Pak. J. Bot., 49(6): 2499-2506, 2017.

ANTIBACTERIAL, ANTIOXIDANT AND PHENOLICS COMPOUND ANALYSIS OF *ABRUS PRECATORIUS* SEED COAT EXTRACT AND ITS DIFFERENT FRACTIONS

LUBNA MOBIN[1*], SYED ASAD SAEED[1], RASHIDA ALI[1, 2] SYED MUHAMMAD GHUFRAN SAEED[1] AND RAHIL AHMED[2]

[1]Department of Food Science and Technology, University of Karachi, Pakistan
.[2]English Biscuit Manufacturers (Pvt.) Limited, Plot 1-4, Sector 23, Industrial Area, Karachi-749 00, Pakistan
[*]Correspondence author's email: lubna.mobin@hotmail.com; Tel.: +923002772186

Abstract

Chromatographic (DAD-HPLC) analysis of purified fractions from *Abrus precatorius* seed coat extract has been performed; the predominant phenolic compounds found were delphinidin, epicatechin, syringic acid, caeffic & vanillic acid. The purification was done by solid phase extraction (SPE) through using C18 silica bonded sorbent; two main fractions (non-anthocyanin I & anthocyanin II) were elucidated from extract and three sub fractions (neutral Ia, neutral Ib, acidic Ic) were separated from non-anthocyanin fraction. Antibacterial activity was evaluated by an agar well diffusion method against three Gram-negative & two Gram-positive bacteria. Result showed that *A. precatorius* seed coat extract were active against a panel of bacteria. Moreover fractionation of seed coat extract increased the antibacterial effect. Among sub-fractions, fraction Ic was found more active against gram positive bacteria whereas gram negative bacteria was found more sensitive towards fraction Ia. Antioxidant screening was done by four different methods; diphenyl-1-picrylhydrazy, reducing power, phenanthroline and lipoxygenase assay. Result showed that *A. precatorius* seed coat extract have excellent free radical scavenging activity and reduction potential. The order of antioxidant activity of the fractions was equivalent to their sequence of total phenolic and flavonoid contents i.e. fraction I>fraction Ic >fraction Ib>fraction Ia (except fraction II). The antioxidant activities of crude extract were highly correlated with the total phenolic content ($p<0.01$). Current finding suggest that along with the total phenolic content, the structure of polyphenols direct its antimicrobial and antioxidant potential. This study highlighted the medicinal importance of the seed coat of *A. precatorius* and its potent phenolic constituent. It suggested that the seed coat of *A. precatorius* could potentially be used for the isolation of potent antibacterial and antioxidant compounds.

Key words: *Abrus precatorius*, Antibacterial, Antioxidant, Seed coat, Polyphenol fractions, DAD HPLC.

Introduction

Discovery of natural occurring antimicrobial and antioxidant compounds has always been of interest because of their importance in food and medicinal substance to replace artificial compounds. The curative effect of many plants suggests the presence of anti-oxidative and antimicrobial constituents in their cells. Phenolic compounds are the most abundant secondary metabolite of the plants that have the therapeutic role in the treatment of many human diseases. Thus identification of phenolic compounds from plants and plant parts has become a major area of health- and medical-related research (Adaramola & Onigbinde, 2017; Asif, 2015; Hamid *et al.*, 2016; and Ashraf *et al.*, 2015).

The experimental plant *Abrus precatorius* L., belongs to family leguminosae with typically red and black seeds. The different part (seed, stem, leaves, and root) of this plant has been traditionally used for medicinal purpose (Garaniya & Bapodra, 2014). The study of *A. precatorius* seeds has showed that it is a good source of polyphenolic (Vadivel *et al.*, 2011a) and thus could have therapeutic potential. Reported activities of plant seeds are antibacterial, antiplatelet, antitumor, anti-inflammatory, immunomodulating, anti-allergic, insecticidal, antidiarrheal, molluscicidal, male antifertility and female abortifacient (Pal *et al.*, 2009). The phenolic content and antioxidant property of *A. precatorius* seeds have also been evaluated in a recent few years (Pal *et al.*, 2009; Marimuthu *et al.*, 2014 & Tabasum *et al.*, 2016). The seed coats of legumes are an excellent source of the phenolic compound (Troszyńska *et al.*, 2002) yet very sparse research is available on the phenolic profile and remedial effect of the specifically seed coats of *A. precatorius* (De Britto *et al.*, 2012).

Current study has focused on the phenolic compounds of seed coat of *A. precatorius* and health relevant functionalities associated with them. Thus supports the use of *A. precatorius* seed coat as an alternative for synthetic drugs in the field of medicine. The research involved the determination of antibacterial and antioxidant activities of *A. precatorius* 80% methanolic seed coat extract and identifies the phenolic compounds present in it by DAD-HPLC. To explore the potent antioxidant and antibacterial compound in the seed coat of plant the activities of individual phenolic fractions, which represent a specific class of polyphenols, from the extract have also been studied. To best of our knowledge, not much work is done on the medicinal aspect of the seed coat of this plant. From our research, it is suggested that the seed coat of *A. precatorius* could potentially be used for the isolation of potent antibacterial and antioxidant compounds.

Experimental section

Reagent and standards: The phenolic standards of syringic acid (PubChem CID: 10742), vanillic acid (PubChem CID: 8468), myricetin (PubChem CID: 5281672), p coumaric acid (PubChem CID: 637542) were purchased from Sigma-Aldrich (St. Louis, MO, USA) while catechin (PubChem CID: 73160), epicatechin (PubChem CID: 72276), kaempferol (PubChem CID: 5280863), delphnidin (PubChem CID: 68245), petunidin

(PubChem CID: 73386), malvidin (PubChem CID: 159287) were obtained from ChromaDex (Irvine, California, US). The solvents such as methanol (CH_3OH), ethyl acetate ($CH_3COOC_2H_5$), acetonitrile [(NH_4) PO_4] were of HPLC grade (99.9% ±1) and purchased from Fisher Scientific. The reagent such as ammonium [$(NH_4)H_2PO_4$], phosphoric acid (H_3PO_4), silver nitrate ($NaNO_2$), aluminium chloride ($ALCL_3$), potassium chloride (KCL), sodium acetate ($C_2H_3NaO_2$), hydrochloric acid (HCL), sodium hydroxide (NaOH) were ACS grade and purchased from Fisher Scientific, Surrey and Merck.

Sample preparation: *Abrus precatorius* seeds (red variety) were purchased from the local Market (Ghousia Dawakhana, Jdi herb shop, Shah Faisal Colony Karachi-25) in the month of March, 2015. The samples were verified by a taxonomist, at Herbarium, Centre for Plant Conservation, Department of Botany, University of Karachi and a voucher specimen was deposited in its herbarium. Seeds were washed, air dried and dehulled by a pulse grinding method (Black & Decker, US) for a minute then manually red black seed coat was removed from the inside legume. The collected seed coats were packed and immediately stored in a freezer at -4ºC.

Extraction: The extract of *Abrus precatorius* seed coat was obtained by the method described by Sultana *et al.* (2009) with modification. Briefly the collected seed coats were further ground (Black & Decker, US) to get the particle size of 80 mesh. The ground particles with a ratio of 1:10 (sample: solvent) were macerated with 80% methanol (methanol: water, 80:20 v/v) for around 24 hrs at room temperature (30±2ºC)**.** The filtrate separated from the residue by using what man No. 1 filter paper. The combined extracts were concentrated under a vacuum at 45ºC, using rotary evaporator (Buchi, R-200, Flawil, Switzerland). The concentrated extracts were stored at -4º C until utilized for investigation.

Fractionation

Fractionation of a seed coat extract into anthocyanin and non-anthocyanin: Extract obtained by above method was dissolved in distilled water. The sample was filtered (0.45 µm PVDF) and loaded onto the C18 sorbent bonded on silica (Sep-Pak Cartridge: 500 mg sorbent, Waters) which was preconditioned before. Washing of cartridges were carried out with 10 mL of 0.01N HCl then dried by passing N_2 for 10 minutes. Ethyl acetate (15 mL) was used to elute polyphenols other than anthocyanin (Fraction I). The acidified methanol (10 mL) was used to elute absorbed anthocyanin (Fraction II) from the column. Both fractions solvent were removed under vacuum at 40ºC respectively. Each concentrate then re dissolved in distilled water. To prevent decay the fractions were flush with nitrogen and stored at -4ºC (up to 1day) (Kim and Lee, 2002a).

Separation of a non-anthocyanin fraction: Non anthocyanin were separated in three sub fractions neutral Ia (flavanols and other polyphenol), neutral Ib (flavonols), acidic Ic (phenolic acids). The pH of non-anthocyanin fraction was adjusted to 7 and passed through preconditioned C18 cartridges. The acidic portion (Fraction Ia) was at first eluted by washing with water (7 mL), followed by 0.01 N HCl (7 mL). Neutral Ib (flavanols) retained was recovered with 5 mL acetonitrile (10% acidified, pH 2). The neutral Ic fraction portion consisting flavonols was eluted with 5 mL of 40% (v/v) acetonitrile. The pH of acidic fraction was maintained to with HCl to 2.0. It was then passed through preconditioned C18 cartridge methanol (5 mL) and 0.01 (5 mL) HCl. The retained phenolic was then obtained with 5 mL of 40% (v/v) acetonitrile. The fractions were concentrated under a vacuum at 40ºC and were solubilized in 5 mL deionized distilled water, nitrogen flushed to prevent decay and for 24 hours stored at -4ºC (Oszmianski & Lee, 1990).

Identification and quantification

HPLC analysis: The isolated fractions I and II were filtered first through PVDF filters (0.45 µm pore size) (Millipore, US) for consequent measurement by DAD-HPLC Agilent Technology LC series 1260 infinity. The injection volume was 20 µm for each fraction. The chromatographic separation was carried out on a reverse-phase C18 column (5-µm × 250-mm × 4.6-mm) provided by Agilent and was thermostated at room temperature (e.g. 23ºC). The chromatographic conditions were set according to basic protocol described by Kim & Lee, (2002b). Briefly, the solvent was (A) 50 mM Ammonium $(NH_4)H_2PO_4$, pH 2.6, (B) 80:20 (v/v) acetonitrile/50 mM (NH4)H2PO4, pH 2.6 and (C) 200 mM H_3PO_4, pH 1.5. The gradient was set at a flow rate of 1ml/min in a ratio given below:

Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)
0	100	0	0
4	92	8	0
10	0	14	86
22.5	0	16.5	83.5
27.5	0	25	75
50	0	80	20
55	100	0	0
60	100	0	0

Spectra were recorded in UV/Vis range. The compounds were identified by comparing with the phenolic standards on the basis of retention times and characteristic UV–Vis spectra.

Total phenol content (TPC): Total phenolic content were estimated using Folin-Ciocalteu method as described by Gutfinger, (1981). Gallic acid was used as a standard and result are expressed as mg of gallic acid equivalent per gram of extract on a dry matter basis (DM).

Total flavonoid content (TFC): The total flavonoid content was measured by a method previously reported by Dewanto *et al.* (2002). Briefly describing, each fraction was diluted with 4ml of water, 0.3 ml of $NaNO_2$ (5%) was added to it. After 5 min, 0.3 ml of $ALCL_3$ (10%) was then added, after 6 min 2 ml of NaOH (1.0 M) was added. Later on 2.4 ml of water was mixed well in it. Absorbance

of a mixture was read at 510 nm. Quercitin was used as standard. Results are expressed as mg quercitin equivalent per gram of extract on a dry matter basis (DM).

Total anthocyanin content (TAC): Total monomeric anthocyanin content was measured by the method previously reported (Wrolstad *et al.*, 1995). Sample was diluted with potassium chloride and sodium acetate buffers to maintain the pH 1.0 and pH 4.5 and kept for 15 min. Then absorbance of each was determined at 520 nm and at 700 nm. The TAC calculation is based on molecular weight (MW) and the molar extinction coefficient (ε) of cyanidin-3-glucoside (449.2g). DF represents dilution factor. The results are expressed in mg of cyanidin-3- glucoside per gram of dried extract. The absorbance (A) was calculated as follows:

$$A = (A520 - A700)\,pH1.0 - (A520 - A700)\,pH4.5 \qquad Eq\,(1)$$

$$TAC\left(\frac{mg}{\square}L\right) = \frac{A \times MW \times DF \times 1000}{\square}(\epsilon \times 1) \qquad Eq\,(2)$$

Antibacterial property

Microbial strains: The microbial strains *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (27853), *Staphylococcus aureus* (ATCC 25931) and *Salmonella typhi* (6539) used were obtained from Microbiology Department, University of Karachi.

Zone of inhibition: The antibacterial activity of *A. precatorius* seed coat extract/fractions was evaluated by agar well diffusion method (Ferreira *et al.*, 1996 and Ortega & Julian, 1996). The seed coat extract and all fractions were filtered by a syringe filter (pore size 0.45µm, Millipore). For assay 1 ml of culture suspension of each strain (gave 25% transmittance at 530nm) was added in 100ml antibiotic agar No.11 (temperature became 45°C) and mix well. Poured 25ml of inoculated agar in each petri dish (20×100mm) and kept for solidification. After solidification 4 holes were made using sterile borer of 8mm diameter with 6mm internal diameter. Holes were marked and each extract (100µl) was poured in the respective well and incubated for 24hours at 37°C. The experiment was performed in triplicate under aseptic conditions. After incubation the zone of inhibition (mm) produced by each extract was measured and antibacterial activity was expressed in terms of percent inhibition. Gentamycin (0.3%) was used as a standard antibiotic.

Antioxidant property

Reducing power: Reducing power was determined according to the procedure proposed by Yen *et al.* (2000) with a slight modification. To sample 5ml of 0.2M sodium phosphate buffer (pH 6.6) and 5ml of 1% potassium ferricyanide was added then kept for incubation at 50°C for 20 min. To that 5ml of 10% trichloroacetic acid was mixed and centrifuged at 980 *g* for 10 min at 5°C using a centrifuge (Beckman Coulter, Inc USA). Around 5ml of upper layer from the centrifuge solution was taken out and 5 mL distilled water was added to it. Then ferric chloride (1.0 mL, 0.1%) was added, and absorbance was read at 700 nm using a spectrophotometer (Perkin Elmer, Shelton CT, lambda 25, USA). Readings were taken in thrice and the average was used for calculation. Percent reduction ability was determined in terms of percentage considering butylated hydroxytoluene as a standard.

$$Perceny\ Reduction\ Activity = \frac{As}{Ast}X100 \qquad Eq(3)$$

where,
As = Absorbance of sample
Ast= Absorbance of standard

Phenanthroline test: Phenanthroline test was performed according to previously described method (Aleksandra *et al.*, 2008). Briefly 0.6ml sample was mixed with 1ml of $FeCl_3$ (0.2%) and 0.5mL of phenanthroline (0.5%), both solution were prepared in methanol. The volume of a mixture was then made up with methanol up to 10 ml, mixed and kept at room temperature (i.e. 25-27° C) in a dark for 20 min. The absorbance of colored complex solution was measured at 510 nm against a blank, comprised of 1mL of $FeCl_3$ (0.2%) and 0.5 mL Phen (0.5%) diluted upto 10 ml with methanol. The results are expressed in µmol of $FeSO_4$per gram of dried extract.

Free radical scavenging activity: Radical scavenging activity of the seed coat extract and fraction were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Hossain *et al.*, 2011.

Statistical analysis: All samples were analyzed in triplicate, and the results were pooled to express the means ± standard error. Analysis of variance procedure (ANOVA) was performed. The results were significant when $p<0.01$ and $p<0.05$. Correlations between total phenolic content and tested activities were established using Pearson test. SPSS software (version 17.0) was used for statistical analysis.

Results and Discussion

Total phenolic content, total flavonoid content and HPLC analysis: The total phenolic content (TPC) of extract depends on the shifting of an electron from a phenolic compound present in extract towards the phosphomolybdic acid in an alkaline medium. The higher the phenolic compound, the more will be the transfer of electrons and the greater will be the absorbance measured spectroscopically. Total phenolic content in the *A. precatorius* seed coat crude extract was found to be 140.10±4.25mg GAE g^{-1} of dried extract. Variation in TPC was observed while comparing the obtained result with the previous researches *i.e.*, 219.96 mg GAE/g in hydro-methanolic extract (Tabasum *et al.*, 2016), 31 mg GAE/g in aqueous extract (Marimuthu *et al.*, 2014), 142 mg catechin/g in methanolic extract (Vadivel *et al.*, 2011b) and 95 mg GAE/g in ethanolic extract (Pal *et al.*, 2009). Total flavonoid content (TFC) represents the amount of flavonoids in extract, which are the largest class of plant

phenolic. The flavonoid content found in the *A. precatorius* seed coat crude extract was 88.19±4.60 mg quercitin/ g of dried extract, which is much higher than the TFC values previously reported (Marimuthu *et al.,* 2014 and Pal *et al.,* 2009). This dissimilarity in TPC and TFC could be due to the different type of solvents used in study, as the yield of phenolic compound greatly depends on number of factors such as solvent type, extraction method, the measuring bases (wet or dry bases) (Cheng *et al*., 2012). In addition by considering that the seed coat of legume grains contains a variety of phenolic compound (Troszyńska *et al.,* 2002); the sample used in current study is exclusively the outer coat of *A. precatorius* seed while the previous researches are on the whole seed of *A. precatorius*. The analysis of variance showed a significant difference (p<0.05) between the TPC and TFC of *A. precatorius* seed coat crude extract and its fractions. The percent yield of phenolic compounds in seed coat extract was found to be the highest as it represents the total of all phenolic compounds present in the seed coat. Total phenolic and flavonoid content in a non-anthocyanin fraction I was found higher than the anthocyanin fraction II. It can be concluded that total phenolic content in the seed coat of *A. precatorius* is largely comprised of polyphenols other than anthocyanin (i.e. phenolic acids, flavanol). Among sub fractions statistically, the content of total phenolic were in order of Ic> Ib> Ia (Table 1).

The phenolic compounds identified in non-anthocyanin (I) and anthocyanin (II) fractions by chromatographic study are shown in the Table 2. Polyphenols have recently been reported in the whole seed of *A. precatorius* (Jain *et al.,* 2015). In current study, the variety of phenolic compounds at their maximum strength was identified in the seed coat. This might be due to the purification step performed prior to HPLC analysis that had maintained the integrity of polyphenols and eliminated the interfering substance. Syringic acid, caeffic acid and vanillic acid were the phenolic acids identified in the non-anthocyanin fraction I. Epicatechin was the predominant flavanol identified. HPLC chromatographs of the identified compounds in non-anthocyanin and anthocyanin fractions of the *A. precatorius* seed coat are shown in Figure 1. Mostly similar phenolic constituents (except vanillic acid) were reported by Vadivel *et al.* (2011b) in a whole seed of *A. precatorius.* The delphinidin was found as an active anthocyanin in fraction II. The delphinidin derivatives in the whole seeds of *A. precatorius* have already been previously reported by Garaniya & Bapodra (2014). It could be related that among the total anthocyanin content (Table 1) the delphinidin and its derivative are the predominant anthocyanidin and anthocyanin present in the seed coat of *A. precatorius.*

Antibacterial activity: The antibacterial activity of *A. precatorius* seed coat crude extract and fractions from *A. precatorius* against food-borne and pathogenic bacteria were determined. The results are presented in Table 3. The antibacterial activity was evaluated by agar well diffusion method against three Gram-negative bacteria (*S. typhi, E. coli, P. aeruginosa*) and two Gram- positive bacteria (*B. subtilis, S. aureus*). A result revealed that the *A. precatorius* seed coat extract and all fractions except anthocyanin have shown a broad antibacterial spectrum. A significant zone of inhibition was found against *S. aureus* and *S. typhi* while activity was moderate against *E. coli, P. aeruginosa* and *B. subtilis* by crude seed coat extract. While comparing the obtained inhibitory zone against *P. aeruginosa, B. subtilis, S. aureus* and *S. typhi* was much greater than the result previously reported by Roy *et al.* (2012) of aqueous and chloroform whole seeds extract. The antimicrobial activity of phenolic compounds is widely known world over (Pereira *et al.,* 2007). The mechanism has revealed that the antimicrobial action of phenolic compounds was related to the inhibition of enzymes that depends on the rate of penetration of the phenolic into the cell or due to other membrane permeability changes (Moreno *et al.,* 2006). The inhibitory action of phenolic components against each microbe is different depending on the nature of microbes.

Table 1. Total content of phenols, flavonoids and anthocyanin in the crude extract and fractions isolated from crude extract of *A. precatorius* seed coat[a].

Crude/Fractions	TPC (GAE/ gm)	TFC (QE/ gm)	TAC (CYN-3-GLU/100gm)
Extract	140.10 ± 4.25^{f}	88.19 ± 4.60^{e}	93.40 ± 5.40^{b}
Non Anthocyanin (I)	111.53 ± 6.62^{e}	62.83 ± 3.61^{d}	ND
Anthocyanin (II)	24.25 ± 1.01^{d}	20.02 ± 1.83^{c}	60.44 ± 0.50^{a}
Acidic (Ia)	14.22 ± 0.83^{a}	-	ND
Neutral A (Ib)	18.31 ± 0.9^{b}	13.67 ± 0.4^{a}	ND
Neutral B (Ic)	21.94 ± 1.45^{c}	17.55 ± 2.96^{b}	ND

(-) estimated value

[a]Values are mean and ± standard deviation of three separate determinations. Values in the same column with different alphabet superscripts are significantly different at (p<0.05)

Table 2. Phenolic compounds identified in the seeds coat of *A. precatorius*[a].

Fractions	Compounds	Retention time (min)	Identification wavelength (nm)
Non-Anthocyanin (I)	Vanillic acid	21.2 ± 0.34	280
	Caffeic acid	21.5 ± 0.31	320
	Syringic acid	22.3 ± 0.21	280
	Epicatechin	25.5 ± 0.14	320
Anthocyanin (II)	Delphinidin	29.9 ± 0.5	520

[a]Values are mean and ± standard deviation of three separate determinations

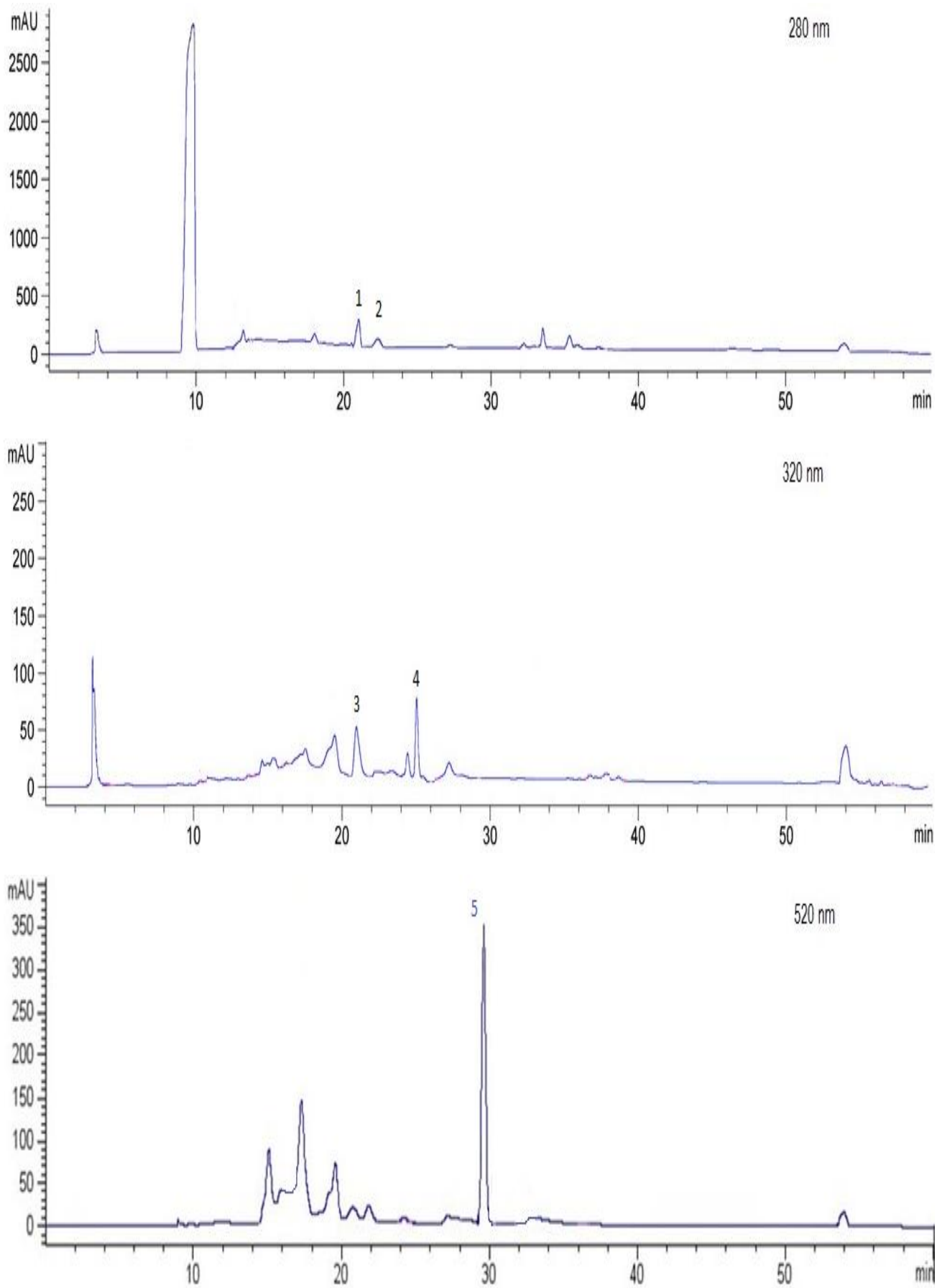

Fig. 1. HPLC chromatograph of the non-anthocyanin and anthocyanin fractions of the *Abrus precatorius* seed coat monitored at 280, 320 and 520 nm. Peaks no are the compound shown in Table. 2 i.e. vanillic acid (1), syringic acid (2), caffeic acid (3), epicatechin (4) and delphinidin (5).

Table 3. Antimicrobial Activity of seeds coat extract and fractions of *A. precatorius*[a].

Sample	Zone of inhibition (mm)				
	S. typhi	***B. subtilis***	***E. coli***	***P. aerugenosa***	***S. aureus***
Extract	35 ± 0.32^{c}	20 ± 0.17^{c}	20 ± 0.12^{b}	24 ± 0.15^{c}	33 ± 0.12^{c}
Non anthocyanin (I)	47 ± 0.53^{e}	25 ± 0.00^{d}	31 ± 0.02^{e}	40 ± 0.02^{e}	40 ± 0.2^{e}
Anthocyanin (II)	-	-	-	-	25 ± 0.91^{b}
Acidic (Ia)	45 ± 0.21^{d}	15 ± 0.25^{a}	28 ± 0.14^{d}	36 ± 0.17^{d}	22 ± 0.91^{a}
Neutral A (Ib)	25 ± 0.18^{a}	22 ± 0.14^{b}	15 ± 0.22^{a}	17 ± 0.18^{a}	33 ± 0.17^{c}
Neutral B (Ic)	29 ± 0.15^{b}	30 ± 0.18e	22 ± 0.25^{c}	25 ± 0.21^{b}	39 ± 0.13^{d}

[a]Values are mean and ± standard deviation of three separate determinations. Values in the same column with different alphabet superscripts are significantly different at (p<0.05)

Table 4. Correlation analysis between phenolic content and antioxidant *Abrus precatorius* seeds extract and fractions.

	Pearson coefficient (r)		
	PHEN	**RED**	**DPPH**
Total phenolics	0.875**	0.712**	0.700**

** Correlation is significant at 0.01 (p two-tailed)

PHEN-phenanthroline activity; RED-reducing power; DPPH- α, α, diphenyl –β, picrylhydrazyl

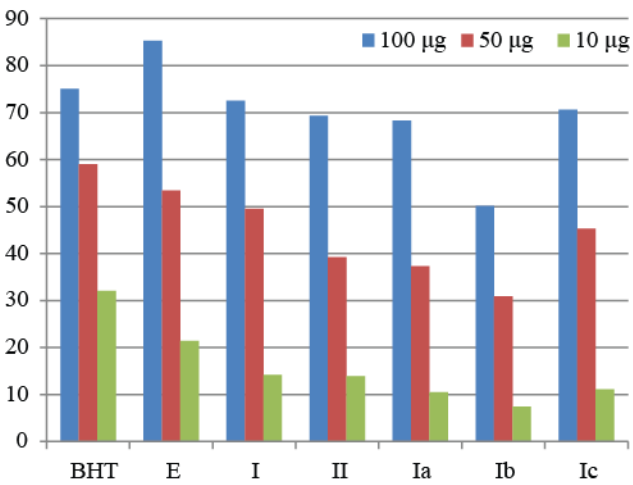

Fig. 2. Reducing activity of *A. precatorius* seed coat extract and its fractions compared with BHT.

Results have shown that the fractionations of seed coat crude extract increases the antibacterial effect against all tested strains. Mostly all fractions except anthocyanin has shown significantly higher activity (p<0.05) against all bacterial than the seed coat extract. It can be elucidated from the results that individual fractions that are supposed to be comprised of specific class of polyphenols at considerable amount have higher antibacterial property than a mixture of polyphenols present in an extract. The non-anthocyanin fractions (Ia, Ib and Ic) derived from the crude extract of seed coat of *A. precatorius* have significantly shown antibacterial activity towards all tested species. The purified anthocyanin fraction (II) was found active only towards *S. aureus*. The antibacterial activity of fractions derived from extracts were in order of fraction Ic> fraction Ib> fraction Ia for gram positive bacteria and fraction Ia > fraction Ic> fraction Ib for gram negative bacteria. The fraction Ic, though higher in phenolic and flavonoids content, was found more active against gram positive bacteria whereas gram negative bacteria was found more sensitive towards fraction Ia (Table 3). These variation in the fractions antibacterial potential could be explained on the basis of the fact that the inhibitory action of polyphenolic components against each microbe was different depending on the nature of microbes and structure of polyphenols. In particular, the membrane complexity of gram negative acts as a protective wall against lipophilic components (Helander *et al.,* 1998). Thus high antibacterial activities of fraction (Ia) of both seed coat extract against gram negative bacteria was due less lipophilic nature of phenolic acid. This increases the membrane permeability and more uptake of phenolic acids (Ia) as compared to flavanol (fraction Ib) and flavonol (fraction Ic) in the cell. After which they successfully inhibit the growth of microbes by interfering with the electron transfer, reacting with nucleic acid and protein and/or other enzymic activity (Dorman & Deans, 2000).

Antioxidant capacity: Antioxidant screening of *A. precatorius* seed coat crude extract and its fractions was carried out by four different methods: diphenyl-1-picrylhydrazy (DPPH), reducing power, phenanthroline and lipoxygenase assay. Result showed that *A. precatorius* seed coat crude extract had high inhibition of DPPH free radical i.e. 91%. This value is much higher than the previous reported results by Vadivel *et al.* (2011b) on the DPPH activity of a whole seed extract of *A. precatorius* i.e., 62.13%. Diphenyl-1-picrylhydrazy test measured the free radical scavenging activity of sample, which depends on the presence of antioxidant in it. Antioxidants transform the DPPH free radical into more stable molecule by denoting its electron. Therefore the more the antioxidant in the extract, the higher would be the scavenging of DPPH radical. In the phenanthroline test, the antioxidants present in the sample are added to the methanolic solution of iron (III), which causes reduction of iron (III) into iron (II). The reduced iron (II) form colored complex with the phenanthroline compound i.e. Iron $(Phen)^{+2}_3$. The intensity of complex was measured spectroscopically at 510 nm. According to results, highly intense color complex [Iron $(Phen)^{+2}_3$] was formed due to the reduction of iron by the antioxidant present in the crude extract (i.e. 1143.01±6.94 µmol of $FeSO_4$ per gram of extract). A relation between antioxidant ability and reducing potential of certain plant and plant part extracts has been reported (Singh *et al.,* 2016). Reducing power is found to be associated with the

presence of hydrogen denoting compounds, which behave as an antioxidant by denoting a hydrogen atom to a free radical and break the chain reaction of it (Elmastaş *et al.*, 2006). The greater reducing power (89.76± 0.5 percent of BHT) of crude extract was obtained using potassium ferricyanide reduction method. Phenolic compounds have proved themselves as powerful antioxidant in food and/or isolated medium (Yu & Ahmedna, 2013; Qasim *et al.*, 2016; Abideen *et al.*, 2015). Either by scavenging free radical or preventing radical formation by inhibiting enzyme or chelating pro oxidant metals, phenolic compound regulates the antioxidant defense system. High reduction potential of *A. precatorius* seed coat crude extract was due to the presence of polyphenols that synergistically produced high antioxidant effect. When reducing power of seed coat extract and its fraction were compared at increasingly concentrations, it was increased as the dose got high similar to standard BHA (Fig. 2). A study on the whole seed of *A. precatorius* has shown its total phenolic content relation with potent antioxidant activity (Pal *et al.*, 2009; Marimuthu *et al.*, 2014). In accord with previous researches, positive co relation was found between the total phenolic content and antioxidant activity of *A. precatorius* seed coat crude extract ($p<0.01$) (Table 4).

All fractions derived from the seed coat crude extract showed antioxidant activities which differ significantly ($p<0.05$) from one another (Table 5). The order of antioxidant potential followed by the fractions (I> Ic> Ib> II> Ia) can be demonstrated on the basis of total phenolic contents and the structure of polyphenolic compounds. Fraction I had a main contribution in the total phenolic compound of seed coat thus possessed the highest antioxidant activity. Among sub fractions, Neutral fraction B (Ic) showed a significantly higher antioxidant activity then acidic (Ia) and Neutral fraction A (Ib). This could be explained with respect to the structure function related property of polyphenols. Along with the content of polyphenols, the chemical structure plays a key role in attributing the antioxidant property. The polysubstituted phenols with OH groups have higher antioxidant abilities than the phenols with single electron donating groups (Nascimento *et al.*, 2014). The high efficacy of Neutral fraction B was supposed to be due to the presence of flavonol i.e., quercitin. The multiple OH substitution and presence of conjugation in the polyphenols present in fraction Ic, it has much high free radical scavenging activity and reduction potentials. On the other hand, the acidic fraction (Ia) comprised of phenolic acids i.e. hydroxybenzoic and hydroxycinnamic acid. The phenolic acid are the mono substituted OH phenols with single electron donating system therefore showed less antioxidant activity. However, among the phenolic acids, the number and position of OH determined their degree of effectiveness as an antioxidant. The comparatively low antioxidant activity in fraction II (anthocyanin fraction) was possibly due to the presence of the glycosylated anthocyanins in the fraction. Hopia & Heinonen (1999) reported that flavonoids glycosylation at 3 position reduces its activity compared to aglycones structure. Result of lipoxygenase inhibition assay showed that there was a decrease in the oxygenation of linoleic acid by lipoxygenase in the presence of crude extract and all fractions from the seed coat extract of *A. precatorius.* The activity order was same as in radical scavenging and reduction potential results.

Table 5. Antioxidant screening of *A. precatorius* seeds coat extract and its fractions[a].

Fractions	DPPH (% inhibition)	Phenanthroline ($FeSO_4$ µmol/g)	Reducing activity (percent compared with BHT)	Lipoxygenase (% inhibition)
Extract	91.31 ± 1.3 [f]	1143.01 ± 6.4 [e]	89.76 ± 0.5 [f]	85.3 ± 0.1 [f]
Non-Anthocyanin (I)	84.53 ± 4.1 [e]	1598.33 ± 0.1[f]	82.36 ± 0.2 [e]	72.5 ± 0.1 [e]
Anthocyanin (II)	54.12 ± 2.1 [b]	395.11 ± 2.3 [b]	63.03 ± 0.6[b]	65.1± 0.1 [b]
Acidic (Ia)	36.20 ± 3.4 [a]	320.52 ± 1.2 [a]	59.10 ± 0.1 [a]	68.3 ± 0.2 [a]
Neutral A (Ib)	77.05 ± 2.4 [c]	542.14 ± 2.1[d]	80.20 ± 0.3[d]	69.3 ± 0.2 [d]
Neutral B (Ic)	80.84 ± 1.3 [d]	431.54 ± 1.9 [c]	79.17 ± 0.4 [c]	70.6 ± 0.1 [d]

[a]Values are mean and ± standard deviation of three separate determinations. Values in the same column with different alphabet superscripts are significantly different at ($p<0.05$)

Conclusion

Abrus precatorius seed coat has been found to be a considerable source of variety of phenolic compounds which plays very active role against reactive oxygen species and has broad spectrum against disease causing bacteria. Current findings suggest that *A. precatorius* seed coat can be used as a natural source of preservative for curing number of human ailments. However, more research should be performed on such bioactive plant seed coat to explore its other possible biological and industrial applications.

References

Abideen, Z., M. Qasim, A. Rasheed and M.Y. Adnan, M.Y. 2015. Antioxidant activity and polyphenolic content of Phragmites karka under saline conditions. *Pak. J. Bot*., 47(3): 813-818.

Adaramola, B. and A. Onigbinde. 2017. Influence of extraction technique on the mineral content and antioxidant capacity of edible oil extracted from ginger rhizome. *Chemistry International*, 3(1): 1-7.

Aleksandra, S.C., D. Csilla, R. Katalin, K. Gyorgy and S. Edward. 2008. Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. *Talanta*, 76(4): 899-905.

Ashraf, M.W., M. Bilal and M. Iqbal. 2015. Antiglycation activity of vegetables aqueous and methanolic extracts. *Current Science Perspectives*, 1: 12-15.

Asif, M. 2015. Antiviral and antiparasitic activities of various substituted triazole derivatives: A mini. *Chemistry International*, 1(2): 71-80.

Cheng, V.J., A.E.-D.A. Bekhit, M. McConnell, S. Mros and J. Zhao. 2012. Effect of extraction solvent, waste fraction and grape variety on the antimicrobial and antioxidant activities of extracts from wine residue from cool climate. *Food Chem*., 134(1): 474-82.

De Britto, A.J., P.B. Jeya, R. Kumar, S. Gracelin and D. Herin. 2012. *Abrus precatorius* L.: A medicinal plant with potential as antibacterial agent. *A. J. Pharmcy Res*., 5(2): 1207-1209.

Dewanto, V., X. Wu, K.K. Adom and R.H. Liu. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem*., 50(10): 3010-3014.

Dorman, H. and S. Deans. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol*., 88(2): 308-316.

Elmastaş, M., L. Gulcin, S. Beydemir, K. Irfan and Y. Hasan. 2006. A study on the *In vitro* antioxidant activity of juniper (*Juniperus communis* L.) fruit extracts. *Anal. Lett*., 39(1): 47-65.

Ferreira, M.J.U., A. Daurte and J.R. Ascenso. 1996. Antimicrobial and phytochemical studies of *Euphorbia tuckeyana. Fitoterapia*, 67(1): 85-86.

Garaniya, N. and A. Bapodra. 2014. Ethno botanical and Phytophrmacological potential of *Abrus precatorius* L.: A review. *Asian Pac. J. Trop. Biomed*., 4*:* 27-34.

Gutfinger, T. 1981. Polyphenols in olives oils. *J. Am. Oil Chem. Soc*., 58(11): 966-968.

Hamid, A.A., S.O. Oguntoye, S.O. Alli, G.A. Akomolafe, A. Aderinto, A. Otitigbe, A.M. Ogundare, Q.M. Esinniobiwa and R.O. Aminu. 2016. Chemical composition, antimicrobial and free radical scavenging activities of *Grewia pubescens*. *Chemistry International*, 2(4): 254-261.

Helander, L.M., H.-L. Alakomi and K. Latva-Kala. 1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. *J. Agric. Food Chem*., 4(9): 6, 3590-3595.

Hopia, A. and M. Heinonen. 1999. Antioxidant activity of flavonol aglycones and their glycosides in methyl linoleate. *J. Am. Oil Chem. Soc*., 76(1): 139-144.

Hossain, M.S., M.B. Alam, M. Asadujjaman, M. Islam, M.E. Mazumdar and M. Haq. 2011. Antidiarrheal, antioxidant and antimicrobial activities of the *Musa sapientum* seed. *Avicenna J. Med. Biotech.*, 3: 95-105.

Jain, A., P. Sinha and S. Vavilala. 2015. Estimation of flavonoid content, polyphenolic content and antioxidant potential of different parts of *Abrus precatorius* (L.). *Intern. J. Pharm. Pharm. Sci*., 7(8): 157-163.

Kim, D.O. and C.Y. Lee. 2002a. Extraction and isolation of polyphenolics. Current protocols in food analytical chemistry, Unit 11.2, John Wiley & Sons Inc

Kim, D.O. and C.Y. Lee. 2002b. HPLC separation of polyphenols. Current protocols in food analytical chemistry, Unit 11.3, John Wiley & Sons, Inc

Marimuthu, K., N. Nagaraj and D. Ravi. 2014. Analysis of phytochemical constituents, antioxidant potential of *Abrus precatorius* L. seeds. *Int. J. Pharm. Sci. Rev.*, 28: 43-46.

Moreno, S., T. Scheyer, C. Romano and A. Vojnov. 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radic. Res.*, 40(2): 223-231.

Nascimento, A.P., T.C.E.S. Nascimento, N.S.M. Ramos, G.R. Silva, J.E.G. Gomes, R.E.A. Falcao, K.A. Moreira, A.L.F. Porto and T.M.S. Silva. 2014. Quantification, antioxidant and antimicrobial activity of phenolics isolated from different extracts of *Capsicum frutescens* (Pimenta Malagueta), *Molecules*, 19(4): 5434-5447.

Ortega, M.G. and H.R. Julian. 1996. Antimicrobial agents in *Dalea elegant*, *Fitoterapia*, 67(1): 81-85.

Oszmianski, J. and C.Y. Lee. 1990. Isolation and HPLC determination of phenolic compounds in red grapes. *Am. J. Enol. Vitic*., 41(3): 204-206.

Pal, R.S., G. Ariharasivakumar, K. Girhepunje and A. Upadhyay. 2009. In-vitro antioxidative activity of phenolic and flavonoid compounds extracted from seeds of *Abrus precatorius. Int. J. Pharm. Pharm. Sci*., 1(2): 136-140.

Pereira, A.P., I.C.F.R. Ferreira, F. Marcelino, P. Valentão, P.B. Andrade, R. Seabra, L. Estevinho, A. Bento and J.A. Pereira. 2007. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. *Cv.* Cobrançosa) leaves. *Molecules*, 12(5): 1153-1162.

Qasim, M., I. Aziz, M. Rasheed, B. Gul and M.A. Khan. 2015. Effect of extraction solvents on polyphenols and antioxidant activity of medicinal halophytes. *Pak. J. Bot.*, 48(2): 621-627.

Roy, S., R. Acharya, N.C. Mandal, S. Barman, R. Ghosh and R. Roy. 2012. A comparative antibacterial evaluation of raw and processed *Guñjā* (*Abrus precatorius* Linn.) seeds. *Ancient Sci. Life*, 32(1): 20-23.

Singh, J.P., A. Kaur, N. Singh, L. Nim, K. Shevkani, H. Kaur and D.S. Arora. 2016. *In vitro* antioxidant and antimicrobial properties of jambolan (*Syzygium cumini*) fruit polyphenols. *LWT - Food sci. Technol*., 65: 1025-1030.

Sultana, B., F. Anwar and M. Ashraf. 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 14(6): 2167-2180.

Tabasum, S., S. Khare and K. Jain. 2016. Spectrophotometric quantification of total phenolic, flavonoid, and alkaloid contents of *Abrus precatorius* L. seeds. *Asian J. Pharm. Clin. Res*., 9(2): 371-374.

Troszyńska, A., I. Estrella, A. López, M. Luisa and T. Hernández. 2002. Antioxidant activity of pea (*Pisum sativum* L.) seed coat acetone extract. *LWT- Food Sci. and Tech*., 35(2): 158-164.

Vadivel, V., A. Nandety and H.K. Biesalski. 2011b. Antioxidant, free radical scavenging and type II diabetes-related enzyme inhibition properties of traditionally processed Jequirity bean *(Abrus precatorius* L.). *Int. J. Food. Sci. Tech*., 46(12): 2505-2512.

Vadivel, V., W. Stuetz, V. Scherbaum and H.K. Biesalski. 2011a. Total free phenolic content and health relevant functionality of Indian wildlegume grains: Effect of indigenous processing methods. *J. Food Compos. Anal*., 24(7): 935-943.

Wrolstad, R.E., V. Hong, M.J. Boyles and R.W. Durst. 1995. Use of anthocyanin pigment analysis for detecting adulteration in fruit juices. In: *Methods to Detect Adulteration in Fruit Juice and Beverages*, Vol. I (Eds.): Nagy, S. and R.L. Wade. Ag Science Inc., Auburndale, Fla

Yen, G.C., P.D. Duh and D.Y. Chuang. 2000. Antioxidant activity of anthraquinones and anthrone. *Food Chem*., 70(4): 307-315.

Yu, J. and M. Ahmedna. 2013. Functional components of grape pomace: Their composition, biological properties and potential applications. *Int. J. Food. Sci. Tech*., 48(2): 221-237.

(Received for publication 13 October 2016)