

Mineral composition, Antioxidants and Antimicrobial activities of freshwater algae (spirogyra genus) from Jomo Kenyatta University of Agriculture and Technology (JKUAT).

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Abstract: Dry biomass of algae is a good source of nutrients and biologically active substances, which in the recent years attracted the interest of the specialists in their search for natural, ecologically and healthy sound foods for the animals. The aim of the present study was to determine the mineral composition, the antioxidant and antimicrobial activities of the dry biomass of freshwater algae (spirogyra genus). The freshwater algae were collected in February and March 2010 at the pond of Jomo Kenyatta University of Agriculture and Technology (JKUAT). The Mineral content by acid digestion (HNO₃:HClO₄) in mg /100g was : 387.22 ± 38.72 Ca, 384.99 ± 39.17 Mg, 244.44±17.10 Fe, 2.53 ± 0.48 Cu, 7.25 ± 0.55 Zn, Phytochemical screening of methanolic and water extract showed the presence of alkaloids, steroids, flavonoids, tannins, terpenoids, but only the water extract showed the presence of total reducing sugars. The content of phenolic compounds and tannins was 10.63 ± 0.05g gallic acid/100g of extract and 0.399 ± 0.02g tannic acid/100g of dried sample and the level of flavonoids was 6.86 ± 0.14g Quercetin/100g of extract. The algal antioxidant activity expressed in a dose able to decrease the initial DPPH concentration by 50%, or IC₅₀ was 0.078 mg/ml. The antimicrobial activity of methanolic extract was efficient against *Escherichia coli* and *Candida albicans*. The water extract didn't show any activity. The freshwater algae used in this study were identified as *Spirogyra nitida* associated *Spirogyra weberi* both filamentous green algae. The mineral composition showed that they contain minerals which give the possibility of using them as food for fish and also by human as food supplement. The levels of Ca, Mg and Fe were very high as the *Spirulina* and *Chlorella* are used as food supplement. The strains showed a relative antioxidant and antimicrobial activities.

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1. Introduction

Algae are simple organisms that are mainly aquatic and microscopic. Spirogyra is a filamentous algae, its cells form long, thin strands that, in vast numbers, contribute to the familiar green, slimy 'blanket weed' in ponds. Seen under the microscope, each filament consists of an extensive chain of identical cells. *Spirogyra* is the most common genus of Zygnemataceae and member of the freshwater algae. It exhibits the greatest diversity of the 12 to 13 genera recognized in this family of green algae (Transeau 1951, Randhawa 1959, Kadlubowska 1972). More than 400 species are presently recognized based on morphological characteristics: filament width, chloroplast number, type of endwall, details of the conjugation process, and size, shape and ornamentation of zygospores. All algae contain proteins, carbohydrates and lipids in varying proportions. They contain chlorophyll and make their food by photosynthesis.

The aim of the present study is to determine the

mineral composition, the antioxidants and antimicrobial activities of the dry biomass of freshwater algae (spirogyra genus).

2. Materials and methods

2.1 Algal materials

The freshwater algae were collected from a pond in Jomo Kenyatta University of Agriculture and Technology (JKUAT) in small plastic container first for identification by Dr Lukhoba Catherine, taxonomist from University of Nairobi. Algal samples for analysis were collected using a small funnel of net attached to a metal frame, then air dried.

2.2 Methanolic Extraction

The powdered of dried algae mixed with methanol was allowed to stand for 72 hours with shaking. It was then filtered through Whatman filter paper No. 1 and distilled using rotary evaporator (Bibby Sterilin Ltd, RE 100B, UK) at 60°C. The resulting extracts were then subsequently labelled as methanol extracts and preserved in airtight containers

until further use (Alanis *et al.*, 2005).

2.3 Water extraction

Dried sample were mixed with hot distilled water in a conical flask for 1 hour. The mixture was first filtered through double layered muslin cloth and then centrifuged. The supernatant was then filtered through Whatman No.1 filter paper and then preserved aseptically in an airtight bottle at 5 °C for later use.

2.4 Minerals analysis

Samples for mineral analysis were dissolved in hydrochloric acid and analyzed through Atomic Absorption Spectrophotometer (AAS) following the procedures described by (AOAC, 1995); (Gupta, 1999).

2.5 Phytochemicals analysis

Qualitative analysis was carried out to ascertain the presence of the different phytochemicals activities as described by (Trease and Evans, 1989) and (Harborne, 1998) before quantitative analysis was done. The various extracts were tested for reducing sugars, terpenoids, steroids, flavonoids, tannins and alkaloids, total phenols.

2.6 Antioxidants activities

The radical-scavenging activity of the methanolic extract was determined using diphenyl picryl hydrazyl radical (DPPH) according to (Ayoola *et al.*, 2006).

2.7 Antimicrobial activities

The extracts were tested for antimicrobial activity using the Agar Well Diffusion Method (Ruiz, 2009).

2.8 Statistical analysis

Each data point was obtained by making at least 3 independent measurements. The results were expressed as mean \pm SD. Statistical analysis were done using Microsoft Excel.

3. Results and Discussion

3.1 Mineral content of dried algal biomass by acid digestion

The Figure 1 showed the results of minerals content from acid digestion. The Calcium showed the highest content for acid digestion, 387.22 ± 38.72 followed by 384.99 ± 39.17 mg/100g for Mg, then 244.44 ± 17.10 mg/100g and 39.84 ± 0.52 mg/100g for Fe, followed by 7.25 ± 0.55 mg/100g and 0.40 ± 0.02 mg/100g for Zn and finally 2.53 ± 0.48 mg/100g and 0.32 ± 0.04 mg/100g for Cu. The high value obtained of Ca from acid digestion is in range of other freshwater algae like *Spirulina* 700 mg/100g and *Chlorella* 266mg/100g (Mason, 2001). The value obtained for Mg is also in the range of *Spirulina* 400 mg/100g and *Chlorella* 333.3 mg/100g (Mason, 2001).

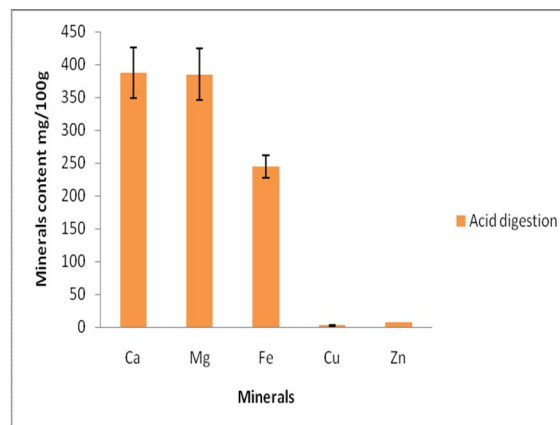


Figure 1: Mineral content of dried algal biomass by acid digestion

Acid digestion using acid mixture gives good results of minerals found in the dried algal biomass as shown in Figure 1 Calcium is one of the most important elements in the diet because it is a structural component of bones, teeth, and soft tissues and also essential in many of the body's metabolic processes. According to the results, calcium level are higher than other minerals in the *Spirogyra* genus but less in comparison with *Spirulina* as shown in table 1. The magnesium levels are as high as calcium content because the Magnesium is found in chlorophyll in green algae. It is even higher than other green algae (*Chlorella*) as shown in table 1. Iron is an essential component of haemoglobin, transporting oxygen in the blood to all parts of the body. It also plays a vital role in many metabolic reactions. Iron deficiency can cause anaemia resulting from low levels of haemoglobin in the blood. Iron deficiency is the most widespread mineral nutritional deficiency both in Britain and worldwide. The iron level are high (244 mg/100 g) which is more than the iron content of *Spirulina* a well know in food supplement as shown in table 1. It means that *spirogyra* can be a provider of iron supplement for food.

Table 1: Comparison of minerals levels in freshwater algae

Minerals	Freshwater algae		
	<i>Spirogyra</i> mg/100g	<i>Spirulina</i> ¹ mg/100g	<i>Chlorella</i> ¹ mg/100g
Ca	387.22 \pm 38.72	700	266.67
Mg	384.99 \pm 39.17	400	333.3
Fe	244.44 \pm 17.10	100	93
Zn	7.25 \pm 0.55	3	2.33
Cu	2.53 \pm 0.48	1.2	-

3.2 Phytochemical analysis of methanolic and aqueous extract

Qualitative evaluation of phytochemicals was aimed at simply identifying the presence of individual group of substances. The presence of phytochemical compounds suggests the pharmacological activities of algal biomass (table 1).

Phytochemicals act as antioxidants, suppressors of tumor growth, anti-mutagens, enzyme modulators, chemical inactivators, and free radical scavengers. It is also reported that they have anti-aging properties. Therefore, their presence in algal extracts may justify the potential medicinal value of the freshwater algae. Flavonoids and tannins have been reported to have antimicrobial and antioxidant properties Stephen *et al.* (2009).

The total flavonoid contents were 6.863 ± 0.14 g quercetin equivalent/100g of extract while the total tannins were formed to be 0.399 ± 0.02 g tannic acid/100g of dried sample. Total phenol compounds, as determined by Folin Ciocalteu method, are reported as gallic acid equivalents were 10.63 ± 0.05 g gallic acid/100 g of extracts.

Table 2: Phytochemicals screening of extracts

active principle	methanolic extract	aqueous extract
Alkaloids	+	+
Steroids	+	+
Flavonoids	+	+
Tannins	+	+
Terpenoids	+	+
Total reducing sugars	-	+

- : Absent

+: Present

The result obtained in the current study are in the same range with (Ghasemi *et al.*, 2009) who evaluated the total phenolic content of the *citrus spp.* samples (based on Folin Ciocalteu method) and flavonoids content (based on colorimetric $AlCl_3$ method). He found that the total phenolic content varied from 6.65 to 39.68 g gallic acid equivalent/100 g of extract while the flavonoids content varied from 0.03 to 3.11 g quercetin equivalent/100g of extract.

Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Van Acker *et al.*, 1996).

Alkaloids have proved to have pharmacological properties such as hypotensive activity, anticonvulsant activity, antiprotozoal, antimicrobial and antimalarial activities (Mallikharjuna *et al.*, 2007). They also have pronounced physiological effects particularly on the nervous system (Stephen *et al.*, 2009). Plants have the limitless ability to synthesize phenols or their derivatives. These phenolic compounds give the vegetable an astringent taste. They also bind proteins and may lower protein digestibility and quality as well as reducing the risk of heart disease and certain types of cancers.

3.3 Antioxidant activities

Free radical scavenging activity of methanolic extracts of dried algal biomass was confirmed in this study. Figure 2 illustrates the percentage of Inhibition of DPPH versus concentration curve of methanol extracts. These results were compared with the standard curve of ascorbic acid (Figure 2). The decrease in absorbance as the concentration increase was taken as a measure of the extent of radical scavenging activity (Kizhiyathu *et al.*, 2005).

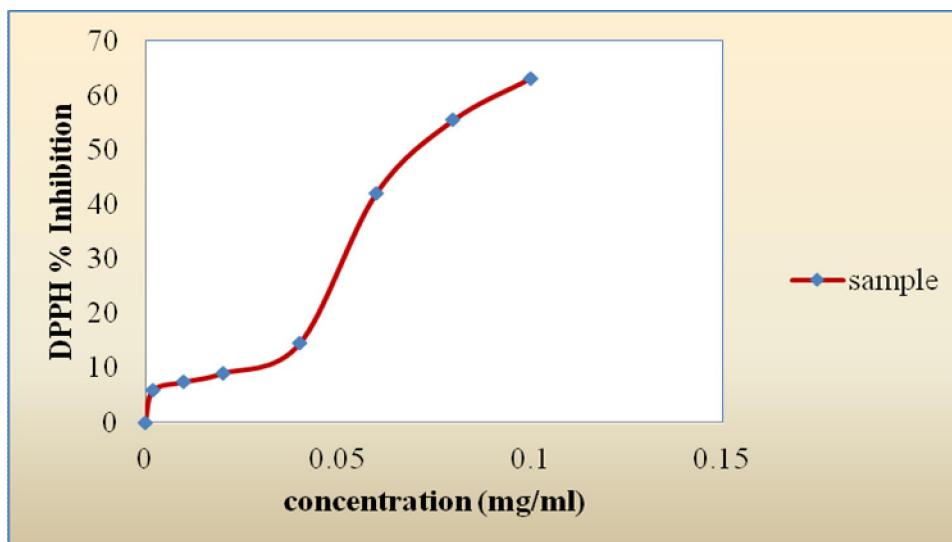


Figure 2: Free radical scavenging of methanolic extract.

The results of the free radical scavenging effect of *Spirogyra* showed an IC_{50} of the sample of 0.078 mg/ml (Figure 2) which is lower in comparison to IC_{50} of Ascorbic acid (0.008 mg/ml). The IC_{50} is the concentration of extract which corresponds to 50% of inhibition. The free radical scavenging activity is valued according to the percentage of inhibition of DPPH radical which is beyond 50% according to our results. The methanolic extract was found to have the maximum activity of 63.16 % at concentration of 100 μ g/ml. The result of the free radical scavenging effect of Ascorbic acid was 96.96% at a concentration of 20 μ g/ml. This good antioxidant activity of *Spirogyra* might be attributed to the presence of phytochemicals such as flavonoids (Kumar *et al*, 2005). Phenolic compounds are commonly found in plants, including algae, and have been reported to have a wide range of biological activities including antioxidant properties (Cox *et al*, 2010).

3.4 Antimicrobial activity

The antimicrobial activity of methanolic extract and aqueous extract was carried out by determining the zone of inhibition diameter. Only methanolic extract showed zone of inhibition against *E.coli* and *C. albicans* as shown on plates 1 and 2. The two extract did not show any inhibition against *S. aureus* as shown in Figure 3.

The highest zone of inhibition of methanolic extract against *E.coli* was 19.83 mm which corresponds to the concentration of 40 mg/ml. For *C. albicans*, the highest zone of inhibition was 15 mm which corresponds to the concentration of 80 mg/ml. In comparison to the positive controller, zone of inhibition were found by most of them against *E. coli*. Only two of them were active against *S. aureus*. Four out of eight of controllers show zone of inhibition against candida albicans. For the negative controls (water and methanol), there was no inhibition zone as shown on plates 3 and 4. It means that the inhibition of methanolic extract shown against *e-coli* and *C. albicans* was due to the extracts and not of the solvent.

The antimicrobial activity of *Spirogyra* genus is in accordance with other freshwater algae as *Spirulina* and *Chlorella* (Mason, 2001). Algae are generally known to have antimicrobial activities and the result showed that the methanol extract possessed a strong antimicrobial activity against gram-positive (*E. coli*) and fungi (*C. albicans*) as did antibiotics (standards) (plate 5 and 6). However, the exact mechanism and the compound responsible for the antimicrobial activities were not investigated.

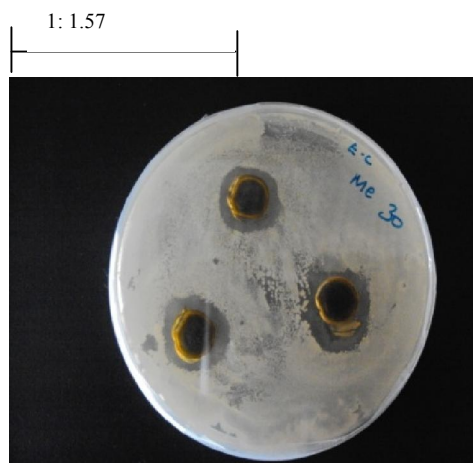


Plate 1 : Inhibition of methanolic extract (30 mg/ml) against *E. coli*

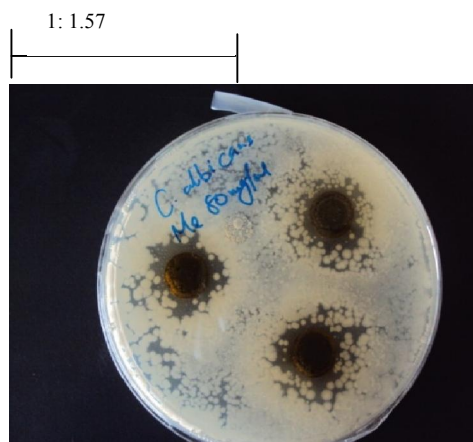


Plate 2: Inhibition of Methanolic extract (80 mg/ml) against *C. albicans*

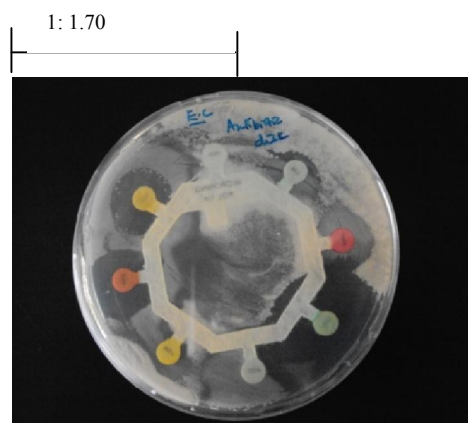


Plate 3 : Inhibition of positive control against *E. coli*.



Plate 4 :Inhibition of positive control against *C. albicans*

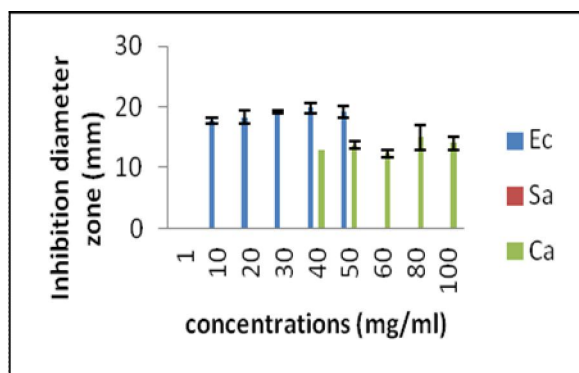


Figure 3: Antimicrobial activity of methanolic extract

Conclusion

The freshwater algae used in this study were identified as *Spirogyra nitida* associated *Spirogyra weberi* both filamentous green algae. The mineral composition showed that they contain minerals which give the possibility of using them as food for fish and also by human as food supplement. The levels of Ca, Mg and Fe were very high as the *Spirulina* and *Chlorella* are used as food supplement.

The antioxidant and antibacterial activities of the freshwater algae found in this study were explained by the presence of fatty acid and phytochemicals such as flavonoids, tannins which are phenolic compounds.

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