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Antimicrobial activities of Six Types of Wheat Bran

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Abstract: *Six types of wheat bran (Emam, Pohean, Wadielneel, Argeen, Pladi and Debeira) investigated for their biological and antioxidant activities. Physiochemical properties carried for their oil. The polar and nonpolar extracts showed antioxidant activity. These results showed the importance of the wheat bran types as nutritive and medicinal plant. All plant bran types investigated for its biological activity as antifungal and anti-bacterial. Some types of the plant bran extracts showed antibacterial activity towards Escherichia coli, Pseudomonasaeruginosa, Bacillus subtilis, and Staphylococcus aureus. In addition, some types of the extracts showed antifungal activity towards Candida albicans, and Aspergillusniger. Polar and nonpolar extracts of the plant bran types prepared. The petroleum ether extracts subjected to determination by GC/ MS. It showed different major fatty acids as Linoleic acid followed by Linolelaidic acid and then Palmatic acid. The ethanolic extract of Plaid type separated by column chromatography. The isolated constituents were structurally determined using spectrophotometric analysis as IR, UV, and GC/MS. The ethanolic extract components may be esters and ketones as suggested by GC/MS and their functional groups appeared in the IR readings.*

Key Words: ***Wheat Bran (****Emam, Pohean, Wadielneel, Pladi, Argeen, And Dibera****),*** *Antimicrobial activity (Antibacterial and* ***Antifungal****), Antioxidant activity.*

I. Introduction

Wheat is a type of grass grown all over the world for its highly nutritious and useful grains. The wheat kernels have three parts: (sometimes called a wheat berry):

1. The Bran is the hard outer covering of the wheat kernel, high in fiber and nutrients.
2. The Germ is the nutrient rich embryo that will sprout and grow into a new wheat plant.
3. The Endosperm is the biggest part (83%), the "insides" of the kernel mostly starch[1].

Wheat bran is not only a good source of dietary fibers[2-3]. But also rich source of various natural antioxidants including tocopherols, phenolic acids[4,5]. Antioxidants modulate cellular oxidative status, and prevent biologically important molecules such as DNA, proteins, and membrane lipids from oxidative damage and consequently reduce the risk of several chronic diseases including cancer and cardiovascular disease[6-8]. Tocopherols, phenolic acids and other antioxidants in wheat bran generally believed to be primarily responsible for its positive effects on cardiovascular diseases[2-3-7]. The compounds of wheat bran exhibited significant capabilities in scavenging free radicals, chelating metal ion oxidants*;* and reducing lipid oxidation at different conditions[9, 10].

Wheat Bran Oil

The major fatty acids of wheat bran oil were palmitic, oleic, linoleic and γ- linolenic acids. Linoleic acid found in highest amount and it was present in the range from 52.2 to 60% of total identified fatty acids. Another unsaturated fatty acid, oleic acid was also present in higher percentage, ranging from13.3 to 15.9%. Within saturated fatty acids, palmitic acid was present in the highest concentration, ranging from16.8 to 18.6% of total identified fatty acids[11]. In addition, α- and *β*- tocopherols were found significant amount in wheat bran oil obtained by SC - CO_2 extraction. The identification of species cultivars with high level of antioxidants, as well as in tracing of biological activities is important for human and other living organisms. Therefore, antioxidants in wheat bran might be quantified by SC - CO_2 extraction for proper using in biological purposes[12,11].

Nutritionally, bran fractions produced by milling are rich in fiber, minerals, vitamin B6, thiamine, folate and vitamin E and some phytochemicals; in particular, antioxidants such as phenolic compounds[13]. There are increased interest in the use of plant extracts as natural antimicrobial and antioxidant agents, especially in food, cosmetic, medical and agrochemical areas[14-16]. Phenolic compounds including tannins, terpenoids, alkaloids, and flavonoids in plant extracts have or show good antioxidant and antimicrobial abilities[14, 17.]

II. Material and Methods

Collection of Plant Materials

Six types of Sudanese wheat bran from Poaceae family collected for this study. The samples Emam, Pohean, Wadielneel, Pladi and Diberawere obtained from Northern state while Argeen sample obtained from Gzeera state.

Preparation of Crude Plant Extracts

Fifty grams (50gm) of each of the six types of the wheat bran extracted with 250 ml of different solvents as petroleumether, ethyl acetate, chloroform using a standard soxhlet apparatus for 8 hours at 40℃, 60℃, and 50℃ respectively. The residue of petroleum ether was dried and extracted with ethanol using a standard soxhlet apparatus for 8hours at 60^{O}C. A water extract was also prepared using cold extraction for 2 hours. All samples kept forfurther analysis. Five (5) μg from each extract sample taken for testing the antioxidant activity using 2.2 Di-(4-tert-octylphenyl)-1-picryl-hydrazyl. For testing the antimicrobial activity, 1 mg from each extract sample taken. The physiochemical propertieswere done for the most two active of the petroleum ether extracts.

Procedure of Biological Activities

Antioxidant Activity using Di Phenyl Picrayl Hydrazine Radical Scavenging Assay

The DPPH radical scavenging was determined according to the method of Shimada ET. al. (1992)[18]. With modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4- tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37*oC*. The concentration of DPPH kept as (300µm). The test samples dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis run in triplicate[19].

Preparation and Test for Antimicrobial and Antibacterial Activity

The bacterial suspensions were prepared by aseptically distributing One ml aliquots of a 24 hours broth culture of the test organisms onto nutrient agar slopes and incubated at $37^{o}C$ for 24 hours. The bacterial growth harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^{8}-10^{9}C.F.U/ ml. The suspension was stored in the refrigerator at $4^{o}C$ until used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Milesand Misra, 1938). Serial dilutions of the stock suspension made in sterile normal saline solution and 0.02ml volumes of the appropriate dilution transferred by micropipette onto the surface of dried nutrient agar plates. The plates allowed to stand for two hours at room temperature for the drops to dry and then incubated at $37^{o}C$ for 24 hours. After incubation, the number of developed colonies in each drop counted. The average number of colonies per drop (0.02 ml) multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared; all the above experimental conditions were maintained constant so that suspensions with very close viable counts obtained. For testing the antibacterial Activity, the cup-plate agar diffusion method (Kavanagh, 1972) adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10^{8}- 10^{9} C .F.U/ml were thoroughly mixed with100 ml of molten sterile nutrient agar which was maintained at $45^{o}C$. Twenty (20) mlaliquots of the inoculated nutrient agar distributed into sterile Petri-dishes. The agar left to set and in each of these plates 4 cups (10 mm in diameter) cut using a sterile corn borer (No. 4) and agar discs removed. Alternate cups filled with 0.1 ml sample of each extracts using automatic micro liter pipette, and allowed to diffuse at room temperature for two hours. The plates then incubated in the upright position at $37^{o}C$ for 18 hours. Two replicates carried out for each extract against each of the test organisms. After incubation, the diameters of the resultant growth inhibition zones measured. Averaged and the mean values were tabulated. Preparation and Test for Antifungal Activity The fungal suspensions were prepared by aseptically distributing One ml aliquots of a 48 hours broth culture of the test organisms onto sabourd agar slopes and incubated at $25^{o}C$ for 48 hours. The van gall growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^{8} -10^{9} C.F.U/ ml. The suspension was stored in the refrigerator at $4^{o}C$ until used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried nutrient agar plates. The plates allowed to stand for two hours at room temperature for the drops to dry and then incubated at $25^{o}C$ for 48 hours. After incubation, the number of developed colonies in each drop counted. The average number of colonies per drop (0.02 ml) multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a

fresh stock suspension was prepared; all the above experimental conditions were maintained constant so that suspensions with very close viable counts obtained. For testing the antifungal Activity, the cup-plate agar diffusion method (Kavanagh, 1972) adopted with some antifungal activity of the prepared extracts. One ml of the standardized fungal stock suspension 10^8-10^9 C.F.U/ml were thoroughly minor modifications to assess the mixed with 100 ml of molten sterile nutrient agar which was maintained at 45*oC*. Twenty (20) mlaliquots of the inoculated nutrient agar distributed into sterile Petri-dishes. The agar left to set and in each of these plates 4 cups (10 mm in diameter) cut using a sterile corn borer (No. 4) and agar discs removed. Alternate cups filled with 0.1 ml sample of each extracts using automatic micro liter pipette, and allowed to diffuse at room temperature for two hours. The plates then incubated in the upright position at 25^{o}C for 18 hours. Two replicates carried out for each extract against each of the test organisms. After incubation, the diameters of the resultant growth inhibition zones measured. Averaged and the mean values were tabulated. Bacterial and Fungal Test microorganisms[19]:
Bacillus subtilis ..... NCTC 8236 (Gram + ve bacteria)
Escherichia coli..... ATCC 25922(Gram -ve bacteria)
Pseudomonas aeruginosa ..... ATCC 27853 (Gram -ve bacteria)
Staphylococcus aureus ..... ATCC 25923(Gram +ve Bacteria)
Candida albicans .... ATCC 7596
Aspergillusniger .... ATCC 9763

Preparation and Separation Of Ethanolic Extract of the Wheat Bran Using a Laboratory Column

250 g of Pladi type of the wheat bran extracted with ethanol 95 by soxhlet extractor. The ethanolic extract was concentrated for separation with a laboratory column. The eluted solvents were petroleum ether, ethyl acetate, chloroform, acetone and ethanol. The fractions collected into fifteen (15) ones and all of them analyzed by Infrared Spectrophotometer. Three (3) of the fractions were selected after observing the IR charts for GC/MS analysis. The results of separation and the suggested structure of the MS detector recorded in charts.

III. Result and discussion

Biological Activities of Wheat Bran Extracts

Antioxidant Activity of Wheat Bran Extracts

Tables (3.1-3.5) exhibited the antioxidant activities of different extracts (petroleum ether, ethanol, ethyl acetate, chloroform, and water) of six types of the wheat bran.

Table 3.1: Antioxidant activity of Petroleum ether

Sample Cod	%RSA ± SD (DPPH)
Emam	54 ± 0.1
Pohean	D.N
Wadielneel	66.4 ± 0.01
Argean	Inactive
Pladi	9.3 ± 0.03
Dibera	6.3 ± 0.05

The highest antioxidant activity of the Petroleum ether extract was in Wadielneel followed by Emam and Pladi.

Table3.2: Antioxidant activity of Ethanol extract

Sample Cod	%RSA ± SD (DPPH)
Emam	17.7 ± 0.01
Pohean	21.6 ± 0.01
Wadielneel	35.4 ± 0.01
Argean	34.6 ± 0.02
Pladi	50.7 ± 0.02
Dibera	10.4 ± 0.01

In ethanolic extract, Pladi is the highet in antioxidant activity followed by Wadielneel and then Argeen. It has well established that free radical scavenging activity of wheat extracts is mainly due to phenolic compounds.

Table 3.3: Antioxidant activity of Ethyl acetate extract

Sample Cod	%RSA ± SD (DPPH)
Emam	26.9 ± 0.07
Pohean	18.5 ± 0.004
Wadielneel	21.3 ± 0.03

Argean	13.2 ± 0.07
Pladi	14.1 ± 0.2
Dibera	42.8 ± 0.03

Dibeira showed the highest antioxidant activity in ethyl acetate extract.

Table3.4: Antioxidant activity of Chloroform

Sample Cod	%RSA ± SD (DPPH)
Emam	Inactive
Pohean	14
Wadielneel	Inactive
Argean	61.2 ±0.02
Pladi	Inactive
Dibera	8.6 ±0.01

The most antioxidant active in chloroform extract is Argeen extract.

Table 3.5: Antioxidant activity of Water extract

Sample Cod	%RSA ± SD (DPPH)
Emam	17.7 ± 0.06
Pohean	Inactive
Wadielneel	6.6 ±0.03
Argean	5.7 ± 0.01
Pladi	12.7 ±0.01
Emam	17.7 ± 0.06

The Water extract showed negligible value for the antioxidant activity.

Antimicrobial Activity of the Wheat Bran Extracts

Antimicrobial Activity of Wheat Bran Petroleum ether Extract

As Shown in table 3.6. Pladi and Dibera petroleum ether extracts showed various antibacterial and antifungal activities (sensitive and intermediate) towards the selected bacteria and fungi in the study, these activities were the most active ones of the wheat bran extracts. While Argeen showed intermediate antibacterial activity towards *Pseudomonas aeruginosa*. All of the petroleum ether extracts of the other types of the wheat bran were inactive towards the selected bacteria and fungi in the study.

Table 3.6: Microbial Activity of the Petroleum ether Extract.

Plants name	Antimicrobial activities					
	Antibacterial activity				Antifungal activity	
Extract of	S.a	B.s	P.s	E.c	A.s	Ca
Emam	-	-	-	-	-	-
Pohean	-	-	-	-	-	-
Wadielneel	-	-	-	-	-	-
Argean	-	-	17	-	-	-
Pladi	15	20	18	23	21	34
Dibera	17	18	16	24	22	30

E.C: Escherichia coli, P.S: Pseudomonas aeruginosa, B.S: Bacillus subtilis, S.a: Staphylococcus aureus; Ca:Candida albicans, A.S: Aspergillus niger.

Scale of interpretation:

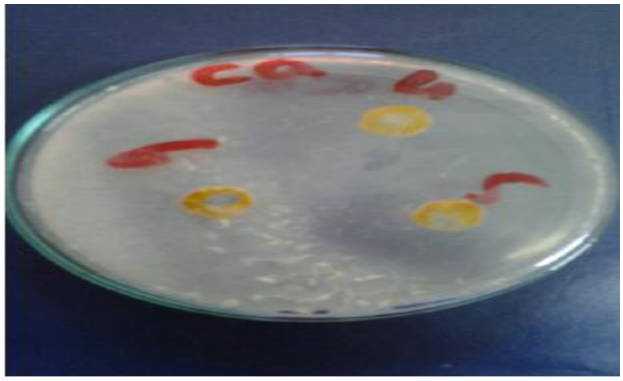
>18 mm (MIZD) = sensitive

14-18mm (MIZD) = intermediate

<14 mm (MIZD) = resistant

Escherichia coli ATCC 25922(Gram -ve bacteria)

Petroleum ether (Debera (4))
Petroleum ether (Pladi (5))
Petroleum ether (Argeen (6))



Candida albicans (ATCC 7596)

Petroleum ether (Debera (4))
Petroleum ether (Pladi (5))
Petroleum ether (Argeen (6))

Microbial Activity of Wheat Bran Ethanolic Extract

In table 3.7 Dibera ethanolic extract showed intermediate antibacterial activity towards *Escherichia coli* and *Staphylococcus aureus.* Whereas the other types were found to be resistant to the other selected bacteria. Emam, Argeen and Wadielneel showed intermediate antifungal activity towards *Candida albicans*. While the extract of the other types were found to be resistant to the fungus. Wadielneel, Argeen, Emam and Dibera ethanolic extracts showed intermediate antifungal activity to *Aspergillus niger* while Pohean and Pladi were found to be resistant to the fungus.

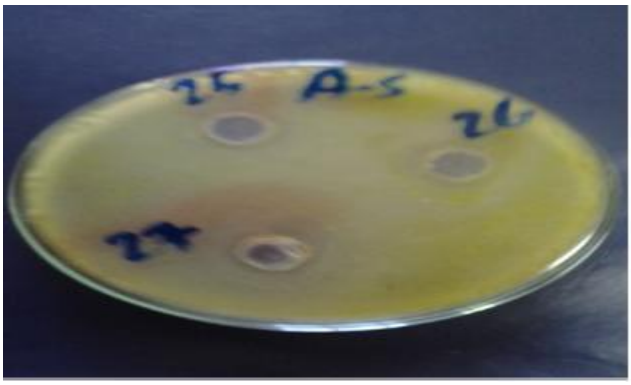
Table 3.7: Microbial Activity of Ethanolic Extract

Plants name	Antimicrobial activities					
	Antibacterial activity				Antifungal activity	
Extract of	S.a	B.s	P.s	E.c	A.s	Ca
Emam	12	13	12	13	17	16
Pohean	13	13	12	13	13	13
Wadielneel	12	12	13	13	18	15
Argean	12	12	12	12	18	16
Pladi	12	15	12	13	12	13
Dibera	14	12	12	14	14	11

E.C: Escherichia coli, P.S: Pseudomonas aeruginosa, B.S: Bacillus subtilis, S.a: Staphylococcus aureus; Ca:Candida albicans, A.S: Aspergillus niger

Scale of interpretation:

>18 mm (MIZD) = sensitive
14-18mm (MIZD) = intermediate
<14 mm (MIZD) = resistant



Extract of Ethanol (Pohean (1))

Extract of Ethanol (Debera (2)

Extract of Ethanol (Pladi (3))

Aspergillus niger ATCC 9763

Microbial Activity of Wheat Bran Ethyl acetate Extract

In Table 3.8. The types Pladi, Dibera, and Argeen found to be sensitive antifungal activity to Aspergillusniger. While Pladi was found to be sensitive antibacterial to Pseudomonasae ruginosa and Dibera was found to be sensitive as antibacterial to Staphylococcus aureus.Emam showed
Sensitive antibacterial activity to seudomonas aeruginosa. The extracts of the other types showed intermediate and resistant as antifungal and antibacterial.

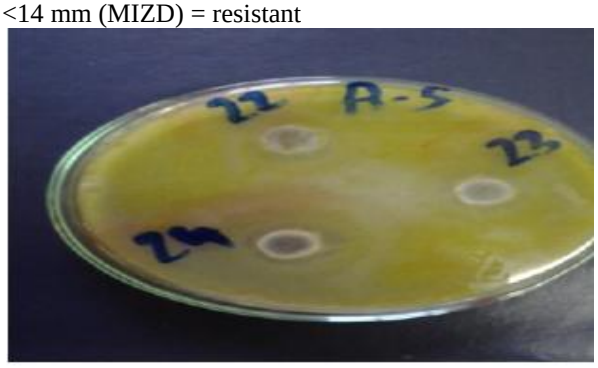
Table 3.8: Microbial Activity of Ethyl acetate Extract

Plants name	Antimicrobial activities					
	Antibacterial activity				**Antifungal activity**	
Extract of	S.a	B.s	P.s	E.c	A.s	Ca
Emam	15	18	21	-	18	14
Pohean	15	-	12	12	18	-
Wadielneel	14	15	18	-	17	16
Argean	16	16	16	15	21	15
Pladi	17	17	19	14	22	16
Dibera	20	15	18	14	24	18

E.C: Escherichia coli, P.S: Pseudomonas aeruginosa, B.S: Bacillus subtilis, S.a: Staphylococcus aureus; Ca:Candida albicans, A.S: Aspergillus niger .

Scale of interpretation:

>18 mm (MIZD) = sensitive
14-18mm (MIZD) = intermediate
<14 mm (MIZD) = resistant



Extracts of ethyl acetate (Argeen (25))

Extracts of ethyl acetate (Pladi (26))

Extracts of ethyl acetate (Debera (27))

Figure 45: Aspergillusniger (ATCC 9763)

Microbial Activity of Wheat Bran Chloroform Extract

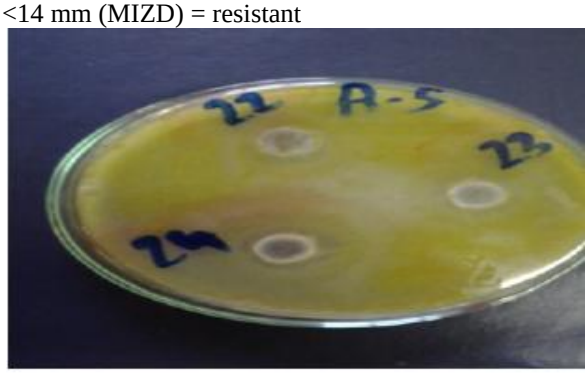
In table 3.9. Pladi showed antibacterial activity (sensitive) to Pseudomonas aeruginosa and antifungal activity (sensitive) to Aspergillusniger. The other types of wheat bran chloroform extract showed antibacterial and antifungal activities ranging from intermediate to resistant for the selected bacteria and fungi.

Table 3.9: Microbial Activity of Chloroform Extract

Plants name	Antimicrobial activities					
	Antibacterial activity				Antifungal activity	
Extract of	S.a	B.s	P.s	E.c	A.s	Ca
Emam	15	13	14	12	17	15
Pohean	13	-	-	-	-	-
Wadielneel	15	13	15	13	16	16
Argean	14	14	14	12	15	17
Pladi	15	16	19	-	19	12
Dibera	14	-	-	-	-	-

E.C: Escherichia coli, P.S: Pseudomonas aeruginosa, B.S: Bacillus subtilis, S.a: Staphylococcus aureus; Ca:Candida albicans, A.S: Aspergillus niger .

Scale of interpretation:
>18 mm (MIZD) = sensitive
14-18mm (MIZD) = intermediate
<14 mm (MIZD) = resistant



Extracts of chloroform (Pladi (22))

Extracts of ethyl acetate (Emam (23))

Extracts of ethyl acetate (Wadielneel (24))

Aspergillus niger ATCC 9763

Microbial Activity of Wheat Bran Water Extract

Table 3.10. Showed that Dibera water extract was the most active as antifungal and as antibacterial to the all selected bacteria and fungi. Pladi showed antibacterial activity (sensitive) to Escherichia coli and to Bacillus subtilis. In addition, it showed antifungal activity (sensitive) to the two fungi Candida albicans and Aspergillusniger. Emam extract showed antibacterial activity (sensitive) to Escherichia c oli,B acillus subtilis, and Staphylococcus aureus, and antifungal activity (sensitive) to Aspergillusniger. The water extract for the remaining types showed intermediate activities to the selected bacteria and fungi.

Table 3.10: Microbial Activity of Water Extract

Plants name	Antimicrobial activities					
	Antibacterial activity				Antifungal activity	
Extract of	S.a	B.s	P.s	E.c	A.s	Ca
Emam	22	23	17	19	30	-
Pohean	17	15	13	15	21	-
Wadielneel	-	-	-	-	20	-
Argean	16	15	15	17	20	18
Pladi	17	19	16	23	24	19
Dibera	22	20	20	20	30	19

E.C: Escherichia coli, P.S: Pseudomonas aeruginosa, B.S: Bacillus subtilis, S.a: Staphylococcus aureus; Ca:Candida ,albicans, A.S: Aspergillus niger

.Scale of interpretation:
>18 mm (MIZD) = sensitive
14-18 mm (MIZD) = intermediate
<14 mm (MIZD) = resistant

Extracts of Water (Argeen (13))
Extracts of Water (Pladi (14))
Extracts of Water (Debera (15))

Figure 47 Escherichia coli ATCC 25922(Gram -ve bacteria)

Extracts of Water (Argeen (13))
Extracts of Water (Pladi (14))
Extracts of Water (Debera (15))

Aspergillus niger ATCC 9763)

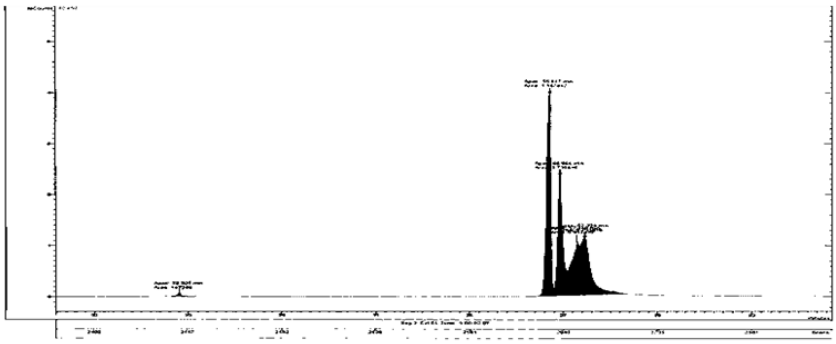


Chart 3:1 G.C of Petroleum ether Fraction 1

Chart 3:2 G.C of Petroleum ether Fraction 2

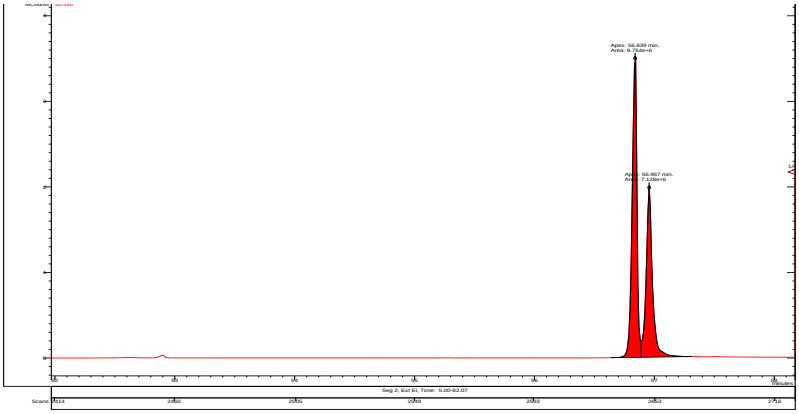
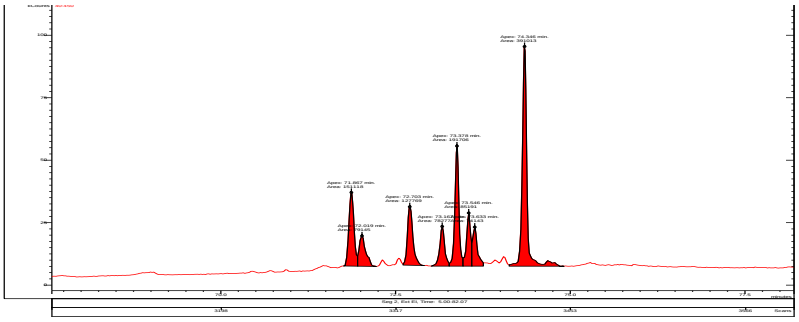


Chart 3:3 G.C of Acetone Fraction 2

Chart 3:4 G.C of Ethanol Fraction 2



IV. Conclusion

The results conclude that the Wheat bran contains Antioxidant Activity. Using DiPhenyl Picrayl Hydrazine Radical Scavenging Assay, the highest antioxidant activity of the Petroleum ether extract found to be in Wadielneel. In ethanolic extract, Pladi is the highest in antioxidant activity followed by Wadielneel and then Argeen. The free radical scavenging activity of wheat extracts is mainly due to phenolic compounds. It is also notable that this product is a potential source of antimicrobial activity that act mainly on Gram-positive and Gram-negative bacteria and fungous. Thus, it can be an efficient protective agent as antioxidant and antibacterial additives in food systems. The overall results shows that, these by-products are very good source of bioactive compounds of beneficial effects on health.

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References

[1]. Surget, A.; Barron, C. histology du grain du ble. Industrie des cereals. **145**: 3-7, (2005).
[2]. Alabaster, Z. Tang, and N. Shivapurkar. "Inhibition by wheat bran cereals of the development of aberrant crypt foci and colon tumors". Food and Chemical Toxicology, **35**, pp. 517-522**, (**1997).
[3]. Moller, M.E. R. Dahl, and O. C. Bockman. "A possible role of the dietary fibre product, wheat bran, as a nitrite scavenger", Food Chemistry and Toxicology, **26**, pp**.** 841-845, (1988).
[4]. Halliwell. B, "Antioxidants in human health and diseases," Annu. Rev. Nutr., **16**, pp. 33-50, (1992).
[5]. Truswell. A. S. "Cereal grains and coronary heart disease," Eur. J. Clin. Nutr, **56**, pp**.** 1-14, (2003).
[6]. Haley. L. Yu, Perret S., J.; Harris M. 'Antioxidant properties of hard winter wheat extracts,' Food Chem, **78**, pp. 457-461, (2002).
[7]. Andreasen. M. F, Kroon, P. A., Williamson. G. and M. T. Garcia-Conesa. "Intestinal release and uptake of phenolic antioxidant diferulic acids , Free Radical Biol. Med., **31**, pp. 304-314, (2001).
[8]. Haley. L. Yu. Perret S., J, Harris M., Wilson J.; M. Qian. –Free radical scavenging properties of wheat extracts, 'J. Agric. Food Chem., **50**, pp. 1619-1624, (2002).
[9]. Yu, L.; Haley, S.; Perret, J.; Harris, M.; Wilson, J.; Qian, M.; 'Free radical scavenging properties of wheat extracts' , J. Agric. Food Chem., **50**, pp. 1619-1624, (2002).
[10]. Adom, K. K.; R. H. Liu. 'Rapid peroxyl radical scavenging capacity(PSC) assay for assessing both hydrophilic and lipophilic antioxidants', J. Agric. Food Chem., **53**, pp. 6572-6580,(2005).
[11]. Kwon, K-T; Uddin, M- S; Jung, G-W; Sim, J-E; Chun. B-S; 'Supercritical Carbon Dioxide Extraction of Phenolics and Tocopherols Enriched Oil from Wheat Bran' World Academy of Science, Engineering and Technology, **4**: 04-27, (2010).
[12]. Gelmez, N. S. Kincal, ; M. E. Yener, Optimization of supercritical carbon dioxide extraction of antioxidants from roasted wheat germ based on yield, total phenolic and tocopherol contents, and antioxidant activities of the extracts, J. Supercrit. Fluids, **48**, pp. 217-2240, (2009).
[13]. shewry P. The Healthgrainprogramme opens new opportunities for improving wheat for nutrition and health. Nutr Bull. 2009;34:225–231
[14]. Gurjar M S, Ali S, Akhtar M, et al., Efficacy of plant extracts in plant disease management. Agricultural Sciences, 2012; 3: 425-433.
[15]. Merkl R, HrádkoVá I, Filip V, et al., Antimicrobial and antioxidant properties of phenolic acids alkyl esters. Czech J Food Science, 2010; 28: 275-279.
[16]. Roby M H H, Sarhan M A, Selim K A H, et al., Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), and marjoram (Origanummajorana L.) extracts. Industrial Crops and Products, 2013; 43: 827-831.
[17]. Gutiérrez-Larraínzar M, Rúa J, Caro I, et al., Evaluation of antimicrobial and antioxidant activities of natural phenolic compounds against foodborne pathogens and spoilage bacteria. Food Control, 2012; 26: 555-563.
[18]. Shimada, K.; Fujikawa, K.; Yahara, K.; Nakamura, T. Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem; 40:945-8. (1992).
[19]. Kavanagh, F. Analytical Microbiology. Academic Press, York, London, **11:11**, (1972).