TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF *Hydnora abyssinca* FLOWER METHANOL AND AQUEOUS EXTRACTS

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DECLARATION

I declare that this is my original work and has not been submitted in any institution for

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Supervisor’ s approval

I confirm that this research project has been conducted and submitted with my approval as the student supervisor.

Signature…… ... Date ……

Dr. JARED ONYANCHA

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DEDICATION

I would like to dedicate this work and give special thanks to my parents; Mr. AHMED NOORand Mrs. AMINA ABDALLA, my elder brother's Abdikarim and Abdihakim who supported me throughout my entire education. Their affection, unconditional love, encouragement and dedication for the upliftment in my life is incredible, I owe them a lot for their selfless support towards my education.

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ABSTRACT

Antioxidants a group of compounds that are naturally found in various sources such as plants and are responsible for preventing or reducing the oxidative stress of the body. The body constantly produces free radicals as result of regular use of oxygen. The free radicals cause oxidative damage that may result into cell and tissue death. The oxidative damage contributes to genesis of many health threatening conditions such as diabetes and cancer. Plants are abundant source of these antioxidants which are helpful in preventing the deleterious effect of free radicals. Very few studies on plants have been conducted. The current study aimed at evaluating the total flavonoid content and antioxidant activity of methanol and aqueous flower extract of *Hydnora abbyssinica*. Total flavonoid content was conducted by the aluminum chloride method while the antioxidant activity was conducted by 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging assay. L-ascorbic acid and catechin were used as the standard antioxidant and flavonoid respectively. The methanol and aqueous extracted recorded total flavonoid content of 7.884±0.025 and 7.940±0.024 mg CE/g dw respectively ($p>0.05$). The flavonoids were significantly not different from each other. The antioxidant activity results showed methanol extracted recorded significantly higher antioxidant activity between 1000 ug/ml to 10 ug/ml as compared to aqueous extract($P<0.05$). Between 1 ug/ml to 0.01 ug/ml no significant difference was noted between methanol and aqueous extract ($P>0.05$). L-ascorbic acid recorded significantly higher antioxidant activity of 1000 ug/ml to 1 ug/ml ($p<0.05$) and significantly lower activity between 0.1 ug/ml and 0.01 ug/ml ($p>0.05$). The methanol and aqueous extract recorded IC_{50} values of 4.50 ug/ml and 5.580 ug/ml respectively while L-ascorbic acid recorded IC_{50} values of 4.12 ug/ml. in conclusion, *Hydnora abbyssinica* flower has antioxidant activity.

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CHAPTER ONE: INTRODUCTION

1.1 Background information

Oxygen is very important and unreplaceable molecule as far as metabolism and energy production for most of the life process. During key physiological processes such as inflammation and metabolism, reactive oxygen species is formed as free radicals are generated as by products. These reactive oxygen species under considerable concentration that is in balance with the antioxidants and are beneficial to the body as they aid in cell signaling. However, when their concentration is more as compared to the antioxidants, they result into harmful effects that include the attack on the important cellular structures such as proteins, lipids and nucleic acids hence oxidative damage (Wu et al., 2013). This damage is as a result of the imbalance with the reactive oxygen species and the antioxidant and results into state known as oxidative stress. This condition arises due to the imbalance that results into shift redox status that favor the reactive oxygen species. Research evidences has shown that oxidative stress plays an important part in genesis and progression of various disorders such as cancer, diabetes, metabolic disorders, atherosclerosis, cardiovascular diseases as well as aging (Pizzino et al., 2017).

The prevention and treatment of the oxidative stress related disorders mainly is achieved in reducing the free reactive oxygen species. The body usually has a defense mechanism in place to check the levels of is these reactive oxygen species are in the right concentration. This defense mechanism is made up of the endogenous antioxidants that counteract the detrimental effects of the reactive free radicals. This antioxidant defense system consist of both the enzymatic antioxidants such as catalase, superoxide dismutase and glutathione and non-enzymatic such as lipoic acid, glutathione, arginine, and coenzymeQ10 (Deponte, 2013). However, this antioxidant defense system of the body is usually overwhelmed or in some instance it may relapse. To aid in this condition conventional agents that include the synthetic antioxidants such as BHT and BHA are used. Other neurodegenerative diseases such as Alzheimer's disease is managed by drugs such as donepezil or rivastigmine. However, these synthetic antioxidants

and conventional drugs have been associated with side effects on addition to being costly.

Complementary medicine that involves use of medicinal remedies from plants and other natural sources has been embraced in the recent days. Their use dates back many years as they were the only source of medicine for the ailments that affected humans. Medicinal plants are in the recent years the most prescribed medicine for the many ailments that have attacked man. This has seen many countries turn on medical plants as the source of remedy due to the high potency, safety and cheapness of obtaining plant remedies. Additionally, these medicinal plants contain bioactive compounds identified as phytochemicals that are responsible for the various therapeutic effects such as antioxidant, anti-inflammatory, analgesic and memory enhancing activity.

1.2 Problem statement and justification

Even though oxygen is necessary for survival, its metabolic processes results into the generation of the free reactive species of oxygen origin as the by-products. These products affect the organs such as brain when in excess. The brain is prone to the oxidative damage as a result of the lower level of the antioxidant defense systems, densely pollution of the polyunsaturated fatty acids and its high rate of oxygen consumption. The oxidative damage arises due to the imbalance between the reactive oxygen species and counteractive components. When this imbalance happens, it immensely contributes to the slow onset and progressive nature of the neurodegenerative diseases as well as the age- related cognitive decline (Moriasi et al., 2020). This condition is as well described as a key factor in the genesis of cancer, diabetes, atherosclerosis, cardiovascular diseases, aging and neurodegenerative diseases such as Alzheimer's diseases and Parkinson's disease (Sun et al., 2012). Most of these disorders such as Parkinson disease and Alzheimer's disease, despite being in existence for a long time no proven agent that is able to treat then has been identified. The available therapy is only efficient in relieving symptoms and improving quality of life and is as well very costly (Strafella et al., 2018). Therefore the desire to have alternative agents of natural

origin has risen. These alternatives are usually potent more than the artificial agent and as well less toxic.

Complementary medicine is the way to go in the recent days. This form of medicine has shown potential to take over from the allopathic medicine. Medicinal plants that make up the complementary medicine have been reported to contain various bioactive compounds that have varied therapeutic value. These compounds are responsible for the various pharmacological activities such as antioxidant, analgesic and anti-inflammatory activities. Additionally the remedies from plants are usually safer and potent at the same timer. The less cost of obtaining them is an added advantage. Despite all these benefits, the studies on various components and pharmacological activities of plants is still low. Very few plants have been studied to the full end and documented. Therefore studies that has intention of evaluating the pharmacological importance off medicinal plants is really need as early as yesterday. This is with the aim of filling the existing gap in the herbal medicine that is a guide in obtaining newer medicine. Hence this studies was designed to determine the total flavonoid content and the free radical scavenging ability of both methanol and aqueous flower extract of *Hydnora abysinica*.

1.3 Objectives

1.3.1 General objective

To evaluate total flavonoid content of methanol and aqueous extract of *Hydnora abbysinica* flowers

1.3.2 Specific objective

I. To determine the total flavonoid content of methanol and aqueous extract of *Hydnora abbysinica* flowers

II. To evaluate the DPPH free radical scavenging activity of methanol and aqueous flower extract of *Hydnora abbysinica* flowers

1.4 Research questions

I. What are the total flavanoid content of methanol and aqueous flower extract *Hydnora abbysinica?*

II. Does methanol and aqueous flower extract *Hydnora abbysinica* have free radical scavenging properties?

CHAPTER TWO: LITERATURE REVIEW

2.1 Free radicals

Molecules or atoms characterized by unpaired electrons in their outermost orbitals. These molecules include both the oxygen and nitrogen reactive species. These free radicals are usually unstable and ready to react by gaining an electron to become stable (Azad et al., 2019). During this period, they may attack the biomolecules including proteins, lipids and nucleic acid causing damage. Additionally, they have been reported to take a center stage in the pathogenesis of many conditions including aiding in aging, degenerative diseases and inflammatory diseases. Free radicals commonly known include hydrogen peroxide, hypochlorite, hydroxyl and superoxide anion radicals as well as peroxynitrite and nitric oxide radicals (Fang *et al*., 2002).

2.1.1Reactive oxygen species

These comprises of the reactive species that are of molecular oxygen origin and are the by-products of the normal cellular metabolism. They are grouped into two main categories, the free radicals and the non-radicals. The free radicals are those that have either one or more unpaired electrons hence having the reactivity status while the non-radicals are those that results after the combination of two free radicals and in the process sharing the unpaired electrons. The free radical ROS include the superoxide anion (O^{-2}) and the hydroxyl radical ($\cdot OH$) while the non-radical include the hydrogen peroxide (H_20_2). The reactive oxygen species are both of endogenous and exogenous origin. The endogenous source of reactive oxygen species are the mitochondria, plasma membrane, endoplasmic reticulum and the peroxisomes (Chang & Kim, 2018). The ROS from these sources are generated via a variety mechanism such as enzymatic reactions. The in vivo sources of ROS are through the exogenous stimuli such as ionizing radiation, agricultural chemicals, tobacco smoke, infections due to pathogens and ultraviolet radiations (Halliwell, 2012).

2.2 Sources of free radicals

2.2.1 Endogenous sources

The normal physiological processes in the body are very vital for the survival of the human beings. These processes such as normal metabolism and inflammation are quit important for generation of energy and eradication of the noxious stimuli including infections respectively. However, these processes are accompanied by generation of the free radicals such as peroxides, superoxide and nitric oxide. The degradation of the fatty acids by peroxisome generates hydrogen peroxide as the by-product. Additionally, the free radicals are produced during the inflammatory response, cell activation, ischemia, infection and during excessive mental and physical stress (Sahab Uddin, 2016).

The body protects itself from the dangers of infections, infection causing organisms and foreign substances through the process of inflammation (Bala and Haldar, 2013). This process involves recruiting of the immune system cells such as neutrophils and macrophages to the site of infection. These immune cells are activated and in the processes, they burst leading to production of reactive molecules like nitric oxide and superoxide that help in destroying foreign substances or organism. These free radicals are thought to increases in concentration than the available endogenous antioxidants in the body (Bala and Haldar, 2013).

Additionally, during the process of phagocytic, the leukocytes produces a lot of superoxide radical that offer antibacterial activity. However, this superoxide radical is transformed enzymatically by superoxide dismutase to hydrogen peroxide. This hydrogen peroxide may later on partially dissociate to form a more potent hydroxyl radical (Knight, 2000; Wu and Cederbaum, 2003).

2.2.2 Exogenous sources

The external sources more so from environment are able to generate free radicals. These sources include polluted water and air, radiations, cigarette smoking, industrial waste, sunbeams, heavy metals, alcohol, unsaturated fat and certain drugs

(Arika *et al*., 2019). Upon their entrance in the body they are converted to free radicals that serve as exogenous supply of oxidants to the body. The continuous exposure of the body to different pollutant in the environment including air and water pollution triggers the immune cells to fight the foreign substances. Additionally, these cells generates free radicals that helps in the elimination of the foreign materials and in turn the free radicals accumulates in the body increasing their population.

Foods that people consume on their daily basis have greatly contributed to the number of free radicals in the body. These food products contain chemical residues that may have come from the used pesticides and fertilizers. Some of the chemicals are able to produce free radicals once ingested in the body or others are capable of generating free radicals through various means such as radiations. These free radicals contribute in generation of other more destructive free radicals through chain reactions increasing their population in the body. The free radicals later on react with the cellular components resulting into oxidative damage (Wu and Cederbaum, 2003). Smoke as a result of tobacco smoking contain more oxidants that cause damage to the lungs by depleting the antioxidants contained in the lungs. This results into loss of the redox balance between the free radicals and antioxidants. Smokers, as well have the respiratory tract that is characterized by high number of neutrophils that help fight the foreign substances. These high number of neutrophils as well contribute to increased levels of free radicals through activation of physiological processes such as inflammation (Lobo *et al*., 2010).

2.3 Herbal management of oxidative stress

Herbal medicine is regarded as the oldest form of healthcare to humanity as its use dates back to many centuries (Petrovska, 2012). The therapeutic value of the herbal medicine and its ability to treat majority of the emerging diseases has been boosted by support from the WHO (Olela et al., 2020). Due to its affordability, efficacy and safety it has been regarded as the most sought for means of achieving total health when compared to the conventional medicine (S. Antwi-Baffour, 2014). The medicinal plants are widely used with about 80 % of the global population relying

on them as the primary source of healthcare (WHO 2015). In most of the African countries herbal medicine is part of their culture hence they are more accepted than the modern medicine (Obakiro et al., 2020).

Through various studies, medicinal plants have shown to be potential source of medicine for management of various ailments. This has been linked to the many phytoconstituents that plants contain (Obakiro et al., 2020). These bioactive compounds that are present in the various plant are thought to be the leads to medicine in use currently such as the anticancer and analgesics (Misonge et al., 2015). Various medicinal plants that are potent antioxidant are available and used in compacting oxidative stress and its related disorders. These include *Hypericum keniense, Piliostigma thonningii* and many more. The potential antioxidant activities of these plants have been shown through the various studies (Cai et al., 2004). The pure compounds or crude extracts from these plants have shown to be effective antioxidants as compared to even vitamin E and BHT via the invitro antioxidant assays (Gu & Weng, 2001). Additionally, the medicinal plants have shown to exhibit much higher antioxidant activity that is directly proportional to the higher levels of phenolic compound as compared to some of the common vegetables and fruits (Cai et al., 2004).

2.4 *Hydnora abysinica*

2.4.1 Pant morphological description.

Hydnora abyssinica belongs to the family Hydnora that if found in the holoparastic genus of *Hydnora* (Al-Fatimi et al., 2016). This herb is entirely hypogenous and is characterized by reduced vegetative body (Fig 2.1). Both rhizome and flower are fleshy in nature and very succulent. The flower is the only part of the *Hydnora abbysinica* herb that is exposed to the surface as all the other part remain in the ground. This herb has only the fleshy rhizome and the flower which is the only part of this plant that is exposed outside the soil surface. The *Hydnora abbysinica* herb is only visible upon flowering and with one flower per rhizome. The flowering only happens during the rainy seasons. The flower is inform of a tube like structure that has four lobes all originating from a single central point. The dimensions of the

lobes are 25-39 mm long and 15-39 mm wide. The flower is colored orange to brownish while the inner part of the flower is reddish to orange pinkish.

Figure 2. 1 *Hydnora abyssinica* flower emerging from the soil (Al-Fatimi et al., 2016).

2.4.2 Plant distribution

It's distributed from southern Africa, across east Africa and Arabian Peninsula. In these localities the *H. abyssinica* is the only representative species of the genus *Hydnora*.

2.4.3 Traditional uses of the *Hydnora abyssinica*

The *Hydnora abbyssinica* herb is traditionally used for treatment of different conditions among many communities in the countries it's present. In Sudan the Hydnora herb is locally identified as "Tartous" and it's used as a remedy for treating swellings, tonsillitis and dysentery. The whole plant is boiled in water and then applied (Koko et al., 2015). Other communities use it as food in addition to being medicine. The flowers for example have been reported to be used as source of food and a remedy for various ailments among the residents of Lodwar and Dathin districts of South Yemen. The flowers are consumed as food while still fresh. Similarly, as a remedy for treating the various stomach related diseases, gastric ulcers and cancer, the fresh flower buds are dried and grounded into fine powder that is dissolved in either milk are water and then taken orally (Al-Fatimi et al., 2016).

In eastern Ethiopia, roots of *Hydnora abbysinica* are used to manage disorders such as haemorrhage, diarrhea, wound and mouth infections in the parts of the eastern Ethiopia. In Kenya the flowers are used as a remedy for various ailments and complications such as throat complications. It's as well used in helping in the removal of the placenta in case of delayed placenta after birth (Kokwaro J.O., 20 09).

2.4.4 Phytochemistry and bioactivity of Hydnora abyssinica

2.4.4.1 Phytochemistry

Hydnora abyssinica roots and flowers have been reported in the previous studies to contain various secondary metabolites. The phytochemical screening of the *Hydnora Abyssinica* identified presence of various phytochemicals that have varied bioactivities. In the ethyl acetate, ethanol and methanol extracts, phenols that include the tannins and flavonoids were present. In the n-hexane and dichloromethane extracts lipids, essential oils, fixed oils triglycerides and monoterpenes have been identified. The rhizome and the flower revealed the presence of the alkaloids, glycosides, tannins, phenols, steroids, flavonoids, terpenoids and fatty acids in the study by (Onyancha et al., 2015).

In both rhizome and the flower, *Hydnora abbyssinica* many compounds have been isolated. These compounds include cirsliol (3′, 4′, 5-trihydroxy-6-7-dimethoxy-flavone), trans 3'5-dihydroxy-4'7-dimethoxydihydroflavone), oleic acid vanillin (4-hygroxy-3-methoxybenzaldehyde) and protocatechuic acid (3, 4 dihydroxybenzoic acid) and catechin that were isolated from the root extract (Koko et al., 2015).

2.4.4.2 Bioactivity of Hydnora abyssinica

The many phytochemicals and the isolated compounds, are responsible for the vast pharmacological properties of the *Hydnora abyssinica*. N the various previous studies these bioactivities have been investigated and found to be excellent.

2.4.4.3 Antimicrobial activity

The aqueous root extract of the *Hydnora abyssinica* is reported to be significantly active against many infection causing microbes that are considered a threat to the human health (Yagi et al., 2012). The root extracts against the *E. faecalis* had an MIC value of 16 µg/ml. The root extract is as well reported to have an antidiarrheal activity a pharmacological activity that is due to the very high levels of tannins and flavonoids. In other previous studies the aqueous, chloroform and methanol root extract significantly inhibited the growth of *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*.

2.4.4.4 Cytotoxic activity

In the study done by Yagi et al.(2012) indicated that the aqueous and the ethanolic root extracts of the *Hydnora abyssinica* was infective against the MRC5 cells at the study concentrations of 10 µg/ml and 1 µg/ml. however, Yagi et al.(2012) noted that at concentration of 10 µg/ml of both the aqueous and ethanolic rot extracts had moderate activity against the KB cells. The pure compounds (phenolics) isolated from the aqueous and the ethanolic extract showed no cytotoxicity effect on both the MRC5 KB cell. This was a clear indication that the moderate activity on the KB cells is not as a result of the phenols and tannins.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Source of plant methanol and aqueous extracts

My research project supervisor Dr. Jared Onyancha provided the aqueous and methanol flower extracts of *Hydnora abbysinica*. The already extracted extracts were maintained at 6 ^{0} C in the fridge until the analysis day.

3.2 Reagents and chemicals

The reagent used in this study included sodium hydroxide, catechin, garlic acid, methanol, aluminum chloride, sodium nitrate, L-ascorbic acid and 2.2-diphenyl-2-picryl-hydrazil.

3.3 Determination of *in vitro* antioxidant activities of *Hydnora abbysinica*

3.3.1 Total flavonoid content of methanol and aqueous flower extract *Hydnora abbysinica*

The total flavonoid quantity in methanol and aqueous extract of *Hydnora abbysinica* was determined by the aluminum chloride calorimetric method. The protocol of Al-Rifai et al.(2017) was adopted in this study with some modifications. The methanol and aqueous extracts were first dissolved in methanol to make the stock solution of 1mg/ml. The standard flavonoid catechin was prepared to obtain six concentrations (0.625 mg/ml to 20 mg/ml). The reaction mixture was then constituted by adding 125 ul of the sample/ catechin into clean test-tubes. This was followed by 100ul of sodium nitrate, mixed and then incubated at room temperature for 6 minutes. To the resultant reaction mixture 75 ul of 4 % sodium hydroxide solution was added followed by 750 ul of aluminum chloride to form final volume of 1 ml. The volume was made to 2.5 ml using distilled water and the absorbance measured at 510 nm using UV-Vis spectrophotometer double beam (Labtech). The calibration curve of catechin was drawn and an equation of the straight line used to calculate the concentration of total flavonoid. The concentration of total flavonoid in the dry weight samples was calculated following the equation below and expressed as milligram of catechin per gram of dry weight (CE, mg/g dw).

$$Concentration\ \left(\text{CE}, \frac{\text{mg}}{\text{g}}\text{dw}\right) = \frac{c \times v}{m}$$

Where c is the concentration from the standard catechin curve, v is the volume of the sample and m is the mass of the extract weighed.

3.3.2 Determination of the DPPH free radical scavenging activity of *Hydnora abbysinica*

The 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging activity of methanol and aqueous *Hydnora abbysinica* was evaluated following the method of Sousa et al.(2016) with minor modifications. Exactly 10mg of both methanol and aqueous *Hydnora abbysinica* were weighed and dissolved in 10 ml of pure analytical grade methanol to make a stock solution of 1000 ug/ml. This stock solution was then serially diluted to obtain the working concentrations of 100, 10, 1, 0.1, and 0.01 ug/ml. The L-ascorbic acid which was used as the standard antioxidant and was prepared in the same concentrations as that of the study extract. Clean 36 test tubes were labeled as per the concentrations of the extract and other 18 for the standard. Three test tubes were labeled as the control. Into all the 18 test tubes of both plant extracts and L-ascorbic acid exactly 1.4 ml of plant extract/ standard at different concentrations were added. This was followed by 2.6 ml of 0.3 mM methanolic 2.2-diphenyl-2-picryl-hydrazil (DPPH) solution in all the test tubes. In the blank test tubes 1.4 ml of methanol and 2.6 ml of DPPH solution was added instead of extract or L-ascorbic acid. All the reaction mixtures were carefully mixed and incubated in the dark for 15 minutes. The absorbance of the L-ascorbic acid/ methanol and aqueous *Hydnora abbysinica* were measured at 517 nm and the free radical scavenging activity calculated following the formula.

$$\%\ RSA = \frac{Abs.C - Abs.T}{Abs.C} \times 100$$

Where Abs.C is the absorbance of the control (methanol + DPPH), Abs.T is the absorbance of the test (sample/L-ascorbic + DPPH).

The half extract concentration that is able to scavenge 50 % (IC_{50}) of the 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radical was computed from the percentage radical scavenging activity against concentration curve.

3.4 Data management and statistical analysis

The antioxidant data which consisted of both triplicate values of concentration of total flavonoid and the percentage radical scavenging activity were noted in the laboratory book. These was followed by tabulating the results in the excel spread sheet office 2013. This data (% RSA) and that total flavonoid was then imputed in the graph pad statistical analysis software version 9.0.1 and descriptive statistic conducted. The results were then expressed as Mean ± SEM (standard error of the mean). Comparison of both the extract and standard for the 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging assay was done using two way anova followed by tukeys post hoc test to establish the level of significance at $p<0.05$.

CHAPTER FOUR: RESULTS AND DISCUSSION

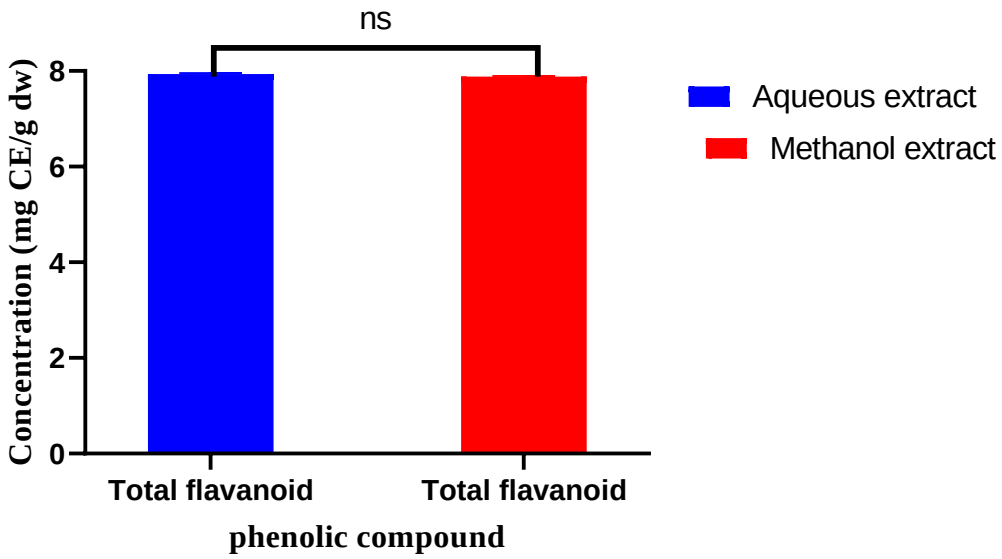
4.1 Total flavonoid content of methanol and aqueous flower extract of *Hydnora abbysinica*

The results for the total flavonoid content present in the methanol and aqueous flower extract of *Hydnora abbysinica* are summarized in table 4.1 and fig 4.1. The concentration were calculated from the standard catechin curve (y=42.33x-3.308). The methanol extracted recorded total flavonoid content of 7.884±0.025 mg CE/g dw while aqueous extract recorded 7.940±0.024 mg CE/g dw. The total flavonoid obtained from methanol extract was not significantly different from that in aqueous extract ($p>0.05$; fig 4.1).

Table 4. 1Total flavonoid content of methanol and aqueous flower extract *Hydnora abbysinica*

Phytochemical compound	Methanol extract	Aqueous extract
Total flavonoid content	7.884±0.025	7.940±0.024

Figure 4. 1 Comparison of the total flavonoid content in methanol and aqueous flower extract of *Hydnora abbysinica*



4.2 Antioxidant activity of methanol and aqueous flower extract of Hydnora abbysinica.

The antioxidant results for both methanol and aqueous flower extract of *Hydnora abbysinica* are in table 4.2, figure 4.2, 4.3 and 4.4. The results showed that the antioxidant activity of both extract and standard (L-ascorbic acid) increased with the increase in the concentration (table 4.2). The methanolic flower extract recorded significantly higher percentage radical scavenging activity. The 100 and 10 concentration were second and third respectively. However, no significant difference was noted in the percentage free radical scavenging activity recorded at 1, 0.1 and 0.01 of the methanol flower extract ($p>0.05$; fig 4.2). The aqueous extract at 1000 concentration recoded significantly higher percentage radical scavenging activity as compared to the other concentration levels. Also, significant difference was noted in the percentage free radical scavenging activity recorded at 100 as compared to the other concentrations. However, no significant difference was noted in the percentage free radical scavenging activity recorded at concentration levels 10, 1, 0.1 and 0.01 (fig 4.2). L-ascorbic acid the standard antioxidant recorded no significant difference in the percentage free radical scavenging between concentrations 1000 and 10 and concentrations 0.01 and 0.1. However, the percentage free radical scavenging activity recorded between concentration levels 1 and 10 were significantly different from each other (fig 4.2).

At concentration levels 1000, 100 and 100 significant difference was noted in the percentage free radical scavenging activity recorded by methanol, aqueous and L-ascorbic acid. At this particular concentration levels, L-ascorbic acid recorded significantly high percentage free radical scavenging activity followed by methanol extract. Aqueous extract recorded significantly low percentage free radical scavenging activity ($p<0.05$; fig 4.3). At 1 there was significant difference between L-ascorbic acid, methanolic and aqueous extract. However, there was no significant difference in the percentage free radical scavenging activity recorded by methanol and aqueous extract. At concentration 0.01 and 0.1, L-ascorbic acid recorded significantly lower percentage free radical scavenging activity as compared to both

methanol and aqueous extract. However, no significant difference was noted in the percentage free radical scavenging activity recorded at the both concentration (fig 4.3).

L-ascorbic acid recorded IC_{50} value of 4.12 while methanol and aqueous flower extract of *Hydnora abysinica* recorded IC_{50} values of 4.50 and 5.580 respectively (fig 4.4).

Table 4. 2The 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging activity of methanol and aqueous flower extract of *Hydnora abysinica*

Concentration (ul/ml)	% radical scavenging activity (Mean ± SEM)		
	Methanol extract	Aqueous extract	L-ascorbic acid
0.01	17.676±0.398	17.277±0.413	7.437±0.296
0.1	18.042±0.340	18.212±0.210	9.680±0.397
1	20.667±0.094	19.180±0.305	36.400±1.111
10	31.428±1.163	19.840±0.410	43.693±3.365
100	67.734±0.363	24.311±1.284	93.313±0.887
1000	80.695±0.312	66.482±3.112	95.960±0.968

Figure 4. 2 Concentration wise comparison of the percentage radical scavenging activity of individual extracts and L-ascorbic acid

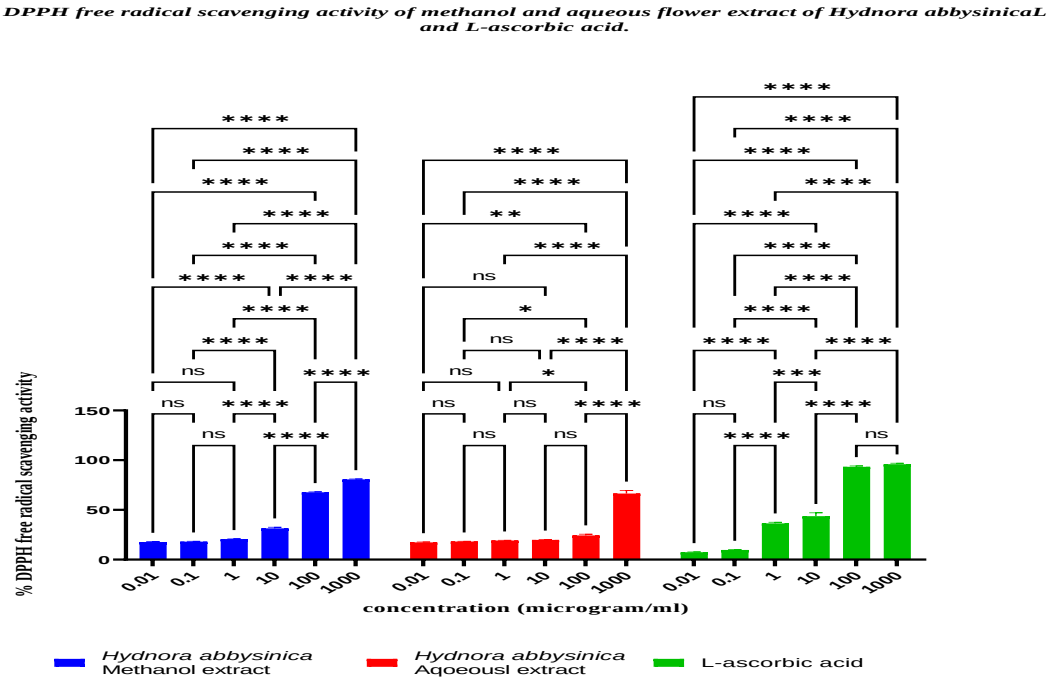


Figure 4. 3 Comparison of the percentage radical scavenging activity of the extracts and L-ascorbic acid at each concentration

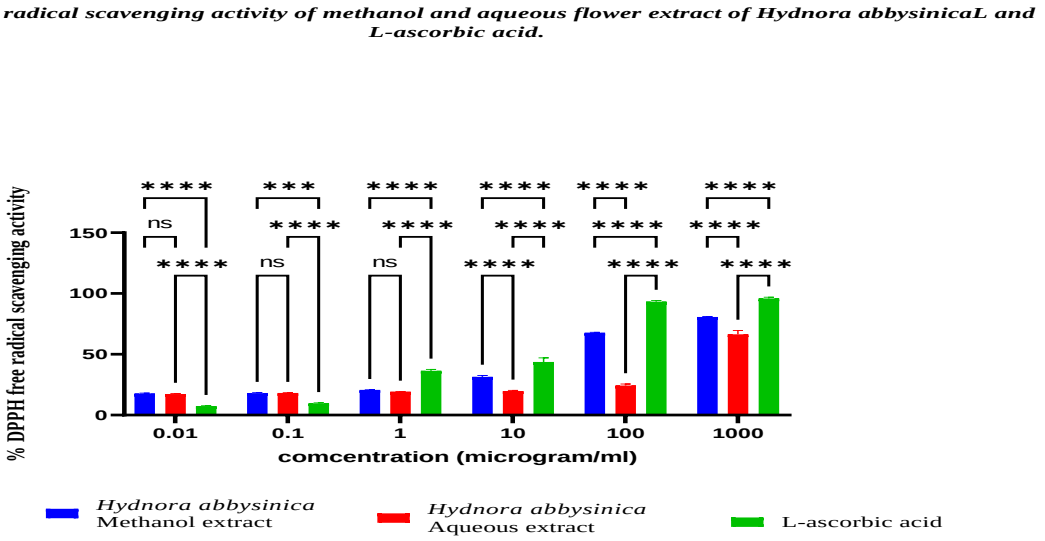
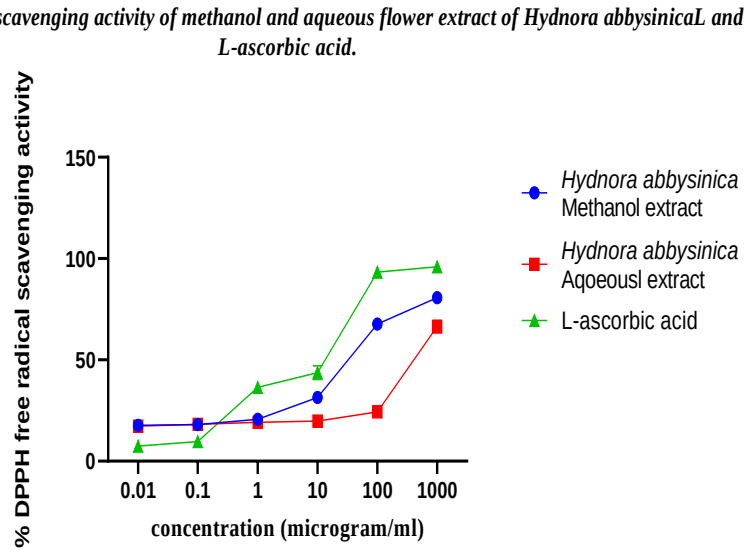


Figure 4. 4 IC $_{50}$ concentration of methanol and aqueous flower extract of Hydnora abbysinica and L-ascorbic acid



In this study, the total flavonoid content and free radical scavenging activity of the methanol and aqueous flower extracts of the *Hydnora abyssinica* was evaluated. The 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging assay was adopted to determine the radical scavenging activity of the *Hydnora abyssinica* extracts. The 2.2-diphenyl-2-picryl-hydrazil (DPPH) is a stable free radical that is organic nature with wavelength maximum absorbance ranging between 515-528 nm. When dissolved in either ethanol or methanol, a solution with an intense pink colour is formed. The resultant solution turns to yellow in the presence of the hydrogen donating antioxidant (Sakat et al., 2010). The 2.2-diphenyl-2-picryl-hydrazil (DPPH) scavenging activity of both studied methanol and aqueous flower extract of *Hydnora abyssinica* revealed that they are potently active as antioxidants. Methanol and aqueous flower extracts at concentration levels 0.01 ug/ml and 0.1 ug/ml significantly scavenged the extracts to scavenge the 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radicals more as compared to the standard L- ascorbic acid. However, the ability of the methanol extract to scavenge the free radical was not different from that of the aqueous extract. At concentration levels 1, 10, 100 and 1000 ug/ml, L-ascorbic acid significantly scavenged the 2.2-diphenyl-2-picryl-

hydrazil (DPPH) free radical as compared to both methanol and aqueous root extract. However, at concentration 10, 100 and 1000 ug/ml the methanol flower extract was able to scavenge the free radical more than aqueous extract. The minimum conconcentration required to scavenge the free radical was gotten from the curve. The IC 50 values of L-ascorbic acid was 4.12 ug/ml followed by methanol with 4.50 ug/ml and aqueous extract with 5.580 ug/ml. From this IC_{50} values the methanol extract had higher antioxidant activity as compared to the aqueous extract. However, both methanol and aqueous extract were less potent as compared to L-ascorbic acid. These results are in agreement with those reported by Onyancha et al.(2015) in the study of the antioxidant activity of the *Hydnora abyssinica* flower methanol extract.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From this study based on the findings, it can be concluded that *Hydnora abyssinica* has antioxidant properties. This is as a result of its ability to scavenge the free 2.2-diphenyl-2-picryl-hydrazil (DPPH) radical. Likewise, the *Hydnora abyssinica* can be used as a potent antioxidant as demonstrated by the lower IC_{50} obtained in the methanol and aqueous extracts. The antioxidant activity can be linked to the phenolic compounds such as flavonoids that were quantified in this study. However, the methanol extract is more potent than the aqueous extract and this is due to the more flavonoids quantified in the methanol extract.

5.2 Recommendation

From the current study, the following recommendations were made

I. A guided isolation of the bioactive compounds with the antioxidant activity.
II. Safety studies to be done to determine the toxicity of both the extracts and the compounds isolated.
III. The *in vivo* antioxidant of the methanol and the aqueous extracts showed be done to find out if the reported activity can be replicated in the *in vivo* setting

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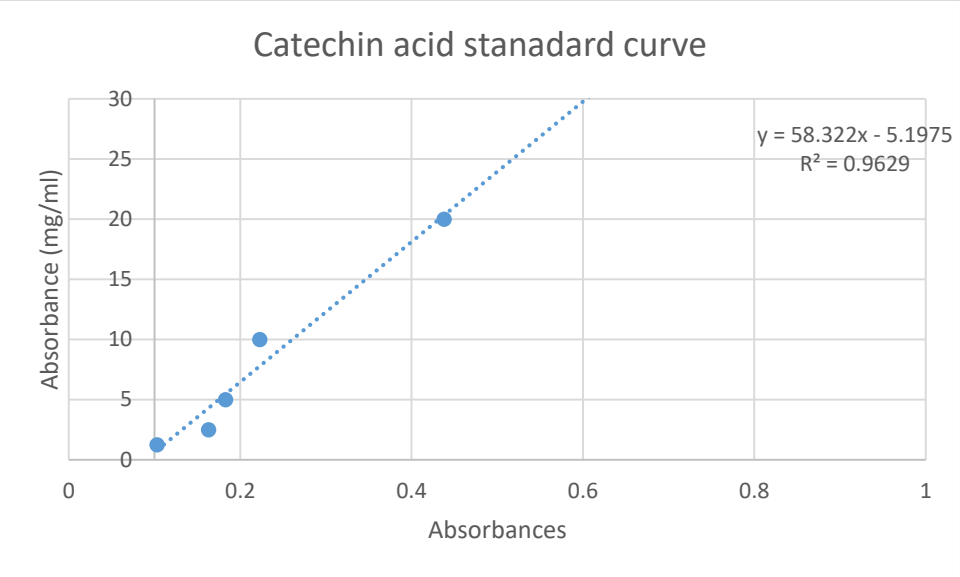
APPENDICES

Appendix 1. 1Catechin standard calibration curve



Appendix 1. 2Absorbance of Catechin and *Hydnora abyssinica* flower methanol and aqueous extracts

Concentration mg/ml	Absorbance at 510 nm		
20	0.438	0.553	0.274
10	0.223	0.240	0.188
5	0.183	0.176	0.179
2.5	0.163	0.224	0.150
1.25	0.103	0.113	0.113
HAW	0.039	0.061	0.061
HAM	0.039	0.022	0.029

Appendix 1. 3Antioxidant raw data

L-Ascorbic acid

Concentration	Absorbance at 517 nm		
	T1	T2	T3
1000ug/ml	0.046	0.025	0.022

100ug/ml	0.041	0.043	0.065
10ug/ml	0.418	0.395	0.484
1ug/ml	0.472	0.495	0.496
0.1ug/ml	0.689	0.695	0.696
0.01ug/ml	0.711	0.704	0.717
0.00	0.769	0.764	0.770

L-Ascorbic acid

Concentration	% RSA		
	T1	T2	T3
1000ug/ml	94.04%	96.72%	97.14%
100ug/ml	94.02%	94.37%	91.55%
10ug/ml	45.64%	48.30%	37.14%
1ug/ml	38.62%	35.20%	35.58%
0.1ug/ml	10.40%	9.03%	9.61%
0.01ug/ml	7.54%	7.89%	6.88%

Hydnora abbysinica flower methanol extract (% RSA)

Concentration	Absorbance at 517 nm		
	T1	T2	T3
0.01ug/ml	17.197	17.364	18.468
0.1ug/ml	17.452	18.631	18.043
1ug/ml	20.509	20.659	20.834
10ug/ml	29.531	31.21	33.545
100ug/ml	68.28	67.046	67.878
1000ug/ml	80.382	81.321	80.382

Hydnora abbysinica flower aqueous extract (% RSA)

Concentration	Absorbance at 517 nm		
	T1	T2	T3
0.01ug/ml	17.197	17.364	16.56
0.1ug/ml	17.452	18.631	18.631
1ug/ml	20.509	20.659	18.598
10ug/ml	29.531	31.21	20.636

100ug/ml	68.28	67.046	22.548
1000ug/ml	80.382	81.321	61.216

Hydnora abbysinica flower methanol extract (Absorbance)

Concentration	Absorbance at 517 nm		
	T1	T2	T3
1000ug/ml	0.154	0.154	0.147
100ug/ml	0.249	0.260	0.255
10ug/ml	0.540	0.556	0.523
1ug/ml	0.624	0.628	0.623
0.1ug/ml	0.648	0.642	0.651
0.01ug/ml	0.650	0.652	0.707
0.00	0.785	0.789	0.787

Hydnora abbysinica Aqueous extract (Absorbance)

Concentration	Absorbance at 517 nm		
	T1	T2	T3
1000ug/ml	0.265	0.306	0.219
100ug/ml	0.608	0.603	0.576
10ug/ml	0.623	0.638	0.631
1ug/ml	0.639	0.638	0.635
0.1ug/ml	0.644	0.642	0.645
0.01ug/ml	0.655	0.647	0.651
0.00	0.785	0.789	0.787