Effect of fermentation and processing of *Sorghum bicolor* Grains to produce traditional Sudanese Hulu-Mur on phytochemicals and their biological activities

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Abstract

Sorghum plays an important role in the diet of millions of people in semi-arid regions of sub-saharan Africa, Asia, central America and the middle east. Sorghum grains (feterita) were subjected to processing operations. Ethanolic extraction was done for raw grains, germinated grains, fermented porridge and cooked fermented porridge. All extracts were fractionated to petroleum ether, chloroform and ethyl acetate extracts and also All extracts subjected to phytochemicals profiling, total phenolic contents, antioxidant activity and antimicrobial properties. Thin layer chromatography (TLC) revealed the presence of polyphenols, Flavonoidal acids, coumarin, anthrone, anthraquinones and Terpenoids. Flavonoid mainly accumulated in the ethyl acetate fraction in all extracts before and after fermentation. Flavonoidal acids, coumarin, anthrone and anthraquinones were detected in the chloroform fraction in all extracts before and after fermentation. Terpenoids were accumulated in the petroleum ether fraction. From our finding the fermentation and cooking of the sorghum grains increased the total phenolic contents, the fermented porridge showed the highest phenolic content (305.17 mg GAE/g). The radical scavenging activity of fermented and non-fermented sorghum grain fractions were screened by using 1, 1 diphenyl-2- picrylhydrazyl (DPPH), the ethyl acetate fraction of cooked fermented porridge at a concentration of 1mg/ml had the high inhibition of DPPH radicals (57.33%). Antimicrobial activity of different extracts was recorded against different pathogenic bacteria and fungi, the petroleum ether extract showed antimicrobial activity against *Bacillus subtiles* and *Staphylococcus aureus* at concentration of 40mg/ml. These findings proved that fermentation and cooking could increase the bioavailability of phenols, which subsequently may improve the antioxidant and antimicrobial activity so the Sudanese Traditional Hulu-Mur may be a valuable health and nutrition promoting and hence it can consider one of the functional foods.

Keywords: *Sorghum bicolor*; DPPH; TLC profiling; Antimicrobial activity

1. Introduction

Fermentation in food processing typically is the conversion of carbohydrates to alcohols and carbon dioxide or organic acids using yeasts, bacteria, or a combination thereof, under anaerobic conditions. Fermentation by micro-organisms enhance produce of enzymes which degrade anti-nutritive compounds by render them available and may enhance flavour and aroma. Also, might enrich food with essential amino acids, vitamins, mineral and bioactive compounds.

Grains are commonly cooked before being consumed, and it is known that, cooking induces significant changes in chemical composition that affect the bio accessibility and concentration of nutrients and health-promoting compounds
such as vitamin C, carotenoids and polyphenols [1]. To date, there is limited information on the effect of processing on phytochemical compounds, particularly on the phenolic profile, and total antioxidant capacity (TAC) of cereals, like sorghum [2].

In Sudan cereals which are commonly fermented include sorghum for making kisra, nasha, aceda, hulu-mur and millet for red and white damirga. Sorghum is one of the cereals that constitute a major source of protein, calories, and minerals for millions of people in Africa and Asia. Sorghum grains are unusually rich in health-promoting phytochemicals, especially polyphenols, and as consequently they are considered as being health-promoting foods, such as tannins, phenolic acids, anthocyanins, phytosterols and policosanols [3]. Studies have shown that sorghum has antioxidant activity [4], anticarcinogenic effects [5], and cholesterol lowering effect [6]. And can reduce the risk of cardiovascular disease [7]. Hulu-mur is a traditional non-alcoholic beverage prepared from fermented sorghum grains, used by Sudanese during the fasting month of Ramadan [8]. The main aim of this study was to determine the effects of fermentation on the phytochemicals of sorghum and the exerted changes in their corresponding biological activities namely antioxidant and antimicrobial activities.

2. Material and methods

2.1. Sorghum bicolor fermentation

2.1.1. Preparation of malt (zurrria)

Preparation of malt (zurrria) from sorghum seeds in Sudan has been described by [9]. First (feterita) grain were steeped in water for about 24 hours, the softened grains were then spread out as a 2-cm thick bed, on damp sacks and covered with another set of sacks. From time to time, during the germination period of 2–6 days, the sacking are sprinkled with water figure (1). The duration of the germination stage of the grain depends largely on the environmental conditions usually 4-5 days are required for hulu-mur production.

2.1.2. Preparation of the dough

The fermented dough (Ajin) was prepared in traditional way as described by [10]. Equal quantities (200 g) of sorghum malt and sorghum grains are milled separately. The un-germinated flour is cooked into porridge (aceda) and then mixed with the malted-floor while the former is still hot and then (20g) fermented dough called al khumara was added to act as a starter. Fermentation was carried out 48 hours figure (2).

2.1.3. Preparation of baked thin flakes (hulu-mur)

The process of baking the fermented dough is known as Owasa. Accordingly, a small amount of the fermented dough was spread over a hot plate forming a very thin flake within 1–2 s and then taken out and considered baked ready for consumption. Fermented baked samples were dried at shade, then ground to pass a 0.4 mm screen and stored at 4°C in polyethylene bags.

2.1.4. Fermented and non-fermented sorghum grains Preparation and Extraction

Extraction was done according to [11], 200 grams of semi powdered plant materials were extracted by maceration overnight in 80% ethanol. The ethanol extracts were then fractionated (liquid/liquid) in sequence using solvents with increasing polarities Petroleum ether (PE), Chloroform (CH Cl3) and Ethyl acetate (EtOAc). The solvents were evaporated to dryness using rotary evaporator figure (3).

2.2. Chromatographic analysis of Phytochemicals in Sorghum grains

2.2.1. Thin Layer Chromatography (TLC) Analysis

Thin layer chromatography analysis was carried out for the Petroleum ether chloroform and ethyl acetate fractions of fermented and non-fermented sorghum extracts using stationary phases Aluminum silica gel plates 60F254 Merck5554 and pre-coated TLC plates Sil, NP–18W/UV254 (Macherey/Nagel). Standard chromatograms were prepared by applying 20µl solution (5mg/ml) to a silica gel plate and developing it in mobile phases which was ratios of solvent systems (toluene/ethyl acetate/formic acid) and (petroleum ether: ethyl acetate) depending on the type of fraction. Chromatograms were detected under UV lamp (Camag), at different values (254 & 366 nm) and sprayed with diagnostic reagents, which include:KOH Reagent, Vanillin - H2SO4 Reagent and Natural Product(polyethlenglycol)(NP/PEG)
Reagent (NPR). The retention factor value (Rf) of the visible bands were marked under daylight. $Rf = \frac{\text{distance moved by analyte (compound)}}{\text{distance moved by solvent}}$ according to [12].

**Figure 1** Preparation of malt (zurrria) from sorghum seeds.

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**Figure 2** The fermented dough (Ajin) and hulu-mu
Figure 3 Extraction and fractionation of fermented and non-fermented sorghum grains.

2.3. Total phenolic contents

The concentration of phenolics in fractions was determined using spectrophotometric method with some lab modification. Samples solutions of the fractions at concentration of 1 mg/ml were used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of samples solutions of fractions, 2.5 ml of 10% Folin-Ciocalteu’s reagent dissolved in water and 2.5 ml 7.5% NaHCO3. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu’s reagent dissolved in water and 2.5 ml of 7.5% of NaHCO3. The samples were thereafter incubated in a thermostat at 30°C for 90 min. The absorbance was determined using spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration curve was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration curve, by the following Equation Y = 1328.4 X - 243.38 with R2 = 0.9553 then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

2.4. Antioxidant test

Extracts of sorghum grains were screened for their antioxidant activity applied adopting [13] assay with minor modifications.

2.5. Antimicrobial activity of fermented and non-fermented sorghum grain

The extracts and fractions of fermented and non-fermented Sorghum bicolor were tested in vitro for their antimicrobial activities against different pathogenic organisms. Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC
25922) Staphylococcus aureus (ATCC 25923), Bacillus subtitles and Candida albicans (ATCC 7596) according to [14] with minor modifications.

2.6. Statistical analysis

All data were expressed as the mean ± standard deviation (SD) of triplicate analysis. Statistical differences between samples were evaluated by ANOVA. p < 0.05 was considered to be significant.

3. Results and discussion

3.1. Total phenolic content of ethanolic extracts of fermented and non-fermented sorghum grains

Levels of phenolic content were expressed in terms of gallic acid equivalent (GAE). As calculated the equation of the right hand side of the proportioning of total phenolic content by the method of Folin-Ciocalteu [15] gave Y = 1328.4 X - 243.38 with R² = 0.9553. Which showed significant differences in their content in total phenols of fermented and non-fermented sorghum extracts. The ethanolic extract of raw grain, germinated grain, fermented porridge and cooked fermented porridge showed increase in phenolic content (249.61, 256.56, 290.20, and 305.17 mg GAE/g), respectively. Table (1) shows that there is insignificant difference between raw grain, germinated grain (249.61, 256.56 mg GAE/g), respectively but significant different due to fermentation and heating. The highest phenolic content (305.17 mg GAE/g) was for cooked fermented porridge.

The values obtained disagreed with that stated by [16] who found that cooking reduced total phenolic content in sorghum grains (242.93, 117.03 mg/gm) for raw extract and cooked extract respectively, although other findings showed that fermentation and heating increased the total phenol content of sorghum grains (13.34, 13.76 and 17.83 mg GAE/g) respectively for unfermented dough fermented dough and fermented baked reported by [17].

| Extract                  | Phenolic content (mg GAE/g extract) |
|--------------------------|-------------------------------------|
| Raw grain                | 249.61 ± 6.16                      |
| Germinated grain         | 256.56 ± 4.06                      |
| Fermented porridge       | 290.20 ± 7.35                      |
| Cooked fermented porridges | 305.17 ± 6.25                    |

Values are mean ±SD
Mean value (s) bearing different superscript(s) differ significantly (P≤0.05).

3.2. Antioxidant activity of fermented and non-fermented sorghum grains (Radical Scaving Activity (RSA%±SD))

Table (2) and Figure (4) show significant differences in antioxidant activity. When the fermented and non-fermented sorghum grain “cooked fermented porridges, fermented porridge, germinated grains and raw grains” fractions were tested for their antioxidant potential using DPPH assay, the ethyl acetate fractions was the most active among other fractions (57.33 > 50.00 > 45.67 > 31.00), respectively this could be attributed mainly to presence of polyphenols e.g. flavonoids [18].

The cooked fermented porridge extracts showed the highest activity (57.32 > 53.00 > 15.00) when compared to the raw grain extracts which showed the lowest activity (49.33 > 31.00 > 19.67) Ethyl acetate fraction of cooked fermented porridge had significantly higher antioxidant activity (57.33 ± 2.52). Petroleum ether fraction of fermented porridges had significantly low antioxidant activity (11 ± 1.00). The cooked fermented porridges extract showed the highest phenolic content (305.17 mg GAE/g). A clear relationship between antioxidant capacity and total phenolic content was
observed, indicating that phenolic compounds are the major contributors to the antioxidant properties of sorghum as reported by [19].

These findings are in agreement with [17] who reported that fermentation and heating improved the antioxidant capacity of sorghum grains with values of DPPH scavenging activity of the unfermented dough, fermented baked dough and fermented dough was 18.5%, 52.79%, 46.23% respectively.

These results do not agree with those found by [16]. Who found that cooking decreased the antioxidant capacity in ascorbic acid equivalents in mg/gm for raw grain followed by cooked grain (21.9±0.23 and 8.53±0.18), respectively.

Table 2 Antioxidant activity of fermented and non-fermented sorghum grains (RSA%±SD)

| Sample                  | Petroleum ether | Chloroform | Ethyl acetate |
|-------------------------|-----------------|------------|---------------|
| Raw grain               | 19.67fg ±1.53   | 49.33c ±2.08 | 31.00e ±2.00  |
| Germinated grain        | ±2.00f          | 34.33e ±1.53 | 45.67d ±1.53  |
| Fermented porridge      | ±1.00i          | 48.00cd ±2.00 | 50.00bc ±2.00 |
| Ccooked fermented porridges | ±2.00h          | 53.00b ±2.65 | 57.33a ±2.52  |

Values are mean ±SD
Mean value(s) bearing different superscript(s) are differ significantly (P≤0.05).

Figure 4 Antioxidant activity of fermented and non-fermented Sorghum bicolor grains (RSA%±SD)(DPPH)
3.3. Antimicrobial activity of fermented and non-fermented Sorghum grains

The antimicrobial activity of different fractions of fermented and non-fermented sorghum grains were assessed in Table (3). The petroleum ether extract of raw grain, germinated grain and fermented porridge showed high significant difference (p≤0.05) antimicrobial activity with inhibition zone ≥ 14 mm against Bacillus subtilis with inhibition zone sizes (15, 14.75, 14.50 mm) respectively. Similar high significant (p≤0.05) antimicrobial activity obtained by petroleum ether of fermented porridge with inhibition zone (15 mm) against Staphylococcus aureus at concentration 40 mg/1ml. Similar findings were reported by [20], who found that a methanolic extract from type III tannin sorghum exhibited higher antimicrobial activity against B. subtilis and Listeria monocytogenes than extracts from white non-tannin sorghum. On the other hand, all chloroform and ethyl acetate fractions of fermented and non-fermented sorghum grains showed weak antimicrobial activity against Staphylococcus aureus and Escherichia coli.

All tested extracts and fractions of raw grains and germinated sorghum grains showed no antimicrobial activity at concentration 40 mg/1ml against Pseudomonas aeruginosa and Candida albicans

Table 3 Antimicrobial activities of the different fractions of fermented and non-fermented

| Sample              | Extract      | Measurement of inhibition zones diameter (MIZD) in (mm) |
|---------------------|--------------|--------------------------------------------------------|
|                     |              | **Bacteria**                                           | **Fungi** |
|                     |              | E. coli      | P. a    | S. a    | B. s     | C. a      |
| Raw grain           | Petroleum ether | 12.25abcdef | 0.00n   | 8.75gh  | 15.00a   | 0.00n     |
|                     | Chloroform   | 0.00n        | 0.00n   | 0.00n   | 11.75bcdef | 0.00n     |
|                     | Ethyl acetate | 0.00n        | 0.00n   | 0.00n   | 11.25cdef | 0.00n     |
|                     | Ethanol      | 0.00n        | 0.00n   | 10.50def | 10.75def  | 5.50kl    |
| Germinated grain    | Petroleum ether | 0.00n        | 0.00n   | 0.00n   | 14.75abc  | 0.00n     |
|                     | Chloroform   | 0.00n        | 0.00n   | 0.00n   | 9.75fgh   | 0.00n     |
|                     | Ethyl acetate | 0.00n        | 2.00n   | 0.00n   | 12.00abcdef | 0.00n     |
|                     | Ethanol      | 0.00n        | 0.00n   | 5.00kln | 0.00n     | 0.00n     |
| Fermented porridge  | Petroleum ether | 4.25klm      | 2.00mn  | 15.00a  | 14.50abc  | 11.00cdef |
|                     | Chloroform   | 3.25lm       | 0.00n   | 9.50fgh | 12.00abcdef | 0.00n     |
|                     | Ethyl acetate | 11.00cdef    | 2.00mn  | 6.50ijk | 12.25abcdef | 0.00n     |
|                     | Ethanol      | 0.00n        | 3.25lm  | 13.00abcde | 15.00a  | 0.00n     |
| Cooked fermented porridge | Petroleum ether | 0.00n      | 4.25klm | 12.00abcdef | 13.00abcde | 7.25bijk |
|                     | Chloroform   | 0.00n        | 8.50gh  | 6.75bijk | 13.50abcde | 0.00n     |
|                     | Ethyl acetate | 9.75fgh      | 10.50def | 0.00n   | 10.25def  | 0.00n     |
|                     | Ethanol      | 0.00n        | 0.00n   | 12.00abcdef | 14.25abc  | 0.00n     |

* S. aureus = Staphylococcus aureus, B. s = Bacillus subtilis, E. coli = Escherichia coli, C. a = Candida albicans, P. a = Pseudomonas aeruginosa; MIZD mm ≥18mm: Sensitive, MIZD mm 14-18mm: Intermediate, MIZD mm <14mm: Resistant.

Sorghum grains at concentration 40 mg/1ml

4. Conclusion

Thin layer chromatography (TLC) revealed the presence of flavonoidal acids, coumarin, anthraquinones, terpenoids and anthrone and polyphenol in all fractions. Flavonoids were mainly. The fermentation and cooking increased the phenolic content expressed in terms of gallic acid equivalent (GAE), cooked fermented porridge showed highest phenolic content.
In vitro antioxidant activity showed the highest inhibition of DPPH radicals at a concentration of 1mg/1ml, ethyl acetate fraction of cooked fermented porridges showed higher antioxidant activity (57.33%). Different extracts of fermented and non-fermented of Sorghum bicolor at concentration 40 mg/1ml showed highest antimicrobial activity against different pathogenic bacteria and fungi.

Compliance with ethical standards

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