Phytochemical Composition and Antioxidant Property of *Mandillo, Crassocephalum macropappum* (Sch.Bip. ex. A.Rich.) S.Moore

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**ABSTRACT:** The object of this study was to analyze the major bioactive components and to evaluate biological activity of *Mandillo [Crassocephalum macropappum* (Sch.Bip. ex. A.Rich.) S. Moore], an Ethiopian endemic herbaceous plant. The stem, leaf, and aerial parts of this plant were separately extracted using different solvents before which various biological assays were performed. The ethanolic extract of aerial part showed the highest total phenolic and flavonoid contents (101.48 mg gallic acid equivalents/g and 293.25 mg quercetin equivalent/g, respectively). Interestingly, a phytochemical screening assay revealed the presence of saponins, tannins, anthraquinones, steroids, terpenoids, and flavonoids in the aerial part. The aerial part was also shown to have a strong 2,2-diphenyl-1-picryl hydrazyl scavenging potential (IC₅₀ ≤ 100 μg/mL) and a promising protective activity against oxidative DNA damage. Thus, the results of the present study reveal *Mandillo* contains highly bioactive components, and these properties may be as an antioxidant and to prevent oxidative DNA damage.

**Keywords:** antioxidant activity, *Mandillo*, phytochemical composition, DNA damage

**INTRODUCTION**

Human health may be impacted by chemical substances presents in plants, which produce physiological actions on the human body (Ooi et al., 2012). The most important of these plant bioactive constituents are alkaloids, tannins, flavonoids, phenolic compounds, saponins, anthraquinones, glycosides, steroids, and cardiac glycoside, in addition to other bioactive compounds, have a large importance in human drugs and food (Oloyede and Ogunlade, 2013; Wu et al., 2009).

According to Proestos et al. (2013), the use of certain plant products in food processing is recommended to minimize the undesirable effects of synthetic food preservatives in human health. Aromatic plants are well known as natural food preservatives due to their antioxidant and antimicrobial properties (Wu et al., 2009), which arises from their high phenolic contents and prevent food degradation (Alsabri et al., 2012; Proestos et al., 2013). A strong positive correlation between antioxidant activity and the contents of phenolic acids was also studied by Vladimir-Knežević et al. (2011); this study showed that antioxidants can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals. Antioxidants may also play an important role in human health, through adding flavor to food, without the addition of extra ingredients such as excess fat or salt.

In Ethiopia, different plant parts are used as food preservatives, or flavoring agents to improve organoleptic properties. The stem of *Mandillo [Crassocephalum macropappum* (Sch.Bip. ex. A.Rich.) S. Moore] is a herb added to *Enset* fermentation processes, believed to shorten the fermentation period, to improve sensory qualities, and to increase the shelf-life of *Kocho*, which has been in use by Shekacho society for a long time. Despite its traditional use as a food preservative, there are no prior data on phytochemical profile and antioxidant activity of *Mandillo*. This study therefore aimed to evaluate the phytochemical content and antioxidant of activities of *Mandillo* extracts.
MATERIALS AND METHODS

Plant material and extraction
Fresh Mandillo was collected on November 2015 at Masha, Sheka, Southern Nation Nationalities and Peoples Regional State of Ethiopia. The plant samples were grouped into stem, leaf, and aerial parts. The three parts were dried at room temperature under shade for three days. The dried samples were powdered in a blender. Moisture contents of fresh and dried samples were analyzed in a drying oven (DHG-9055A, Zenith Lab Inc., CA, USA) at 105°C until a constant weight was obtained. The stock samples were kept in air tight bottles at 4°C for future extraction (Sibanda and Okoh, 2008).

The plant materials were extracted following the procedure described by Sultana et al. (2009) and Eom et al. (2008). The powder of the parts of Mandillo were extracted three times by incubating 100 g sample in 1,000 mL of 70% ethanol on an orbital shaker at 130 rpm at 40°C for 24 h. The extracts were filtered using Whatman No. 1 filter paper (Sigma-Aldrich Co., St. Louis, MO, USA), and concentrated by a rotary evaporator (RE300; Wolf Laboratories Ltd., York, UK) at 40°C. The crude extracts were kept at 4°C for further study.

Qualitative phytochemical analysis
Qualitative phytochemical analysis was carried out using the methods described by Tiwari et al. (2011), Akinyemi et al. (2005) and Sofowora (1993). Extracts were dissolved in diluted hydrochloric acid and then filtered. The presence of alkaloids was examined by Wagner’s test. Filtrates were treated with iodine in potassium iodide (Wagner’s reagent), following which a brown to reddish precipitate indicated the presence of saponins. Gelatin solution (1%) containing sodium carbonate was added to the extracts, following incubation for 30 min at room temperature, comparative to the blank sample. The total flavonoid content was determined following the equation: $y=23.95x+0.111$; $R^2=0.991$, through using a quercetin standard curve (Sigma-Aldrich Co.) ranging from concentrations of 0.001 ~ 40 µg/mL dissolved in methanol. The total phenolic content was calculated using the following equation: $y=11.25x+0.068$; $R^2=0.998$. The values were expressed as gallic acid equivalents (GAE) in milligrams per gram of dry material (mg GAE/g).

Determination of total phenolic contents
Total phenolic contents were determined according to the Folin-Ciocalteu’s procedure as described by Alimpić et al. (2014). The stock Mandillo extract solutions were diluted with 0.1 mg/mL in methanol, and 100 µL was added to test tubes in triplicates. Folin-Ciocalteu’s reagent (Sigma-Aldrich Co.) (1 mL; diluted 1:10) was dissolved in deionized water and added to each test tube. Sodium carbonate (1 mL; 7.5% reagent) was then added to the mixture. The tubes were vortexed and incubated at 25°C for 90 min. Absorbance was measured at 765 nm by an UV/VIS Lambda 950 (Perkin Elmer, Liantrisant, UK). The total phenolic content was determined using a standard curve of gallic acid (SD Fine-Chem Ltd., Mumbai, India), with concentrations ranging from 0.001 ~ 1.00 mg/mL dissolved in methanol. The total phenolic content was calculated using the following equation: $y=11.25x+0.068$; $R^2=0.998$. The values were expressed as gallic acid equivalents (GAE) in milligrams per gram of dry material (mg GAE/g).

Determination of total flavonoid contents
Total flavonoid contents were determined using the method described by Sultana et al. (2009). Plant extract stock solutions (1 mg/mL) were prepared, and 2 mL of each solution was added to 2 mL of 2% aluminum chloride. Methanol (2 mL) was used as a blank. The absorbance of each sample was measured at $\lambda_{max}$ 415 nm following incubation for 30 min at room temperature, comparative to the blank sample. The total flavonoid content was determined following the equation: $y=23.95x+0.111$; $R^2=0.991$, through using a quercetin standard curve (Sigma-Aldrich Co.) ranging from concentrations of 0.001 ~ 40 µg/mL dissolved in 2 mL of 2% aluminum chloride, in triplicate. The result was expressed as milligram of quercetin equivalent (QE) per gram of dry samples (mg QE/g).

Evaluation of antioxidant scavenging capacity by 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay
Antioxidant scavenging capacity was analyzed according to the procedure described by Oloyede and Ogunlade (2013), with slight modification. Extracts were mixed with methanol to prepare the stock solution at a concentration of 1 mg/mL. Aliquots of 5, 10, 20, 35, 50, and 60 µL added to test tubes in triplicate. Serial dilution of the solution was carried out by adding 995, 990, 980, 965, 950, and 940 µL of methanol, respectively, to leave a final volume of 1 mL. The final concentrations were calculated as 0.005, 0.01, 0.02, 0.035, 0.05, and 0.06 mg/mL, respectively. Two mL of freshly prepared DPPH solution (0.06% w/v) in methanol was added to each test tube.
The reaction mixtures and the control (2 mL; DPPH in methanol) were vortexed and incubated at room temperature in the dark for 30 min. The absorbance was measured at $\lambda_{\text{max}}$ 517 nm using a spectrophotometer. Ascorbic acid was used as the reference compound. The control was prepared by adding 1,000 $\mu$L of methanol to 2 mL of DPPH solution. The DPPH radical scavenging capacity of the plant extracts was calculated as follow:

$$\text{DPPH radical scavenging capacity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

### Evaluation of DNA-protective activity

The DNA protective activity of Mandillo extracts was evaluated relative to DNA-damaging chemicals according to the method of Kim et al. (2012) as cited by Rafiquzzaman et al. (2013). Hydroxyl radicals were generated using a mixture of 30 $\mu$L of ascorbic acid (10 mM final concentration) and 1 $\mu$L of copper sulfate (II) (1 mM final concentration). Bacteriophage $\lambda$ DNA (40 $\mu$L, 0.1 $\mu$g/mL) was exposed to the solution in both the absence and presence of Mandillo extract (100 $\mu$L, 1 mg/mL). The mixture was incubated at 37°C for 1 h after which the samples were loaded onto 1% agarose gels and the fragments were separated by agarose gel electrophoresis (Rafiquzzaman et al., 2013). The result was compared to the control group.

### Statistical analyses

All experiments were carried out in triplicate and the results were expressed as mean $\pm$ standard deviation (SD). Statistical analyses were carried out with SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) by using one-way ANOVA followed by Duncan’s multiple range test ($P<0.05$).

### RESULTS AND DISCUSSION

#### Phytochemical secondary metabolites in Mandillo extracts

We conducted a preliminary qualitative phytochemical analysis to identify the secondary metabolites present in Mandillo extracts. Phytochemical analysis revealed that ethanol extracts of Mandillo leaf, stem, and aerial parts contained numerous bioactive compounds, including anthraquinones, flavonoids, saponins, steroids, tannins, and terpenoids (Table 1). These secondary metabolites possess many biological properties, such as antioxidant, antitumor and anti-inflammatory activities, suggesting that Mandillo may have many medicinal applications (Sengutuvan et al., 2014). However, alkaloids (an abundant secondary plant metabolite) were not detected in Mandillo extracts (Chen et al., 2014; Compean and Ynalvez, 2014).

#### Quantification of total phenolic and flavonoid compounds in Mandillo extracts

The total phenolic and flavonoid contents of Mandillo ethanolic extracts were determined (Table 2). Aerial extract of Mandillo contained the highest phenolic contents (101.48 $\pm$9.11 mg GAE/g) and flavonoid content (293.25 $\pm$3.48 mg QE/g) of the three parts, followed by leaf extracts and stem extracts. The phenolic and flavonoid contents of the stem extracts were relatively low compared to those of the aerial and the leaf extracts. The phenolic and flavonoid contents of aerial extract were 2.9-fold and 6.2-fold higher than those of stem extracts, respectively.

In contrary to the current report, most plants exhibit higher phenolic and flavonoid contents in their leaves and stems than their roots, indicating that the Mandillo plant may be an unique organism with un-known properties (Chai and Wong, 2012; Silva-Beltrán et al., 2015).

#### Antioxidant scavenging potential of DPPH radical activity

The free radical-scavenging potential of Mandillo extracts showed remarkable DPPH radical scavenging abilities compared to the control ascorbic acid (Table 3). Generally, the radical scavenging activity of Mandillo extracts were shown to be dose-dependent. Leaf and stem extracts showed moderately higher DPPH scavenging activities than those of aerial extracts at a concentration of 0.05 $\sim$ 0.10 mg/mL, whereas no significant differences were observed at concentrations above 0.50 mg/mL.

Extracts containing higher phenolic and flavonoid contents exhibit higher antioxidant effects since polypheno-
lic compounds possess antioxidant activity (Eom et al., 2011; Jiménez-Escrig et al., 2001). In contrary to these reports, Mandillo extracts in this study exhibited different patterns. In low concentrations (0.05 to 0.1 mg/mL), stem extracts showed higher antioxidant activities than those of aerial extracts, despite the stem extract contained the lowest phenolic and flavonoid contents. These results suggest that the Mandillo extracts may possess a novel compound acting as a major antioxidant substance. There are many positive correlations between phenolic contents and antioxidant capacity (DPPH method), whereas a few studies also report that this is not the case. Reihani and Azhar (2012) previously reported that there is no significant correlation between antioxidant activity and phenolic compounds, which may be due to steric hindrance or the presence of other reducing agents.

DPPH radical scavenging capacity is widely used to evaluate antioxidant activity of natural extracts (Chen et al., 2014; Oloyede and Ogunlade, 2013). In this study, Mandillo extracts exhibited significant scavenging potential at concentrations of 0.5 mg/mL when comparable to those of the natural standard antioxidant, ascorbic acid. Thus, these extracts possess strong antioxidative activities through inhibiting or preventing the oxidation of free radicals as a result through radical scavenging (Vladimir-Knežević et al., 2011).

Table 3. DPPH scavenging activity of Mandillo ethanolic extracts

| Conc. (mg/mL) | Ascorbic acid | Stem | Leaf | Aerial root |
|--------------|---------------|------|------|-------------|
| 0.05         | 96.11±0.10^aD | 65.81±0.02^bC | 63.13±0.07^bA | 52.09±0.03^aB |
| 0.10         | 97.48±0.30^aD | 72.67±0.03^bB | 78.22±0.20^cA | 62.15±0.04^dB |
| 0.20         | 97.54±0.07^aD | 78.93±0.11^bA | 93.09±0.02^cB | 92.25±0.03^aB |
| 0.35         | 97.59±0.01^aD | 89.20±0.05^dA | 92.30±0.04^eB | 93.85±0.05^fC |
| 0.50         | 97.70±0.20^aE | 93.62±0.03^fA | 91.61±0.02^eB | 93.66±0.03^dA |
| 0.60         | 97.83±0.08^aE | 93.52±0.01^gB | 91.34±0.04^fA | 93.42±0.02^eB |

Means with different small letters (a-f) within each column and capital letters (A-D) within each row indicate significant differences by Duncan’s multiple range test (P<0.05).

In this study, we evaluated the DNA-protective effects of Mandillo extracts against oxidation induced by oxidative stress. Among of Mandillo extracts, the aerial extract was chosen for the further study, through considering the phenolic and flavonoid contents and antioxidant activity of each extract. As expected, λ DNA were damaged by oxidative stress generated by Cu (II)-ascorbic acid (Fig. 1, lane 2). However, λ DNA treated with Mandillo extracts (Fig. 1, lane 3) showed clear bands, similar to the λ DNA control (Fig. 1, lane 1) despite exposure of the λ DNA to oxidative stress. According to Sultana et al. (2009), dietary supplementation of flavonoid compounds reduces oxidative damage to cell membrane lipids, proteins, and nucleic acids. This protective mechanism is due to the strong quenching property of free radicals, which is in line with the DNA protective effect recorded in the present study.

In this study, we analyzed the major photochemical compounds of Mandillo extracts, which is used as a natural food preservative in Ethiopia. We also evaluated the antioxidant capacity of Mandillo, including its DPPH radical scavenging activity and ability to protect DNA from damage caused by oxidative stress. Our results strongly suggest that this African herb possesses strong antioxidant activity, which may be useful as an antioxidant food or food ingredient.
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