Scientific paper

Characterization, Compositional Studies, Antioxidant and Antibacterial Activities of Seeds of *Abutilon indicum* and *Abutilon muticum* Grown Wild in Pakistan

Muhammad Akram Kashmiri, Sammia Yasmin*, Mushtaq Ahmad and Ayesha Mohy-ud-Din

Center for Plant Product Research, Department of Chemistry, GC. University, Lahore-54000, Pakistan

** Corresponding author: E-mail: sammiayasmin@yahoo.com; Phone: 0092-3008864139*

Received: 11-08-2008

Abstract

The Physicochemical properties, macro and micro elements (Al, Cd, Ca,Fe, Pb, Mg, P, K, and Zn), fatty acids, total phenolic content, antioxidant and antibacterial activities of oil from seeds of two *Abutilon* species, grown wild in Pakistan were investigated. *Abutilon indicum* L. (Malvaceae) and *Abutilon muticum* DC. (Malvaceae) were found to contain 5.9 and 6.3% moisture, 25.1 and 28.3% crude fiber, 22.4 and 23.5% crude protein, 2.3 and 3.4% ash and 33.1 and 25.6% carbohydrate respectively. Seed total lipids were extracted, characterized by chromatographic techniques and analyzed by mass spectrometry. The oils were found to be rich in neutral lipids (95.2 and 94.7% of total lipids) and low in polar lipids (3.7 and 3.6% of total lipids) for *A. indicum* and *A. muticum* respectively. GC-MS data showed that the main fatty acid was linoleic acid in both the species. Total phenolic compounds in each sample were determined according to Follin-Ciocalteu's method as, 13.770 and 38.815 mg/g of oil, respectively. Total antioxidant activity of both oils was checked by ABTS, FRAP, DPPH and oleic acid peroxidation methods. These methods indicated the presence of both the slow reacting and fast reacting components in the seed oils of both the herbs. The seed oil of *Abutilon indicum* and *Abutilon muticum* showed broad spectrum activity as they were active against Gram-positive and Gram-negative bacteria. The findings reveal seeds of *Abutilon* species, indigenous to Pakistan to be potentially valuable herb for oil production, delivery of drugs and cosmetic active ingredients.

Keywords: Malvaceae, minerals, oil composition, GC-MS, antioxidant, antibacterial

1. Introduction

The demand for vegetable oil in Pakistan has been increasing progressively and has seen rapid growth in this industry from two factory productive units in 1947 to more than 40 factory productive units in 2008. Cotton seed is the major domestic source of edible oil followed by rape, mustard and canola. Despite of having a predominantly agrarian economy, Pakistan agriculture is unable to meet the national requirement of vegetable oil. The 70% of short fall of edible oil requirement is currently met through import to the tune of Rs.38 billion annually.[1] Alternate approaches to growing crops have to be found to meet the other requirements (edible oil being an imported role) of the human population. This paper tests the hypothesis that some perennial shrubs, wildly growing, produce oil seeds acceptable for human consumption.

Since cotton has been studied in all its aspects, it appeared of interest to us to investigate the properties and compositions of the oils of other wild plants belonging to the same family (Malvaceae), so the characteristics and compositions of seeds and oils of two species, *Abutilon indicum* L. and *Abutilon muticum* DC. of family Malvaceae,[2] are reported here for the first time as a part of our studies to locate new oil seed resources. *Abutilon indicum* (local name; peely booti or karandi) is an erect, woody, shrubby plant, widely distributed in the tropical coun-

tries.[3] This plant is used in indigenous medicine for number of diseases. The oil from the roots of *A. indicum* is reported to have analgesic activity comparable with that of acetyl salicylic acid and it is devoid of CNS depressant activity.[4] The seeds are used as a laxative in piles and in the treatment of cough. According to the Chinese in Hong Kong, the seeds are employed as an emollient and demulcent. The bark and the root are sued as a diuretic, anthelmintic, pulmonary sedative, haematuria and in fever.[5]

Abutilon muticum (local name; chakrabenda) occurs in plains through out Pakistan especially more common in Sindh and abundantly in desert of Cholistan Bahawalpur. *A. muticum* is a tomentose under shrub, bears spherical fruit having about 25 carpals, each of which contains 3 tasteless seeds.[2] All parts of the plant are used medicinally.[6] Seeds are used in the treatment of cold, cough bronchial infection, inflammation of urinary tract, gonorrhea, diarrhea and ulcers. The seeds cakes are also used for dairy cattle and as fertilizer.[7]

Alkaloids, flavonoids, steroids, terpenoids and saponins have been isolated and characterized from genus *Abutilon*.[8,9] Previous phytochemical investigations of *Abutilon indicum* showed it to contain seven flavonoids,[10] two sesquiterpene lactones,[11] gallic acid, β-sitosterol, geraniol and caryophylline.[9] The analysis of phenolic compounds in plants is of considerable commercial importance, since it is known that they contribute to the flavour.[12] A common spectrophotometric method for total polyphenol content according to Follin-Ciocalteu has been widely used in the area of oncology and viticulture.[13] In this study we present for the first time the composition and total polyphenol content of *Abutilon* seeds.

Spoilage of foods due to the presence of bacterial and fungal infection has been a major concern for decades and it causes a considerable loss world wide. The demand for non toxic, natural preservatives has been rising with increased awareness and reports of ill effects of synthetic chemicals present in foods. Further more emergence of food borne pathogens has lately become a major public health concern. Many compounds present in the plants have been reported to be biologically active, antimicrobial, allopathic, antioxidants and have bio regulatory properties. To our knowledge no information is available on the antioxidant and antibacterial properties of *Abutilon* seeds. Therefore, another objective of the present study was to investigate the antioxidant activity of *Abutilon* seed extracts, in vitro models and to determine their antibacterial activity against different food borne pathogens for their potential as a natural preservative for nutraceutical, food, and cosmetic formulations.

2. Materials and Methods

2. 1. Chemicals

Sodium acetate, 2,4,6-triphenylpyridyl-S-triazine (TPTZ), Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS [2,2'-azinobis (3-ethyl benzothiazoline-6-sulphonic acid)],6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 3,5,7,3',4'-pentahydroxyflavone, $FeCl_3 \cdot 6H_2O$, potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), gallic acid, linoleic acid, Tween-20 and ammonium thiocyanate were purchased from Aldrich Chemicals Co. (Gill Ingham, Dorset, UK). All other reagents and solvents were of analytical grade and purchased from either Sigma or Merck representatives.

2. 2. Plant Materials

The fruits of *A. indicum* and *A. muticum* growing wild in Pakistan were collected and identified by Dr. Zaheer-ud-Din Khan, Head of Botany department, GC University, Lahore-Pakistan. Voucher specimens of both the plants were deposited at the herbarium of the department of Botany GC University, Lahore, Pakistan (*A. indicum*; GC. Herb. Bot. 68: A. *muticum*; GC. Herb. Bot. 138). Seeds were obtained after deseeding the fruits by passing through stainless steel sievers (mesh pore size, 0.042 in). Seeds were kept in dark at 5 °C until analysis were carried out (all analysis were completed within 3 months).

2. 3. Proximate Analysis

Percentage of moisture by vacuum oven (method 934.06), total fat by Soxhlet extractive (method 920.39c), protein by kjeldahl nitrogen (method 920.152) and ash by direct analysis (method 940.26) were determined according to the AOAC method.[14] The percentage of crude protein was estimated by multiplying the total nitrogen content by a factor of 5.30.[14] Total carbohydrates were calculated by subtracting the total percentage of other components from 100. Refractive index, acid value, saponification value and iodine number was determined.[14]

2. 4. Mineral Analysis

Grated seeds (5g) were ashed according to the AOAC method 985.35 to obtain ash free from carbon.[14] The ash obtained was dissolved in 5 mL of I M HNO_3 and then heated over a steam bath (at 50–60 °C) for 5 min to help with dissolution. Subsequently, materials were transferred to a 100 mL volumetric flask and made up to final volume of 100 mL with the extraction solvent. Minerals were determined using a Unicam 969 AA spectrometer equipped with a GF 90 furnace and FS 90 furnace auto sampler (Unicam limited Cambridge UK). Minerals were quantified on the basis of peak areas and comparison with a calibration curve obtained with corresponding standards.

2. 5. Total Lipid Extraction

The *Abutilon* seeds were weighed and the total lipids extracted according to Bligh and Dyer, 1959.[15] Solvent

was removed by a rotary evaporator at 30 °C and the remainder (a yellow oil) was weighed and stored in the dark at 4 °C diluted with $CHCl_3/CH_3OH$, 9:1 v/v, to 100 mL

2. 6. Separation of Lipid Classes

Lipid classes were separated by thin layer chromatography (TLC) using plates of 20 cm × 20 cm × 0.25 mm covered with silica gel (F254, Merck, Darmstadt, Germany). Neutral as well as polar lipids were separated using developing system composed of n-hexane/diethyl ether/acetic acid (80:20:2, v/v/v) and chloroform/methanol/ammonium hydroxide/water (60:35:5:2.5, v/v), respectively.[16] The non destructive locating reagent 2, 7 dichlorofluorescein was used, which gave purple yellow colour bands under an ultraviolet light at 366 nm. Neutral lipids were separated into seven classes while polar lipids into two classes by TLC. These classes were identified by comparison of their R_f values with the corresponding standards. Among the neutral lipids, the presence of sterols and sterol esters was also confirmed on TLC by spraying antinomy trichloride reagent. These compounds showed red violet colour after heating the plates in oven at 100 °C for 10min. Similarly, hydroxylamine ferric chloride reagent was sprayed to confirm the presence of different types of acylglycerols which showed purple color under the above mentioned conditions. Polar lipids gave blue spot with molybdate blue reagent.[17]

2. 7. GC-MS Analysis of Fatty Acid Methyl Esters

The fatty acid methyl esters (FAME's) were prepared from the isolated lipids by heating the samples at 80 °C for 20 min in BF_3 methanol.[18] *n*-hexane (5 mL) was added to the prepared FAME's and the organic layer with the FAME's was recovered. The solvent removed under a steam of nitrogen and residue was quantified by GC-MS (QP-2010, Shimadzu CO, Kyoto, Japan) equipped with 30 m × 0.25 mm DB-5MS column (Agilent technologies J & W scientific products, Folsom, CA). The carrier gas was helium. The temperature program was set as follows; 100 °C hold for 5 min, rose at 4 °C/min to 280 °C respectively. The ion source and interface temperatures were set at 200 and 250 °C respectively. The mass range was scanned from 50 to 900 amu. The control of the GC-MS system and the date peak processing were controlled by means of Shimadzu's GC-MS solution software version 2.4. Compound identification was verified based on the relative retention time and mass fragmentation pattern spectra with those of standards and the NIST 147. LIB database of the GC-MS system (Shimadzu). The fatty acid composition was reported as a relative percentage of the total peak area.

2. 8. Phenolic Compound Extraction

The phenolic compounds were extracted from *A. indicum* and *A. muticum* seed oil using liquid-liquid extraction.[19] Five grams of oil was dissolved in 20 mL hexane and extracted three times with 15 mL of methanol/water (60:40 v/v). The combined extracts were evaporated to dryness in a rotary evaporator at 40 °C. The concentration of total phenols in the methanolic extracts was estimated with Folin-Ciocalteau's reagent. The procedure consisted of dilution of 40 μL aliquots from each of the replicates using water to 3.16 mL and addition of 200 μL of 0.2 N Folin-Ciocalteau's reagent. After 8 min, 600 μL of saturated sodium carbonate (75 gL^{-1}) was added. The absorbance of the resulting blue solution was measured at 765 nm after incubation at 40 °C for 30 min with intermittent shaking. Gallic acid served as a standard for preparing the calibration curve (0.05–0.4 mg/mL). The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g of seed oil.

Total Antioxidant Activities

2. 9. ABTS$^{\cdot+}$ Decolourization Assay

Total antioxidant activity in terms of Trolox Equivalent Antioxidant Activity (TEAC) was measured using an improved ABTS method as described by Re *et al.*, 1999.[20] The absorbance reading was taken at 30 °C exactly 1 min after initial mixing up to 6 min. Appropriate solvent blanks were run in each assay. The percentage inhibition of absorbance at 734 nm was calculated using the following formula:

$$\% \text{ Inhibition}_{(\text{at } 734 \text{ nm})} = (1 - A_f/A_o) \times 100$$

Where A_o & A_f are the absorbance's of ABTS$^{\cdot+}$ solution at 734 nm before and after the addition of seed oil or standard solution respectively. The percentage inhibition was plotted as a function of concentration of antioxidants and Trolox for the standard reference data.

2. 10. DPPH Free Radical Scavenging Activity

The DPPH radical scavenging effect was determined according the method described by Sanchez-Moreno *et al.*, 1998.[21] DPPH solution (3.9 mL, 25 mg/L) in methanol was mixed with sample solution (0.1 mL). Upon reduction the violet color of the solution fades proportionate to the amount of antioxidants present in the sample. The reaction progress was monitored by noting absorbance at 515 nm after every minute, for 30 min or until the absorbance was stable. The percentage of the DPPH remaining (% $DPPH_{rem}$) was calculated using the following formula:

$$\% \text{ DPPH}_{rem} = 100 \times [\text{DPPH}]_{T=t}/[\text{DPPH}]_{T=0}$$

Where $[\text{DPPH}]_{T=t}$ and $[\text{DPPH}]_{T=0}$ are the absorbance's of DPPH solution at the start of reaction with sample (*i.e.* T = 0) and at T = t (*i.e* until the absorbance becomes zero, respectively).

2. 11. FRAP Assay

The reducing capacity of plant extracts was measured according to the method of Benzie *et al*., 1999 with some modifications.[22] Freshly prepared FRAP solution contained; 25 mL of 300 mM acetate buffer (pH 3.6) plus 2.5 mL of 10 mM TPTZ solution in 40 mM HCl solution and 2.5 mL of 20 mM ferric chloride solution. The sample was incubated at 37 °C throughout the monitoring period. 3 mL FRAP reagent was warmed to 37 °C and a reagent blank reading was taken at 593 nm. The reagent was then mixed with 100 µL sample and 300 µL of distilled water. Absorbance reading was taken at 593 nm after every minute for 6 min. The sample and blank (the absorbance of the appropriately diluted sample without FRAP reagent) was subtracted from the final absorbance at 6 min, and the results were compared with the standard curve prepared using different concentrations of Trolox.

2. 12. Lipid Peroxidation Value in Linoleic Acid Emulsion System

Lipid peroxidation value of the extracts was determined according to the ferric thiocyanate method in linoleic acid emulsion as described by Mitsuda *et al*., 1966.[23] The solution was incubated at 37 °C. During incubation 100 µL solution was regularly taken off at intervals of 24 h and the lipid peroxidation value was determined spectrophotometrically at 500 nm, after reacting with $FeCl_2$ and thiocyanate. A 5.0 mL solution consisting of 2.5 mL linoleic acid emulsion and 2.5 mL of potassium phosphate buffer (0.04 mol/L; pH 7.0) was used as blank.

2. 13. Antibacterial Test by Pour Plate Method

Both Gram-positive [*Bacillus licheniformis* (PCSIR-B-252), *Bacillus subtilis* (PCSIR-B-248), *Micrococcus luteus* (NRRL-B-287), *Nocardia asteroids* (NRRL-178)], and Gram-negative [*Escherichia coli* (PCSIR-B-67), *Proteus mirabilis* (ATCC 29245), *Salmonella typhimorium* (ATCC 14028)] bacteria were obtained from PCSIR Laboratories Complex Lahore, Pakistan. All the bacterial cultures were grown in nutrient agar (Merck) at 37 °C. Each bacterial strain was transferred from stored slants at 4–5 °C to 10 mL broth and cultivated overnight at 37 °C. A preculture was prepared by transferring 1 mL of this culture to 9 mL broth and cultivated for 48 h. The cells were harvested by centrifugation (1200 g, 5 min) washed and suspended in saline.

The *Abutilon* seed oils were tested against different bacteria by the method of Negi.[24] To flasks containing 20 mL melted cool agar, different concentrations of test material in DMSO were added. Equivalent amounts of DMSO were added as control. 100 µL (about, 10^3 cfu/mL) of each bacterium to be tested were inoculated into the flasks under aseptic conditions. The media were then poured into sterilized Petri plates, in duplicate and incubated at 37 °C for 20–24 h. The colonies, developed after incubations, were counted and expressed as colony forming units per ml of culture (cfu/ml). The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the compound capable of inhibiting the complete growth of the bacterium being tested.

2. 14. Statistical Analysis

Different statistical techniques such as; analysis of variance (ANOVA), Duncan's multiple range method and regression analysis were carried out for analyzing the data obtained from different samples and to study the relationship between antioxidant activity and total phenolic content. Each parameter was measured thrice. Differences at $P < 0.05$ were considered statistically significant.

3. Results and Discussion

3. 1. Proximate Composition

Proximate composition of *A. indicum* and *A. muticum* seeds and oils is presented in Table 1. The moisture content of *Abutilon* seeds are quite close to brassica and linseed as reported by Krishnamurthy *et al.,* 1960.[25] The oil content in the seeds of both the species are rather low (11.2 and 12.9%). The oils can therefore be recovered economically only by solvent extraction. The protein contents were appreciable (22.4 and 23.5%) and similar to that of cotton; 28.72%.[26] Therefore, *Abutilon* seeds could be recommended as protein supplement. However, their use as food supplement depends on interplay of factors like the digestibility of their nutrients and the presence of anti nutritional factors.[27] These contents still need to be investigated with *Abutilon* seed oil. According the literature no report on the toxicology of both *Abutilon* species for humans has been reported. Solmosi *et al*., 1996[28] reported that *Abutilon indicum* has potential for weed control.

The presence of seed coat (shell) in the ground seed used for analysis accounted for the high fiber content of the seeds under study (25.1 and 28.3%). The ash contents were 2.3 and 3.4%; this indicates that seeds of both species contain higher percentage of mineral matter. The Iodine value is the measure of the degree of unsaturation of fat. The iodine value of *Abutilon* seed oil was 110.4 and 116.3 g I_2/100g. This value suggests that it can also be used in nonfood application such as in the production of shoe polish, varnishes etc. The saponification value is directly related to the mean molecular mass. The saponification value of *Abutilon* seed oil was comparable (188.16 and 195.15 mg KOH/g) to those reported in the literature for cotton seed oil (189–198 mg KOH/g).[29]

Table 1: Proximate Composition of *Abutilon indicum* and *Abutilon muticum* seeds and oils

Component	Proximate Composition (%)	
	A. indicum	*A. muticum*
Seeds		
Moisture	5.9 ±0.01	6.3 ±0.06
Fat/Oil	11.2 ±0.01	12.9 ±0.00
Protein	22.4 ±0.08	23.5 ±0.01
Fiber	25.1 ±0.02	28.3 ±0.10
Ash	2.3 ±0.09	3.4 ±0.08
Carbohydrate	22.92 ±0.12	20.80 ±0.06
Oil		
Colour	Pale yellow	Reddish Yellow
n_D^{30}	1.4759 ±0.03	1.4673 ±0.01
Acid Value	2.7 ±0.01	2.1 ±0.06
Iodine Value	110.4 ±0.01	116.3 ±0.03
Saponification Value	188.16 ±0.07	195.15 ±0.02
Unsaponifiable Matter	2.2 ±0.13	1.5 ±0.02

3. 2. Minerals

Nien minerals (seven essential together with aluminium and cadmium) were found for the first time in *Abutilon* seeds (Table 2). Potassium was most abundant (761.01 & 612.32 mg/kg), followed by calcium (437.23 & 520.00 mg/kg), phosphorus (412.15 & 315.02 mg/kg) and magnesium (176.50 & 183.23 mg/kg) for *A. indicum* and *A. muticum respectively*. Although each mineral has its own health benefits, minerals are generally important as constituents of bones, teeth, soft tissues, haemoglobin, muscle, blood and nerve cells. Minerals are also vital to overall mental and physical well being.[30] With regard to human nutritional aspects; *Abutilon* seeds have significant mineral contents.

Table 2: Mineral contents of *Abutilon indicum* and *Abutilon muticum* seeds

Minerals	Concentration (mg/100g)	
	A. indicum seeds	*A. muticum* seeds
Aluminium	4.02 ±0.03	4.13 ±0.00
Cadmium	0.01 ±0.01	0.03 ±0.02
Calcium	237.32 ±0.03	320.00 ±0.02
Iron	3.13 ±0.10	4.97 ±0.08
Lead	0.08 ±0.12	0.04 ±0.21
Magnesium	176.50 ±0.05	183.23 ±0.12
Phosphorous	212.15 ±0.01	235.02 ±0.01
Potassium	261.01 ±0.01	312.32 ±0.04
Zinc	1.94 ±0.03	1.37 ±0.02

3. 3. Lipid Classes of Abutilon Seed Oil

Both the species of *Abutilon* showed the presence of nine lipid classes (Table 3). Neutral lipids were in high amounts (95.2% in *A. indicum* and 94.7% in *A. muticum*) of total lipids and the predominant lipid classes were triacylglycerids and sterol esters, while the polar lipids represented only 3.7 and 3.6% of total lipids in *A. indicum* and *A. muticum* respectively. The percentage composition of nine lipid classes is shown in Table 3. This composition was comparable with the lipid composition of five other Malvaceae species, having similar composition of neutral and polar lipids.[31]

All lipid classes, except hydrocarbons, were converted into their methyl esters by BF_3 methanol reagent and fatty acids composition was determined by GC-MS. The fatty acid range was $C1_{14:0} – C_{18:3}$ containing saturated and unsaturated fatty acid in all the lipid classes. The oleic acid ($C_{18:1}$) was found as the predominant fraction in neutral as wall as in polar lipids in both the species (Table 4). This pattern of fatty acid composition resembles the composition of family Malvaceae.[31] The other fatty acids were myristic, palmitic, stearic and linoleic acids. Unsaturated fatty acids were higher, as compared to saturated fatty acid in all the lipid classes, which is the characteristic of vegetable oils.

Table 3: Lipid Classes in *Abutilon indicum* and *Abutilon muticum* seeds

Lipids	R_f value	Concentration (%) *A. indicum* seeds	*A. muticum* seeds
Neutral lipids			
Hydrocarbons	0.94	1.4 ±0.05	2.1 ±0.00
Sterol esters	0.73	8.3 ±0.03	7.4 ±0.01
Triglycerides	0.64	70.3 ±0.01	68.9 ±0.01
Free fatty acids	0.45	2.8 ±0.08	3.4 ±0.023
Diglycerides	0.37	3.2 ±0.46	2.7 ±0.08
Sterols	0.20	2.7 ±0.33	3.8 ±0.05
Monoglycerides	0.18	6.5 ±0.02	6.4 ±0.03
Polar lipids			
Phospholipids	0.70	2.1 ±0.00	1.7 ±0.02
Glycolipids	0.26	1.6 ±0.01	1.9 ±0.02

3. 4. Total Phenolic Content

The quality and duration of storage of fat and fat products are directly associated with their optimum stabilization by a simple antioxidant. Because synthetic antioxidants such as butylated hydroxytoluene or butylated hydroxyanisol could promote cancer development in rats and the fact that consumers are much interested in natural food additives has resulted in a surge in research on natural antioxidants such as phenolic compounds.[32] Phenolic compounds constitute a part of the unsaponifiable matter also known as "minor constituents" of oils. These compounds are determinant for some characteristics of oils such as flavor, shelf life and resistance against oxidation.

The total phenolic content (TPC) of the extracts obtained from seeds of *A. indicum* and *A. muticum* are 13.770 and 38:815 mg/g of seed oil respectively. The values for phenolic contents are comparable to previously exploited natural sources of antioxidants.[33]

Table 4: Percentages of fatty acids in the principal classes of non polar and polar lipids of *A. indicum* and *A. muticum* seeds.

Lipid Class	Fatty acid composition in weight % methyl ester								
	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Saturated fatty acid (%)	Unsaturated fatty acid (%)
Sterol esters									
A. indicum	2.15	36.21	2.57	2.09	43.28	3.27	10.43	40.45	59.55
A. muticum	3.85	24.32	5.64	7.42	34.25	14.29	10.11	35.59	64.29
Triglycerides									
A. indicum	1.39	26.37	1.72	7.67	44.37	14.78	3.69	35.43	64.56
A. muticum	3.13	26.94	2.49	10.64	36.90	13.77	6.08	40.71	59.24
Free fatty acids									
A. indicum	1.49	34.56	2.45	4.96	44.78	5.68	6.07	41.01	58.98
A. muticum	5.36	32.27	2.81	6.30	34.28	13.02	5.96	43.93	56.07
Diglycerides									
A. indicum	2.24	21.07	1.92	1.38	63.34	3.68	6.27	24.69	75.21
A. muticum	3.39	27.83	3.73	8.56	35.73	12.04	9.17	39.78	60.07
Sterols									
A. indicum	2.56	24.18	1.62	1.41	52.59	13.99	3.49	28.15	71.69
A. muticum	7.40	20.01	6.65	10.42	38.74	12.68	4.00	37.83	62.07
Monoglycerides									
A. indicum	2.72	28.28	2.63	2.51	47.79	12.05	3.93	33.51	66.40
A. muticum	5.02	28.09	2.91	2.73	51.12	7.21	2.82	35.84	64.06
Glycolipids									
A. indicum	3.00	32.05	1.19	2.65	43.07	13.75	4.21	37.70	62.22
A. muticum	traces	31.2	6.4	5.4	35.30	5.30	16.30	36.60	63.30
Phospholipids									
A. indicum	1.19	38.58	1.85	2.40	46.98	5.64	3.36	42.17	57.83
A. muticum	6.1	31.5	5.6	3.92	32.10	9.40	10.80	41.52	57.90

3. 5. Antioxidant Activity

The seed oil of *A. indicum* and *A. muticum* exhibited strong antioxidant activity assayed by four different methods including $ABTS^{·+}$, FRAP, DPPH and linoleic acid peroxidation. *A. muticum* seed oil showed stronger antioxidant activity than *A. indicum* seed oil by different methods.

Antioxidant capacity of both herbs was checked by different assays because of the lack of a validated assay that can reliably measure the antioxidant capacity of foods, herbs and biological samples. In a review by Frankel and Meyer,[34] the authors pointed out that it is problematic to use one dimensional method to evaluate multifunctional food, herbs and biological antioxidants. Different antioxidant assays differ from each other in terms of substrates, probes, reaction conditions and quantitation methods. It is extremely difficult to compare the results from different antioxidant assays as Frankle and coworkers have already concluded; instead different assays can be used to check wide antioxidant capacity.

TEAC values of *A. indicum* and *A. muticum* seed oils are 14.006, 21.375 and 16.369, 18.08 by $ABTS^{·+}$ and FRAP methods respectively (Table 5). $ABTS^{·+}$ and FRAP methods are not much different except that $ABTS^{·+}$ is carried out at neutral pH and FRAP assay under acidic conditions. In DPPH assay graphs (Figure 1) showed a sharp fall in the absorbance of DPPH in the first five min after addition of oils and then became more moderate and gradual for next 35 min. Different concentrations of oils were applied to obtain EC_{50} and T_{EC50} values (Table 5) as function of time. The results of linoleic acid peroxidation determined by the thiocyanate method at 30 °C are shown in Figure 2. Results showed decrease in absorbance of control after an initial increase. This is due to oxidation of linoleic acid that generates hydroperoxides, which are then decomposed to many secondary oxidation products. Both the plant species exhibited inhibition to peroxidation of linoleic acid.

According the results seeds of both the plant species have powerful antiradical and antioxidant components which may be helpful in controlling complications during diseases.

3. 6. Antibacterial Activity

The effects of *A. indicum* and *A. muticum* seed oils on growth of different bacteria are presented in Figure 3. Both the plant species inhibited growth of different bacteria to variable extents; depending on the bacterium in que-

stion *A. muticum* seed oil was found to be more effective than *A. indicum* seed oil. *Salmonella typhimorium was* most resistant to both the seed oils and higher MIC values were obtained for it. Higher resistance of Gram negative bacteria to external agents has been earlier reported, and it is attributed to the presence of lipopolysaccharides in their outer membranes which make them inherently resistant to antibiotics detergents and hydrophilic dyes.[35] Both the plant species inhibited the growth of both Gram positive and Gram negative bacteria, showing their broad spectrum activity.

4. Conclusion

As *Abutilon* species grow wildly in tropical and sub tropical areas under harsh conditions, they may be produced on a large scale as value added products. The distribution of fatty acids in oils examined, reflected their richness in saturated components. Pakistan being agriculture country undoubtedly has the capacity for large scale production of *Abutilon* species. The results may therefore, offer a scientific basis for use of the seeds, both in human diet and other commercial products. We propose that the higher antibacterial and antioxidant activities of seed oils of both the *Abutilon* species may be due to the higher phenolic content. This is a preliminary report on the isolation of antioxidants and antibacterial fractions from *Abutilon* seeds, and further studies are needed for the characterization of individual phenolic compounds, to elucidate the mechanisms underlying bioactive properties and the existence of possible synergies if any among these compounds. Research is in progress to isolate water soluble compounds from both the herbs and to check their medicinal properties. These analytical findings will provide a regional data base for these valuable herbs, which have not been explored so far. The data obtained will be useful to both producers and consumers.

5. Acknowledgements

The authors are grateful to Higher Education Commission of Pakistan for research grant No. 042-121289-PS2-212 and to the plant taxonomist, Dr. Zaheer-ud-Din Khan (Department of Botany, GC University, Lahore. Pakistan) for the collection of plant material

6. References

1. Anonymous: Agricultural Statistics of Pakistan, 2005–2006, Ministry of Food, Agriculture and Live Stock, Government of Pakistan, Islamabad, **2007**.
2. E. Nasir, S. I. Ali: Flora of West Pakistan, No. 130; Malvaceae, Department of Botany, University of Karachi, **1979**, pp. 69–72
3. K. R. Kirtikar, B. D. Basu: Indian Medicinal Plants, 2nd Ed., *Vol.1*, Bishen Singh, Mahendra Pal Singh, India, **1980**, pp. 314–315.
4. M. K. Bagi, G. A. Kalyani, I. J. Dennis, K. K. Akshaya, H. K. Kakrani, *Fitoterapia*. **1985**, *56*, 169–171.
5. M. K. Bagi, G. A. Kalyani, *Indian Drugs.* **1984**, *22*, 69–72.
6. V. Lander, E. A. Morrison: The wealth of India CSIR, India. A-Dictionary of Indian Raw Materials and Industrial Products. **1962**, *6*, pp. 120.
7. S. S. Gutkin, *J. Am. Oil Chem. Soc*. **1950**, *27*, 538–544.
8. S. K. P. Sinha, J. V. V. Dogra, *Int. J. Crude Drug Res*. **1985**, *23*, 77.
9. P. C. Kuo, M. L. Yang, P. L. Wu, H. N. Shih, T. D. Thang, N. X. Dung, T. S. Wu, *J. Asian Nat. Prod. Res*. **2008**, *10*, 689–693.
10. I. Matlawska, M. Sikorska, *Acta Pol Pharm*. **2002**, *59*, 227–229.
11. P. V. Sharma, Z. A. Ahmad. *Phytochemistry*. **1989**, *28*, 3525.
12. U. Vrhovsek, *Research Reports*. **1999**, *73*, 39–47.
13. M. A. Amerina, C. S. Ough: Methods for analysis of musts and wines, John Wiley & Sons, New York, **1988**, pp. 204–206.
14. AOAC. Official Methods of Analysis, 16th Ed., Association of Official Analytical Chemists, Arlington, VA **1995**.
15. E. G. Bligh, W. J. Dyer, *Can. J. Biochem. Physiol.* **1959**, *37*, 911–17.
16. M. Kates: Techniques of Lipidology; Isolation, Analysis and Identification of lipids, North Holland Publishing, Amsterdam, **1972**, pp. 393–465.
17. M. Y. Raie, M. A. Ahmad, I. Ahmad, S. A. Khan, S. A. Jafri, *Fette Seifen Anstrichmittel.* **1983**, *85*, 279–280.
18. AOAC, Official Methods of Analysis, 14th Ed., Association of Official Analytical Chemists, Arlington, VA. **1990**.
19. M. T. Silvia, E. E. Miller, D. E. Pratt, *J. Am. Oil. Chem. Soc*. **1984**, *61*, 928–931.
20. R. Re, N. Pellegrini, A. protegent, A. Pannale, M. Yang, C. Rice-Evans, *Free Radic Biol. Med*. **1999**, *26*, 1231–1237.
21. C. Sanches-Moreno, J. A. Larrauri, F. Saura-Calixto, *J. Sci. Food Agr.* **1998**, *76*, 270–276.
22. I. F. F. Benzie, J. J. Strain, *Anal. Biochem.* **1996**, *239*, 70–76.
23. H. Mitsuda, K. Yasumoto, K. Iwami, *Euyo to Shokuryo*. **1996**, *19*, 210–214.
24. P. S. Negi, G. K. Jayaprakasha, L. J. M. Rao, K. K. Sakariah, *J. Agric. Food Chem.* **1999**, *47*, 4297–4300.
25. K. Krishnamurthy, T. N. Ramakrishnan, R. Rajagopalan, M. Swaminathan, V. Subrahmanyan, *Ann. Biochem*. **1960**, *20*, 73–76.
26. O. U Eka, B. Isbell, *Nig. J. Sci*. **1994**, *18*, 76–78
27. T. A. El-Adawy, K. M. Taha, *Food Chem.* **2001**, *74*, 47–54.
28. P. solymosi, *Novenyvedelem*. **1999**, *32*, 23–34.
29. Codex Alimentarius Commission, Recommended Internal Standards, Edible Fats and Oils, FAO/WHO, Rome, Italy, **1982,** 1st Ed., *Vol-2*.
30. B. L. O' Dell, R. A. Sunde: Introduction in Hand Book of

Nutritionally Essential Minerals; Marcel Dekker, New York, **1997**, pp. 1–12.

31. E. M. Marinova, N. V. Yanishlieva, *Food Chem*. **2003**, *81*, 189–197.
32. S. Mohammadzadeh, M. Shariatpanahi, M. Hamedi, R. Ahmadkhaniha, N. Samadi, S. N. Ostad, *Food Chem*. **2007**, *103*, 1097–1103.
33. G. C. Yen, P. D. Duh, *J. Am. Oil Chem. Soc*. **1993**, *70*, 383–386.
34. E. N. Frankle, A. S. Meyer, *J. Sci. Food Agri*. **2000**, *80*, 1925–1941.
35. H. Nikaido, M. Vaara, *Microbiol. Rev*. **1985**, *49*, 1–32.

Povzetek

Proučili smo fizikalno kemične lastnosti makro in mikroelementov (Al, Cd, Ca,Fe, Pb, Mg, P, K, and Zn), maščobnih kislin, fenola, antioksidantov ter antibakterijsko aktivnost v oljih semen dveh divje rastočih vrst rodu *Abutilon* v Pakistanu. *Abutilon indicum* L. (Malvaceae) in *Abutilon muticum* DC. (Malvaceae). *A. indicum* vsebuje 5.9 % vlage, 25.1 % vlaken, 22.4 % beljakovin, 33.1 % ogljikovih hidratov ter 2.3 % pepela, medtem ko vsebuje *A. muticum*, 6.3 % vlage, 28.3 % vlaken, 23.5 % beljakovin, 25.6 % ogljikovih hidratov in 3.4 % pepela. Celokupna izolirana vsebnost lipidov semen je bila okarakterizirana s kromatografskimi metodami ter analizirana z masno spektrometrijo. Olja vsebujejo visoko količino nevtralnih lipidov (95,2 % *A. indicum* in 94.7 % *A. muticum*) ter nizko količino polarnih lipidov (3.7 % *A. indicum* in 3.6 % *A. muticum*). GC-MS analiza je pokazala linolensko kislino kot glavno predstavnico maščobnih kislin pri obeh vrstah. Celokupno vsebnost fenolov smo določili po Follin-Ciocalteu metodi in znaša pri *A. indicum* 13.770 mg/g ter pri *A. muticum* 38.815 mg/g olja. Aktivnost antioksidantov smo preverili s pomočjo metod ABTS, FRAP, DPPH ter peroksidacije z oleinsko kislino. Vse metode so pokazale prisotnost tako hitro kot počasi delujočih komponent. Olja semen *A. indicum* in *A. muticum* so imela širok spekter delovanja proti Gram pozitivnim in Gram negativnim bakterijam. Dognanja potrjujejo, da sta lahko obe vrsti rodu *Abutilon* potencialno pomembni za pridobivanje olja, zdravil in aktivnih kozmetičnih sestavin.