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Evaluation of antidiabetic, antioxidant, and cytotoxic potential of maize (*zea mays* l.) husk leaf extracts

Muhammad Riaz^{1*}, Sadia Nawaz², Iqra Ilyas¹, Muhammad Misbah ur Rehman³, Rahman Qadir¹, Tahir Mehmood², Muhammad Afzal⁴, Natasha Abdul Rehman⁶, Abid Ali⁵

¹Institute of Chemistry, University of Sargodha, Sargodha-40100, Pakistan

²Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan

³ Department of Chemistry, University of Lahore, Sargodha Campus, Sargodha-40100, Pakistan

⁴Faculty of Life Sciences, University of Central Punjab, Lahore, Pakistan

⁵Department of Allied Health Sciences, The University of Lahore-Gujrat campus, Gujrat

⁶ Higher Education Department, Punjab, Pakistan

*Correspondence to: riaz453@gmail.com, Abid.ali@ahs.uol.edu.pk Received August 10, 2020; Accepted December 7, 2020; Published January 31, 2021 Doi: http://dx.doi.org/10.14715/cmb/2021.67.1.25

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Abstract: In current study, Maize (*Zea mays* L.) husk leave extracts were appraised for biological activities such as cytotoxicity, antidiabetic, antioxidant and antimicrobial. Maceration was performed to collect various fractions of husk leave extracts using a pool of solvents i.e., n-hexane, chloroform, ethyl acetate, butanol and methanol. Antioxidant potential was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging, reducing power and linoleic acid oxidation assay, using butylated hydroxy toluene (BHT) as a positive control. Total phenolic and flavonoid contents were found to be 18.47-425.11 mg/100 g GAE and 5.83-16.72 mg/100 g CE, respectively. The DPPH scavenging assay was exhibited in the range of 76.36 to 88.53%. The percentage inhibition in linoleic acid oxidation was found from 10.16 to 79.51%. Significant antimicrobial activity was demonstrated by husk leaf extracts against bacterial strains and fungal strains using disc diffusion and minimum inhibitory concentration (MIC) method. Amylase alpha assay was employed to analyze the antidiabetic activity which ranged between 9.52-24.81%. Cytotoxicity was evaluated by % age lysis (0.35-9.54%), while thrombolytic activity ranged between 7.67 to 31.27%. The results presented in this study revealed that maize (*Zea mays* L.) husk leaf extracts can be a valuable source of biologically active compounds and may be consumed as a source of potent herbal medicine in pharmaceuticals.

Key words: Maize (Zea mays L.); Antidiabetic; Antioxidant; Antimicrobial; Haemolysis; Thrombolytic activity.

Introduction

Recently, herbal drugs are gaining keen attention of researchers due to safe, efficient, and non- toxic effects. Herbal drugs are considered as a basic part of health care by the people of developing countries. In ancient times, drugs were derived from various parts of plant extracts in simple and refined form. Medicinal plants are being studied due to their pharmaceutical potential such as healing of wounds, antidiabetic, antimicrobial and antioxidant properties. Medicinal plants show medicinal properties as they are reserve of biologically active organic compounds such as terpenoids, steroids, saponins, flavonoids, alkaloids and phenolic compounds. Chemists are taking interest to evaluate these compounds due to their better pharmaceutical performance. Hence, investigation and isolation of bioactive compounds from medicinal plants can be interesting for druggists and researchers (1-2). Zea mays is one of the most productive and earliest crop species and gained well recognition in the modern world due to its multiple applications. Maize plant contains various bioactives such as flavonoids, alkaloids, masonic acid, saponins and nutrients i.e., vitamins B₁, K, potassium, zinc and phosphorous. Presence of such phytochemicals lends a variety of applications in maize such as antioxidant, antidiabetic, anticancer, anti-inflammatory, pain killer and anti-inflammation of prostate gland. Maize plant has many medicinal and industrial uses and has become a source of interest for modern civilizations (3).

Based on inexpensive, non-toxic and protective behavior of natural antioxidants, there is an increasing demand for isolation and identification of plant bioactives as better substitutes of synthetic antioxidant compounds (4). Medicinal plants act as antidiabetic agents due to presence of biologically active chemicals. Researchers also reported that plant bioactives lower blood sugar level by acting on alpha amylase (salivary and pancreatic) and are used for treatment of diabetes (5). Traditional antibiotic drugs are developing resistance for a variety of bacterial strains. Many antimicrobial compounds isolated naturally from plants, terpenoides, xanthones, benzo phenones, coumarins and flavonoids, are used for treatment of microbial diseases (6). Researches have shown that the consumption of maize plant assists in the management of diabetes mellitus due to the presence of phenolic phytochemicals. Vitamin B present in maize regulates diabetes by maintaining lipid, protein and carbohydrate metabolism and phytochemicals present in maize plant regulate the release of insulin.

Maize is a super food having a glycemic index of 58, used for controlling diabetes. Similarly, phenolic phytochemicals present in maize are used for treatment of hypertension (high blood pressure) (7). Keeping in view the medicinal values of maize plant, the present study was designed to evaluate and determine the biological potential and cytotoxic behavior of maize husk leaves.

Materials and Methods

Sample collection

The husk leaves of maize (*Zea mays* L.) were collected from the farmlands of Faisalabad district, Punjab, Pakistan. After collection, leaves were cleaned properly; shade dried and converted into powder with grinder machine.

Preparation of Maize (Zea mays L.) husk leave extracts

Dried powdered plant material was extracted thrice with solvents i.e. n-hexane, chloroform, ethyl acetate, n-butanol and methanol in the order of increasing polarity and soaked for about 72 hours by maceration. The filtrate was concentrated using rotary evaporator. The extracts were filtered with the help of filter paper in a petri dish. Plant extracts were dried and weighed to calculate the extract yield.

Total phenolic contents

Total phenolic contents were measured according to reported method of Chaovanali kitand Wrolstad, 2004. 0.01 g/ml of plant extract was prepared in test tubes for each solvent. About 10% folin ciocalteu reagent (0.5 ml) and 7.5 ml of distilled water was added. All test tubes were covered for 10 minutes. About 1.5 ml of 20% sodium carbonate (w/v) solution was added and heated on hot water bath at 40°C for 20 minutes and then cooled in an ice bath. Gallic acid was used as a standard (20 to 200 mg/L, R^2 =0.998) to prepare curve. The total phenolic contents were calculated by using a calibration curve for gallic acid and absorbance was noted spectrophotometrically at 755 nm.

Total flavonoid contents

Total flavonoid contents were calculated by using explained method of (Dewanto *et al.*, 2002) About 0.01 g/ml of extract was prepared in test tubes for each solvent (8). About 0.3 ml of (5%) sodium nitrite solution (NaNO₂) and5 ml of distilled water was added in all test tubes. About 0.3 ml of AlCl₃ (10%) was added, followed by an addition of 2 ml of sodium hydroxide (1M) solution. The solution was shaken continuously and the absorbance was taken at 510 nm via spectrophotometer. Total flavonoids contents were showed as catechin equivalent (CE) per gram of dry plant extract.

Antioxidant activity

DPPH free radical scavenging activity

The scavenging activity of radicals was performed by employing the reported experiment of (Iqbal *et al.*, 2005) (9). The solution of plant extract was prepared in methanol solvent with different concentrations ranging from 0.1 to 100 μ g/ml by dilution method. Then 1 ml of the sample solution was mixed with 2.5 ml of DPPH solution having a concentration of 0.025 g/L. Extract concentration provided 50% inhibition values that were estimated from graph plotted between extract concentrations against scavenging percentage. After keeping solution for 30 minutes in dark, absorbance was analyzed at 515 nm wavelength spectrophotometrically. Each test was performed in triplicate and percentage scavenging was calculated by following equation: Scavenging (%) = $100 \times [(A_{blank} - A_{sample})/A_{blank}]$

Reducing power activity

Reducing activity of plant extract was examined by using the procedure of (Yen *et al.*, 2000). The sample extract of plant containing 2.5-10 mg/ml of powder material was prepared for each solvent. 5.0 ml of sodium phosphate buffer was added along with 5.0 ml of potassium ferricyanide (1.0%). Mixture was then incubated for 20 minutes at 50°C and 5 ml of trichloroacetic acid (10%) was added. Solution was centrifuged for 10 min at 980 xg and upper 5.0 ml layer of the solution was diluted with about 5.0 ml of distilled water. About 1 ml of 0.1% FeCl₃ was added and the absorbance was analyzed at 700 nm.

Percentage inhibition of linoleic acid oxidation

The measurement of percentage inhibition in the linoleic acid system was determined according to (Iqbal et al., 2005; Yen et al., 2000) (9-10). Butylatedhydroxy-toluene (BHT) at 200 mg/L concentration was used as standard compound. The sample that contained no antioxidant component was used as a blank. The maximum oxidation level in the sample was noted at 360 h (15 days). Blank was referred to as with no sample antioxidant component. At 500 nm, the absorbance of the sample was taken spectrophotometrically. The %age inhibition was calculated by the following formula:

Inhibition (%) = (100 - (Absorbance increase of sample at 360 h / Absorbance increase of control at 360 h) x 100)

Determination of antidiabetic activity by alpha amylase inhibition

Antidiabetic activity was measured through alpha amylase inhibition assay by using reported method of (Lordan et al., 2013) (11). After solution preparation, about 0.01 g/ml of plant extract was prepared in five test tubes for each respective solvent. About 500 µl of plant extracts from each test tube was taken with the help of micropipette. 500 µl of alpha amylase solution was added into each test tube. After incubating the solution at 25°C for 10 minutes, 500 µl of starch solution was added further for incubation at 25°C for 10 minutes. 1 ml of DNSA (3, 5-Dinitrosalicylic acid) was added and the solution was boiled for 5 minutes in boiling water bath. The mixture was allowed to cool. Dilution was done by adding 10 ml distilled water. Finally, the absorbance was measured at 540 nm. Following equation was used to calculate inhibition assay of alpha amylase.

Inhibition (%) = $(A_{blank} - A_{sample} / A_{blank}) \times 100$

Antimicrobial activities

Test microorganisms and preparation of nutrient agar and mullerhinton agar

The microbial strains were collected from the Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacillus subtilis and Escherichia coli were bacterial strains, while fungal species were Aspergillusniger and Fusariumsolani; these strains were used to analyze the antbacterial and antifungal potential of sample extracts, respectively. 28 g/L of the nutrient agar was prepared in distilled water for antibacterial studies and about 3.3 g/L of mullerhinton agar was dissolved in distilled water for antifungal studies. Sterilization of agar, beakers, petri plates, wick paper sheet was done by autoclaving for 15 minutes at 121°C temperature. The safety values were adjusted to the required temperature (121°C) and pressure (15 pound/ sq. inch) in autoclave machine. At the end of this period, heater was switched off and autoclave was allowed to cool.

Disc diffusion method

15 ml of agar media was transferred to each of sterilized petri dishes. Agar media, on cooling, formed a 2-3 mm thick layer in every plate. 6 mm (diameter) discs from wick paper sheet were prepared. Bacterial strains were spread over the nutrient agar and fungal strains were maintained over mullerhinton agar by using micropipette. The discs were impregnated with 100 μ l of tested samples. Respective solvents of plant sample were used as negative controls. Rifampin and terbinafine were used as reference drugs for bacterial and fungal strains at 1000 μ g/mL concentration. Petri plates were incubated at 28°C temperature for 48 hours against fungal strains, while for 24 hours at 37°C against bacterial strains in a microbiology incubator. The diameter of the zone of inhibition was measured in mm.

Minimum inhibitory concentration

Different extracts of maize (Zea mays L.) husk leaf extracts were analyzed by modified resazurinmicrotitreplated assay as reported by Sarker et al., 2007 (12). About 10 μ l of sample extract in 10% (v/v) dimethyl sulfoxide was made and added into 96 µL well plates (first row). Nutrient broth (50 µl) for bacteria and mullerhinton broth (50µl) for fungi were added into each of plate. To prepare the resazurin indicator solution, 270 mg of resazurin tablet was dissolved in sterile distilled water (40ml). Microbial suspension (10 µl) was added to each of the plate. Each plate had two controls (positive and negative), and a column containing all the solution except test sample (broad spectrum antibiotic column). The plates were prepared thrice and incubated at $(37 \pm$ 0.1°C) for 24 hours for bacteria and at $(28 \pm 0.3$ °C) for 48 hours for fungi. The absorbance was measured for fungi at 620 nm and at 500 nm for bacteria. Changes in color were investigated by spectrophotometer. MIC value was taken at lower concentration at which color change took place.

Hemolytic activity

The hemolytic activity was analyzed by method of (Powell *et al.*, 2000) (13). About 3 ml of fresh human blood was obtained in 15 ml falcon tube and heparin was

added in order to avoid coagulation. The blood solution was mixed gently and centrifuged for 5 minutes at 850 g. The upper liquid layer was wasted and the thick pellet blood layer was washed thrice with 5 ml of chilled phosphate buffer solution having pH of 7.4. Then 20 ml of chilled PBS was added to washed cells and to count blood cell heamocytometer was used. Then about 20 µl/ ml of plant extracts was prepared in respective solvents in eppendorfs.0.1% Triton X-100 was used as the standard and PBS as negative control. 180 µl of blood cells were added and incubated at 37°C for 35 minutes and tubes were placed on ice bath for 5 min, and centrifuged eventually for 5 min at 1310 g. After that, 100 µl of the liquid layer was collected from tubes, and diluted with 900 microlitre of PBS. Eppendorfs were further maintained on ice bath. 1% Triton X-100 was taken as standard. At the end, absorbance for each sample and control was measured at 576 nm and %age haemolysis was calculated by following equation.

% haemolysis = Abs (sample absorbance) / Abs (control absorbance) \times 100

Thrombolytic activity

The experiment for clot lysis was carried according to method as reported by (Prasad et al., 2007) (14). About 100 µl of plant extract was prepared in test tubes for each respective solvent. About 3 ml of fresh human blood was obtained in 15 ml falcon tube. Then 500 µl of blood in pre weighed eppendorfs was added. All the eppendorfs tubes were incubated for 45 min at 37°C. As a result of the incubation, clot formed in each Eppendorf tube. Serum was removed with the help of micropipette without disturbing clot and it was weighed. Furthermore, 100 µl of plant extract was added ineppendorfs containing blood clot. Phosphate buffer saline and distilled water were used as positive and negative controls respectively. Percentage of clot lysis was calculated by taking difference obtained in weight taken before and after clot lysis. The test was repeated thrice.

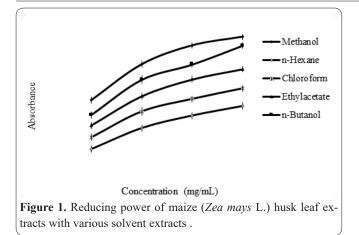
Results and discussion

Percentage yield

The percentage yield obtained from plant extract in respective solvents decreased in the following order: methanol (2.802%) > ethyl acetate (1.048%) > n-butanol (1.039%) > chloroform (0.457%) > n-hexane (0.408%). The highest percentage yield was found in methanol while lowest was found in n-hexane extract. According to these results, methanol solvent showed good potency for extraction of plant constituents from plant extracts as compared to other solvents. It was concluded that the yield of extract depended upon the nature of the respective solvent used. The present results were very close to the previous findings on other medicinal plants (15).

Total phenolics and flavonoids content

Total phenolic contents were found to be in range of 18.47-425.11 mg/100 g. The total phenolic contents (TPC) obtained from maize (*Zea mays* L.) husk leaf extracts in respective solvents were found to be highest in the methanol extract (425.11 \pm 4.25 mg/100 g GAE) and lowest amount was expressed in the n-hexane extract (18.47 \pm 0.23 mg/100 g GAE) of dry material. The total



flavonoid contents obtained from extracts in respective solvents were in the following descending order: methanol (16.72 \pm 0.17 mg/100 g) > n-butanol (14.96 \pm 0.15 mg/100 g) > ethyl acetate (14.37 \pm 0.14 mg/100 g) > chloroform (13.82 \pm 0.14 mg/100 g) > n-hexane (5.83 \pm 0.06 mg/100 g).Obtained results were in consonance with previous reports (16).

The dietary intake of flavonoids containing foods probably lowers the risk of free radical relatedphysiopathology (17). So, the above results depicted that maize (*Zea mays L.*) husk leaf extracts were enriched with antioxidant and flavonoid components.

Antioxidant activity *Reducing power*

The decreasing order of reducing power in accordance to polarity of extract is: methanol > n-butanol> ethyl acetate > chloroform > n-hexane as shown in Fig. 1. The methanolic extract showed highest value due to possessing greater ability of dissolving solute particles as compared to other extracts which is quite similar to work of (Riaz *et al.*, 2012) on another medicinal plant. The high antioxidant activity is indicated by higher absorbance and conclusively high reducing power value. In this activity, test samples changed from yellow colour to bluish green shades by donating a hydrogen atom to test sample or by breaking the free radical chain. The reducing power increased with the increase in the extract concentrations (18).

DPPH free radical scavenging

The percentage value of DPPH activity of maize (*Zea mays* L.) husk leaf extracts was shown as 88.53% in methanol, 85.54% in n-butanol, 81.18% in ethyl acetate, 78.67% in chloroform and 76.36% in n-hexane as

summarized in the table 1. Butylatedhydroxy toluene was used as standard. These results were in consonance with (Jan *et al.*, 2013), indicating that the antioxidant potential was dependent on percentage scavenging.

The free radical scavenging test determines the reduction of DPPH radical by electron transmission antioxidants due to formation of DPPH-H (reduced form). The free radicals are scavenged by transfer of hydrogen atom, the purple color of the methanolic DPPH solution changes to a pale yellow and a characteristic absorbance is seen at 517 nm. This antioxidant action may be associated for the presence of flavonoids, phenolics and tannins and that were found to be present in the extracts. Plant phenolics act as a free radical scavenging group (19).

Percentage inhibition of linoleic acid oxidation

The percentage inhibition of linoleic acid in maize (Zea mays L.) husk leaf extracts ranged between 10.16 to 79.51%. Maximum inhibition (%) was presented by the methanol extract due to existence of high phenolic and flavonoid contents. The minimum % inhibition in n-hexane extract could be clarified by the lesser amount of phenolics. BHT used as a positive control displayed 90.58% inhibition. These results were in consonance with (Velioglu *et al.*, 1998)who reported that extracts with higher TPC also showed strong activity of inhibition of linoleic acid (20). These results recommended that maize (Zea mays L.) husk leaves extract may be used for slowing down lipid oxidation procedures.

Antidiabetic activity

The in vitroantidiabetic activity was determined by assaying the inhibitory activity of the enzyme a-amylase. The antidiabetic activity showed descending order represented as methanol (24.81%) > n-bu-(14.91%) >ethyl acetate (11.78%) >chloroform (10.22%) > n-hexane (9.52%). Acarbose was used as standard and showed higher inhibition (62.47%) of alpha amylase. The present results were very close to the previous findings (21). The remedial ways for reducing postprandial (PP) blood glucose levels for diabetes mellitus patients can be done by preventing absorption of carbohydrate after food intake. By the inhibition of α -amylase and α -glucosidase enzymes, the high postprandial (PP) blood glucose level in diabetic patients can be reduced. In our investigation, highest inhibition of alpha amylase was found in methanolic extract and lowest inhibition was observed in n-hexane extract. According to the present results, it was concluded that

Table 1. Percentage yield, total phenolic content (TPC), total flavonoids content (TFC) and antioxidant potential by maize (Zea mays L.) husk leaves extracts.

Maize husk leaf extracts	Percentage yield	TPC(mg/100 g) GAE of dry matter	TFC (mg/100 g) CE of dry matter	DPPH scavenging assay (µg/ml)	% Inhibition of oxidation in linoleic acid
n-Hexane	0.408 ± 0.004	18.47 ± 0.23	5.83 ± 0.06	76.36 ± 0.76	10.16 ± 0.11
Chloroform	0.457 ± 0.005	54.25 ± 0.51	13.82 ± 0.14	78.67 ± 0.77	33.14 ± 0.31
Ethyl acetate	1.048 ± 0.012	35.19 ± 0.32	14.37 ± 0.14	81.18 ± 0.813	59.29 ± 0.57
n-Butanol	1.039 ± 0.010	103.31 ± 1.07	14.96 ± 0.15	85.54 ± 0.85	67.13 ± 0.65
Methanol	2.802 ± 0.025	425.11 ± 4.25	16.72 ± 0.17	88.53 ± 0.87	79.51 ± 0.80
BHT	_	_	_	89.64 ± 0.89	90.58 ± 0.91

maize (*Zea mays* L.) husk leaf extractscould be used for lowering the rate of absorption of carbohydrates and for the efficient regulation of diabetes by decreasing blood sugar level.

Haemolytic activity

Haemolytic activity of the plant extract was expressed as percentage haemolysis and the percentage (%) of RBCs lysis. Following order was obtained for haemolytic activity from maize husk leaf extracts prepared in different solvents: methanol (9.54%) > n-hexane (8.23%) > ethyl acetate (7.46%) > n-butanol (2.049%)> chloroform (0.35%) as summarized in Table 2. Triton X-100, a synthetic compound was used as positive control that gave maximum (99.49%) percentage lysis value. The present results were very close to the previous findings on other plants as reported by (Zubair et al., 2013). Erythrocytic membrane's stability is a good indicator for *in vitro* studies of cytotoxic effects. The plants extract is considered to have less cytotoxicity, if the percentage haemolysis of erythrocytes remains below 5.0% for sample extracts (22). The above results revealed that methanol, n-hexane and ethyl acetate possessed higher haemolytic activity against human erythrocytes and were toxic while, n-butanol and chloroform possessed less haemolytic activity and could be considered safe for the human erythrocytes.

Thrombolytic activity

Thrombolytic activity of the plant extract was displayed as percentage (%) of clot lysis in solvents and expressed in the following order: ethyl acetate (31.27%)> n-hexane (25.52%) > chloroform (22.27%) > n-butanol (22.26%) > methanol (7.67%) as summarized in Table 2. All of the above results were compared with reference streptokinase that showed about 82.6% lysis.

Antimicrobial activity

The antimicrobial activity of maize (*Zea mays* L.) husk leaf extracts was evaluated by disc diffusion method against *E. coli*, *B. subtilis*, *A. niger* and *F. solani* (Table 3). The results showed that antibacterial activity against *B. subtilis*ranged from 24.54-35.27 mm wherein which ethyl acetate extract showed highest activity and n-hexane extract showed lowest inhibitory activity. According to the results, antibacterial activity against *E. coli* ranged from 15.52-21.52 mm in which methanol extract showed lowest antibacterial activity, while chloroform showed lowest antibacterial activity. Antifungal activity against *A. niger* ranged from 10.22-15.25 mm

in which activities of n-hexane and chloroform extracts were not detected. According to results, antifungal activity against *F. solani*ranged from11.22-21.34 mm in which methanol extract showed the highest and the chloroform extract showed lowest antifungal activity. Antifungal activity of n-hexane extract was not detected. Rifampin and terbinafine were used as reference antibiotics.

In case of minimum inhibitory concentration (MIC), antibacterial activity in B. subtilisstrain ranged from 180-435 µg/ml in which n-hexane extract showed highest while methanol extract showed lowest inhibitory activity. The inhibitory activity against E. coli ranged from 175-415 µg/ml. n-hexane extract showed highest activity against E. coli strain, while the lowest was observed for methanol extract. In case of fungal species such as A. nigerstrain, antifungal activity ranged from 200-365 µg/ml. The ethyl acetate extract showed highest and methanol extract showed lowest antifungal activity, while antifungal activity was not detected in chloroform extract. The antifungal activity in case of F. solani ranged from 215-415 µg/ml. The chloroform extract showed potent inhibitory activity as compared to other extracts.

Maize (*Zea mays* L.) husk leaf extracts showed high zone of inhibition (mm) against *B. subtilis* (gram positive bacteria), while low inhibitory activity was observed against *E. coli* (gram negative bacteria).

The presence of phytochemicals in maize (Zea mays L.) provides protection against various pathologies by acting as antioxidant, antidiabetic, antimicrobial and anticancer agent. The DPPH assay showed that maize plant has potent antioxidant activity which can be an excellent choice for biological and chemical analyses. Plant extracts with higher TPC showed strong reducing power and good activity against linoleic acid peroxidation. Moreover, in line with present results, maize plant husk leaves can be used for lowering absorption of carbohydrates by decreasing high blood sugar level. The results revealed that husk leaves of maize plant are active against tested micro-organisms and may be utilized as herbal medicine against bacterial and fungal infections. The present investigation showed that n-butanol and chloroform possessed less haemolytic activity against human erythrocytes and can be considered safe as compared to other extracts. Hence it can be stated that the presence of potentially active compounds in maize husk leaf extracts paves its use in pharmaceutical industry as a value addition.

Maize husk leaf extracts	Percentage (%) inhibition of alpha amylase	Percentage (%) yield of clot lysis	Percentage (%) of RBC lysis
n-Hexane	9.52 ± 0.09	25.52 ± 0.24	8.23 ± 0.07
Chloroform	10.22 ± 0.09	22.27 ± 0.22	0.35 ± 0.03
Ethyl acetate	11.78 ± 0.11	31.27 ± 0.31	7.46 ± 0.06
n-Butanol	14.91 ± 0.13	22.26 ± 0.22	2.049 ± 0.02
Methanol	24.81 ± 0.25	7.67 ± 0.07	9.54 ± 0.09
Triton X-100	_	_	99.49 ± 0.98
Acarbose	62.47 ± 0.62	_	-
Streptokinase	_	82.60 ± 0.82	-

Maize husk leaf extracts	Tested microorganisms (zone of inhibition in mm)				
-	Bacterial strains		Fungal strains		
-	E. coli	B. subtilis	A. niger	F. solani	
	Disc diffus	sion assay (mm)			
n-Hexane	17.22 ± 0.18	24.54 ± 0.23	N.A*	N.A*	
Chloroform	15.52 ± 0.14	28.52 ± 0.29	N.A*	11.22 ± 0.11	
Ethyl acetate	20.56 ± 0.21	35.27 ± 0.32	10.22 ± 0.11	15.55 ± 0.16	
n-Butanol	16.25 ± 0.15	30.55 ± 0.28	13.45 ± 0.13	18.25 ± 0.17	
Methanol	21.52 ± 0.23	31.21 ± 0.29	15.25 ± 0.17	21.34 ± 0.21	
Standard	33	30	28	32	
	Minin	num inhibitory conce	ntration (µg/ml)		
n-Hexane	415	435	N.A*	N.A*	
Chloroform	385	395	N.A*	415	
Ethyl acetate	315	335	365	380	
n-Butanol	215	245	260	290	
Methanol	175	180	200	215	
Standard	29	78	99	31	

Table 3. Antimicrobial activity in terms of disc diffusion method and minimum inhibitory concentrations (MIC) by maize (*Zea mays* L.) husk leaf extracts against selected strains.

*N.A. = Not active.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Audipudi AV, Mani PG, Pagadala GS, Supriya GNR, Pallavi R. Evaluation of immunostimulating activity in ethanol, ethyl acetate, methanol and chloroform extracts of *Ocimum sanctum* L. Int J Pharmacog Phyto Res. 2013; 5:311-314.

2. Inayatullah S, Prenzler PD, Obied HK, Rehman A, Mirza B. Bioprospecting traditional Pakistani medicinal plants for potent antioxidants. Food Chem 2012; 132:222-229.

3. Milind P, Isha D. Zea maize: A modern craze. Int Res J Pharm 2013; 4:39-43.

4. Meenakshi S, Gnanambigai DM, Mozhi ST, Arumugam M, Balasubramanian T. Total flavanoid and *in vitro* antioxidant activity of two seaweeds of Rameshwaram coast. Global J Pharmacol 2009; 3:59-62.

5. Singh R, Rajasree P, Sankar C. Screening for antidiabetic activity of the ethanolic extract of *Bryonia alba* roots. Int J Pharm Biol Sci 2012; 2:210-215.

6. Belguith H., Kthiri F., Chati A., Sofah AA., Hamida JB. and Ladoulsi A. Inhibitory effect of aqueous garlic extract (*Allium sativum*) on some isolated *Salmonella serovars*. Afr J Micobiol Res. 2010; 4:328-338.

7. Franz MJ, Powers MA, Leontos C, Holzmeister LA, Kulkarni K, Monk A, Wedel N, Gradwell E. The evidence for medical nutrition therapy for type 1 and type 2 diabetes in adults. J Am Diet Assoc 2010; 110:1852-1889.

8. Dewanto V, Wu X Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem. 2002; 50:3010-3014.

9. Iqbal S, Bhanger M, Anwar F. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chem 2005; 93:265-272.

10. Yen GC, Duh PD, Chuang DY. Antioxidant activity of anthraquinones and anthrone. Food Chem 2000; 70:437-441.

11. Lordan S, Smyth TJ, Soler Vila A, Stanton C, Ross RP. The

 α -amylase and α -glucosidase inhibitory effects of Irish seaweed extracts. Food Chem 2013; 141:2170-2176.

12. Jan S, Khan MR, Rashid U, Bokhari J. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. Osong Public Health Res Perspect 2013; 4:246-254.

13. Powell W, Catranis C, Maynard C. Design of self processing antimicrobial peptides for plant protection. Lett Appl Microbiol 2000; 31:163-168.

14. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Effect of *Fagonia arabica* (Dhamasa) on *in vitro* thrombolysis. BMC Complement Altern Med 2007; 7:1-7.

15. Puripattanavong J, Songkram C, Lomlim L, Amnuaikit T. Development of concentrated emulsion containing *Nicotiana tabacum* extract for use as pesticide. J App Pharm Sci 2013; 3:16-21.

16. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern Med 2012; 12:1-12.

17. Duthie G, Duthie SJ, Kyle JA. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nut Res Rev 2000; 13:79-106.

18. Nair VD, Panneerselvam R, Gopi R. Studies on methanolic extract of Rauvolfia species from Southern Western Ghats of India *in vitro* antioxidant properties, characterisation of nutrients and phytochemicals. Ind Crops Prod 2012; 39:17-25.

19. Adedapo AA, Jimoh FO, Afolayan AJ, Masika PJ. Antioxidant activities and phenolic contents of the methanol extracts of the stems of *Acokanthera oppositifolia* and *Adenia gummifera*. BMC Complement Altern Med. 2008; 8:1-7.

20. Velioglu Y, Mazza G, Gao L, Oomah B. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem 1998; 46:4113-4117.

21. Qayyum A, Sarfraz RA, Ashraf A, Adil S. Phenolic composition and biological (antidiabetic and antioxidant) activities of different solvent extracts of an Endemic plant (*Heliotropium strigosum*). J Chil Chem Soc 2016; 61:2828-2831.

22. Sharma P, Sharma JD. *In vitro* hemolysis of human erythrocytes by plant extracts with antiplasmodial activity. J Ethnopharmacol 2001; 74:239-243.