Antioxidant Activity of the *Hulu-mur*

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By

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Dedication

I dedicate this work to my parents...
# Contents

Acknowledgements ........................................................................................................... i
English abstract ................................................................................................................ ii
Arabic abstract ................................................................................................................... iii
List of figures ..................................................................................................................... vi
List of tables ...................................................................................................................... vii

CHAPTER ONE ................................................................................................................... 1

INTRODUCTION AND LITERATURE REVIEW .................................................................. 1
1.1 Introduction ..................................................................................................................... 1
1.2 Indigenous fermented foods of the Sudan ........................................................................ 1
  1.2.1 Classification of Sudan’s fermented foods ............................................................... 2
  1.2.2 Fermented foods in nutrition .................................................................................. 4
1.3 Preparation of Hula-mur ............................................................................................... 6
1.4 Antioxidant property ...................................................................................................... 8
1.5 Objectives of the work ................................................................................................... 9

CHAPTER TWO ................................................................................................................ 10

MATERIALS AND METHODS ............................................................................................ 10
2.1 Materials ....................................................................................................................... 10
2.2 Methods ....................................................................................................................... 10
  2.2.1 Preparation of extracts .......................................................................................... 10
  2.2.2 Phytochemistry ...................................................................................................... 10
  2.2.2.1 Test for flavonoids ............................................................................................ 11
  2.2.2.3 Test for alkaloids ............................................................................................. 11
  2.2.2.5 Test for anthraquinones .................................................................................. 11
2.2.3 Antioxidant activity studies ..................................................................................... 12
  2.2.3.1 DPPH radical-scavenging test .......................................................................... 12

CHAPTER THREE .............................................................................................................. 14

RESULTS AND DISCUSSION ......................................................................................... 14
3.1 Antioxidant activity ..................................................................................................... 14
  3.1.1 DPPH radical scavenging activity ........................................................................ 14
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Abstract

_Hulu-mur_ is a food prepared for the special occasion of _siam Ramadan_. Almost every Muslim household prepares _hulu-mur_ during the few weeks just preceding the lunar month of Ramadan.

In this study, results showed that water and ethanol extracts from the _hulu-mur_ had moderate antioxidant activity on DPPH scavenging system.

Phytochemical screening showed that both extracts contained flavanoids, tannins and saponins.

Further pharmacological studies will be necessary to establish more information on the benefit use of _hulu-mur_ as a drink.
CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The nutritional health and well-being of humans are entirely dependent on plant foods. Plants are critical components of the dietary food chain in that they provide almost all essential mineral and organic nutrients to humans either directly, or indirectly when plants are consumed by animals, which are then consumed by humans.

1.2 Indigenous fermented foods of the Sudan

The Sudan is a melange of races and cultures and fermented foods hold an important position in the nutrition of the Sudanese.

Fermented foods hold a central position in the nutrition of the Sudanese. Dirar (1994) pointed out the general features of these foods and the general characteristics of the procedures followed in their preparations as follows: First, the fermented foods of the Sudan are numerous. Actually the Sudan has the greatest number of fermented foods and the greatest diversity of them in Africa and the Middle East. It has been reported that Africa has over 30 fermented foods (Odunfa, 1985, 1988) and that a country such as Nigeria has over 20 (Odunfa, 1981). The Sudan alone, however, has over 80 different foods and beverages that are distinctly different from each other. The wide diversity of these foods means that the country may have been a centre for the spread of these foods to other African countries.

A second feature of the Sudan's indigenous fermented food is that about 90% of them are of true black African origin but one should not be taken to the exclusion of the Arab influence; one major feature of these foods is that they
now carry Arabic names. Also, a diverse array of starting food materials is used to produce the fermented products. Almost any edible material is subjected to fermentation in one form or other. Sorghum, millet, other cereals, milk, honey, meat, fat, fish, frogs, caterpillars, locusts, bones, skins, hooves, bile, cow urine, press cakes, wild leaves, etc. are all material for fermentation. Again here, the Afro-Arab culture is prominent, as the stating material for fermentation ranges from such typical African foods as cassava and finger millet to such typical Arab foods as camel milk and date fruit.

1.2.1 Classification of Sudan's fermented Foods

The literature of the fermented foods shows a lack of standardization in their classification. However, the most accepted classifications are those lands according to the kinds of microorganisms involved in the fermentation. Ko (1982) and Yokotsuka (1982) divided the traditional fermented foods into six categorized:

1. Alcoholic beverages fermented with yeasts
2. Vinegars fermented with Acetobacter.
3. Fermented milk products treated with Lactobacilli.
4. Picklis fermented with Lactobacilli.
5. Fermented fish or meat treated with enzymes together with Lactobacilli.
6. Fermented plants proteins treated with moulds, with or without Lactobacilli and yeasts.

This classification scheme is based on the processes and microbes involved in the fermentations were found inapplicable to most African fermented foods, including those of the Sudan.
Campbel-Platt (1987) divided fermented foods into nine classes:

1. Beverages.
2. Cereal products.
3. Dairy products.
4. Fish products.
5. Fruit and vegetable products.
8. Starch crop products.

This classification is based on commodity.

Odunfa (1988) grouped the indigenous fermented foods of Africa on a similar basis into five broad categories:

1. Fermented starchy roots.
2. Fermented cereals.
3. Alcoholic beverages.
4. Fermented vegetable proteins.
5. Fermented animal protein.

Kuboye (1985) classified the traditional fermented foods and beverages of Nigeria into four categories also based on commodity:

2. Cereals.
3. Legumes.
The Sudanese traditionally classify their food not on the basis of microorganism or commodity but on a functional basis. Any food should is supposed to fulfill a certain function in the nutritional process and should fall thus in one or other of four distinct food groups of which miscellaneous is not one. These four groups are:

1. Kissar (staples).
2. Milhat (sauces and relishes for the staples).
3. Marayiss (beers and other alcoholic drinks).
4. Akil-munaasabay (food for special occasions).

The Kissar include porridges and breads, such as aceda and kissra respectively. Made from sorghum or bulrush millet, these staple dishes comprise the backbone of human nutrition.

The milhat (singular mullah) are numerous. Sauces and staples always go together (e.g. Kissra bila mullah)

The marayiss (singular merissa) comprise more than 30 types of opaque beer in addition to a clear beer, date wines and meads.

The most common occasions that call for the making of special food or drinks (akil-munaasabay) are wedding, the fasting moth of Ramadan etc..(Dirar 19)

1.2.2 Fermented foods in nutrition

The advantage of food fermentation have been dealt with by many authors (Van Veen et al., 1968; Whitaker, 1976; Bouvy, 1979; Hesselting and Wang, 1979; Steinkraus, 1982; Erichsen, 1983). These advantages may be summarized as follows:

1. Fermentation is a method of preservation.

2. Fermentation may destroy undesirable factors in the raw product.
3. The fermented food may have an enhanced nutritional value and digestibility.

4. Fermented food may have a better flavour than the raw products.

5. Fermentation may be used to salvage some products that could not otherwise be used for food.

6. Fermentation may improve the appearance of some foods.

7. Fermentation reduces cooking time.

8. Some fermented foods, e.g. beers and wines, are enjoyable to some people.

9. Fermented food may be safer.

10. Fermentation may improve the texture of the food.

11. Fermentation helps solubilize some food components.

12. The methods used are inexpensive.

13. The techniques are simple and well understood.

14. The process involves little waste.

15. Products are well established and acceptable.

Hesseltine (1979) enumerated some of the special merits and features of the fermentations characterizing the fermented food product of Africa and the Middle East as follows:

1. The preservation of these fermented foods is by the action of organic acids and low pH.

2. In the Arab world this is usually combined with sun-drying of the fermented product.

3. Dried, fermented foods have a small volume and are therefore easily transported (e.g. by nomads).
4. The foods may keep for months or even years and thus help in cases of drought and famine.

5. The foods have a high (dietary) fibre content (and are therefore good for the functioning of gut).

6. They have varied flavour because of varied ingredients, mixed microorganisms and addition of spices and salt.

7. All are self-inoculated fermentations, the microorganisms being typically those present in or on one of the ingredients.

8. Most of fermentations are based on cereals.

9. Most of fermentations are carried in a liquid menstrum, in contrast to the Far Eastern fermentations which use moist solid substrates.

10. Yeast and bacteria (mainly lactic bacteria) are the only organisms that carry out fermentation; moulds are present only as chance contaminants.

The fermentation time is short, a matter of hours, not weeks or months

1.3 Preparation of Hulu-mur

Hulu-mur is food prepared for the special occasion of siam Ramadan. Almost every Muslim household prepares hulu-mur during the few weeks just preceding the lunar month of Ramadan. The unmistakable caramelized fragrance of hulu-mur in the alleys of villages and towns ushers in the imminent approach of the holy month.

Feterita sorghum is being used as the source of the malt and the flour needed for hulu-mur production. The recipe calls for the use of malt and grain in the ratios of 1:1 and 1:2 respectively. Todays, as well as in the past, the best hulu-mur is said to be produced when equal amounts of malt and grain are used.
The malt and the grain are separately milled into fine flour. The flour from the ingeminated grain is then cooked directly into a rather thick porridge then the hot porridge is transferred from the cooking pot to another pot in which fermentation and amylolysis will take place. The malt flour is now added to the porridge while the latter is still hot. The two ingredients are thoroughly mixed with a wooden stirrer. During this process, liquefaction of the porridge occurs very rapidly so that within minutes is already sweetish and fluid even though water has not been added to it.

The liquefied mixture is left to ferment in a warm corner of the house or out in the sun in a large container, leaving a head space to accommodate the expected increase in batter volume ensuing from fermentation gases. No starter from any kind is used, the correct organisms being provided by the natural flora of the malt flour and to a lesser extent, by the flora of the utensils used.

While fermentation occurs, an assortment of selected spices is ground to powder. The most basic of these are ginger (*Zingiber officinale*), cinnamon (*Cinnamomum zeylanicum*) and galangal (*Alpinia officinarum*). Frequently, other spices such as coriander seeds (*Coriandrum sativum*), cumin (*Cuminum cyminum*), black cumin (*Nigella sativa*) and black pepper (*Piper nigrum*) are used in addition to the basic three spices above. Cloves (*Eugenia caryophyllus*) and cardamom (*Elettaria cardamomum*) are sometimes also used. Some affluent households may add also tamarind water, red roselle calices water and date slurry. The ground spices and the liquid additives are mixed into the fermenting batter at a specified stage. When a 1:1 ratio of malt to grain is used, additives are mixed in after fermentation has proceeded for 12 hours. When a 1:2 ratio is used, the extras are added after 24 hours of fermentation.

After thorough mixing, the whole mixture is further fermented so that the total fermentation time is 24-36 hours and not more as has been reported in the literature (El-Gendy, 1983). The batter is now ready for baking; it is sour and
sweetish, and has a red-brown colour, and a strong bouquet of malt and spices. An important precaution often mentioned is that hands should never be introduced into the fermenting batter until the time it is backed.

1.4 Antioxidant property

Currently antioxidant activity is one of the most common in vitro parameter used to assess or predict potential benefits of plant phytochemical compounds. Antioxidants, which scavenge active oxygen species (free radicals), are found in a variety of foodstuffs and are commonly referred to as scavengers. (Beckman et al., 1990; Bohme et al., 1993). Free radicals are known to be the major cause of various chronic and degenerative disease, inflammation, stroke, diabetes mellitus, tissue injury skin, aging and cancer (Beckman, 1994; Cheng et al., 2003). Reactive oxygen species (ROS) include free radicals such as $O_2^-$ (superoxide anion), OH• (hydroxyl radical), $H_2O_2$ (hydrogen peroxide) and $\frac{1}{2}O_2$ (single oxygen) can cause cellular injuries and initiate peroxidation of polyunsaturated fatty acids in biological membranes (Halliwell, 1997). The tissue injury caused by ROS may include DNA damage (Halliwell, 1997), protein damage (Bartold et al., 1984) and oxidation of important enzymes (Varani et al., 1985) in the human body. These events could consequently lead to the occurrence of various free radical-related diseases.

The need to replace synthetic antioxidants with natural and properly safe ones, together with the interest of food industry and preventive medicine in the development of bioactive naturally-occurring antioxidants, has fostered research on the screening of plant sources, especially the inexpensive residue sources from agricultural industries (Moure et al., 2001). There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the
incidence of chronic diseases including heart disease and some cancers (Miller, 2000).

1.5 Objectives of the work

1. To evaluate the antioxidant activity of the *hulu-mur*.
2. Preliminary phytochemical screening of secondary metabolites in *hulu-mur*.
CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

Flakes of *Hulu-mur* (250 g) were obtained from home and ground to fine powder using a motor and pistil.

2.2 Methods

2.2.1 Preparation of extracts

Two types of extracts were prepared:

1. Ethanol extract

The powdered *Hulu-mur* (100 g) was repeatedly extracted (3 times) with 1 L of ethanol with stirring at an interval of 4 h for 24 h. After filtration, the solvent was evaporated under reduced pressure in a rotary evaporator at 45 ºC to afford the ethanol crude extract (8 g).

2. Water extract

Water extract was prepared by simple maceration of 100 g of powdered *Hulu-mur* in 100 mL of distilled water maintained at ambient temperature for 4 h. Extract was filtered on filter paper and left to dry under hood to yield 7.5 g.

2.2.2 Phytochemistry

Phytochemical screening for the identification of major groups of chemical constituents using standard procedures (Harborne (1973), Trease and Evans (1989)). The phytochemical components analysed were alkaloids, saponins, flavonoids, tannins, anthraquinones and cardiac glycosides.
2.2.2.1 Test for flavonoids

Plant sample (0.5 g) was suspended in 5 mL of water and 2.5 mL of methanol added to it. After filtration 1 mL of NaOH 10% was added to 1 mL of the filtrate. The appearance of a yellow precipitate indicated the presence of flavonoids.

2.2.2.2 Test for tannins

Water (7.5 mL) was added to plant extract (1 g) and heated in a water bath. It was then filtered upon cooling. Few drops of iron III chloride (FeCl3) 0.5% were added to 2 mL of the filtrate. The appearance of a green or dark-blue precipitate indicated the presence of tannins.

2.2.2.3 Test for alkaloids

Sample (2 g) was heated in a test tube containing 25 mL of HCl (1%) for 15 min in a boiling water bath. The suspension was then filtered and 5 drops of Meyer's reagent (potassium tetraiodomcurate) were added into the filtrate (1 mL). The formation of a precipitate indicated the presence of alkaloids.

2.2.2.4 Test for saponins

A quantity of 0.5 g of extract was introduced into a test tube containing 7.5 mL of distilled water and the mixture heated for 5 min in a boiling water bath. The solution was then filtered and cooled to room temperature. Five milliliters of the filtrate was introduced into a test tube and agitated for 10 seconds. The formation of persistent foam indicated the presence of saponins.

2.2.2.5 Test for anthraquinones

The sample (0.5 g) was boiled with 1 mL of 10% H2SO4 and filtered. 2.5 mL of benzene was added to the filtrate and shaken. The benzene layer was transferred with a pipette to a test-tube and then 2 mL of 10% ammonia solution
was added. The presence of a pink or red–violet colour in the lower ammonia phase indicated the presence of anthraquinones.

2.2.3 Antioxidant activity studies

In these experiments two complementary methods of free radical scavenging activity; DPPH radical-scavenging test and Ferric reducing power (FRAP) assay were used.

2.2.3.1 DPPH radical-scavenging test

The determination of the free radical scavenging activity of each of the crude extract was carried out using the DPPH (1, 1-diphenyl-2- picrylhydrazyl) assay as described by Villano et al. (2007) Test samples were dissolved separately in methanol to get test solution of 1 mg/mL. Series of extract solutions of different concentrations (1, 5, 10, 20, 40, 60, 80 and 100 μg/mL) were prepared by diluting with methanol. Assays were performed in 96-well, microtiter plates. One hundred forty μL of 0.6.10⁻⁶ mol/L DPPH were added to each well containing 70 μL of sample. The mixture was shaken vigorously and left to stand for 30 minutes in dark at room temperature. The absorbance was measured spectrophotometrically at 517 nm using a microtiter plate reader (Synergy HT Biotek®, logiciel GEN5). Blank was done in the same way using methanol and sample without DPPH and control was done in the same way but using DPPH and methanol without sample. The same procedure was applied for the positive controls trolox.

The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) =

\[ 1 - \frac{(\text{Abs}_\text{sample} - \text{Abs}_\text{blank})}{(\text{Abs}_\text{control})} \] \times 100

Where;

Abs_{sample} is the absorbance of DPPH radical + sample;
Abs\textsubscript{blank} is the absorbance of sample + methanol;

Abs\textsubscript{control} is the absorbance of DPPH radical + methanol.
CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Antioxidant activity

3.1.1 DPPH radical scavenging activity

DPPH radical scavenging activity of the water and ethanol extracts of *Hulu-mur* was carried out. The scavenging effects of these compounds were shown in Fig.1. Results showed that both extracts had moderate scavenging activity when compared with two controls (ascorbic acid and propylgallate). Moreover, the ethanol extract gave higher scavenging activity than the water indicating that it might contain more active ingredients.

However, antioxidant activity can be determined with different methods. Beside the DPPH test system there is the ABTS-based test systems. Wang *et al.* (1998) found that some compounds/extracts which have ABTS scavenging activity did not show DPPH scavenging activity. This suggests that the *hulu-mur* extracts might reveal higher antioxidant activity on other antioxidant systems.

3.2 Phytochemical screening

3-2-1 Qualitative analysis of secondary metabolites

Analysis of different classes of major secondary metabolites presents in water and ethanol extracts of the *Hulu-mur* was carried out using the method described by Harbone (1973). Results are presented in Table 1. It was clear that both extracts revealed the same classes of secondary metabolites but not necessarily
the same compounds. Both extracts contained flavanoids, tannins and saponins. Alkaloids and anthraquinones were not detected on both extracts.
Fig. 1: DPPH radical scavenging activity of *hulu-mur* extracts.
Table 1. Preliminary phytochemical screening of *Hulu-mur* extracts.

<table>
<thead>
<tr>
<th>Class of compounds</th>
<th>Water</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+, present; -, absent
The observed antioxidant properties in this study could be due to constituents belonging to the groups of phytochemicals, mainly the phenolic compounds (flavanoids and tannins) that were identified in the two crude extracts.

Polyphenols are the major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties (Zheng and Wang, 2001; Tung et al., 2007)) which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Results obtained in the present study revealed that the level of phenolic compounds in the water and ethanol extracts of Hulu-mur were considerable (Table 1) and thus contributed to the antioxidant capacity of this plant.
Conclusion and Recommendations

In this study, results showed that water and ethanol extracts from the *hulu-mur* had moderate antioxidant activity on DPPH scavenging system.

Phytochemical screening showed that both extracts contained flavanoids, tannins and saponins.

Further pharmacological studies will be necessary to establish more information on the benefit use of *hulu-mur* as a drink.
References


