/g of dry sample) water absorption index, 3.60-24.10% water solubility index, 18.37-44.78 (mg GAE/g of sample) TP, 0.09-1.67 (mg QE/g of sample) TF, 2.97-6.37 (mg CAE/g of sample) CT and 40.8-49.3% AA. Cultivated species had 9.64-13.45% moisture, 2.34- 6.20% ash, 13.55-28.30 water absorption index, 6.40-25.35% water solubility index, 36.70-52.73 (mg GAE/g of sample) TP, 0.41-0.86 (mg QE/g of sample) TF, 11.38-15.29 (mg CAE/g of sample) CT and 53.6-63.7% AA. Infusions prepared from cultivated *Ganoderma* species had higher levels of TP, CT and AA except for TF than those prepared from wild *Ganoderma* species suggesting that they can be potential nutraceutical. Further studies should be done on *in vitro* and *in vivo* medicinal effects of *Ganoderma* species in Namibia.

Keywords: *Ganoderma*; wild; cultivated; infusions; phenolics; antioxidant activity

3.2. Introduction

Ganoderma species are edible (Hapuarachchi *et al*., 2015; Wachtel-Galor *et al*., 2011) and medicinal (Kozarski *et al*., 2012; Obodai *et al*., 2017, Wachtel-Galor *et al*., 2011) mushrooms. They are found in different parts of the world such as Asian and African countries, United States of America and the United Kingdom (Coetzee *et al*., 2015; Hapuarachchi *et al*., 2015; Kim *et al*., 2002). *Ganoderma* is commonly processed into consumable products such as the dietary supplements in the form of for example capsules, tablets, infusions such as coffee and tea (Hapuarachchi *et al*., 2015; Wachtel-Galor *et al*., 2011; Yang *et al*., 2012). These products have been trending mostly in Asian markets contributing a total of U$1628.4 million in 1995 (Hapuarachchi *et al*., 2018).

There are many species of *Ganoderma* discovered worldwide (Kozarski *et al*., 2012; Luangharn *et al*., 2017) that include *Ganoderma lucidum*, *Ganoderma applanatum*, *Ganoderma tsugae*, *Ganoderma neo-japonicum* and *Ganoderma australe.* Their taxonomy (Singh *et al*., 2014a; Hapuarachchi *et al*., 2015), physicochemical parameters and chemical constituents (Singh *et al*., 2014), nutritional composition (Hung & Nhi, 2012; Abdalla *et al*., 2016) have been investigated.

Furthermore, the medicinal effect of *Ganoderma* have been studied especially in *Ganoderma* mushrooms found in Asia and is attributed to its biologically active compounds such as polysaccharides, triterpenoids (Xu & Beelman, 2015) and polyphenolic contents (Kozarski *et al*., 2012). These bioactive compounds have been reported to have health promoting activities such as anti-bacterial (Dharmaraj *et al*., 2015; Fathima & Reena, 2016; Shah *et al*., 2014), anti-cancer (Fathima & Reena, 2016), antitumor and anti-inflammatory (Joseph *et al*., 2011), anti-diabetic (Ma *et al*., 2015) and anti-oxidant (Kozarski *et al*., 2012).

Most of the economic value and medicinal characterisation of *Ganoderma* species are realised in developed countries but not in developing nations. It is however known that *Ganoderma* mushrooms exist in developing countries such as Namibia but their identity and number have so far been barely studied. In Namibia, there are limited studies on *Ganoderma* species on the distribution, genetic diversity and uses of *Ganoderma* mushrooms (Kadhila-Muandingi, 2010; Ekandjo, 2012) as well as the mycochemical screening and anti-bacterial activities of wild *G. lucidum* (Shikongo, 2012). In the northern and north-eastern parts of Namibia, wild *Ganoderma* species live as saprophytes on dead stumps of and as parasites on host tree species such *Croton gratissimus, Colophospermum mopane, Sclerocarya caffra, Terminalia sericea, Mundulea sericea, Terminalia prunioides*, *Acacia erioloba* and *Combretum collinum* (Kadhila-Muandingi, 2010; Shikongo, 2012; Ekandjo, 2012). To contribute to the understanding of *Ganoderma* species in Africa, this study aimed to answer the following questions: What is the identity of *Ganoderma* collected from the wild and from different host trees in north-central Namibia? What are their phenolic composition and antioxidant activities? Can they be cultivated and how does the phenolic content fair between cultivated and wild collected *Ganoderma* species?

3.3. Materials and methods

3.3.1. Sample collection and preparation

Ganoderma fruiting bodies (23 from 6 different host trees species) were collected from three central northern regions in Namibia (Table 5). During collection *Ganoderma* fruiting bodies were identified using pictures of *Ganoderma* mushrooms taken by Kadhila-Muandingi (2010) and Shikongo (2012). The collection was done randomly from any host tree on which a fruiting body was seen (Figure 9 in Appendix 8.1). The host tree species were identified by their local names with the help and voluntary permission of the elderly owners of the plots where the mushrooms were collected. The fruiting bodies were transported to Windhoek a day after collection in khaki paper bags. The following day from the day of transportation the fruiting bodies were cleaned using a dry paper towel to remove foreign matters such soil, grass and dust. After cleaning they were sun dried for at least 8 hours and packaged in clean khaki paper bags which were stored at room temperature until analyses. Out of the 23 collected *Ganoderma* mushrooms 21 were successfully identified through DNA extraction and 15 of the identified species were subjected to physicochemical, mycochemical and Antioxidant analyses.

3.3.2. Sample identification

3.3.2.1. DNA extraction and PCR amplification

Cetyltrimethylammonium bromide (CTAB) extraction buffer (20 g w/v CTAB, 1 M Tris-HCl pH 8.0, 5 M NaCl, 0.5 M EDTA, 2.5 µL 2-mercaptoethanol, 0.02 g polyvinylpolyrrolidone) was used to obtain DNA from wild *Ganoderma* fruiting bodies following a Soltis laboratory CTAB DNA extraction protocol described by Doyle & Doyle (1987). To detect the presence of DNA, 8 µL of DNA solution was mixed with 2 µL loading dye and loaded into the gel in the electrophoresis immersed in TBE (Tris Borate EDTA) buffer (1% 1x TBE agarose gel) at 100V for 20 min, with 1 kb DNA ladder as guidance and viewed under ultra violet light.

The ITS (internal transcribed spacer) region for all isolates was amplified using ITS1 (forward) and ITS4 (reverse) primers. All PCR (Polymerase Chain Reaction) mixtures consisted of 25 μL One Taq Master Mix with standard buffer, 1 μL forward primer, 1 μL reverse primer, 4 μL template DNA, and 18 μL nuclease-free water. PCR cycles consisted of an initial denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 48°C for 1 minute 30 seconds, and extension at 72°C for 1 minute. The final extension was set to 72°C for 10 minutes to complete the reaction and stored at 4 °C. PCR products were visualized using Gel-Green dye under UV light after electrophoresis on agarose gel (1% w/v).

3.3.2.2. DNA sequencing and Analyses

The PCR products were sent to South Africa at Inqaba biotechnology laboratory for sequencing using internal transcribed spacer (ITS 1 and 4) primers. ITS sequences were compared with those in NCBI GenBank using the BLAST search tool (see Appendix 8.2). *Ganoderma* species were identified based on the sequences in the GenBank with 98-100% similarity.

3.3.3. Mushroom cultivation

Cultivation was done following the procedures outlined by Ueitele *et al*. (2014) with few modifications. Mushroom cultivation included pure culture preparation, spawn development, substrate inoculation and lastly the fruiting. In phase 1, pure cultures and sub-cultures were prepared by placing strains of wild fruiting bodies onto Potato Dextrose Agar (PDA) in petri dishes and incubating for 8 – 15 days at room temperature (± 25°C) until petri dishes were fully covered by mycelia (see Appendix 8.4.1). In phase 2, wheat grains were soaked overnight in water and packed in 250 ml plastic honey jars the following day. The prepared jars were autoclaved at 121°C for 15 minutes. After cooling the grains were inoculated with a sub-culture from each mushroom sample and incubated for 3 to 4 weeks at room temperature until the grains were fully colonized by mycelium (see Appendix 8.4.2). In phase 3, 15 kg of wood chips from Kiaat tree (*Pterocarpus angolensis*) was mixed with 3 kg of wheat bran and soaked in water for 10 minutes and were left to drain overnight. The wood chips were packed in autoclavable plastic bags and sterilized at 121 °C for 15 minutes. After sterilisation the wood chips were cooled and transferred into saturated polyethylene bags, followed by inoculation with 3 layers of spawn. The bags were left at room temperature for 4 to 5 weeks until they were completely colonised by mycelium (see Appendix 8.4.3). They were then transferred to the mushroom house which was water sprayed twice a day to keep it humid. In the fruiting phase, fully colonised bags were placed onto shelves in the mushroom house and they were slashed open with a blade to allow mushrooms to start sprouting (see Appendix 8.4.4).

3.3.4. Physicochemical Parameters

The moisture content of the ground fruiting bodies was determined by drying in the oven at 135°C for 2 hours following the Association of Official Analytical Chemists (AOAC, 2005). Ash content was determined by burning in the muffle furnace at 600°C for 2 hours following the AOAC (2005). Water Absorption Index (WAI) and Water Solubility Index (WSI) of the ground fruiting bodies were determined following the method described by

Rweyemamu, Yusuph & Mrema (2015) with modifications. WAI was determined by weighing 0.1 g of sample into a 15 mL centrifuge tube and 10 mL of distilled water was added. The tubes were vortex mixed for 30 minutes and centrifuged at 5000 rpm for 20 minutes. The supernatant was decanted and the weight of water absorbed after decantation was recorded. WAI was calculated according to the following equation:

$$WAI = \frac{\text{Weight of the absorbed water (g)}}{\text{Dry weight of sample (g)}}$$

WSI was determined by drying the supernatant of the sample obtained in analysis of WAI at 105 °C for 3 hours. WSI was calculated according to the following equation:

$$WSI = \frac{\text{Weight of dry solids in supernatant (g)}}{\text{Dry weight of sample (g)}} X\ 100$$

3.3.5. Phenolics composition and Antioxidant Activities

3.3.5.1. Preparation of infusions (hot water extracts)

Hot water infusions were prepared in duplicates from ground fruiting bodies by steeping 0.1 g of ground sample into 40 mL of boiled tap water for 5 minutes and filtered (11 µm, Whatman) following the methods described by Hussein *et al*., (2011) and Herrera *et al*., (2018) with few modifications. Hussein *et al*., (2011) used 15 g into 100 mL of boiled water and Herrera *et al*., (2018) used 1 g into 50 mL of boiled water to prepare their infusions for 5 minutes. After filtration the infusions were stored in the fridge at -4°C for two days prior to phenolics composition and *in vitro* antioxidant activities analyses.

3.3.5.2. Total Phenolic Content

Total phenolic contents were determined using the Folin-Ciocalteu method described by McDonald *et al*., (2001). Determinations were done in triplicates and absorbance was read at 760 nm using a spectrophotometer (MRC, Spectro UV-11). The total phenolic contents were expressed as gallic acid equivalents (GAE) on dry weight of the sample.

3.3.5.3. Total Flavonoids Content

Total flavonoid contents were determined using the aluminium chloride method described by Chang *et al*., (2002). Determinations were done in triplicates and absorbance was read at 415 nm using a spectrophotometer (MRC, Spectro UV-11). The total flavonoid contents were expressed as quercetin equivalents (QE) on dry weight of sample.

3.3.5.4. Condensed Tannins

Condensed tannins were determined using the Vanillin-HCl method described by Price, *et al*., (1978). Determinations were done in triplicates and absorbance was read at 500 nm using a spectrophotometer (MRC, Spectro UV-11). The condensed tannins were expressed as catechin equivalents (CAE) on dry weight of sample.

3.3.5.5. Antioxidant Activity

Antioxidant activity of infusions was done according to the method of McCune & Johns (2002). A mixture consisting of 1 mL of sample extract, 1 mL of 0.3 mM DPPH (2,2-diphenyl-1-picryl-hydrazyl) solution and 1 mL of methanol was incubated for 10 minutes in the dark. Determinations were done at 517 nm using a spectrophotometer. The radical scavenging activity was calculated as a percentage inhibition of DPPH discoloration according to the following equation:

$$\% \, inhibition = \frac{A_0 - As}{A_0} \text{ x } 100$$

Where, A_s is the absorbance of the sample extract or standard and A_0 is the absorbance of the negative control, which is the blank. Quercetin was used as the standard.

3.3.6. Statistical analysis

All determinations for physicochemical properties were done in duplicate. Determinations for phenolics compound and *in vitro* antioxidant activity were done in triplicate following two independent extractions. The results were reported as mean ± standard deviation. Statistical analyses were done using SPSS software version 21. One-way analysis of variance (ANOVA) was done for the comparison of mean values and means that differed significantly ($p<0.05$) were separated using Duncan's Post Hoc test.

3.4. Results and Discussion

3.4.1. Sample collection and identification

A total of 23 wild *Ganoderma* fruiting bodies were collected from host trees of different species and from different trees of the same host species (Table 5). The host tree species were locally identified as Omusati (*Colophospermum mopane*), Omumbanganyana (*Mundelea sericea*), Omupupwaheke (*Combretum collinum*), Oshidimba (*Pechuel-Loeschea leubuitziae*), Omwoongo (*Sclerocarya birrea*) and Omwoonde (*Senegaria erioloba*). Out of the 23 collected fruiting bodies 11 were collected from 11 different *Colophospermum mopane* host trees, 3 were collected from 3 different *Mundelea sericea* host trees, 6 were collected from 6 different *Combretum collinum* host trees and the remaining 3 were collected each from *Pechuel-Loeschea leubuitziae*, *Sclerocarya birrea* and *Senegaria erioloba* host tree species, respectively.

DNA extraction and PCR amplification was successful on 21 fruiting bodies out of the 23 collected fruiting bodies. Their DNA sequences after a comparison with the DNA sequences in the GenBank were found a match of 98 – 100% similarity (see table 8 in appendix) with three *Ganoderma* species namely: *G. enigmaticum*, *G. wiireonse* and *G. lucidum*. The remaining 2 were unidentified as no DNA could be detected. The number of identified *Ganoderma* species per host trees is given in Table 5. Out of the 21 identified *Ganoderma* species 16 were *G. enigmaticum*, 4 were *G. wiireonse* and the remaining species was identified as *G. lucidum*.

Table 5: Wild *Ganoderma* species collected from different host trees of different species and from different host trees of the same species.

Location (region)	**Scientific names of host tree species**	**Local names of host tree species**	**Number of collected samples from host tree species**	**Identity of collected *Ganoderma* samples and number of samples per species**	**Number of samples subjected to analyses**
Oshana	*Colophospermum mopane*	Omusati	11	*G. enigmaticum* (9) *G. lucidum* (1) *G. wiireonse* (1)	3 1 1
Oshikoto	*Mundelea sericea*	Omumbanganyana	3	*G. enigmaticum* (1) *G. wiireonse* (2)	1 2
Ohangwena	*Combretum collinum*	Omupupwaheke	6	*G. enigmaticum* (4)	4
Ohangwena	*Pechuel-Loeschea leubuitziae,*	Edimba	1	*G. enigmaticum* (1)	1
Ohangwena	*Sclerocarya birrea*	Omwoongo	1	*G. enigmaticum* (1)	1
Ohangwena	*Senegaria erioloba*	Omwoonde	1	*G. wiireonse* (1)	1
Total			23	21	15

3.4.2. Mushroom cultivation

C-PA-SBGE (cultivated *G. enigmaticum* collected from *Sclerocarya birrea*), C-PA-$MSGW_1$ (cultivated *G. wiireonse* collected from *Mundelea sericea*), C-PA-CMGW (cultivated *G. wiireonse* collected from *Colophospermum mopane*) and C-PA-CMGL (cultivated *G. lucidum* collected from *Colophospermum mopane*) were the only 4 species that yielded fruiting bodies out of 23 cultivated *Ganoderma* species. The low yield was due to contaminations experienced in the fruiting room. Contaminations were likely to be fungal or bacterial and this could be due to unclean surroundings and lack of fumigation in the fruiting room. Some species failed to yield fruiting bodies even though they were not contaminated which could be due to fluctuations in temperature and humidity since there was no thermometer to monitor the temperature in the fruiting room and watering was done manually. Figure 7 shows a fruiting body of the cultivated *Ganoderma* wiiroense (C-PA-$MSGW_1$).

The weight of the harvested fruiting bodies was record to be 3.65 g, 4.01 g, 5.72 g and 2.62g for samples C-PA-CMGL, C-PA-$MSGW_1$ and C-PA-SBGE, respectively. The yield (0.762 g/kg) and biological efficiency (0.08%) obtained during cultivation of *Ganoderma* species in this 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). This could be due to inadequate nutrients provided by the substrates for mushrooms to sprout as stated by Kadhila-Muandingi *et al*., (2012).

The duration of the cultivation cycle took about 272 days which was longer than that reported (224 days) by Kamra and Bhatt (2013). The difference might be due to more days

(60- 65) taken before primordial initiation (fruiting body emerging from substrate) in this study in comparison to the days (33-40) reported by Kamra and Bhatt (2013) and Roy *et al*., (2015). The delay in the primordial initiation could be due to fluctuations in temperature or humidity thus the optimal growth conditions were not met on time (Kadhila-Muandingi *et al*., 2012).

Figure 7: Cultivated fruiting bodies of *Ganoderma wiiroense* collected from *Mundelea sericea* host tree (C-PA-MSGW$_1$) with approximately 5 cm horizontal diameter.

3.4.3. Physicochemical Parameters

3.4.3.1. Moisture content

Moisture is an important factor in food quality, preservation and resistance to deterioration (Nielsen, 2010). High moisture content in mushrooms can lead to mold formation. The moisture contents of the wild and cultivated *Ganoderma* species ranged between 6.12% and 13.45% (Table 6). This was in the following significant ($p< 0.05$) order: C-PA-CMGL ≥ C-PA-SBGE ≥ C-PA-MSGW = W-SE-GW ≥ W-CM-GE3 ≥ C-PA-CMGW = W-CM-

GL = W-CM-GW = W-SB-GE = W-PL-GE = W-CC-GE4 = W-CC-GE3 = W-MS-GE > W-MS-GW2 = W-MS-GW1 = W-CC-GE2 = W-CC-GE1. All wild species had moisture contents <10%, except W-CM-GE3 and W-PL-GE. All the cultivated species had moisture contents >10% except C-PA-CMGW. Cultivated species had high moisture content than all wild species except for C-PA-CMGW. The differences in moisture content could be due to variation in environmental conditions such as temperature and humidity during the growing period (Roy *et al*., 2015). The moisture contents of these wild and cultivated species were comparable to the moisture contents such as 7.5% (Ogbe *et al*., 2009), 8.10% (Abdalla *et al*., 2016), 10.78% and 11.47% (Slynko *et al*., 2017) reported on wild *G. lucidum*.

3.4.3.2. Ash content

Ash content indicates the amount of total mineral content in foods and it is also used as a parameter for nutritional labelling on food products (Nielsen, 2010). The ash contents of wild and cultivated *Ganoderma* species ranged between 1.91% and 6.20% (Table 6). This was in the following significant ($p<0.05$) order: C-PA-CMGL > W-MS-GE ≥ C-PA-SBGE ≥W-CM-GE ≥ W-MS-GW2 = W-CM-GW ≥ W-CC-GE4 ≥ W-CC-GE2 = W-CM-GE2 ≥ W-CC-GE1 ≥ C-PA-CMGW = W-SE-GW = W-CM-GE3= W-CM-GE1 ≥ C-PA-MSGW = W-SB-GE = W-CC-GE3 ≥ W-MS-GW1 ≥ W-PL-GE. For wild species, the highest ash content was observed in W-MS-GE (5.32%) and the lowest in W-PL-GE (1.91%). The differences in their ash contents could be influenced by host trees on which they were growing. Host tree species influence the chemical composition of *Ganoderma* (Singh *et al*., 2015). Ash contents (1.91-5.32%) of wild species were within the range of ash contents (0.88-9.70%) reported on wild *G. lucidum* and other *Ganoderma* species (Obodai *et al*., 2017; Ogbe *et al*., 2009; Rawat *et al*., 2012; Takshak *et al*., 2014).

For cultivated species, the highest ash content was observed in C-PA-CMGL (6.20%) and the lowest in C-PA-MSGW$_1$ (2.34%). The differences in their ash contents could be influenced by the species type (Wandati *et al*., 2013). Ash contents (2.34–6.20%) of cultivated species were within the range of that (1.40–10.07%) reported on cultivated *G. lucidum* (Hung & Nhi, 2012; Zhou *et al*., 2014). Although the highest ash content was reported in a cultivated species (C-PA-CMGL) the second highest was reported in a wild species (W-MS-GE) and their ash contents were proven to be statistically insignificant ($p>0.05$). The other three cultivated species (C-PA-SBGE, C-PA-CMGW, C-PA-MSGW$_1$) also had insignificant ($p>0.05$) ash content levels with most of the wild species. This could indicate that both cultivated and wild species are potential source of inorganic matter such as minerals.

3.4.3.3. Water Absorption Index

Water absorption index shows how powder particles associates with water molecules and whether the powder can be used in water nutraceuticals and drug formulations (Singh *et al*., 2014a). The water absorption indices of wild and cultivated *Ganoderma* species ranged between 11.55 and 28.30 g of absorbed water/g dry sample (Table 6). This was in the following significant ($p<0.05$) order: W-CM-GE$_2$ = W-PL-GE ≤ W-CC-GE$_1$ W-SB-GE ≤ C-PA-MSGW$_1$ ≤ W-CC-GE$_3$ = C-PA-CMGL < W-CM-GE$_3$ < W-CM-GE$_1$ W-SE-GW ≤ W-MS-GE ≤ C-PA-CMGW ≤ W-CM-GW = W-CM-GL < C-PA-SBGE. For wild species the lowest water absorption index was observed in W-PL-GE (11.55 g of absorbed water/g of dry sample) and the highest was observed in W-CM-GE_1 (21.30 g of absorbed water/g of dry sample).

For cultivated species the lowest water absorption index was observed in C-PA-$MSGW_1$ (13.55 g of absorbed water/g of dry sample) and the highest in C-PA-SBGE (28.30 g of absorbed water/g of dry sample). The water absorption index of some species (W-CM-GE_2, W-CC-GE_1, W-PL-GE, W-SB-GE, C-PA-$MSGW_1$) were comparable to those reported by Singh *et al*., (2014a) and the rest of the species both cultivated and wild had higher water absorption indices. The differences could be due to variation in the amounts of water soluble constituents of the individual *Ganoderma* mushrooms (Singh *et al*., 2014a; Stojković *et al*., 2014).

Low water absorption index could indicate that the species have more hydrophilic (soluble sugars, organic acids, phenolic compounds) constituents and less alcohol soluble constituents which is the opposite for high water absorption index (Stojković *et al*., 2014). Therefore, cultivated (C-PA-$MSGW_1$, C-PA-CMGL) and wild (W-CM-GE_2, W-CM-GE_3, W-CC-GE_1, W-CC-GE_3, W-SB-GE) species that presented low water absorption index could be considered suitable for the formulation of nutraceuticals such as hot water extracts (infusions, tea).

3.4.3.4. Water Solubility Index

Water solubility index determines the amount of soluble polysaccharides released from sample granules when access water is added (Yousf, Nazir, Salim, Ahsan & Sirwa, 2017). Polysaccharides are one of the major bioactive compounds in *Ganoderma* reported to have health promoting properties such as anti-cancer, antibacterial (Fathima & Reenaa, 2016) and antitumor (Chang & Miles, 2004),

The water solubility indices of wild and cultivated *Ganoderma* species ranged between 3.60% and 25.35% (Table 6). This was in the following significant ($p<0.05$) order: C-PA-CMGL = W-SE-GW ≥ C-PA-MSGW = W-CM-GL ≥ W-CM-GE1 > C-PA-CMGW > W-CC-GE3 = C-PA-SBGE = W-CM-GW = W-PL-GE = W-CC-GE1 = W-MS-GE = W-CM-GE2 > W-SB-GE. For wild species the highest water solubility index was observed in W-SE-GW (24.10%) and the lowest in W-SB-GE (3.60%). For cultivated species the highest water solubility index was observed in C-PA-CMGL (25.35%) and the lowest was in C-PA-SBGE (6.40%). Some species (W-SB-GE, W-PL-GE, W-CMGW, W-CM-GE$_2$, and C-PA-SBGE) had water solubility indices comparable to those (5.35–6.70%) reported on wild *G. lucidum* and *G. brownie* (Singh *et al.*, 2014b). The rest of the species had higher water solubility index than that reported by Singh *et al.*, (2014b). Significantly ($p<0.05$) higher solubility indices were observed in both wild (W-SE-GW, W-CM-GE$_1$, W-CM-GE$_3$) and cultivated (C-PA-CMGL, C-PA-MSGW$_1$, C-PA-CMGW). This could mean that both wild and cultivated species have high amounts of water-soluble polysaccharides and phenolic compounds (Sigh *et al*., 2014b; Zhu *et al*., 2015).

Table 6: Physicochemical properties of ground fruiting bodies of wild and cultivated *Ganoderma* species

Sample code	Moisture (%)	Ash (%)	WAI	WSI (%)
W-CM-GE$_1$	7.56 ± 0.08^{e}	2.89 ± 0.21^{fghi}	21.30 ± 0.00^{cd}	19.10 ± 0.14^{b}
W-CM-GE$_2$	7.41 ± 0.54^{e}	3.30 ± 0.42^{efg}	12.55 ± 0.35^{g}	6.50 ± 0.42^{f}
W-CM-GE$_3$	9.56 ± 0.08^{cd}	2.51 ± 0.21^{ghi}	18.70 ± 0.92^{e}	16.60 ± 0.92^{c}
W-MS-GE	9.29 ± 0.64^{d}	5.32 ± 0.62^{ab}	20.75 ± 0.57^{bcd}	7.00 ± 0.50^{ef}
W-CC-GE$_1$	6.12 ± 0.18^{e}	3.19 ± 0.67^{efgh}	13.70 ± 0.92^{fg}	8.95 ± 1.48^{e}
W-CC-GE$_2$	6.18 ± 0.74^{e}	3.33 ± 0.28^{efg}	-	-
W-CC-GE$_3$	9.70 ± 0.79^{d}	2.40 ± 0.13^{ghi}	15.40 ± 1.84^{f}	11.90 ± 0.35^{d}
W-CC-GE$_4$	9.20 ± 0.31^{d}	3.62 ± 0.14^{def}	-	-
W-PL-GE	10.15 ± 0.01^{d}	1.91 ± 0.03^{i}	11.55 ± 0.85^{g}	6.40 ± 1.70^{f}
W-SB-GE	9.14 ± 0.32^{d}	2.48 ± 0.60^{ghi}	13.15 ± 0.28^{fg}	3.60 ± 0.57^{g}
W-CM-GW	9.07 ± 1.42^{d}	4.07 ± 0.43^{cde}	24.40 ± 0.14^{b}	6.20 ± 0.28^{f}
W-MS-GW$_1$	7.08 ± 0.87^{e}	2.19 ± 0.12^{hi}	-	-
W-MS-GW$_2$	6.56 ± 0.16^{e}	4.21 ± 0.03^{cde}	-	-
W-SE-GW	11.70 ± 0.46^{bc}	2.90 ± 0.34^{fghi}	19.55 ± 0.64^{cd}	24.10 ± 1.70^{a}
W-CM-GL	9.64 ± 0.10^{d}	4.50 ± 0.85^{bcd}	24.25 ± 0.35^{b}	19.20 ± 0.28^{b}
C-PA-SBGE	12.32 ± 0.28^{ab}	4.64 ± 0.01^{bc}	28.30 ± 0.57^{a}	6.40 ± 1.06^{f}
C-PA-CMGW	9.64 ± 0.04^{d}	2.83 ± 0.22^{fghi}	22.65 ± 1.48^{bc}	15.00 ± 2.33^{c}
C-PA-MSGW$_1$	11.75 ± 0.28^{bc}	2.34 ± 0.03^{ghi}	13.55 ± 1.98^{efg}	21.90 ± 0.85^{b}
C-PA-CMGL	13.45 ± 0.62^{a}	6.20 ± 1.66^{a}	15.40 ± 2.12^{f}	25.35 ± 1.91^{a}

WAI: Water Absorption Index (expressed as gram of water absorbed per gram of dry sample); WSI: Water Solubility Index; W: Wild; CM: *Colophospermum mopane*; GE: *Ganoderma enigmaticum*; MS: *Mundelea sericea*; CC: *Combretum collinum*; PL: *Pechuel-Loeschea leubuitziae*; SB: *Sclerocarya birrea*; GW: *Ganoderma wiireonse*; SE: *Senegaria erioloba*; GL: *Ganoderma lucidum*; C: Cultured; PA: *Pterocarpus angolensis*; 1-4 on a sample code: are same *Ganoderma* species collected from different host trees of the same species. Values are mean ± SD (n = 2). Means with different superscripted alphabets $^{(a,\ b,\ c,)}$ in the same column differ significantly ($p<0.05$). (-): not analysed.

3.4.4. Phenolics composition and Antioxidant Activities

3.4.4.1. Total Phenolic Content

The total phenolic contents of infusions prepared from wild and cultivated *Ganoderma* species ranged between 18.37 mg GAE/g of sample and 52.73 mg GAE/g of sample (Table 7). This was in the following significant ($p<0.05$) order: C-PA-CMGL > W-MS-GE ≥ C-PA-MSGW ≥ C-PA-SBGE > C-PA-CMGW > W-SE-GW > W-MS-GW2 > W-CC-GE4 ≥ W-CC-GE3 ≥ W-SB-GE = W-CC-GE2 ≥ W-MS-GW1 = W-CC-GE1 > W-CM-GL = W-CM-GE2 > W-CM-GW = W- PL GE = W-CM-GE3 = W-CM-GE1.

For wild species, infusion prepared from W-MS-GE had the highest total phenolic content (44.78 mg GAE/g of sample) and infusion prepared from W-CM-GW had the lowest total phenolic content (18.89 mg GAE/g of sample). For cultivated species, infusion prepared from C-PA-CMGL had the highest total phenolic content (52.73 mg GAE/g of sample) and infusion prepared from C-PA-CMGW had the lowest total phenolic content (36.70 mg GAE/g of sample). The total phenolic contents of infusions prepared from both wild and cultivated species were comparable to those (21.06-46.97 mg GAE/g and 11.55-77.10 mg GAE/g) reported by Cor *et al*., (2017) and Raseta *et al*., (2017), respectively. On the other hand, the findings of this study on the total phenolic contents were higher than those reported (8.44 - 11.60 mg GAE/g) by Rajoriya *et al*., (2015) and Rawat *et al*., (2013). However, they were lower than those (60.72-360.72 mg GAE/g) of Sharif *et al*., (2017). Higher levels of total phenolic content (360.72 mg GAE/g) reported on hot water extracts by Sharif *et al*. (2017) could be influenced by their longer extraction time (overnight) compared to 5 minutes extraction time used in this study.

Infusions prepared from cultivated species had significantly ($p<0.05$) higher levels of total phenolic content than all the infusions prepared from wild species, except for one prepared from W-MS-GE. The collected wild *Ganoderma* fruiting bodies used appeared to be matured compared to the cultivated fruiting bodies. This might explain why the infusions from wild species had lower total phenolic contents compared to those of cultivated species because the level of total phenolic content of a mushroom is influenced by the species, the substrate and by the maturity of the fruiting body (Wandati *et al*., 2013). Furthermore, low total phenolic contents could be a result of defense mechanisms due to aging (Wandati *et al*., 2013). The total phenolic content comprises of compounds such as phenolic acids, flavonoids and tannins and this compounds are known to have health promoting properties such as antioxidants (Kozarski *et al*., 2012), anticancer (Fathima & Reena), antidiabetic (Ma *et al*., 2015), anti-inflammatory (Joseph *et al*., 2011) and antimicrobial (Dharmaraj *et al*., 2015; Fathima & Reena, 2016; Shah *et al*., 2014).

3.4.4.2. Total Flavonoids Content

The total flavonoid contents of infusions prepared from wild and cultivated *Ganoderma* species ranged between 0.09 mg QE/g of sample and 1.67 mg QE/g of sample on dry weight (Table 7). This was in the following significant ($p<0.05$) order: W-MS-GE > W-CC-GE2 > C-PA-SBGE > W-MS-GW1 > C- PA-CMGL = C-PA-CMGW = W-CC-GE3 > C-PA-MSGW > W-SE-GW = W-PL-GE ≥ W-SB-GE ≥ W-MS-GW2 = W-CM-GE2 = W-CM-GE1 ≥ W-CC-GE4 = W-CC-GE1 = W-CM-GE3 > W-CM-GL = W-CM-GW. For wild species, infusion prepared from W-MS-GE had highest total flavonoids content (1.67 mg QE/g of sample) and infusion from W-CM-GL had the lowest total flavonoids content (0.09 mg QE/g of sample). For cultivated species, infusion prepared from C-PA-SBGE had the highest total flavonoids content (0.86 mg QE/g of sample) and infusion from C-PA-MSGW$_1$ had the lowest total flavonoids content (0.41 mg QE/g of sample).

Infusions prepared from W-MS-GE, W-CC-GE$_2$, W-CC-GE$_3$, W-MS-GW$_1$, C-PA-SBGE, C-PA-CMGW and C-PA-CMGL had total flavonoids contents comparable to those (0.62–2.14 mg QE/g) reported by Rajoriya *et al*., (2015) and Rawat *et al*., (2013), and all other infusions had lower levels of total flavonoids content than those reported by Rajoriya *et al*., (2015) and Rawat *et al*., (2013). Low levels of flavonoids could be influenced by their involvement in defense mechanisms due to aging of fruiting bodies which results in decreased contents during extraction as reported by Wandati *et al*., 2013 whose study found high levels of total flavonoid (1129.75 mg/100g) in young fruiting bodies compared to low levels (890.87 mg/100g) in mature fruiting bodies.

Although total flavonoids content was determined in mushrooms in this study and previous studies (Sharif *et al*., 2017; Rajoriya *et al*., (2015); Rawat *et al*., (2013); Islam *et al*., 2016), Gil-Ramirez *et al*., (2016) contends that mushrooms do not contain flavonoids because they lack main enzymes (chalcone synthase and chalcone isomerase) involved in their metabolic pathway. Apparently, what is determined by the aluminium chloride colorimetric method used for detection of flavonoids by most researchers are other phenolic compounds such as chlorogenic acid, o-diphenols, melanin-precursors or ergosterol, which are not flavonoids.

3.4.4.3. Condensed Tannins

The condensed tannins of infusions prepared from wild and cultivated *Ganoderma* species ranged between 2.97 mg CAE/g of sample and 15.29 mg CAE/g of sample on dry weight (Table 7). This was in the following significant ($p<0.05$) order: C-PA-CMGL = C-PA-CM-GW > C-PA-CMGE > C-PA-MSGW > W-SE-GW ≥ W-CC-GE4 ≥ W-SB-GE = W-MS-GE ≥ W-MS-GW1 = W-PL-GE ≥ W-MS-GW2 = W-CC-GE3 ≥ W-CM-GE3 ≥ W-CC-GE2 ≥ W-CM-GL ≥ W-CM-GE2 ≥ W-CC-GE1 ≥ W-CM-GW = W-CM-GE1. For wild species, infusion prepared from W-SE-GW had the highest levels of condensed tannins (6.37 mg CAE/g of sample) and infusion prepared W-CM-GW had the lowest levels of condensed tannins (2.97 mg CAE/g of sample). For cultivated species, infusion prepared from C-PA-CMGL had the highest levels of condensed tannins (15.29 mg CAE/g of sample) and infusion prepared from C-PA-MSGW$_1$ had the lowest levels of condensed tannins (11.38 mg CAE/g of sample). All infusions prepared from cultivated species had significantly ($p<0.05$) higher levels of condensed tannins than those prepared from wild species. Higher levels of condensed tannins in cultivated species could be influenced by the substrate (*Pterocarpus angolensis*) on which they were grown.

Condensed tannin contents of both wild and cultivated species in this study were higher than the condensed tannin contents (1.82–2.43 mg/g of sample) reported on wild *G. lucidum* (2.29 mg/g of sample), *G. applanatum* (2.43 mg/g of sample) and *G. tsugae* (1.82 mg/g of sample) by Rajoriya *et al*., (2015). This suggests that Namibian *Ganoderma* mushrooms are a potential source of condensed tannins.

3.4.4.4. Antioxidant Activity

The DPPH scavenging activities of infusions prepared from both wild and cultivated *Ganoderma* species ranged between 40.8% and 63.7% (Table 7). This was in the following significant ($p<0.05$) order: C-PA-MSGW > C-PA-SBGE > C-PA-CMGL > C-PA-CMGW > W-SE-GW = W-MS-GW2 >W-CM-GE2 ≥ W-MS-GW1 = W-CM-GW = W-CM-GE1 ≥ W-CC-GE4 ≥ W-CC-GE2 = W-MS-GE ≥ W-CC-GE1 > W-CM-GL = W-SB-GE = W-CM-GE3 > W-PL-GE = W-CC-GE3. For wild species, infusion prepared from W-MS-GW$_2$ had the highest DPPH scavenging activity (49.3%) and that prepared from W-PL-GE had the lowest DPPH scavenging activity (40.9%). The higher the percentage, the higher the antioxidant activity. For cultivated species, infusion prepared from C-PA-MSGW$_1$ had the highest DPPH scavenging activity (63.7%) and infusions prepared from C-PA-CMGW had the lowest.

All the infusions prepared from cultivated species had the highest significant ($p<0.05$) DPPH scavenging activities than infusions prepared from wild species. This could be due to high levels of total phenolic content of these infusions which is positively correlated with the radical scavenging activities (Cor *et al*., 2017). Quercetin was used as a standard for comparison. Quercetin had DPPH scavenging activity of 30.6% inhibition at 0.2 mg/ml concentration. Infusions of all wild and cultivated species had antioxidant activities higher than that of Quercetin at the concentration (0.2 mg/ml) that was used. The DPPH scavenging activities of infusion prepared from both wild and cultivated species were within the range of the DPPH scavenging activities (17.1-93.2% inhibition) reported on wild and cultured *G. lucidum* (Cor *et al*., 2017; Rajoriya *et al*., 2015). The high levels of

DPPH scavenging activity observed in the infusions prepared from cultivated species indicates that they are a potential source of antioxidant properties.

Table 7: Phenolics composition and antioxidant activities of infusions from wild and cultivated *Ganoderma* species

Sample code	**TPC (mg GAE/g of sample)**	**TFC (mg QE/g of sample)**	**Condensed Tannins (mg CAE/g of sample)**	**% Inhibition (DPPH Assay)**	**IC (mg QE/ ml of extract)**
W-CM-GE$_1$	19.50 ± 0.95^{k}	0.20 ± 0.02^{jkl}	3.00 ± 0.49^{h}	46.9 ± 0.1^{fg}	0.321 ± 0.001
W-CM-GE$_2$	23.60 ± 1.48^{j}	0.21 ± 0.02^{jk}	3.56 ± 0.67^{gh}	47.4 ± 0.2^{f}	0.324 ± 0.001
W-CM-GE$_3$	19.25 ± 0.94^{k}	0.19 ± 0.02^{jkl}	4.69 ± 0.73^{fgh}	43.1 ± 0.1^{j}	0.294 ± 0.001
W-MS-GE	44.78 ± 2.40^{b}	1.67 ± 0.02^{a}	5.82 ± 2.12^{de}	45.9 ± 0.2^{hi}	0.314 ± 0.001
W-CC-GE$_1$	23.93 ± 1.46^{i}	0.15 ± 0.02^{lm}	3.47 ± 0.80^{gh}	45.0 ± 0.2^{i}	0.308 ± 0.017
W-CC-GE$_2$	24.97 ± 0.55^{gh}	1.17 ± 0.02^{b}	4.73 ± 1.09^{efg}	45.3 ± 0.1^{hi}	0.309 ± 0.001
W-CC-GE$_3$	25.15 ± 1.95^{gh}	0.60 ± 0.13^{ef}	4.85± 1.44^{efg}	40.8 ± 0.2^{k}	0.279 ± 0.002
W-CC-GE$_4$	26.02 ± 0.31^{g}	0.16 ± 0.04^{jk}	6.09 ± 1.46^{de}	46.2 ± 0.2^{gh}	0.316 ± 0.001
W-PL-GE	18.37 ± 0.85^{k}	0.31 ± 0.02^{h}	5.11 ± 1.55^{def}	40.9 ± 0.2^{k}	0.280 ± 0.001
W-SB-GE	24.21 ± 0.17^{hi}	0.25 ± 0.07^{ij}	5.71 ± 1.05^{de}	43.3 ± 0.2^{j}	0.296 ± 0.002
W-CM-GW	18.89 ± 0.77^{k}	0.10 ± 0.01^{m}	2.97 ± 0.49^{h}	47.2 ± 0.2^{fg}	0.323 ± 0.001
W-MS-GW$_1$	23.27 ± 0.12^{i}	0.69 ± 0.02^{d}	5.40 ± 0.93^{de}	47.1 ± 0.2^{fg}	0.322 ± 0.002
W-MS-GW$_2$	28.78 ± 2.74^{f}	0.20 ± 0.01^{jkl}	4.99 ± 1.54^{def}	49.3 ± 0.4^{e}	0.337 ± 0.002
W-SE-GW	31.53 ± 1.38^{e}	0.30 ± 0.01^{hi}	6.37 ± 0.89^{d}	49.2 ± 0.2^{e}	0.337 ± 0.001
W-CM-GL	20.86 ± 0.54^{j}	0.09 ± 0.01^{m}	3.90 ± 0.73^{fgh}	43.4 ± 0.1^{j}	0.297 ± 0.001
C-PA-SBGE	42.11 ± 2.11^{c}	0.86 ± 0.08^{c}	12.99 ± 1.01^{b}	61.7 ± 2.6^{b}	0.422 ± 0.018
C-PA-CMGW	36.70 ± 1.34^{d}	0.57 ± 0.08^{f}	14.89 ± 1.71^{a}	53.6 ± 0.8^{d}	0.367 ± 0.005
C-PA-MSGW$_1$	43.40 ± 0.33^{bc}	0.41 ± 0.03^{g}	11.38 ± 1.33^{c}	63.7 ± 2.5^{a}	0.436 ± 0.017
C-PA-CMGL	52.73 ± 1.67^{a}	0.63 ± 0.09^{e}	15.29 ± 0.92^{a}	55.1 ± 0.4^{c}	0.377 ± 0.002
Quercetin				30.7 ± 0.1^{l}	0.2 ± 0.0

TPC: Total Phenolic Content; GAE: Gallic Acid Equivalent; TFC: Total Flavonoids Content; QE: Quercetin Equivalent; CAE: Catechin Equivalent; W: Wild; CM: *Colophospermum mopane*; GE: *Ganoderma enigmaticum*; MS: *Mundelea sericea*; CC: *Combretum collinum*; PL: *Pechuel-*

Loeschea leubuitziae; SB: *Sclerocarya birrea*; GW: *Ganoderma wiireonse;* SE: *Senegaria erioloba*; GL: *Ganoderma lucidum*; C: Cultivated; PA: *Pterocarpus angolensis*; 1-4 on a sample code: same *Ganoderma* species collected from different host trees of the same species. Values are mean ± SD on dry weight basis (n = 6). Means with different superscripted alphabets (a, b, c…) in the same column differ significantly ($p<0.05$).

3.5. Conclusions

Two species of *Ganoderma* (*G. enigmaticum* and *G. wiiroense*) were for the first time identified in Namibia. This suggests that there is possibly still unidentified *Ganoderma* species in Namibia. W-CM-GE$_1$, W-CM-GE$_3$, W-SE-GW, W-CM-GL, C-PA-CMGW, C-PA-CMGL, C-PA-MSGW$_1$ had high water solubility index suggesting that they have more water soluble constituents and thus can be potentially used in formulation of hot water extracts. 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. Although wild species had relatively lower levels of total phenolics, condensed tannins and antioxidant activity than those of cultivated *Ganoderma* species, they still had comparable levels to those reported in literature which makes both wild and cultivated species investigated in this study potential candidates for use as nutraceuticals and sources of possibly healthful antioxidants.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 4: General discussion

This chapter firstly discusses the cultivation potential of *Ganoderma* which includes the technology involved in each cultivation step. This discussion also highlights the principles, strengths and weaknesses of major methods used in this study such as phenolics compounds extraction, Folin-Ciocalteu method for total phenolics determination, Aluminium chloride method for total flavonoids determination and DPPH assay for antioxidant activity determination. Furthermore, this chapter notes the safety, sustainability of wild *Ganoderma*, underutilisation of *Ganoderma* in Namibia as well as the potential of *Ganoderma* for job creation.

4.1. Cultivation potential of *Ganoderma*

Cultivation of *Ganoderma* involves four stages as depicted in Figure 8. Each stage has its own requirements in terms of materials, equipment and growth parameters such temperature, humidity, moisture, aeration and light. The first stage (selection of pure culture) requires a culture room or laboratory, growth media (potato dextrose agar or malt extract agar), weighing scale, petri dishes, autoclave for cooking and sterilising the agar, laminar flow under which the agar is inoculated with culture or strain, parafilm or paper cello tape for sealing the inoculated petri dishes, methylated spirit or 70% ethanol for sterilisation of hands and working environment and a Bunsen burner to sterilise the inoculating blade. This stage can be cost effective in terms of constructing the culture room with all the necessary equipment and materials.

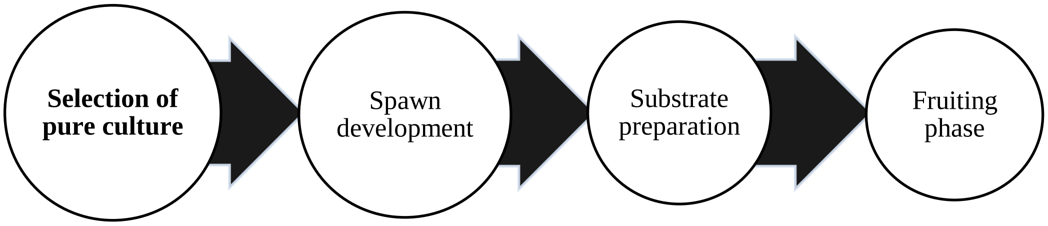

Figure 8: General flow process of *Ganoderma* cultivation cycle.

The second stage (spawn development) is carried out in a culture room as it requires a laminar flow hood, an autoclave for sterilising spawning materials, bucket for soaking spawning materials and sealable bottles or jars in which spawn is going to be developed. Spawning materials that can be used for *Ganoderma* cultivation include wheat grains (Ueitele *et al*., 2014), rice grains, maize grits and sorghum grains (Magday *et al*., 2014). Maize grits and sorghum grains are usually locally available for example in Namibia compared to rice grains which are grown on a small scale in Zambezi region and to wheat grains which are imported from South Africa. Therefore, anyone keen to venture into *Ganoderma* cultivation especially in Namibia can considered using maize grits or sorghum as spawning materials. Noteworthy is that small scale growers or beginners of *Ganoderma* cultivation can opt to skip stage 1 and 2 by purchasing already developed spawn by spawn producers. Currently there are no spawn growers, but decentralized university campuses or graduates in the field of biological sciences can take up this entrepreneurial chance. It is also noteworthy that farmers can be involved as collectors of the *Ganoderma* fruiting bodies and they can sell it to pharmaceutical companies or possibly to UNAM to be used in the products as supplements. Equally important, communities can be trained by UNAM on how to cultivate the mushrooms and then be supplying from their growing houses.

The third stage (substrate preparation) requires an inoculation room with built-in shelves on which the substrate bags can be placed for incubation. The stage also requires an autoclave to sterilise the substrate however, a drum heated with fire wood can be used as an alternative which can be an advantage to individuals that have no electricity to operate the autoclave. Different substrates such as saw dust, wood chips, sorghum straws, barely straws, rice straws, mahangu straws can be used for cultivation of *Ganoderma*.

The final stage (fruiting phase) requires a fruiting room with built-in shelves on which slashed open substrate bags can be placed to allow for mushroom sprouting and growth. The fruiting room or mushroom house can be made up of concrete, or thatch (for wall and roof) as long as there are built-in windows to allow adequate aeration and lighting. Therefore, beginners who are keen to start *Ganoderma* cultivation can start off with thatch which is inexpensive compared to concrete. The fruiting room also requires a concrete floor for easy cleaning and water drainage.

Overall, *Ganoderma* can be cultivated in any part of the world as long as a suitable mushroom house can be constructed in that area and growth parameters such as temperature, humidity, aeration and light are monitored and kept optimal especially in the fruiting room. *Ganoderma* growers or beginners should at all times ensure sanitary measures such as sterilisation of hands and all utensils before use at every stage of cultivation to minimize contamination. Beginners in *Ganoderma* cultivation may require basic training on how to carry out each step of cultivation in terms of handling, cleaning, inoculation and monitoring the growth parameters especially where no calibrated instruments are available to automatically monitor these parameters.

4.2. Principles, strengths and weaknesses of phenolic compounds extraction, quantification by chemical methods and antioxidant activity analyses

For phenolics composition analyses to be performed, phenolic compounds had to be extracted from crushed *Ganoderma* fruiting bodies. The extraction yield of phenolic compounds is influenced by the extracting solvents used (Cor *et al*., 2017). There are several solvents such as water, ethanol, methanol, ethyl acetate and n-hexane that can be used in the extraction of phenolic compounds (Sharif *et al*., 2017; Cor *et al*., 2017). The solubility of phenolic compounds depends on the polarity of the solvent (Sharif *et al*., 2017; Cor *et al*., 2017). Hot water was used as an extracting solvent in this study. The grinded fruiting bodies were steeped into boiled water for 5 minutes. Boiled water was used because high temperature increases extraction yield (Cor *et al*., 2017). The advantage of hot water extraction is that it is a simple process with low extraction time. It is environmental friendly and the extract can be consumed without harm since it simply involves the use of water. However, some phenolic compounds are less soluble in water and more soluble in organic solvents which can influence the concentration in the extract (Pereira, Arruda & Pastore, 2018).

There are two common methods used in the determination of total phenolic content in plants namely Folin-Ciocalteu assay and Prussian-Blue assay (Huda-Faujan, Rahim, Rehan, & Ahmad, 2015). However, for mushrooms especially *Ganoderma* Folin-Ciocalteu assay is the commonly used method to determine total phenolic content in those mushrooms (Rajoriya *et al*., 2015; Rawat *et al*., 2013; Sharif *et al*., 2017; Cor *et al*., 2017).

Folin-Ciocalteu method was used to determine the total phenolic contents in infusions of wild and cultivated *Ganoderma* species used in this study. The method involves the reduction of Folin-Ciocalteu reagent via transfer of an electron from a phenolic compound to phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMo_{12}O_{40}$) acids of Folin-Ciocalteu reagent. Sodium carbonate is added in the reagent mixture to adjust pH ~10 to form an alkaline medium in which a phenolic proton becomes dissociated leading to a phenolate anion that enables a reduction of Folin-Ciocalteu reagent. This reaction results in the oxidation of phenolic compound by phosphotungstic and phosphomolybdic acids to blue oxides of tungstene (W_8O_{23}) and molybdene (Mo_8O_{23}) forming a blue colour which presents wavelength of maximum absorption close to 760 nm (Pereira *et al*., 2018). On the other hand, the Prussian Blue method involves the formation of ferricyanide-ferrous [(FeCN)6)3-Fe2+] ion complex through the reduction of red ferric (Fe3+) to blue ferrous (Fe2+) ion by phenolic compounds. Addition of HCl to the reagent mixture during assay preparation increases the stability of ferric chloride (FeCl3), therefore speeding up the reaction (Huda-Faujan *et al*., 2015).

The advantage of Folin-Ciocalteu assay is that it is more stable and reproducible method in comparison to Prussian-Blue assay method which is unstable due to the formation of undesirable precipitation of extract with the reagent that increases with incubation time (Huda-Faujan *et al*., 2015). However, Prussian-Blue assay is more rapid and sensitive as it enables detection of phenolic contents at a lower concentration of 1.10^{-6} mg/mL (Huda-Faujan *et al*., 2015).

Total flavonoids content can be determined using two methods namely; Aluminium Chloride (Al_3Cl) Colorimetric method and Ceric Sulfate [Ce (IV)] method. Determining the total flavonoids by using aluminum chloride method is based upon the formation of stable complex between aluminum chloride and keto and hydroxyl groups of flavones and flavonoids. The principle of Aluminium Chloride Colorimetric method is based upon formation of acid stable complexes between aluminium chloride and the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. The method is also based upon aluminum chloride forming acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids (Hassan, Aqil & Attimarad, 2013). On the other hand, Ce (IV) method is based upon the oxidation reaction between Ce (IV) and flavonoids. Ce (IV) is a non-luminous oxidizing agent capable of reacting quantitatively with flavonoids (Hassan *et al*., 2013).

Although the above mention methods can be used in the determination of total flavonoids in plants, the Aluminium Chloride Colorimetric is the commonly used method. The Aluminium Chloride method used for determination of total flavonoids in plants has also been used to determine total flavonoids in mushrooms by several researchers (Islam, Yu & Xu, 2016; Rajoriya *et al*., 2015; Rawat *et al*., 2013). The same method was used to determine total flavonoids content in infusions of wild and cultivated *Ganoderma* species used in this study. The Aluminium Chloride Colorimetric method takes twice the incubation time (30 minutes) compared to the Ceric Sulfate [Ce (IV)] method with the incubation time of 15 minutes. However, Ceric Sulfate [Ce (IV)] method is an oxidation reaction between ceric sulfate and flavonoids which might result in the formation of free radicals making the end product not safe for consumption.

Although total flavonoids have been detected in mushrooms using Aluminium Chloride method, Gil-Ramirez *et al*., (2016) contends that mushrooms do not contain flavonoids. This is because they lack the main enzymes involved in their metabolic pathway (Gil-Ramirez *et al*., 2016). Gil-Ramirez *et al*., (2016) claimed that this method is not specific and can therefore may have detected any other phenolic compounds such as chlorogenic acid, o-diphenols, melanin-precursors or ergosterol that are not flavonoids and might be what previous researchers have quantified as total flavonoids. Since Gil-Ramirez *et al*., (2016) stated that the quantified total flavonoids in mushrooms by some researchers could be other types of phenolic compounds, this does not exclude those compounds from having potential health promoting properties such as antioxidant activity like the flavonoids.

There are several methods that can be used in determination of antioxidant activity such as DPPH (2,2’-Diphenyl-1-picrylhydrazyl) radical scavenging assay, Ferric ion reducing antioxidant power (FRAP) assay, ABTS (2,2’-Azinobis (3-Ethylbenzo Thiazoline)-6-Sulphonic Acid) radical cation decolourisation assay, Cupric ion reducing antioxidant capacity (CUPRAC) assay (Rafi, Febriany, Wulandari, Suparto, Ridwan, Rahayu, & Siswoyo, 2018), phosphomolybdenum assay, ferric thiocyanate assay and thiobarbituric acid assay (Attanayake, Jayatilaka & Malkanthi, 2016). DPPH, FRAP and ABTS assays are however, the commonly used methods by many researchers (Rebaya, Belghith, Baghdikian, Leddet, Mabrouki. Olivier, Cherif, & Ayadi, 2014; Rafi *et al*., 2018; Cor *et al*., 2017; Rajoriya *et al*., 2015; Rawat *et al*., 2013).

The principle of the DPPH radical scavenging assay is based on the transfer of an electron or hydrogen atom to DPPH free radical therefore, neutralizing its free radical by converting it to 1 - 1 diphenyl- 2 - picryl hydrazine. DPPH is a stable free radical that accepts an electron or hydrogen to become a stable diamagnetic molecule (Amoussa, Sanni & Lagnika, 2015). The decrease in absorbance of DPPH radical is due to the reaction between antioxidant molecules and the radical resulting in the scavenging of the radical by hydrogen donation (Amoussa *et al.*, 2015). FRAP assay is based on the reduction of a ferric-tripyridyltriazine complex in presence of antioxidants to form a coloured ferrous (Rebaya *et al.*, 2014). On the other hand, ABTS radical cation decolourisation assay is based on the nitrogen atom of ABTS (2, 2’-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) losing an electron to form ABTS cation radical (ABTS•+) through quenching a hydrogen atom from any hydrogen donating antioxidant in the sample extract, yielding the solution decolourisation. In addition, ABTS can be oxidized by potassium persulphate to generate ABTS cation radical (Pisoschi & Negulescu, 2011).

In this study, DPPH assay was used to determine the antioxidant activity of infusions from wild and cultivated *Ganoderma* species. This was because DPPH assay is a most commonly used spectrophotometric method to determine antioxidant activity (Rebaya *et al.*, 2014; Rafi *et al.*, 2018; Cor *et al.*, 2017; Rajoriya *et al.*, 2015; Rawat *et al.*, 2013). The advantage of DPPH assay comes with its operational simplicity during chemicals preparation and performing the analysis. The assay is also said to be stable and reproducible, however it can be costly (Shah & Modi, 2015). For the FRAP assay, it is simple, reproducible and low-cost, however it is more wearisome and requires a lot of time during chemicals preparation (Shah & Modi, 2015). In terms of ABTS assay, it is very time consuming compared to DPPH and FRAP assays as it uses ABTS radicals preformed through oxidising ABTS with potassium persulphate which takes 12 to 16 hours for the radicals to be generated and for ABTS to react with potassium persulphate. The only advantage of ABTS assay is that once the radicals are generated it is very simple and easy to perform the analysis (Shah & Modi, 2015).

4.3. Safety, quality and sustainability of wild *Ganoderma*

Wild mushrooms are exposed to insect infestation, soil and dust contamination which can affect their quality (Venturini, Reyes, Rivera, Oria & Blanco, 2011). Quality of *Ganoderma* and its products can also be influenced by change 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). Therefore, the chance of wild *Ganoderma* to be contaminated with bacteria including pathogenic ones such as *Listeria monocytogenes* and *Yersinia enterocolitica* (Venturini *et al*., 2011) is higher than that of cultivated *Ganoderma.* This is because wild mushrooms are harvested from the natural habitat, an open vegetation on which livestock and wild animals graze and browse (Venturini *et al*., 2011).

It is difficult to collect wild *Ganoderma* species in nature due to their irregular distribution and they can easily become extinct if unsustainably exploited commercially (Wachtel-Galor *et al*., 2011). Wild species experience different parameters such as temperature, humidity, aeration and light during their growth depending on geographical location. Furthermore, the age or maturity of wild *Ganoderma* species is generally unknown. All of these can result in variable quality of the *Ganoderma*. Therefore, cultivation of wild

Ganoderma can help sustain and maintain the quality of *Ganoderma* in terms of monitoring the growth parameters and minimise if not eliminate pathogenic bacteria, insects' infestations, soil, and dust contaminations.

4.4. Underutilisation of *Ganoderma* in Namibia

Ganoderma has long been used for over 2000 years especially in countries like Asia as nutraceuticals, dietary supplements and for medical purposes. They have been attributed to treat chronic diseases such as cancer, HIV/AIDs, diabetes, stroke, hepatitis (Wachtel-Galor *et al*., 2011). The medicinal effect of *Ganoderma* mushrooms is attributed by the presences of bioactive compounds such as polysaccharides and triterpenoids, glycoproteins (lectin), immunomodulatory proteins (Ling Zhi-8) and phenolic compounds (Chang & Miles, 2004). *Ganoderma* contributed to about 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). Annual production of *Ganoderma* in China was estimated at 36700 MT in 2002 and 49200 MT in 2003 (Hapuarachchi *et al*., 2018).

Nevertheless, in a developing country such as Namibia the market value and annual production of indigenous *Ganoderma* is nonexistent. Indigenous *Ganoderma* is exclusively still limited to traditional use. Lack of awareness and knowledge on the health potential of *Ganoderma* mushrooms might be the reason why it is underutilised in Namibia. Another reason could be that people regard *Ganoderma* as inedible due to its thick, corky, non-fleshy texture and tough fruiting body (Chang & Miles, 2004). Therefore, documentation of knowledge on ways to process *Ganoderma* for easy ingestion can also promote the consumption of *Ganoderma* mushrooms.

4.5. Potential of *Ganoderma* for job creation

Although *Ganoderma* is one of the prized mushrooms in developed countries such as in Asia, UK and USA, it is still an untapped resource in Namibia. *Ganoderma* in Namibia is not utilised for economic value. Import market value of *Ganoderma* in Namibia is also not clearly documented but there are a number of imported dietary supplements present in local pharmacies which contain *Ganoderma*. Therefore, with the reported potential of *Ganoderma* from its health benefits, cultivation capabilities as well as its market value for export and import substitution, this could be an opportunity for the youth to create employment through gaining basic training on how to start mushroom farming and how to process different *Ganoderma*-based products. This can contribute to reducing the high rate (over 40%) of unemployment among the youth in Namibia (Mulama & Nambinga, 2016).

Chapter 5: General conclusions and recommendations

5.1. Conclusions

The research undertaken compared the moisture, ash, WAI, WSI and phenolic compounds (total phenolic content, total flavonoids content, condensed tannins) and *in vitro* antioxidant activities of wild and cultivated *Ganoderma* species. The highest levels of moisture, ash, WAI, WSI were reported in cultivated species. Cultivated species had significantly higher levels of total phenolic contents, condensed tannins and *in vitro* antioxidant activities than the mushrooms collected from the wild. On the other hand, the highest levels of total flavonoids contents were reported in wild *Ganoderma* species.

Cultivation of *Ganoderma* can be a way of ensuring sustainable supply for commercialisation of *Ganoderma* mushrooms. Although *Ganoderma* is a prized mushroom in some countries, it is still an untapped resource in Namibia and most likely in other undeveloped countries in Africa. Furthermore, *Ganoderma* mushrooms both wild and cultivate can potentially be used as nutraceuticals due to their ability to be processed into consumable products such as hot water extracts (infusions).

5.2. Recommendations

More studies need to be done to identify *Ganoderma* species diversity in Namibia. I*n vitro* and *in vivo* studies should be conducted on medicinal effects of *Ganoderma* species in Namibia. Advanced studies e.g. using HPLC techniques to identify the bioactive compounds of *Ganoderma* species in Namibia are recommended. Microbial, allergens and toxins analyses should also be investigated on *Ganoderma* species in Namibia to ensure their safety. Since only two parameters of proximate composition (moisture and ash) were determined on the wild and cultivated *Ganoderma* species in this study, it is recommended that further research be done on the nutrient composition (protein, fat, fiber, carbohydrates) of the wild and cultivated *Ganoderma* species in Namibia .

A further recommendation is that more studies be done to optimise cultivation process to improve the yield during production and shorten the cultivation cycle. There is also a need to confirm whether or not mushrooms contain flavonoids and identify the compounds that are currently quantified as flavonoids.

Chapter 7: References

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8. Appendices

8.1.

Figure 9: Photos of the Namibian wild *Ganoderma* species in their natural habitats. (A-B) *Ganoderma* fruiting bodies from Ohangwena region growing on dead stump of Omupupwaheke, (C) *Ganoderma* fruiting body from Ohangwena region growing on a trunk of a dead Edimba, (D) *Ganoderma* fruiting body from Ohangwena region growing on a dead stump of Omwoonde, (E) *Ganoderma* fruiting body from Ohangwena region growing on a trunk of live Omupupwaheke tree, and (F) *Ganoderma* Fruiting body from Oshikoto region growing on dead stump of Omumbanganyana.

8.2. Blast search results of some indigenous wild *Ganoderma* species

Ganoderma enigmaticum strain I160004 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: gi|1562950582|MK453308.1Length: 602Number of Matches: 1

Alignment statistics for match #1

Score	**Expect**	**Identities**	**Gaps**	**Strand**
931 bits(1032)	0.0	516/516(100%)	0/516(0%)	Plus/Plus

```
Query  1    TTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCAATCCACTCTACACCTGTGCAC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  24   TTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCAATCCACTCTACACCTGTGCAC  83

Query  61   TTACTGTGGGTGACGGATCGCAAAGCGGGCTTCTTGTCCGTTATAAAGCGCATCTGTGGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  84   TTACTGTGGGTGACGGATCGCAAAGCGGGCTTCTTGTCCGTTATAAAGCGCATCTGTGGC  143

Query  121  CTGCGTTTATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATATAATAGATCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  144  CTGCGTTTATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATATAATAGATCT  203

Query  181  ATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204  ATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAAT  263
```

```
Query  241   GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGC  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  264   GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGC  323

Query  301   GCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACTTGCAAC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  324   GCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACTTGCAAC  383

Query  361   CTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTCGGCCTTTAACGGTCGGCTC  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  384   CTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTCGGCCTTTAACGGTCGGCTC  443

Query  421   CTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTGTCGGTGTGATAAAATGTCTAC  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  444   CTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTGTCGGTGTGATAAAATGTCTAC  503

Query  481   GCCGTGACCGTGAAGCGTTTGGATGAGCTTCCAACC  516
             ||||||||||||||||||||||||||||||||||||
Sbjct  504   GCCGTGACCGTGAAGCGTTTGGATGAGCTTCCAACC  539
```

Ganoderma wiiroense MIN 938704 ITS region; from TYPE material

Sequence ID: gi|1467229757|NR_158480.1Length: 722Number of Matches: 1

Alignment statistics for match #1

Score	**Expect**	**Identities**	**Gaps**	**Strand**
940 bits(1042)	0.0	524/526(99%)	0/526(0%)	Plus/Plus

```
Query  1    AGCTGGACTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTCACT  60
            |||||| |||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  38   AGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTCACT  97

Query  61   GTGGGCTTCAGGCATTATTGCGGAGCGCGTTCCTCACTGGACTTGTGGAAGCAGTGTGTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  98   GTGGGCTTCAGGCATTATTGCGGAGCGCGTTCCTCACTGGACTTGTGGAAGCAGTGTGTC  157

Query  121  TGTGCCTACGTTTATCACAAACTCTATAAAGTATCAGAATGTGTATTGCGATGTAACGCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  158  TGTGCCTACGTTTATCACAAACTCTATAAAGTATCAGAATGTGTATTGCGATGTAACGCA  217

Query  181  TCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  218  TCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGA  277

Query  241  AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  278  AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCT  337
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Query  301  TGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  338  TGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTAC  397

Query  361  GAGCTTTTGTGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCGTTCTTGGTCGGCTTC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  398  GAGCTTTTGTGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCGTTCTTGGTCGGCTTC  457

Query  421  TCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCGGTGTGATAATGTCTACGCT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  458  TCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCGGTGTGATAATGTCTACGCT  517

Query  481  GCGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCTCTGAGACA  526
            |||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  518  GCGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCTTTGAGACA  563
```

Ganoderma lucidum strain RMK1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: gi|1417767876|MH553170.1Length: 685Number of Matches: 1

Alignment statistics for match #1

Score	**Expect**	**Identities**	**Gaps**	**Strand**

259 bits(286)   3e-65   146/148(99%)   0/148(0%)   Plus/Plus

```
Query  1    CTGGACTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTCACTGT  60
            |||| |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  92   CTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTCACTGT  151

Query  61   GGGCTTCAGGTATTATTGCGGAGCGCGTTCCTCACTGGACTTGTGGAAGCAGTGTGTCTG  120
            |||||||||| |||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  152  GGGCTTCAGGCATTATTGCGGAGCGCGTTCCTCACTGGACTTGTGGAAGCAGTGTGTCTG  211

Query  121  TGCCTACGTTTATCACAAACTCTATAAA  148
            ||||||||||||||||||||||||||||
Sbjct  212  TGCCTACGTTTATCACAAACTCTATAAA  23
```

Table 8: Genomic Identification of wild *Ganoderma* species from Oshana, Oshikoto and Ohangwena regions in the northern part of Namibia based on ITS region.

Sample	**NCBI Identified species name**	**Similarity**	**Accession Number**
A_1	*Ganoderma enigmaticum*	100.%	MK453308.1
A_2	*Ganoderma enigmaticum*	98.64%	NR132918.1
A_3	*Ganoderma lucidum*	98.65%	MH553170.1
A_4	*Ganoderma enigmaticum*	99.67%	NR132918.1
A_5	*Ganoderma wiiroense*	99.42%	NR158480.1
A_6	*Ganoderma enigmaticum*	99.05%	MK453308.1
A_7	*Ganoderma enigmaticum*	99.83%	NR132918.1
A_8	*Ganoderma enigmaticum*	99.04%	MK453308.1
A_9	*Ganoderma enigmaticum*	99.06%	MK453308.1
A_{10}	*Ganoderma enigmaticum*	100%	KR150678.1
A_{11}	*Ganoderma enigmaticum*	100%	MK453308.1
B_1	*Ganoderma wiiroense*	98.01%	NR158380.1
B_2	*Ganoderma wiiroense*	99.03%	NR158480.1
B_3	*Ganoderma enigmaticum*	100%	MK453308.1
C_1	*Ganoderma enigmaticum*	99.51%	MK453308.1
C_2	*Ganoderma enigmaticum*	99.50%	NR132918.1
C_3	*Ganoderma enigmaticum*	100%	MK453308.1
C_4	-	-	-
C_5	-	-	-
C_6	*Ganoderma enigmaticum*	99.83%	NR132918.1
C_7	*Ganoderma enigmaticum*	100%	NR132918.1
C_8	*Ganoderma enigmaticum*	99.59%	MK453308.1
C_9	*Ganoderma wiiroense*	99.62%	NR158480.1

*(-): not identified

8.3. Photos of collected wild *Ganoderma* species from Oshana, Oshikoto and Ohangwena regions in the northern part of Namibia.

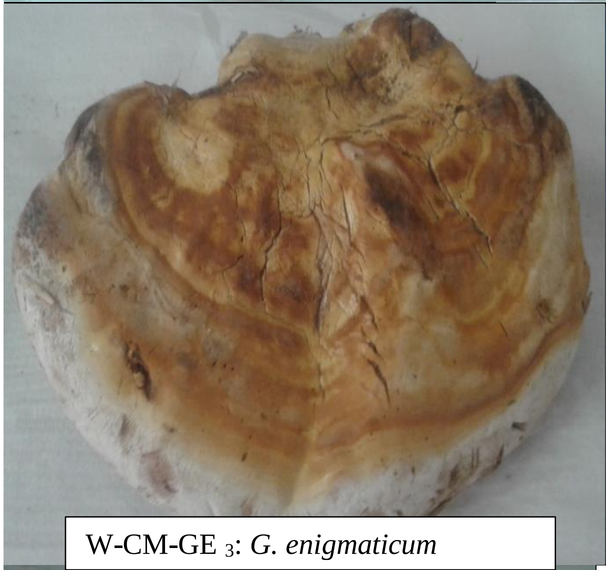
W-CM-GE$_1$: *G. enigmaticum*

W-CM-GE$_2$: *G. enigmaticum*

W-CM-GL: *G. lucidum*

W-CM-GE$_4$: *G. enigmaticum*

W-CM-GW: *G. wiiroense*



W-CM-GE $_3$: *G. enigmaticum*

W-MS-GW$_1$: *G. wiiroense*

W-CC-GE$_1$: *G. enigmaticum*

W-PL-GE: *G. enigmaticum*

W-CC-GE $_4$: *G. enigmaticum*

W-SE-GW: *G. wiiroense*

8.4. Outcomes of each cultivation stage

8.4.1. Stage 1: Pure culture preparation, mycelia growth on PDA

8.4.2. Stage 2: Spawn development, mycelia colonize wheat grains

8.4.3. Stage 3: Substrate preparation, mycelia colonizing woodchips.

8.4.4. Stage 4: Fruiting phase, *Ganoderma* fruiting bodies emerging from substrate bags