GSC Biological and Pharmaceutical Sciences, 2020, 10(03), 076–084

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

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

Salma A Salih [1], Khogali E Ahmed [2], Lana T Ezzdeen [3] and Abdelhalim A Hamza [1,*]

[1] *Commission of Biotechnology and Genetic Engineering, National Center for Research, Biochemistry Department, Khartoum, Sudan.*
[2] *Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Sudan.*
[3] *Commission of Biotechnology and Genetic Engineering, National Center for Research, Microbial Biotechnology Department, Khartoum, Sudan.*

Publication history**:** Received on 12 February 2020; revised on 25 February 2020; accepted on 04 March 2020

Article DOI: https://doi.org/10.30574/gscbps.2020.10.3.0040

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 subtitles* 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

* Corresponding author: Abdelhalim A Hamza

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 CL_3) and Ethyl acetate (Eto_2Ac). The solvents were evaporated to dryness using rotary evaporator figure (3).

2.2. Chromatographic analysis of Phytochemicals in Sorghum grains

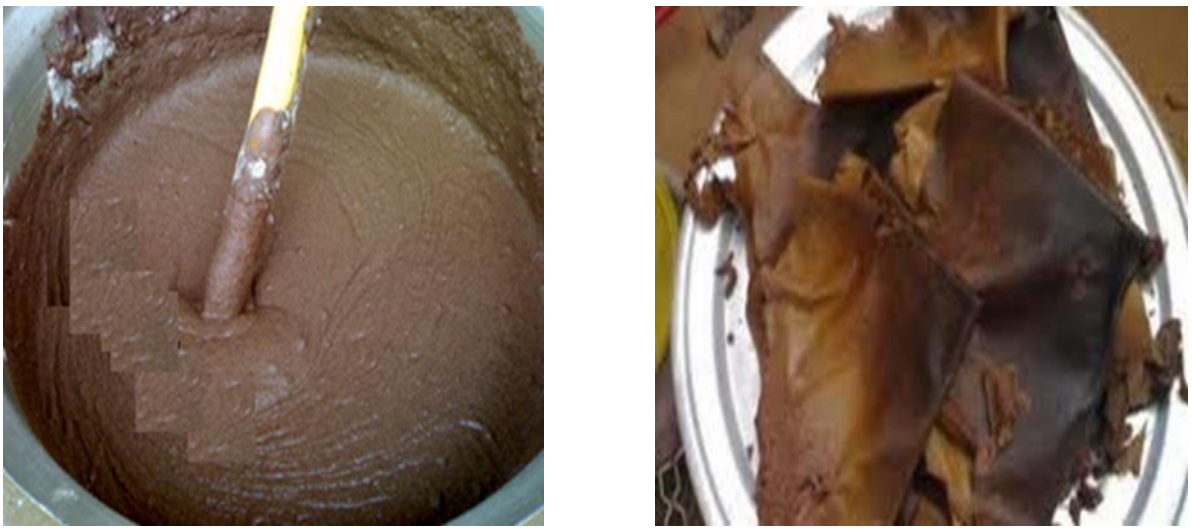
2.2.1. Thin Layer Chromatography (TLC) Analysis

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 $60F_{254}$ Merck5554 and pre-coated TLC plates SII, 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 - H_2SO_4 Reagent and Natural Product(polyethlenglycol)(NP/PEG)

Reagent(NPR). The retention factor value (Rf) of the visible bands were marked under daylight. Rf = distance moved by analyte (compound) distance moved by solvent according to [12].

Feterita Zurrria

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



Fermented dough Baked fermented dough

Figure 2 The fermented dough (Ajin) and hulu-mu

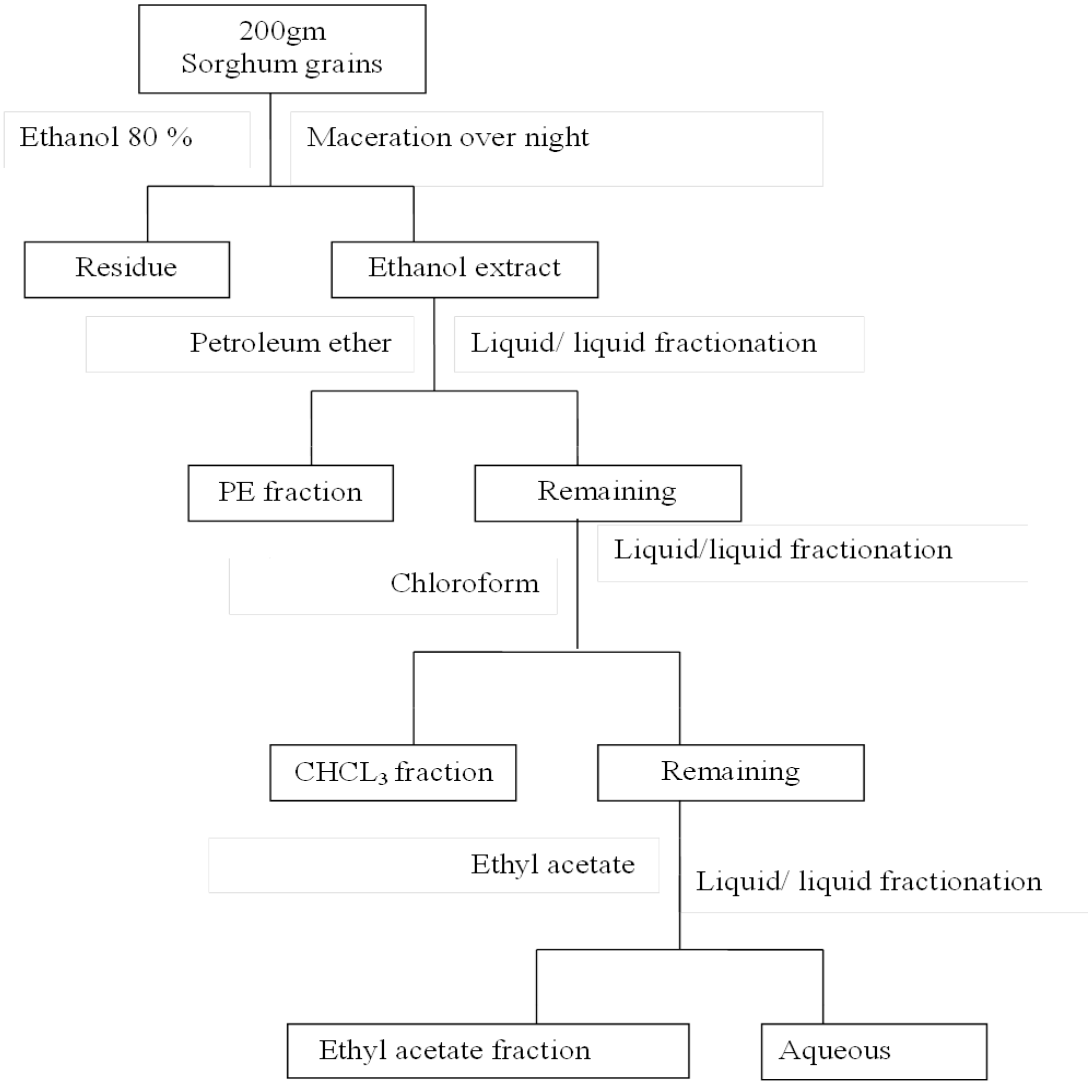


Figure 3 Extraction and fractionation of fermented and non-fermented sorghum grains.

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].

Table 1 Total phenolic content of the ethanolic extract of fermented and non-fermented sorghum (mg GAE/g extract)

Extract	Phenolic content (mg GAE/g extract)
Raw grain	249.61c ±6.16
Germinated grain	256.56c ± 4.06
Fermented porridge	290.20b ± 7.35
Cooked fermented porridges	305.17a ± 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 Scavinging 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.g1. 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)

Extracts / sample	Petroleum ether	Chloroform	Ethyl acetate
Raw grain	19.67fg ±1.53	49.33c ±2.08	31.00e ±2.00
Germinated grain	21.00f ±2.00	34.33e ±1.53	45.67d ±1.53
Fermented porridge	11.00i ±1.00	48.00cd ±2.00	50.00bc ±2.00
Ccooked fermented porridges	15.00h ±2.00	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).

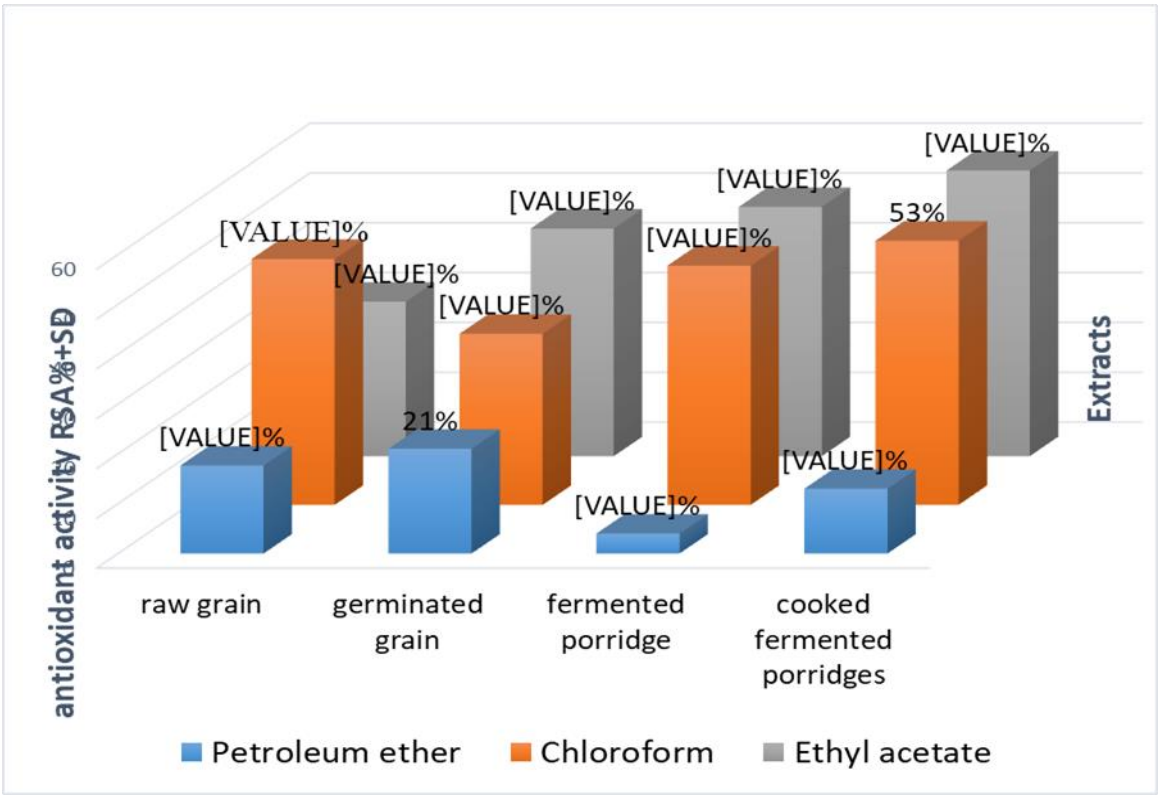


Figure 4 Antioxidant activity of fermented and non-fermented *Sorghum bicolor* grains (RSA%+SD)(DPPH)

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 ($p \leq 0.05$) antimicrobial activity with inhibition zone ≥ 14 mm against *Bacillus subtitles* with inhibition zone sizes (15, 14.75, 14.50 mm) respectively. Similar high significant ($p \leq 0.05$) 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	12.25^{abcdef}	0.00^{n}	8.75^{ghi}	15.00^{a}	0.00^{n}
	Chloroform	0.00^{n}	0.00^{n}	0.00^{n}	11.75^{bcdef}	0.00^{n}
	Ethyl acetate	0.00^{n}	0.00^{n}	0.00^{n}	11.25^{cdef}	0.00^{n}
	Ethanol	0.00^{n}	0.00^{n}	10.50^{defg}	10.75^{defg}	5.50^{jkl}
Germinated grain	Petroleum ether	0.00^{n}	0.00^{n}	0.00^{n}	14.75^{ab}	0.00^{n}
	Chloroform	0.00^{n}	0.00^{n}	0.00^{n}	9.75^{fgh}	0.00^{n}
	Ethyl acetate	0.00^{n}	2.00^{n}	0.00^{n}	12.00^{abcdef}	0.00^{n}
	Ethanol	0.00^{n}	0.00^{n}	5.00^{klm}	0.00^{n}	0.00^{n}
Fermented porridge	Petroleum ether	4.25^{klm}	2.00^{mn}	15.00^{a}	14.50^{ab}	11.00^{cdef}
	Chloroform	3.25^{lm}	0.00^{n}	9.50^{fgh}	12.00^{abcdef}	0.00^{n}
	Ethyl acetate	$11.00c^{defg}$	2.00^{mn}	6.50^{ijk}	12.25^{abcdef}	0.00^{n}
	Ethanol	0.00^{n}	3.25^{lm}	13.00^{abcde}	15.00^{a}	0.00^{n}
Cooked fermented porridge	Petroleum ether	0.00^{n}	4.25^{klm}	12.00^{abcdef}	13.00^{abcde}	7.25^{hijk}
	Chloroform	0.00^{n}	8.50^{ghi}	6.75^{hijk}	13.50^{abcd}	0.00^{n}
	Ethyl acetate	9.75^{fgh}	10.50^{defg}	0.00^{n}	10.25^{efg}	0.00^{n}
	Ethanol	0.00^{n}	0.00^{n}	12.00^{abcdef}	14.25^{abc}	0.00^{n}

* S. aureus = Staphylococcus aureus, B. s = Bacillus subtitles, 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

(305.17 mg GAE/g). *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

Acknowledgments

We are grateful for the Commission for Biotechnology and Genetic Engineering, National Centre for Research Specially Department of Biochemistry and Microbiology for Kindly permitting to conduct this Research.

References

[1] Pellegrini N, Chiavaro E, Gardana C, Mazzeo T, Contino D, Gallo M and Porrini M. (2010). Effect of Different Cooking Methods on Color, Phytochemical Concentration, and Antioxidant Capacity of Raw and Frozen Brassica Vegetables. Journal of Agricultural and Food Chemistry, 58(7), 4310–4321.

[2] Denis N Teresa M, Rosalia F, Vincenzo F and Nicoletta P. (2012). Effect of cooking on the total antioxidant capacity and phenolic profile of some whole-meal African cereals. J. Sci. food and Agri, (92), 13.

[3] Awika JM and Rooney LW. (2004). Sorghum phytochemicals and their potential impact on human health. Phytochemistry, 65, 1199-1221.

[4] Choi YM Jeong HS and Lee JS (2006). Antioxidant activity of methanolic extracts from some grains consumed in Korea. Journal of Food Chemistry, 103, 130-138.

[5] Kwak CS, Lim SJ, Kim SA, Park SC and Lee MS. (2004). Antioxidative and antimutagenic effects of Korean buckwheat, sorghum, millet and job's tears. Journal of Korean Society of Food Science and Nutrition, 33, 921-929.

[6] Ha TY, Cho IJ and Lee SH. (1998). Screening of HMG-CoA reductase inhibitory activity of ethanol and methanol extracts from cereals and legumes. Journal of Korean Society of Food Science and Technology, 30, 224-229.

[7] Cho SH, Choi Y, and Ha TY. (2000). In vitro and in vivo effects of pros millet, buckwheat and sorghum on cholesterol metabolism. Journal of the Federation of American Societies for Experimental Biology, 14, 249.

[8] Dirar HA. (1993). The indigenous fermented foods of the Sudan. A study in African food and nutrition CAB International Wallingford, UK.

[9] Dirar HA. (1987). Fermented foods and beverages in the Sudan. A paper presented at the UNISCO training course on fermented foods of the word, Khartoum, Sudan.

[10] El-Tinay AH, El-Mahdi ZM and El-Soubki A. (1985). Supplementation of fermented sorghum Kisra bread with legume protein isolates. Journal of Food Technology, 20, 679-687.

[11] Fyhrquist P, Mwasumbi L, Haeggstrom CA, Vuorela H, Hiltunen R and Vuorela P. (2002). Ethnobotanical and antimicrobial investigation on some species of Terminalia and Combretum (Combretaceae) growing in Tanzania. Journal of Ethnopharmacology, 79, 169-77

[12] Robard K and Antolovich M. (1997). Analytical chemistry of fruit bioflavonoid Journal of the Analyst, 122(2), 11 -34.

[13] Shyur L, Tsung J, Chenb J, Chiua C and Loa C. (2005). Antioxidant properties of extracts from medicinal plants popularly used in Taiwan. International journal of applied science and engineering, 3, 195-202.

[14] Kirby WMM, Yoshihara GM, Sundsted KS and Warren JH. (1957). Clinical usefulness of a single disc method for antibiotic sensitivity testing. Antibiotics Annu, 892.

[15] Singleton V, Orthofer R and Lamuela-Raventos R. (1999). Analysis of total phenols and other oxidation substrates. Journal of Methods in enzymology, 299, 152-178.

[16] Polumahanthi S and Nallamilli SM. (2014). Comparative studies on raw and cooked extracts of Sorghum Cultivars for their Bio-active Constituents. International Journal of Advanced Research, 2(2), 804-813.

[17] Zaroug MA. (2008). Antioxidant activity of traditional Sudanese Kisra prepared from two sorghum (Sorghum bicolor) cultivars. M.Sc. Thesis. Faculty of Science in Botany, University of Khartoum, Sudan.

[18] Estevinho L, Pereira AP Moreira L Dias LG and Pereira E. (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food and Chemical Toxicology, 46, 3774–3779.

[19] Awika JM, Rooney LW, Wu X, Prior RL and Cisneros-Zevallos L. (2003). Screening methods to measure antioxidant activity of sorghum (Sorghum bicolor) and Sorghum Products. Journal of Agricultural and Food Chemistry, 51 6657–6662.

[20] Svensson L, Sekwati-Monang B, Lopes-Lutz D, Schieber A and Gänzle MG. (2010). phenolic acids and flavonoids in nonfermented and fermented red sorghum (Sorghum bicolor (L.) Moench). J Agric Food Chem, 58 9214-9220.

How to cite this article

Salma AS, Khogali EA, Lana T Ezzdeen and Abdelhalim AH. (2020). Effect of fermentation and processing of *Sorghum bicolor* grains to produce traditional Sudanese Hulu-Mur on phytochemicals and their biological activities. GSC Biological and Pharmaceutical Sciences, 10(3), 76-84.