Pure Appl. Biol., 7(2):765-774, June, 2018
http://dx.doi.org/10.19045/bspab.2018.70095

Research Article

Nutritional, physicochemical, antimicrobial and DPPH free radical scavenging potential of the biotech and conventional hybrids of seeds and seed oils from *Gossypium hirsutum* L.

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Citation
Fazia Ghaffar, Ikhtiar Khan, Hamidullah Shah and Karishma Iqbal. Nutritional, physicochemical, antimicrobial and DPPH free radical scavenging potential of the biotech and conventional hybrids of seeds and seed oils from *Gossypium hirsutum* L. Pure and Applied Biology. Vol. 7, Issue 2, pp765-774.
http://dx.doi.org/10.19045/bspab.2018.70095

Received: 11/03/2018 Revised: 01/06/2018 Accepted: 05/06/2018 Online First: 28/06/2018

Abstract

This comparative study investigated the differences in the proximate and mineral composition of seed and fatty acid profiles, physiochemical, antimicrobial properties, and antioxidant characteristics of seed oils of FH-942 (NBt) and MNH-886 (Bt) of *Gossypium Hirsutum* (upland cotton). Proximate analysis of seeds of both the hybrids showed ash content (7.74% conventional & 6.69% BT), crude fat (14.4% conventional & 12.2% BT), crude protein (29.9% conventional & 28.49% BT) and organic compounds like alkaloids of the conventional hybrid being comparatively better than the biotech hybrid. The carbohydrates and crude fiber of the Bt variety were on the higher side. The Bt genotype had comparatively higher content of calcium, copper and manganese. The physical properties of the crude oil showed density (g/ml) 1.046 & 1.119, specific gravity 0.981 & 1.043, and refractive index 1.467 & 1.468 for conventional and biotech variety respectively. Chemical properties of the crude oils indicated iodine value (conventional 101.2, BT 95.2g of I2/100g of oil), Saponification value (conventional 189.28, BT 175.30mg of KOH/g oil) of the conventional hybrid was better than the biotech hybrid. Fatty acid composition from both genotypes showed insignificant variations except for linoleic acid and linolenic acid being significantly higher in MNH-886. Crude oil of both the hybrids showed lower antibacterial activity against streptomycin and good antifungal potential. Oils of both the hybrids also showed good antioxidant potential in DPPH free radical scavenging indicating greater potentials of both varieties to be used as an edible oil & in the manufacturing of various industrial products.

Keywords: Antibacterial activity; Antifungal activity; Antioxidant potential; Fatty acids composition; Physico-chemical properties; Proximate compositio

Introduction

Belonging to the *Malvaceae* family and tribe of Gossypieae, *Gossypium* (cotton) grow naturally as a perennial shrub or tree. However for commercial purposes it is grown as an annual crop. *Gossypium*

hirsutum L. (upland cotton) is a major cash crop known to be the source of world's most important textile fiber, the second best source of plant protein after soybean, and the fifth oil producing plant. Cotton fiber is used to make a number of textile products. In addition to textile industry cottonseed is used to produce oil which after refining can be used like any other vegetable oil. Traditionally four major species of cotton are being grown: *hirsutum, barbadense, arboretum,* and *herbaceum* around the world [1].

Cotton accounts for 8.2 percent of the value added in agriculture and about 3.2 % to GDP; around two thirds of Pakistani export earnings are from the cotton made-up textiles which in turn adds about $ 2.5 billion to the national economy. While hundreds of ginning factories and textile mills in the country heavily depend upon cotton, life of millions of farmers is dependent upon this crop [2]. In Pakistan nearly all of the cottonseed oil is processed commercially to be utilized for edible purposes .It contribute 70% in the Pakistani edible oil production as compared to 13% sunflower, 11 % rapeseed/mustard seed, and 5.6% Canola [3]. It has high level of antioxidant-Vitamin E- that contributes to its long life in the cooking and on the shelf. This processed cottonseed oil is the fifth leading vegetable oil in the world. Cotton seed oil is generally considered as healthy vegetable oil. It is cholesterol free and hence termed as "Heart oil". Cotton seed meal which is left after oil extraction is used as a source of fodder protein in livestock industry [4]. Constituting nearly half of a seed's weight the meal contains 23% high biological value proteins [2].

A number of different cotton varieties have been developed from the conventional varieties. These *Bacillus thuringiensus* (BT) cotton are variety of cotton genetically modified that confers resistance to boll worm complex. These proteins have specific insecticidal activity but are nontoxic humans and other vertebrates [5-7]. Year 2012 was third year of commercialization of Bt Cotton in Pakistan. At an adoption rate of 82% on the total 3.4 million hectares of land was used for cotton based on the assumption that this surplus production in 2012-2013 of 15.5 million bales will allow increased cotton exports by 77% [2]. Cotton cultivars (both Bt and conventional) are substantially similar in chemical composition however such type of investigations were being not done previously in Pakistan. The current study aimed to compare nutritional composition of cottonseed and physiochemical, and antimicrobial activities of one biotech variety against one conventional variety as a control.

Materials and methods

Two hybrids of cottonseeds FH-942 (CLCUV- Heat tolerant, conventional) and MNH-886 (FH-207×MNH770×Bollgard-1, Biotech) were obtained from the seed unit of Cotton Research Institute, Ayub Research Institute, Faisalabad, Pakistan. The samples were thoroughly cleaned. Seed were ground and kept in dark dry conditions.

Proximate composition and mineral content determination

After oil extraction the seed cake /residue/flour samples were tested for proximate and elemental composition. By using AOAC official methods, total protein content of the seed cake was estimated by a micro Kjeldhal apparatus. Crude oil was estimated by Soxhlet method while crude fiber and ash contents were determined by the AOAC official methods [8, 9]. For minerals analysis samples were first subjected to acid digestion and later were analyzed by atomic absorption spectrophotometer (AAS) methods [10].

Oil extraction

To determine percent oil seeds were extracted with n-hexane, chloroform and petroleum ether. Seed were soaked separately for 24 hours and the extracts were then concentrated with rotary

evaporator under reduced pressure at 40-60^{o}C.

Physiochemical tests

Based on the methods described by the association of Official Analytical Chemists both of oil samples were tested for refractive index, specific gravity, viscosity, iodine value, saponification value, peroxide value, total acid number and free fatty acids value **[9]**.

Fatty acids analysis

Preparation of fames

About25-40 mg of oil sample were weighed in FAMEs tubes and 1.5 ml methanolic Sodium hydroxide (0.5 N) was added. The tubes were Stoppard with screw capes. The mixture was heated in a boiling water bath for 05 minutes. Tubes were cooled to room temperature and 2.5 ml BF_3 (10 % in MeOH) was added. Tubes were again heated in boiling H_2O for half an hour. Cooled at room temperature & and 5 ml brine solution + 1 ml n-hexane was added. The tubes were shaken vigorously on vortex and then allowed the layers were allowed separate. The upper (n- hexane) layer was taken through pasture pipette. For GCMS analysis about 1 ml n-hexane was added again to repeat the above steps to separate the hexane layer. The volume was adjusted to 2 ml, filtered through 45 µm membrane filter and transferred to GC vial for injection into GC-MS.

Identification of fatty acids by GC-MS

The methyl esters of the oil were analyzed for the respective fatty acid composition by Gas Chromatography Mass Spectrometry. The equipment used for this purpose was Shimadzu GC-MS- QP 2010 Plus using a capillary column TRB FRAP (30 m x 0.25mm i.d). The temperature programming of the column oven was set as 50 °C – 220 °C with rise of 5 °C/ min. Helium was used as the carrier gas and its total flow was adjusted to 77.1 ml/min while column flow was 3.29 ml/min at split ratio of 20.0. The temperatures of injector, interface and ion source were set at 240 °C, 240 °C and, 250 °C respectively. The peaks were identified by comparison of their retention time with those of the standard methyl esters (FAMEs standard mix, 37 components, Sigma Aldrich) analyzed under the same conditions.

Antimicrobial assay

The test for susceptibility was done using agar Well Diffusion method **[11]**. Two stains of gram positive bacteria (*Staphylococcus aureus*, and *Bacillus subtilis*) and one gram-negative bacterium (*Klebsiella Pneumonia*) against streptomycin as a standard. Antifungal effect of the oils against three fungal strains (*Alternaria solania, Aspergilus niger* and *Aspergillus flavus*) were determined by Tube Dilution methods using miconazole as a standard **[7]**.

Antioxidant activity by 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging methods

Antioxidant activity was measured from the bleaching of methanol solution of 2, 2-diphenyl-1-picrylhyrazyl (DPPH) using optima UV-Visible Spectrophotometer. DPPH radical scavenging activity was performed by following the method of Rauf et al **[11]**. About 25mg of oil was dissolved in distilled methanol and was diluted up to 50 mL. From the stock solution 10, 20,40,60,80,100 mg/ ml dilutions were prepared. Of each solution 5 mL was taken in a test tube to which 1 mL of 0.01 M of DPPH solution was added. They were kept in a dark for test minutes along a control (5 methanol+ 1 mL DPPH solution). After the incubation for 30 minutes the antioxidant activity was measured by using Optima U-V- Visible Spectrophotometer at wave length of 517 nm. The experiments were performed in three replicates. Quercetin was taken as a standard for the purpose of comparison. Percent radical scavenging activity was calculated as:

% DPPH= Control absorbance- Extract absorbance × 100/ Control absorbance.

Statistical analysis

Data collected from the triplicate analyses was analyzed for means, standard deviations and one way ANOVA at P=< 0.05 level of significance wherever needed.

Results

Cotton as "white gold" monopolizes global trade market as a cash crop. Cotton plant give four main important products-fibers, linters, seed oil, and seed cake. Proximate composition of both seed cakes (Table 1) showed that percent moisture of conventional variety was 16.53% and that of Biotech variety was 8.67% percent ash content of the conventional variety (7.74 %) was higher than the biotech variety (6.69%). Crude protein content of the conventional variety was 29. 98% while that of Biotech variety was 28.49% the results of crude fat (14.4, 12.2), and total alkaloids (12.14, 10.96) content of the conventional variety were higher than Bt variety, However crude fiber content (11.79% and 15.84%) and percent pectin (3.7 and 4.7) of the Bt variety were higher than conventional FH – 942 hybrid of the cotton. Results of the mineral concentrations showed that indigenous variety had significantly higher concentrations of iron and zinc while Bt hybrid contained much higher value of calcium, phosphorus and manganese.

Table 1. Proximate composition of cotton seeds

Properties	FH-942	MNH-886
Proximate Values (g/100g)		
Moisture (%)	16.53±0.02	8.67±0.91
Ash (%)	7.74±0.82	6.69±0.05
Crude protein (%)	29.98±0.09	28.49±0.05
Crude fat (%)	14.4±1.04	12.2±0.05*
Crude fiber (%)	11.79±0.71	15.84±2.13*
Pectin (%)	3.7±0.84	4.7±1.02*
Total alkaloids	12.140±0.56	10.96±0.91*
Minerals (mg/kg^{-1})		
Calcium	0.445±0.006	0.932±0.04*
Phosphorus	1.154±0.04	1.862±0.007*
Copper	13.74±0.003	13.04±0.005
Iron	129.62±0.02	111.54±0.03*
Zinc	67.08±0.02	62.53±0.06
Manganese	12.2±0.04	15.87±0.02*

*****values are different significantly at P≤0.05

Results of the percent oil extraction indicated (Table 2) highest percent oil being extracted (28.83% and 24.07%) with chloroform followed by *n*-hexane (21.29% and 19.29%) and petroleum ether (14.76 and 12.38%) for both of the hybrids respectively. The data also indicated that conventional hybrid (FH-942) had comparatively higher percent oil content as compared to biotech hybrid (MNH-886). Physiochemical properties of both crude oils showed moisture content 1.35% in conventional variety and (2.39%) in biotech variety (75 and 63), density (1.046 gm/ml and 1.119 g/ml), specific gravity (0.981 and 1.043), and refractive index (1.467 and 1.468) of both varieties were almost similar with slight higher values for the conventional variety. Crude oil of conventional variety had a reddish brown color while BT variety had a dark yellowish -to orange oil. Iodine value of conventional hybrids oil was 101.2 while biotech hybrid had an iodine value of 95.2. Saponification value of the conventional variety's oil was 189.28 (mg of KOH/g

Oil) and that of BT variety was 175.30 (mg of KOH/g oil). Both of oil samples had zero peroxide value free fatty acid value was 2.086 and 1.579 and total acid number was 4.1 and 3.1 for both the varieties respectively signifying the oil has edibility and less chances of getting rancid steadily.

Results of the fatty acid composition of the oils from the two *gossypium*, genotypes showed (Table 3, Figures 1 & 2) insignificant differences. The only differences found were in the oleic acid concentrations which were quite high in the conventional variety as compared to the Bt variety. The Bt Variety however contained much higher proportions of linoleic, linolenic acids and arachidic acid. The data showed that when the fatty acids were pooled for the estimation of percent unsaturated and saturated fatty acids and the ratio of 16 carbons containing fatty acids to 18 carbon fatty acids the differences between the two genotypes were non-significant.

Results of the antibacterial assay showed (Table 4) that oil sample of conventional variety had higher activity against *staphylococcus* (18 mm) followed by *Bacillus subtilis* (16 mm) as compared to Biotech sample which showed no resistance against *staphylococcus*, 12 mm against *Bacillus subtilis* and 10mm against *klebsiella pneumonia* respectively. As far the antifungal potential (Table 5)of the oils conventional variety showed excellent activity as compared to the biotech variety against *Aspergillus flavus* (105 mm and 90mm) followed by *Aspergillus Niger* (95mm and 85mm), and *Alternaria Solania* (80 and 70mm) respectively as against the standard DMSO.

The results of the antioxidant potential of the oil samples (Table 6) showed maximum free radical scavenging effect were demonstrated by the conventional oil having 20.88, 20.50, 19.37, 17.86, 16.86 and 13.96% at the tested doses of 100, 80, 60, 40, 20 and 10 ppm respectively. Biotech hybrids oil showed lesser potential 11.57, 11.59, 9.59, 9.11, 8.39, and 6.59% at test doses of 100, 80, 60, 40, 20, and 10 ppm respectively. However both of the oils showed low antioxidant activity as compared to Quercetin as a standard.

Table 2. Physiochemical properties of crude oils of *Gossypium hirsutum*

S. No	Physicochemical Properties	FH-942	MNH-886
1.	% Oil		
	i-Pet. Ether	14.76±0.08	12.38±1.12
	ii- N. Hexane	21.29±0.02	19.29±0.53
	iii-Chloroform	28.83±0.21	24.07±0.08
2.	Moisture (%)	1.35±0.02	2.39±0.56
3.	Viscosity at 21^{o}C (%)	75±0.03	63±0.78
4.	Density (20^{o}C kg/ml)	1.046 ±0.04	1.119±0.04
5.	Specific Gravity (%)	0.981±0.12	1.043±0.03
6.	Refractive index at 25^{o}C	1.467±1.23	1.468±0.91
7.	Color (against light with eye sighting)	Reddish Brown	Dark Yellowish to Orange
8.	Iodine value (g of I2/100 g of oil)	101.2±0.78	95.2±1.02
9.	Saponification value (mg of KOH/g of oil)	189.28±0.03	175.30±0.03
10	Peroxide value (MEq of O2/kg of oil)		0
11.	Free fatty acid value (g/100g of oil)	2.086±0.91	1.579±0.02

Table 3. Fatty acids profiles of the seed oils

Fatty acids %	FH-942	MNH-886	P Level
C 10:0; Capric acid	0.017±0.02	0.01+0.15	0.23
C 12:0; Lauric acid	0.034±0.01	0.04+0.05	0.16
C 14:0; Myristic acid	1.099±0.01	1.18+0.03	0.067
C 15:0; Pantadecanoic acid	0.034±0.01	0.03+0.11	1.02
C 16:0; Palmitic acid	24.670±0.17	23.88+0.09	0.23
C 16:1; Palmitoleic acid	0.685±0.2	0.72+0.61	0.078
C 17:0; Margaric acid	0.102±0.01	0.09+0.11	0.045*
C 18:0; Stearic acid	3.222±0.04	3.17+0.03	0.067
C 18:1c; Oleic acid	15.77±0.01	12.34+0.01	0.046*
C 18:1n9t; Elaidic acid	1.17±0.01	1.30+0.02	0.068
C 18:2c; Linoleic acid	46.69±0.12	49.76+1.73	0.049*
C 18:2t; Octadecadicnoic acid	0.36±0.01	0.33+0.02	1.002
C 18:3n6; g-linolenic aicd	5.67±0.02	6.17+0.02	0.052*
C 18:3n3; Linolenic acid	0.34±0.05	0.37+0.09	0.91
C 20:0; Arachidicacic acid	0.09±0.01	0.37+0.03	0.039*
C 22:0; Behenic acid	0.017±0.02	0.12+0.01	0.061
C 23:0; Tricosanoic acid	0.051±0.02	0.03+0.02	0.071
C 24:0; Lignoceric acid	0.098	0.56+0.09	0.051
% saturated	29.38±0.03	28.79+0.44	0.068
% unsaturated	70.83±0.012	70.68+0.09	0.91
18/16 ratio	0.348±0.003	0.33+0.25	0.081
20-24 ratio	0.5±0.001	0.58+0.02	0.087

*****Differences are significant at P≤0.05

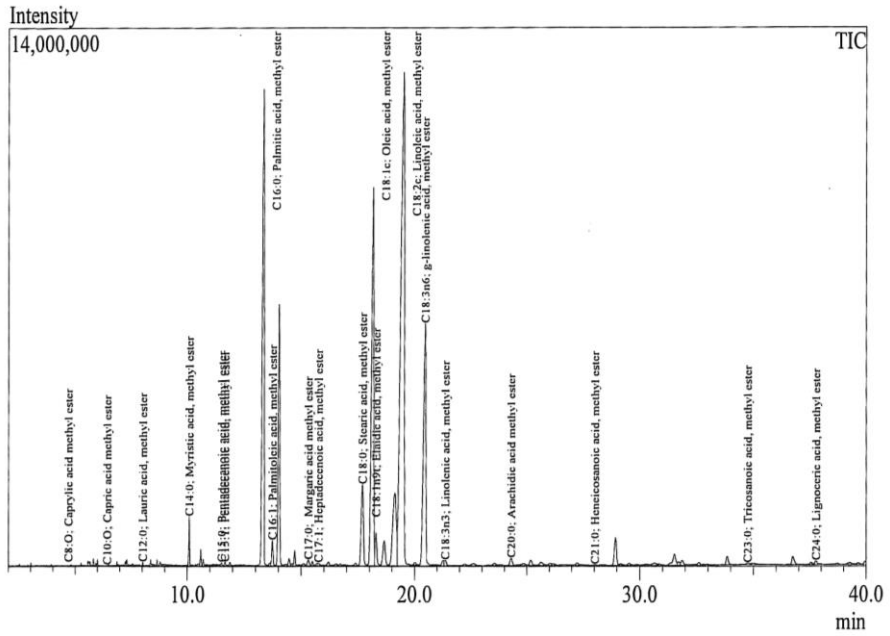

Figure 1. GC MS chromatogram of fatty acids for FH-942

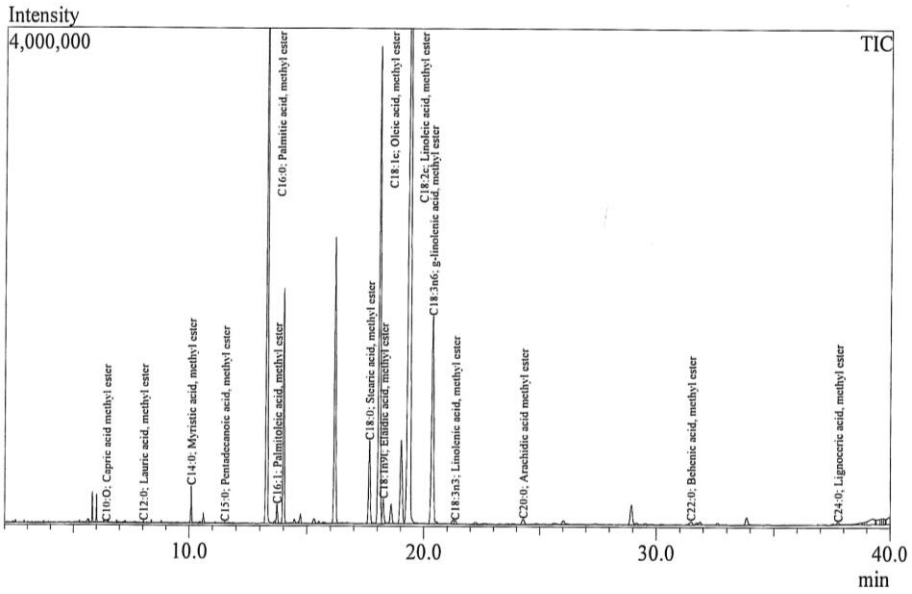

Figure 2. GC MS chromatogram of fatty acids for MNH-886

Table 4. Antibacterial assay of the oils of *G. hirsutum*

Bacterial Strains	Percent inhibition (mm)		
	FH-942	MNH-886	Streptomycin (2mg/ml)
Bacillus subtilus	16	12	28
Staphylococcus aureus	18	0	30
Klebsiella pneumonia	14	10	30
DMSO	0	0	0

Table 5. Antifungal activity of the oils extracted from *G. hirsutum*

Fungi strain	Percent inhibition (mm)		
	FH-942	MNH-886	DMSO (Standard)
Aspergillus flavus	105	90	115
Alternaria solania	80	70	115
Aspergillus niger	95	85	115

Table 6. Antioxidant profile of the oil extracted from *G. hirsutum*

Conc. (ppm)	% DDPH Inhibition	
	FH-942	MNH-886
10	13.69±0.02	6.95±0.02
20	10.86±0.02	8.39±0.04
40	17.86±0.04	9.11±0.04
60	19.37±0.01	9.59±0.03
80	20.50±0.03	11.59±0.05
100	20.88±0.02	11.75±0.03
Quercetin	100±0.001	100±0.002

Discussion

In the present study two samples of cotton seeds were initially studied for various physicochemical characteristics to identify the practical importance and provide basis for suitability and utility of Biotech hybrids seeds. The fixed oils obtained from *G. hirsutum* were extracted with

three different solvents i.e. n- hexane, petroleum ether and chloroform. Oil extraction (Table 2) with the soxhlet method by using chloroform gave highest percent yield (28.83% and 24.07%) from both varieties. This could be due to the increased ability of the solvent to overcome forces that bind lipids within the sample matrix [12]. The oil content of conventional hybrid with all the solvents was greater than that of the Biotech hybrid. Fixed oil yield of cotton seed in the preset was more than being reported in Nigeria which ranged 15.05-24%. This difference in the yield might be attributed to the ecological/ environmental factors in which the respective varieties were grown [13]. Similarly, as reported variations in the oil yield are expected when oils are extracted with different solvents [14-16]. However, the percent oil values of the samples under this study fell well within the recommended standards for cotton seed oil [17].

The moisture content of conventional sample was low as compared to BT sample. Low moisture content increases the shelf life of the oil by preventing oxidation and rancidity [13]. The higher moisture content will assist in hydrolysis while low moisture content in oil have been reported to increase its storability and suitability to be preserved for a longer period [12, 13]. The conventional seed oil was more viscose than its BT counterpart indicating longer chain lengths and the values were found to be closer to values in literature [18].

Specific gravity and density of conventional seed oil were less than the BT variety seed oils confirming that specific gravity less than 1 (one) means the substance will float on water [19]. The value 0.981 specific gravity is also closer to specific gravity of groundnut and neem seed oil [18]. Refractive index values of both the sampled oils (1.467 and 1.468) were in close agreement with values reported for soybean oil (1.466-1.470) and palm kernel (1.449-1.451). The high refractive index of these oils seems to confirm the high number of carbon atoms in their fatty acids and increase in the double bonds [19].

Iodine value of the BT hybrid seed oil was 95.2 g/100g and that for conventional hybrid's seed oil was 101.2g/100g. The values obtained are closer to that of pumpkin seed oil with an iodine value of 105.53/100g [13]. Iodine value above 100 indicates high proportion of instauration and suggests that the oil may be used as drying agent and as a cooking oil [19]. The zero peroxide oxide value of both the oil indicates the oil is stable and would not easily go rancid [1]. Saponification value of the conventional oil was 175.30 mg KOH/g. Both of these values are lower than 213mg KoH/g in neem seed oil and coconut oil 253 mg KoH/g [19]. This indicates that these oils can be used in soap production [20]. Acid value of the sampled oils (Table 2) was found to be 4-1 and 3.1 mg of KoH/g of oil for conventional and BT varieties respectively. There values are lower than that of olive oil (17 mg KoH/1g) and peanut fat (10.49 mg KOH/g) being reported [19]. Results of the current data show that the oil is edible and can stay for a long time without getting rancid readily.

The fatty acid composition of both the oils were according to the standards for cotton seed oil and were at par with the composition of cottonseed oil from Turkey and Nigeria [22, 23]. The differences in the omega fatty acids might be attributed to the adaptability of the Bt variety to the local environment or as suggested might be due to the transcriptional technique adopted [24, 25].

Antioxidant potential of both oils (Table 6) as tested by DPPH radical scavenging activity showed low activity for both the oils (22.88% --6.95%) in contrast to the standard [26]. Antibacterial essay against these bacterial strains (*Bacillus Subtilus, Staphylococcus aureus, and klebsiella Pneumonia)* showed 60-33% lower resistance against standard of

streptomycin. However both of oils showed maximum antifungal activities (*aspergillus flavus, alaternaria solania, and aspergillus Niger*) as tested against standard DMSO indicating the use of cotton seed oils as pharmaceutical fungicide **[27, 28]**.

Proximate composition of the cakes showed conventional variety to be better nutritionally than the BT variety, however the differences were not significant so far. Cotton seed cake of both varieties contained appreciable amount of proteins (29.98 and 28.49 g/100g) and crude fiber (11.79 and 15.84 g/100g) ash (7.74-6.69%) and fats (14.4-12.2g/100gm) suggesting it to be a potential source in the animals feed. The data suggest that nutrient composition of the whole seeds from both the genotypes were at par with the international standards for cottonseed **[29, 30]**.

Conclusion

This study can be concluded on the facts that conventional variety of *G. hirsutum* possess better Physicochemical, antioxidant and antimicrobial potential of oils and nutritional composition of seed cakes, however the differences so far are not significant for the selected samples. The desirable characteristics indicate potential uses of cotton seeds as an edible oil, and have utility in the preparation of pharmaceutical products, soap making, cosmetic products and as an animal feed and protein fortification source in human food.

Authors' contributions

Conceived and designed the experiments: F Ghaffar, I Khan & H Shah, Performed the experiments: F Ghaffar & K Iqbal, Analyzed the data: F Ghaffar, Contributed materials/ analysis/ tools: H Shah, Wrote the paper: F Ghaffar & I Khan.

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