

Full Length Research Paper

Antioxidant activity, total phenolic and flavonoid content of selected Kenyan medicinal plants, sea algae and medicinal wild mushrooms

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Medicinal plants are good sources of bioactive compounds that guards the body against cancer and chronic ailments. A lot of studies have been done on medicinal values of higher plants but less on marine sources and wild non-edible mushrooms. The study aimed to assess the antioxidant activity, total flavonoid and total phenolic content from selected Kenyan medicinal plants, sea algae and mushrooms. The plants were selected based on their availability and folklore medicinal value. The antioxidant activity of the extracts was assessed using 2, 2-diphenylpicryl-1-hydrazyl (DPPH) free radical scavenging method. The total phenolic analysis was achieved using the Folin-Ciocalteu technique while the flavonoid content was determined by the use of aluminium chloride calorimetric method. The total phenolic content was expressed as gallic acid equivalent (GAE) and flavonoid content as quercetin equivalent (QE). *Ganoderma applanatum* had the highest scavenging ability (95.56%), while *Urtica dioica* leaves had the lowest (11.99%) at 0.3 mg/ml of extract. *G. applanatum* also had the lowest IC₅₀ (<0.025 mg/ml), an indication that it had the highest antioxidant potential. *Ganoderma lucidum* showed the highest total phenolic content (GAE/g) of 156 ± 3.45 mg and *U. dioica* showed the lowest. *G. lucidum* also showed the highest total flavonoid content (QE/g) of 31.16 ± 0.04 mg. The study reveals that the Kenyan plant species can be potential sources of new natural antioxidants.

Key words: Antioxidant activity, DPPH, Folin-Ciocalteu, IC₅₀, flavonoids, phenolic content.

INTRODUCTION

Medicinal plants are vital in the survival of living organisms. Phytochemicals from the medicinal plants can be used as an organism defense mechanism and source of vital medicines. Medicinal plants are used for management of human diseases due to the presence of

phytochemicals (Wadood, 2013). Approximately 80% of the population in developing countries depends on medicinal plants for their primary health care needs (Evans, 1997; Farnsworth and Soejarto, 1991). The use of medicinal plants has been motivated by several factors,

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including the belief that they have few side effects, easy accessibility and are cheaper as compared to modern synthetic drugs as well as the effectiveness of some plant remedies (Zheng and Wang, 2017). Phenolic compounds are important constituents present in plants and act as natural antioxidants (Ngueyen et al., 2009). They are composed of a large number of metabolites including flavonoids, polyphenols, tocopherols, tannins and lignins. Secondary metabolites of phenolic nature can act as part of plant's defense against insects, animals or plant pathogens and can protect the body from excess free radicals and retard the progress of many chronic diseases (Lai et al., 2001; Pengelly, 2004).

In humans, they act as antibacterial, anti-inflammatory, anthelmintic and cytotoxicity (Dore et al., 2014). An important function of phenolics is the antioxidant activity. Antioxidants have been reported to act as cardiovascular protectants, anti-aging agents and possible anti-cancer activity (Liu, 2003). The antioxidant properties of phenolic compounds such as flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelating of metal ions and inhibition of enzymes responsible for the generation of free radicals (Acker et al., 1996). Plants, sea algae and mushrooms have varying levels of total phenolic content and antioxidant activity (Kajal et al., 2017; Krishnendu A. 2010). These plants have potential to be good sources of antioxidants and arrest free radical damage. Oxidation is a very useful process in the metabolism of living organisms. During the process, there is production of free radicals. These radicals abstract electrons from other molecules to attain stability hence the damages. Antioxidants reduce free radicals in living organism's cells, hence are useful in the treatment of many human diseases such as cancer (Soni and Sosa, 2013). Antioxidants can neutralize free radicals by two major mechanisms, hydrogen atom transfer (HAT) and single electron transfer (SET) (Aqil et al., 2006). The end result is the same, regardless of the mechanism (Ismail and Tan, 2002; Wright et al., 2001).

The aim of this study was to determine antioxidant activity, total phenolic and flavonoid content of selected Kenyan medicinal plants, mushrooms and seaweeds. The plants were selected based on the availability of plant materials and due to several studies on medicinal potency, such as antimicrobial, anti-inflammatory, anti-diabetic, malaria, fever and anti-cancer (Iwu, 2004; Kokwaro, 2009). The plants will include the following: *Eucheuma denticulatum*, *Ganoderma applanatum*, *Ganoderma lucidum*, *Trametes elegans*, *Prunus africana* *Bridelia micrantha* and *Urtica dioica*. *P. africana* commonly known as African cherry has a wide distribution in Africa growing in mountainous regions. The stem bark is of commercial value (Gulcin et al., 2004). It has several bio components such as polyphenols fatty acids, esters and alkanols (Kadu et al., 2012). *B. micrantha* is a medium-tall tree, growing up to 20 m and belonging to the family of Phyllanthaceae. (Mburu et al.,

2016; Munayi RR 2016) *U. dioica* is a herbaceous perennial flowering plant in the family of *Urticaceae* commonly known as stinging nettle or common nettle (Ilhami et al., 2004).

Both edible and non-edible mushrooms are regarded as good sources of bioactive compounds in human diets for the antioxidant and anti-inflammatory purpose. They store secondary metabolites, which includes compounds like flavonoids, polyphenols, polyketides, terpenes and steroids with pharmacological and nutritional value (Abugri and Mcelhenney, 2013; Nguyen et al., 2016).

Studies on non-edible *G. applanatum* and *G. lucidum* mushrooms have secondary metabolites responsible for antioxidants and anti-inflammatory activity (Rašeta et al., 2016, Jeon et al., 2008). Anti-oxidant and anti-inflammatory activities in marine algae are attributed by high content of hydrophilic polyphenols and soluble polysaccharides (Delgado et al., 2013; Balakrishnan et al., 2014).

In this study, a method based on the scavenging of the stable radical, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was used to measure the antioxidant activity potential of plant extracts from Kenya (Brand et al., 1995; Kulisic et al., 2004; Ansari et al., 2013). Folin-Ciocalteu reagent, a commonly used complexing coloring reagent was used for measuring the total phenolic content while the flavonoid content was determined by the use of aluminium chloride calorimetric method.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent, 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid, and quercetin were purchased from Aldrich Co. Germany. Methanol, sodium carbonate, aluminum chloride, sodium nitrate, and ascorbic acid were of analytical grade.

Sample collection

Roots, stem bark, leaves and whole plant were collected from their natural habitats in Kenya between January and September 2018. Mushrooms: *G. applanatum* and *T. elegans* were collected in February while *G. lucidum* was collected in September from Kitale, Trans-Nzoia County. The samples were collected in the afternoons from dead acacia tree along the river. The mushrooms were transported on the same night in polythene bags. *B. micrantha* bark, *U. dioica* leaves and roots and *P. africana* bark were collected in July from Manyatta constituency, Embu County. The plants and mushrooms were identified and voucher specimens deposited in the herbarium at the National Museums of Kenya, Nairobi. Sea algae: *Eucheuma denticulatum* was collected in August from the Kenyan South Coast, Shimoni, Kwale County and the specimen was identified at Kenya Marine and Fisheries Research Institute (KEMFRI), Mombasa, Kenya.

Sample preparation

All plants, mushrooms and seaweeds were washed thoroughly

three times with tap water, shade dried and ground to fine powder. The fine powder of different samples was stored in air-tight polyethylene bags before extraction.

Extraction

100 g of each ground plant sample was weighed, 500 ml methanol was added and soaked overnight and then sonicated at 60°C over ultrasonic bath for two hours to enhance extraction. The extract was filtered using Whatman filter paper No.1. The clear filtrate obtained was concentrated at reduced pressure by rotary evaporations to produce a crude extract.

DPPH free radical scavenging activity assay

Antioxidant activity was performed according to the method described by Brand et al., 1995 and Jaita et al., 2010 with slight modifications. Series of dilutions of the methanolic extract (0.025-0.3 mg/ml) was prepared. A measure of 2 ml of the extract was mixed with 3 ml of 0.3 mg/ml of DPPH radical. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance of the mixture was read at 517 nm using UV-VIS Spectrophotometer (Analytik Jena model). The absorbance of the resulting solution was converted into a percentage of antioxidant activity (% inhibition) by the use of the following formula:

$$\% \text{ Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A_0 = Absorbance of the control solution containing only DPPH solution; A_1 = Absorbance in the presence of extract in DPPH solution; and Ascorbic acid was used as a standard.

Determination of total phenolic content

The number of total phenolics was determined using the Folin-Ciocalteu method (Jaita et al., 2010) with some modification. 0.1 g of the extract was dissolved in 5 ml methanol. 200 μ l of the extract solution and 1 ml Folin-Ciocalteu reagent were mixed and 1 ml of 7.5% of sodium carbonate solution was added after 3 min. The mixture was shaken and allowed to stand for 2 h in the dark. The absorbance of the solution was read in triplicate using UV-VIS Spectrophotometer (analytic Jena model) at a wavelength of 515 nm. A blank solution was prepared and read similarly. A calibration curve of gallic acid was obtained from serial dilutions of various concentrations of gallic acid prepared from its stock solution. The results were expressed as GAE (gallic acid equivalents/g) of the extract.

Determination of total flavonoid content

The total flavonoid content was determined spectrophotometrically (Quettier-Deleu et al., 2000). 4 ml of distilled water was added to 1 ml of the extract in a 10-ml volumetric flask, followed by 1 ml of 5% sodium nitrate. 1 ml of 10% aluminum chloride was added after 5 min. This was left to settle for 5 min where 2 ml of sodium hydroxide was then added and topped up to the mark with distilled water. The absorbance readings were taken at 510 nm against a blank (water). The flavonoid content was determined using a standard curve with quercetin (10-180 mg/ml). The mean of three readings was used and expressed as milligrams of quercetin equivalents (QE/g of extract).

RESULTS AND DISCUSSION

Antioxidant activity

The effect of antioxidant on DPPH radical is thought to be due to their hydrogen donating ability. The extracts of plant samples are allowed to react with stable DPPH radical in methanol solution. The reduction capability of DPPH radicals is determined by the decrease in its absorbance at 515 nm, induced by antioxidant. The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% is termed as inhibition concentration (IC_{50} or EC_{50}). This parameter is widely used to measure the antioxidant activity (Sanchez-Moreno et al., 1998). A lower IC_{50} value corresponds with a higher antioxidant ability. The plant extracts showed significant DPPH scavenging activity (59.01 – 95.56%) as compared with values obtained for standard ascorbic acid (94.32%) at 0.3 mg/ml. The antioxidant activity increased with the concentration of extracts as shown in Figure 1.

G. applanatum showed the highest scavenging activity of 95.56% ($IC_{50} < 0.025$ mg/ml), higher than that of ascorbic acid 94.32% ($IC_{50} = 0.03$ mg/ml) followed by *P. africana* bark 93.6% ($IC_{50} = 0.033$) at 0.3 mg/ml. This was in agreement with the scavenging ability of 84% reported for methanol extract of *P. africana* bark by Edwin et al. (2018). *U. dioica* leaves showed the lowest scavenging activity of 11.99% ($IC_{50} > 0.3$ mg/ml) as shown in Figure 1. The results of antioxidant activity of other plant extracts were as follows: *B. micrantha* 92.67% ($IC_{50} = 0.038$ mg/mL), *G. lucidum* 91.24% ($IC_{50} = 0.04$ mg/ml), *E. denticulatum* 90.21% ($IC_{50} = 0.042$ mg/ml) *U. dioica* roots 84.32% ($IC_{50} = 0.045$ mg/ml) and *T. elegans* 59.01% ($IC_{50} = 0.22$ mg/ml). In this study, mushroom showed a good scavenging activity. This could be due to the absorption of nutrients from the components they grow from. The scavenging ability of *G. applanatum* was higher than the one reported of 71% (Nagaraj, 2014). *T. elegans* had a scavenging activity value of 59.01% which was lower than the one reported of 65% (Awala, 2015). Antioxidant activity of *B. micrantha* (92.6%) was lower than the one reported of 97.70% (Nwaheujo 2014). This variation may be due to the variation in geographical area and the bioactive compounds present. Different parts of the plants showed variation in antioxidant activity due to the presence of different types of phytochemicals which have different antioxidant activity.

Total flavonoid content

The highest total flavonoid content was observed in *G. lucidum* (31.16 ± 0.04 mg QE/g), which was higher than the reported value of 10.82 mg QE/mg (Raseta, 2016). *B. micrantha* bark had a value of 30.47 ± 0.03 mg QE/g and *T. elegans* had a value of 103.19 ± 1.23 mgQE/g, higher than the reported value of 0.97 mg QE/g (Awala, 2015). It was observed that *U. dioica* leaves had the lowest total flavonoid content (3.97 ± 0.06 mg QE/g) as shown in

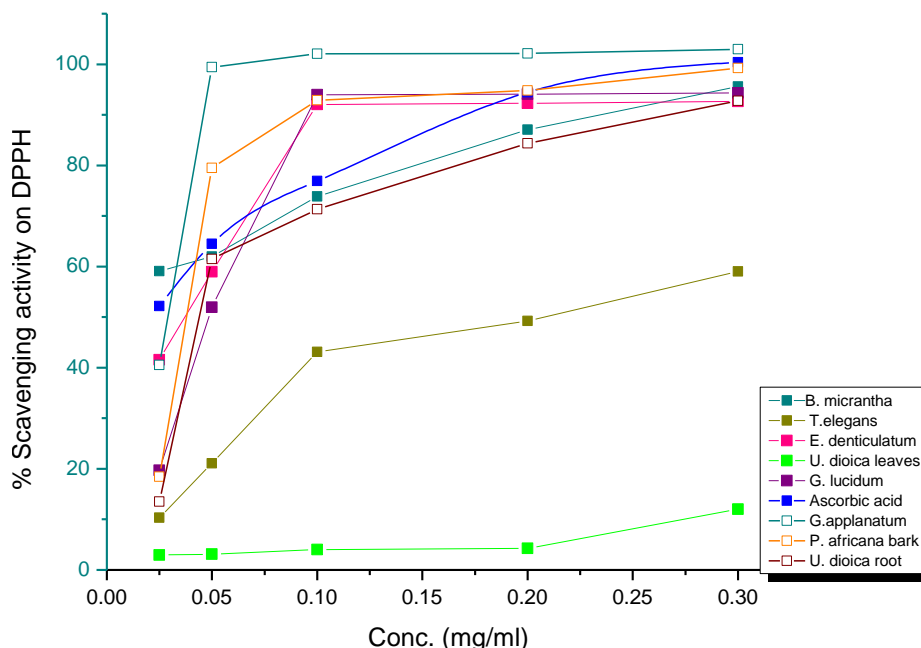


Figure 1. % radical Scavenging activity of extracts on DPPH. Determination of antioxidant activity with concentration.

Table 1. Total phenolic and flavonoid content and antioxidant activity of extracts.

Extract	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)	Antioxidant activity on DPPH at 0.3 mg/ml of extract %	IC ₅₀ (mg/ml)
Ascorbic acid			94.32	0.03
<i>G. applanatum</i>	127.23 ± 0.64	14.53 ± 0.28	95.56	< 0.025
<i>P. africana</i> bark	148.55 ± 4.05	9.29 ± 0.06	93.60	0.033
<i>B Micrantha</i> bark	128.79 ± 1.54	30.47 ± 0.03	92.67	0.038
<i>G. lucidum</i>	156.07 ± 3.45	31.16 ± 0.04	91.24	0.04
<i>E. denticulatum</i>	146.15 ± 1.11	9.36 ± 0.12	90.21	0.042
<i>U. dioica</i> roots	144.04 ± 3.89	28.54 ± 0.67	84.32	0.045
<i>T. elegans</i>	103.19 ± 1.23	9.97 ± 0.32	59.01	0.22
<i>U. dioica</i> leaves	43.19 ± 1.15	3.97 ± 0.06	11.99	> 0.3

Table 1. The extracts with high antioxidant activity also had high total flavonoid content. According to literature, high flavonoid content may provide protection against oxidative stress along with other oxidative defenses such as vitamins and enzymes (Tripathy et al., 2014).

Total phenolic content

All the extracts under the study contained noticeable phenolic content with significant variations (P-value = 0.0004). The highest phenolic content was observed in *G. lucidum* (156.07 ± 3.45 mg GAE/g) > *P. africana* bark (148.55 ± 4.04) > *E. denticulatum* (146.15 ± 1.11) > *B.*

micrantha bark (12879. ± 1.54) > *G. applanatum* (127.23 ± 0.64 mg GAE/g) as shown in Table 1. The highest phenolic content in *G. lucidum* may be due to different polyphenolic content. The results obtained for *G. applanatum* were lower compared to 191.76 mg GAE/g reported by Raseta (2016). The value for *G. lucidum* was higher than the reported value of 60.41 mg GAE/g (Raseta, 2016). *T. elegans* had a higher value (9.97 ± 0.32 mg GAE/g) than reported (4.79 mg GAE/g) (Awala, 2015). The total phenolic content was lowest in *U. dioica* leaves (3.97 ± 0.06 mg GAE/g). The results showed a correlation between antioxidant activity and phenolic content (r = 0.9467) as observed from the results. The higher the phenolic content the higher the antioxidant

activity. Natural extracts with proven antioxidant activity usually contain compounds with a phenolic moiety such as flavonoids, tocopherols, carotenoids, tannins (Dapkevicius et al., 1998). This implies that phenolic components are among the compounds responsible for reducing the DPPH radical.

Conclusion

Results of this study indicate that methanolic plant extracts have a significant effect on the scavenging of free radicals. The antioxidant activity increases with concentration. Most of the extracts had high total phenolic and flavonoid content. *G. lucidum* extracts had the highest total flavonoid and phenolic content, with *G. applanatum* showing the highest antioxidant activity. *B. micrantha* and *P. africana* showed high antioxidant activity of 92.67 and 93.60 respectively. Their total phenolic and flavonoid content were relatively high. The study revealed that these Kenyan plant species can be potential sources of new natural antioxidants.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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