

Phytochemicals in Edible Wild Mushrooms From Selected Areas in Kenya

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Abstract

Mushrooms have been used as food for centuries all over the world because of their characteristic soft texture and mild flavor. They are documented as being good source of nutrients and bioactive compounds that are beneficial to the human body (Chang, 2011). While the exotic varieties have been extensively analyzed, local edible wild mushrooms have not and yet they are consumed by communities living near the forests. This research aimed at screening and determination of phytochemicals compounds in wild edible mushroom found in selected areas in Kenya Two commercially grown mushrooms, oyster (*Pleurotus florida*) and button (*Agaricus bisporus*), and ten edible wild mushrooms species were collected from different locations of the country. They were analyzed for total polyphenols, flavonoids and radical scavenging activity using standard methods. All the analysis was done in triplicate. Phytochemical screening showed presence of saponins, polyphenols and terpenoids. Total polyphenols values obtained ranged between 210-1614 mg Gallic Acid equivalent (GAE)/100g, dry weight basis (dwb) and flavonoids 214-1695 mg Quercetin Equivalent (QE)/100 g dwb. Total polyphenols ($R^2 = 0.82$, $P \leq 0.05$) and flavonoids values showed a positive correlation with the radical scavenging activity.

The results show that cultivated and wild edible mushrooms are rich in health-promoting phytochemical compounds.

Keywords: mushrooms, phenolic compounds, flavonoids, radical scavenging activity

1. Introduction

Mushroom has been defined as ‘a macro-fungus with a distinctive fruiting body, which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand (Chang & Miles, 1989) They are documented as being rich in proteins, minerals, vitamins while they are low in lipids (Pathak et al., 1997).

Documented literature indicates that mushrooms have phytochemicals and other compounds which are strong antioxidants (Fang et al., 2002; Liu, 2004). Phenolic compounds, alkaloids, saponins, flavonoids, tannins, sterols, triterpenes, coumarins and cyanogenic glycosides have been detected in wild mushrooms analyzed in Sudan and in Nigeria (Adebayo et al., 2012; Egwim et al., 2011; Ehssan & Saadabi, 2012).

The compounds seem to mop the free radicals generated in the normal natural metabolism of aerobic cells, mostly in the form of reactive oxygen species (ROS). These include superoxide (O_2^-) and hydroxyl (OH^\cdot) radicals among several others. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides (Barja, 2004). Once in circulation, most of the free radicals are neutralized by cellular antioxidant defense enzymes e.g. Superoxide dismutase (SOD) or catalase (CAT). Non-enzymatic molecules like ascorbic acid and carotenoids are reported to be present in mushrooms and they also act as antioxidants (Fang et al., 2002; Isabel et al., 2004). Maintenance of equilibrium between free radicals production and antioxidant defenses is an essential condition for normal organism functioning (Hollman & Arts, 2000). The disequilibrium, excess free radicals in the system, is known as oxidative stress. It interferes with cell integrity hence normal functioning is altered leading to many stress-related diseases like cancers and diabetes.

Mushroom nutraceuticals describe a new class of compounds extractable from either the mycelium or fruit body of mushrooms and embodies both their nutritional and medicinal features. They are consumed as a dietary supplement which has potential therapeutic applications (Chang & Miles, 1989). Mushroom Nutraceuticals are

enriched food materials which are used for Maintenance of healthy diet. These are part of a meal (Chang & Miles, 1989; Shiuan, 2004). Infusion of mushrooms has been used to prevent beriberi. In addition, the decoction has been used for the treatment of abscesses and wounds (Yu et al., 2009).

2. Materials and Methods

2.1 Materials

Mushrooms used in this research comprised of wild mushrooms (Figure 1) collected from natural habitat and included 7 species from Arabuko Sokoke, one species from Aberdares and Mt Elgon forests, 2 species from Kisumu and Kakamega counties. Two cultivated species; oyster (*Preurotus florida*) and button (*Agaricus biporus*) were included for comparison. To determine the effect of maturity and mushroom parts on phytochemicals, oyster was segregated into young and mature fruit bodies while *oruka* was segregated into caps (pilei) and stipes. Samples of fresh wild mushrooms were taken to the Museums of Kenya, Botany/Herbarium department for scientific identification which is still in progress. Consequently, local mushroom names will be used in this report for uniformity.

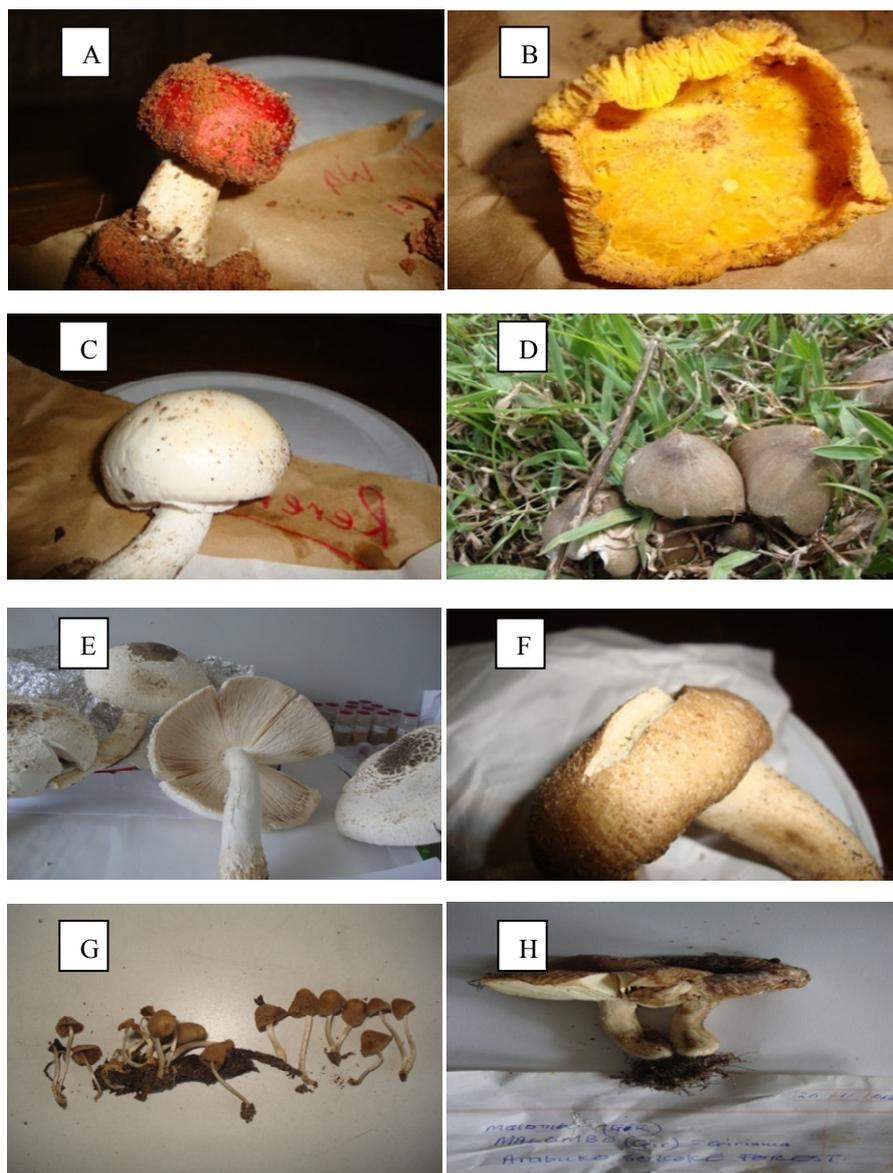


Figure 1. Some of the wild mushrooms collected: A Mkundu wa nyani; B, Masikiro meruhe; C, Rerema; D, Mariondonic/makunu ma mutitu; E, Oruka; F, Joga muhama; G, Kadzodzo; H, Malombo

2.2 Sample Preparation

At the university, Jomo Kenyatta University of Agriculture and Technology (JKUAT), they gently brushed off dirt and were all dried at 70°C, ground using laboratory mill. The Milled mushroom was put in labeled air-tight bottles and stored at 4°C.

2.3 Preparation of Mushroom Extracts

Extraction, screening and quantification was done according to the method described by Harbone (1998) with minor modifications. Known weight of the dried mushroom powder was mixed with 100 ml ethanol in a conical flask. The content was put on a shaker for 24 hours at room temperature. The liquid part was decanted and stored at 4°C. The solid was re-suspended in ethanol and procedure repeated. The liquid was then combined, filtered using whatman paper no. 4. The filtrate was concentrated in vacuum evaporator to 10 ml. This was put in sample bottles and stored at 4°C to await further analysis. Similarly, known weights of mushroom powder were mixed with hot water (50°C), put on a shaker for 24 hours. The rest of the procedure was as described.

2.5 Quick Tests for Phytochemicals

Ethanol and water extracts were subjected to preliminary phytochemical screening for the identification of various classes of active chemical constituents using standard methods (References).

2.5.1 Quick Test for Saponins' Presence

Foam test: To 1 ml of the extracts 5ml distilled water was added and shaken vigorously. Formation of foam indicated presence of saponins.

2.5.2 Quick Test for Total Polyphenols

Ferric Chloride test: To 1 ml of the extract, 2 ml of distilled water, 3 drops of 10% aqueous ferric chloride (FeCl_3) and 3 drops of potassium ferrocyanide were added. Formation of blue or green color showed the presence of polyphenols.

2.5.3 Quick Test for Anthraquinones

Weighed mushroom powder, 0.5 g, was boiled in 10% hydrochloric acid and filtered hot. To this, 2 ml chloroform and 10% ammonia solution each were added. Formation of pink color in the aqueous layer indicated presence of anthraquinones.

2.5.4 Quick Test for Terpenoids

Water extract, 5 ml, was mixed with 2 ml chloroform followed by sulfuric acid along the tube wall. Formation of brown color at interface was a positive indicator.

2.5.5 Quick Test for Tannins

To 3 ml ethanolic extract was added 3 ml 10% ferric chloride (FeCl_3). Formation of blue/black color was a positive indicator.

2.5.6 Quick Test for Alkaloids

On silica gel-coated plates, 10 μl extract was spotted equidistance from each other and eluted with methanol-sulfuric acid solution. The dried plates were sprayed with Dragendroff reagent. Formation of red-brown color was positive indicator.

2.6 Determination of Total Polyphenols

Phenolic compounds in the mushroom extracts were estimated by a colorimetric assay, based on standard procedures described by Harbone (1998) with minor modifications.

To 5 ml distilled water was added 0.5 ml Folin Ciocalteu's reagent. After 3 min, 1 ml 7.5% sodium carbonate solution, 1 ml extract were added to the mixture and made to 10 ml with distilled water. The mixture was kept in water bath maintained at 50°C for 16 minutes. UV Visible spectrophotometer (UV-Vis Shimadzu) was used to read the absorbance at 765 nm. Gallic acid was prepared in different concentrations and the absorbance equally read at 765 nm. The values obtained were used to generate the standard curve against which polyphenols in the mushrooms were calculated and expressed as Gallic acid equivalents (GAEs) per 100 g dwb.

2.7 Determination of Flavonoids as Quercetin Equivalent

Flavonoids contents in the extracts were determined by standard colorimetric method with minor modifications. To 1 ml mushroom extract was added 0.3 ml 5% sodium nitrite; 4 ml distilled water and held for 5 minutes. To the mixture 0.3 ml 10% aluminium chloride was added and held for 6 minutes. Finally 2 ml 1 M sodium

hydroxide was added and the content made to 10 ml with distilled water. Using UV spectrophotometer, (UV-Vis) the intensity of pink color was measured at 415 nm. Pure quercetin was prepared in different concentrations and absorbance read at same wavelength. The readings were used to make standard curve against which flavonoids in the sample were calculated and expressed as mg of quercetin equivalents (QE)/100 g dwb.

2.8 Determination of Radical Scavenging Activity (RAS) Using DPPH

The radical scavenging activities of the mushroom extracts against 2, 2-Diphenyl-1-picryl hydrazyl radical were determined by UV visible spectrophotometer, UV-Vis-SDD-10AV SHIMADZU, at 517 nm. Radical scavenging activity was measured using standard procedures. The following concentrations of the extracts were prepared, 0.05, 0.1, 0.5, 1.0, 2.0 and 5 mg/ml in ethanol. Ascorbic acid was used as the antioxidant standard at concentrations of 0.02, 0.05, 0.1, 0.2, 0.5 and 0.75 mg/ml.

To 1 ml of the extract in a test tube 3 ml ethanol was added followed by 0.5 ml 1 mM DPPH in ethanol. Incubation was done for 5 minutes. A blank solution was prepared containing the same amount of ethanol and DPPH. The absorbance of the blank was read at 517 nm and a standard curve generated using the values. The absorbance of solutions with extract was similarly read at 517 nm.

The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: % RSA = [(ADPPH-AS)/ADPPH] 100, where AS is the absorbance of the solution when the sample extract has been added at a particular level, and ADPPH is the absorbance of the DPPH solution.

The extract concentration providing 50% inhibition (EC₅₀) was calculated from the graph.

2.9 Statistical Analysis

Results are given as mean of triplicate ± SD. Correlation coefficient between total polyphenols and radical scavenging activity was done by regression using Genstat statistical software version 14.

3. Results and Discussion

3.1 Mushrooms Collected

Result of the mushroom collected and analyzed is shown in Table 1.

Collection is normally done early in the morning. A major problem encountered in collection is competition with wild animals like the baboons. It is common to find they started earlier and have eaten them, so one comes back empty handed. Encounter with dangerous animals like elephants is a reality which makes it very risky to the communities that would benefit from this freely available food. Entry to the forest is therefore highly restricted by Kenya Wildlife Service (KWS). The volume picked is therefore very low and no conservation is done. Where collection is from disturbed land e.g. Kakamega and Kisumu, drying is done and it is possible to buy dry mushroom in far off places.

Table 1. Mushrooms collected and analyzed

Botanical name	Common/Local name (community)	Region	Habitat
<i>Agaricus bisporus</i>	button/makunu (kikuyu)	Central	Commercially grown
<i>Preurotus florida</i>	Oyster/Makunu (kikuyu)	Central	Commercially grown
<i>Termitomyces sp</i>	Makunu ma mutitu(kikuyu)	Central	Grows on farms/ forest/ anywhere
<i>Termitomyces sp</i>	Mariondonik (Sabaot)	Rift valley	Grows on farms/ forest/ anywhere
-	Olando (Dholuo)	Nyanza	Grows on farms
-	Obulando (Luhya)	Western	Grows on in farms
<i>Termitomyces sp</i>	Oruka (Dholuo)	Nyanza	Grows on ant-hills
-	Joga muhama (Giriama)	Coast	Grows on disturbed land.
	Joga misinga (wadtha)		
<i>Termitomyces sp</i>	Joga utuwe (Giriama)	Coast	Grows on ant -hills
	Dugo dhinthu (Wadtha)		
-	Malombo (Giriama)	Coast	Mixed & Brachystegia forests

<i>Russula Compressa</i>	Mkundu wa nyani (Giriama)	Coast	Brachystegia forests
-	Joga kadzonzo (Giriama)	Coast	Mixed & Brachystegia forests
<i>Amanita zambiana</i>	Rerema (Giriama)	Coast	Mixed & Brachystegia forests
-	Masikiro meruhe (Giriama)	Coast	Mixed & Brachystegia forests

- Name not identified.

Key-Mixed forest is composed of *Afzelia quanzensis*, *Manikara sansibarensis*, *Hymenaea verrucosa*. Trees;

Brachystegia forest is composed of *Brachystegia speciformis*, *Manikara sansibarensis* and *Hymenaea verrucosa* and Patches of grass present. ASF: Arabuko Sokoke Forest in Kenyan coast;

Olando of Kisumu same species as Obulando of Kakamega;

Makunu ma mutitu of Aberdares =same species with mariondonik of Mt Elgon.

3.2 Quick Assays for Presence of Phytochemical Compounds

The results of phytochemical screening are given in Table 2. The screening indicated presence of saponins, polyphenols and terpenoids. Alkaloids, tannins and anthraquinones were absent in all the species. The Phenolic compounds include different subclasses (flavonoids, phenolic acids, stilbenes, lignans, tannins, oxidized polyphenols) that display a large diversity of structures (Nijveldt, 2001).

The compound detected will depend on the method used, the pH and the interaction with other compounds. As a result it is not possible to conclude that those that were absent would not be detected using different reagents or methods.

Saponins comprise a large family of structurally related compounds containing a steroid or triterpenoid aglycone. They are reported to have a wide range of pharmacological properties that exert various benefits, such as anti-inflammatory and anti-diabetic properties (Lee et al., 2012).

Terpenoids (isoprenoids) are secondary metabolites with molecular structures containing carbon backbones made up of isoprene. The compounds have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti-inflammatory and anti-cancer among others (Beattie, 2011; Roslin & Annular, 2011).

Table 2. Phytochemical compounds detected in the mushrooms

Mushroom	Saponins	Polyphenols	Alkaloids	Tannins	Terpenoids	Anthra-quinones
<i>A bisporus</i>	+++	+	-	-	+	-
<i>P florida</i>	++	+	-	-	+	-
<i>Makunu-ma mutitu</i>	+	+	-	-	+	-
<i>Mariondonik</i>	+	+	-	-	+	-
<i>Obulando</i>	+	+	-	-	+	-
<i>Olando</i>	++	+	-	-	+	-
<i>Oruka</i>	+++	+	-	-	+	-
<i>Joga Muhama</i>	+	+	-	-	+	-
<i>Joga utuwe</i>	++	+	-	-	+	-
<i>Malombo</i>	+++	+	-	-	+	-
<i>Mkundu wa nyani</i>	+++	+	-	-	+	-
<i>Joga kadzonzo</i>	++	+	-	-	+	-
<i>Rerema</i>	+++	+	-	-	+	-
<i>Masikiro maruhe</i>	+++	+	-	-	+	-

+ = Presence; ++ = increasing concentration; - = absence;

Olando of Kisumu same species as Obulando of Kakamega;

Makunu ma mutitu of Aberdares =same species with mariondonik of Mt Elgon.

3.3 Polyphenols, Flavonoids and Radical Scavenging Activity Values Are Shown in Table 3

The figures obtained for polyphenols range between 210-1614 mg GAE/100g, dwb. Flavonoids values obtained are 214-1695 mg QE/100 g and the RSA values obtained ranged between 58.07-458.01

The total polyphenols content show a positive correlation with flavonoids, high polyphenols accompanied by high flavonoids although not in direct proportionality ($P \leq 0.05$). The levels of these compounds are influenced by species, substrate on which mushrooms grew, maturity and the part of the mushroom analyzed (Oboh & Shodehinde, 2009). High levels of flavonoids were accompanied by high RSA (low value). The radical scavenging activity of phenolic compounds has been correlated to their chemical structures (Nijveldt, 2001). RSA is also influenced by other factors, such as presence of other H-donating groups like $-NH$ or $-SH$. Other compounds which have RSA include ascorbic acid, tocopherols, and carotenoids. All these are reported to be present in mushrooms at different levels (Barros et al., 2007). This probably explains why there is no obvious trend that relates levels of TP and TF with RSA. The antioxidant level would therefore be influenced by the nature and levels of compounds elucidated. The RSA values obtained range between 58.07-458.01. The reported values are in the same range of 76-1000 mg/100 g dwb in Portuguese wild mushrooms (Barros et al., 2007).

The Young Fruit Bodies (YFB) have higher TP and total flavonoids (TF) than the mature fruit bodies (MFB). This is in conformity to reported figures (Isabel et al., 2004). The author suggested that the compounds in mature stages could be involved in defense mechanism as a result of the aging process; hence reduced content on extraction.

The TP, TF and RSA values are dependent on the part of the mushroom analyzed. This is exemplified by values obtained for *oruka* cap and stipe. The TP, TF and RSA mean values in cap were 1332.24, 1511.08 and 58.07 respectively. Those for the stipe were 872.57, 648.20 and 59.59 respectively. Similar but lower figures have been reported for total polyphenols in cap 677-1066 and 400-760 for stipe (Isabel et al., 2004). Other reports indicate that polyphenol extracts from the stipes had a significantly ($p < 0.05$) higher free radical scavenging ability and reducing power than those from the caps (pilei) (Oboh & Shodehinde, 2009). Whole *oruka* values were 788.52, 979.64 76.65 for TP, TF and RSA respectively. This indicates that the values obtained for the whole mushroom depend on the proportion of stipe or cap present in the analyzed sample. This research used a whole mushroom.

When all factors that influence the nature and content of phytochemical compounds are considered, the differences are expected. However, although the stipe may have lower TP and TF the RSA may be same or higher than that of cap (Barros et al., 2007). This is the case where values obtained for RSA in cap and stipe is 58.07 and 59.59 respectively. The reason may be that different compounds or compounds with different structures accumulate in stipe but contribute to RSA

All mushrooms from Arabuko sokoke forest exhibited high levels of total polyphenols than the rest from highlands. The flavonoids levels compare well with the mushrooms from other places. However the RSA is not high. This seems to suggest that these compounds are involved in stress-related reactions rather than RSA. This would suggest that the geographic region of coast has influenced the synthesis and bioaccumulation of compounds different from elsewhere. The high temperatures and salt concentration may have influenced production of high levels of polyphenols to cope with environmental stress.

Table 3. Polyphenols, flavonoids and radical scavenging activity values of the mushrooms

Mushroom	TP GAE (mg/100g)	TF QE (mg/100g)	RSA IC ₅₀ (mg/100g)
<i>Button</i>	460.42±1.02	801.34±0.50	156.83±0.89
<i>Oyster-MFB</i>	675.56±0.97	890.87±0.90	61.86±0.56
<i>Oyster-YFB</i>	836.2±0.59	1129.75±0.33	62.64±0.32
<i>*Makunu ma mutitu</i>	798.57±1.03	730.20± 0.55	68.62±0.48
<i>*Mariondonik</i>	728.05±1.05	798.66±0.65	70.02±0.21
<i>#Obulando</i>	432.66±0.41	740.77±0.14	185.10±0.14
<i>#Olando</i>	773.43±1.54	726.36±0.20	205.05±0.05
<i>Oruka whole</i>	788.52±0.45	979.64±0.60	76.65±0.57
<i>Oruka cap</i>	1332.24±0.67	1511.08±0.85	58.07±0.67
<i>Oruka stipe</i>	872.57±0.90	648.20±0.79	59.59±0.60

<i>Muhama</i>	1543.22±1.22	921.30±1.02	68.29±0.45
<i>Joga utuwe</i>	1580.08±0.66	944.55±0.58	122.45±0.55
<i>Malombo</i>	1080.45±0.50	528.52±1.56	112.65±0.56
<i>Mkundu wa nyani</i>	947.95±0.75	463.50±0.46	116.25±0.85
<i>Joga kadzodzo</i>	1250.24±0.56	748.01±0.55	379.10±0.70
<i>Rerema</i>	1058.05±0.54	433.10±0.73	99.15±0.65
<i>Masikiro meruhe</i>	331.54±0.91	214.15±0.57	458.01±0.85

Values expressed as means, mg/100 g ± SD of triplicates on dry weight basis. Correlation coefficient between TP and RSA = 0.82;

TP = Total polyphenols; GAE=Gallic Acid Equivalent; TF = Total flavonoids; QE = Quercetin Equivalent; RSA = Radical scavenging activity; IC₅₀ = Inhibition concentration for 50%; YFB = Young fruit body; MFB = Mature fruit body;

*Makunu ma mutitu of Aberdares = same species with mariondonik of Mt Elgon; #Olando of Kisumu same species as Obulando of Kakamega.

4. Conclusion

The data obtained from this research clearly show that exotic and wild mushrooms contain phytochemical compounds that are necessary for a healthy body.

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