Research Paper

Comparative Phytochemical Investigation and Antioxidant Activity in Different Parts of *Acacia nilotica* Seed

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Kumar *et al.*: Phytochemical Investigation and Antioxidant Activity of *Acacia nilotica* Seed

***Acacia nilotica*, indigenously known as 'Babool' of family Leguminosae has many traditional and ethnobotanical uses. All the parts of tree are important and seeds are most abundantly available which are rich in different phytochemicals. Thus, the aim of this study was qualitative and quantitative analysis of phytochemicals and evaluation of the antioxidant activity in *Acacia nilotica* seed along with their parts *viz.* cotyledon and endosperm as a potential source in nutritional and medicinal applications. Phytochemicals as carbohydrate, protein, alkaloid, flavonoids, phenolics, saponin and tannin were present in seed, cotyledon and endosperm. Cotyledon was rich in sugar, carbohydrate, protein and oil, i.e. 4.73 %, 17.83 %, 30.45 % and 11.78 % respectively whereas endosperm was rich in phenolics and flavonoids i.e. 2.66 mg/g gallic acid equivalent and 7.66 mg/g quercetin equivalent respectively. Antioxidant activity studies showed that endosperm exhibited maximum percentage inhibition i.e. 74.07 % compared to the standard butylated hydroxyanisole having 82.88 % inhibition of 2,2-diphenyl-1-picrylhydrazyl radicals. The study revealed that the specific utilization of *Acacia nilotica* seed have scope in nutritional supplements and as useful bioactive extracts for sustainable health benefits.**

Key words: *Acacia nilotica*, 2,2-diphenyl-1-picrylhydrazyl, gallic acid equivalent, quercetin equivalent, nutritional, phytochemicalsuse

Plants and their parts are good source of effective medicines and nutritional supplements for human health and well-being. Nearly three quarter population of the world depends upon the plants and their products for healthcare management and about 30 % plant species are in medicinal use. In Indian medicine system such as Ayurveda and Unani, drugs of herbal origin have been used traditionally and ethnbotanically since ancient times[1].

Acacia nilotica (*A. nilotica*), indigenously known as 'Babool', belonging to subfamily Mimosoideae[2,3] and family Leguminosae[4,5], is an important and evergreen tree of Indian dried regions. Tree is generally 10 m in height, bark is longitudinally fissured with dark brown or black color and young plant exhibit terete, pubescent and slender branchlets[6]. *A. nilotica* recognized as a pioneer species for high bioactive compounds and economically important source of gum, tannins, fodder, fuel and timber from long time[7,8]. The species are widespread throughout Asia, Africa and America[9], and naturally found in Botswana, Kenya, Egypt, Zimbabwe, India, Saudi Arabia, Burma, Tanzania, Nepal, Pakistan, Nigeria, Sri Lanka, Indonesia, Sudan, Iraq, Iran, Namibia, Yemen and Ethiopia[10]. The plant *A. nilotica* has been used in traditional medicines and their all parts *viz*. gum, seeds, pods, roots, leaves, flowers and bark are medicinally important[11-13]. The plant is rich in different phytochemicals *viz*. carbohydrates, alkaloids, proteins, fatty acids, phenolics, flavonoids, tannins, saponins, cyanogenic glycosides, diterpenes, phytosterols, triterpenegenins, amino acids *viz*. cystine, methionine, threonine, lysine, tryptophan and macro elements such as potassium, phosphorus, magnesium, iron and manganese[14,15]. *A. nilotica* possesses antioxidant, antimicrobial, antifungal, antibacterial, antipyretic, antiinflammatory, antidiarrhoeal, antiulcer, antihelmintic, wound healing, diuretic,

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Accepted 05 May 2022
Revised 26 October 2021
Received 24 December 2020
Indian J Pharm Sci 2022;84(3):552-559

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antispasmodial, antihypertensive, antitumor and anticancer properties[16-19] and effective in coughs, cold, congestion, nerve stimulation, leucorrhea, dysentery, ophthalmia, hemorrhages and sclerosis relief[20]. Antioxidant property plays a vital role for the treatment of inflammation and cancer which are caused by free radicals[21] and presence of tannin which is beneficial for activation of glucose transfer and prevents lipolysis[22]. Phenolic compounds in *A. nilotica* makes the species potential for antioxidant property due to free radical scavenger activity[23,24].

A. nilotica seed exhibit edible characteristics and has nutritive and medicinal values due to various phytochemicals. Although, some studies are available on seed[25], but part specific study of seed on phytochemicals and bioactivity has not been reported yet. Thus, *A. nilotica* seed was exploited for phytochemical investigation and antioxidant property.

MATERIALS AND METHODS

Plant material:

Seeds were collected from Kalli Pashchim region of Lucknow district (Uttar Pradesh), India. The material was authenticated (LWG No.102994) and specimen was deposited in the herbarium at Council of Scientific and Industrial Research (CSIR)-National Botanical Research Institute (Uttar Pradesh), India. Different parts of the seed *viz*. cotyledon and endosperm (seed gum) were separated mechanically using grinder (Philips grinder) and sieved by 40 mesh sieve to obtain 40 mesh powder of each part for study.

Chemicals *viz*. Gallic acid, Quercetin, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and Butylated Hydroxyanisole (BHA) of Merck, solvents *viz*. n-hexane, chloroform, acetone, ethanol and methanol of Qualigens and glasswares made of Borosil were used.

Preliminary study:

Powdered materials were examined for organoleptic characteristics *viz*. colour, odour, taste and texture. The solubility was studied in solvents *viz*. hexane, chloroform, acetone, ethanol, distilled water and boiling water[26].

Extraction and phytochemical screening:

Materials were extracted under cold and hot conditions using different polarity gradient solvents *viz*. n-hexane, chloroform, acetone, alcohol and water. Cold extraction was carried out using percolation method by exhausting 50 g powder materials each in 250 ml solvents for 24 h at room temperature (25°-30°). Extracts were then filtered using Whatman filter paper (No. 42). Hot extraction was carried out through successive soxhlet extraction method by taking 50 g powder materials and 250 ml different solvents in soxhlet apparatus on water bath. The extracts were concentrated through Rotary Evaporator (IKA-RV 10 digital) at 40°-55° and dried at low temperature (-80°) and pressure (0.200 millibar (mBar)) using lyophilizer (Labconco- FreeZone Plus 4.5). Dried extracts were used in qualitative tests for the identification of various phytochemicals *viz*. carbohydrates, sugar, starch, glycosides, alkaloids, phenolics, flavonoids, saponins, tannins and sterols which were estimated quantitatively[27,28].

Phytochemical estimation:

Phytochemicals identified in different polarity gradient solvent extracts, *viz*. n-hexane, chloroform, acetone, ethanol and water were estimated quantitatively according to standard estimation methods.

Total sugar:

0.5 g powdered material was homogenized with 5 ml 80 % ethanol and centrifuged using centrifuge (Eltek TC 4100 F) for 15 min at 2000 rpm. The supernatant volume obtained was adjusted to 10 ml with 80 % ethanol. 0.1 ml sample was taken, mixed 0.1 ml 80 % phenol and 5 ml concentrated sulphuric acid respectively and made up the volume to 10 ml using 80 % ethanol. Thereafter, prepared samples were kept in an ice bath for 30 min. The D-glucose stock solution of 0.1 mg/ml concentration was prepared as standard and 1, 2, 3, 4 and 5 ml of stock solutions were taken for dilutions. The calculation of total sugar was carried out by measuring the absorbance at 490 nm double beam Ultraviolet-Visible (UV) spectrophotometer (Thermo Scientific-Evolution 201 UV spectrophotometer)[29].

Total carbohydrate:

100 mg material was taken into different boiling tubes, added 5.0 ml of 2.5 N Hydrochloric Acid (HCl) in each tube and boiled for 3 h on water bath. Samples were cooled at room temperature, neutralized by adding sodium carbonate and centrifuged. Dilutions were prepared by mixing 0.2 ml of sample, 1 ml 5 % phenol and 5 ml of 96 % sulphuric acid. Samples kept for 10 min at room temperature, then heated on water bath for 15 min and cooled. Stock solution of D-glucose as standard was prepared in 0.1 mg/ml concentration

and dilutions were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml from stock solution. Total carbohydrate was calculated by measuring the absorbance at 630 nm[30] through UV spectrophotometer.

Total protein:

500 mg powdered materials was homogenized with 5 ml of phosphate buffer of pH 7.4 and centrifuged for 20 min at 4000 rpm. The supernatant was filtered and volume was maintained to 10 ml with phosphate buffer. 0.1 ml of sample was taken and made up to 1 ml with distilled water. Then 5 ml of Reagent-C was added which was prepared by adding 50 ml Reagent-A, i.e. 2 % sodium carbonate in 0.1 N sodium hydroxide and 1 ml Reagent-B, i.e. 0.5 % copper sulphate in 1 % potassium sodium tartrate. Mixed the solutions and let stand for 10 min. Thereafter, 0.5 ml 1 N Folin-Ciocalteu Reagent (FCR) was added, mixed the samples and kept in the dark for 30 min. The stock solution of Bovine Serum Albumin (BSA) as standard of 1 mg/ml concentration was prepared. 0.2, 0.4, 0.6, 0.8 and 1 ml of BSA stock solution taken for dilutions. Total protein was calculated by measuring the absorbance at 660 nm[31] through UV spectrophotometer.

Oil content:

Seed, cotyledon and endosperm powder was extracted with 250 ml n-hexane through soxhlet apparatus for 8 h on water bath. The excess solvent from the extract was evaporated through rotary evaporator at 40°, dried in vacuum and oil content was gravimetrically quantified[32].

Total starch:

0.5 g powdered material was homogenized with 5 ml of 80 % ethanol and centrifuged for 15 min at 2000 rpm. The centrifugate was separated and added 4 ml distilled water, heated for 15 min on water bath and macerated using a glass rod. 3 ml of 52 % perchloric acid was then added in the macerated sample, homogenized and centrifuged for 15 min at 2000 rpm. Thus, the supernatant obtained was made up to the volume with distilled water. In 0.1 ml of sample, mixed 0.1 ml of 80 % phenol, 5 ml of concentrated sulphuric acid, make up the volume up to 10 ml with distilled water and kept in an ice bath for 30 min. The dilutions of standard were prepared by taking 1, 2, 3, 4 and 5 ml of D-glucose stock solution of 0.1 mg/ml concentration. Total starch was calculated by measuring the absorbance at 490 nm[29] through UV spectrophotometer.

Total tannin:

1 g powdered material was extracted with 50 ml distilled water by boiling on the water bath for 6-8 h, filtered the extracts and volume was made up to 50 ml in volumetric flask. Then mixed 0.5 ml of FCR, 1 ml saturated sodium carbonate solution in 0.1 ml of sample and made the volume to 10 ml with distilled water. The stock solution of tannic acid at 0.1 mg/ml concentration was used as standard and different dilutions were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution. Total tannin was calculated through tannic acid standard curve with the help of absorbance using UV spectrophotometer at 760 nm[28].

Total phenolics:

Total phenolic content was estimated by extracting 50 g of powdered materials with 250 ml methanol. Extracts were filtered after 24 h, concentrated by rotary evaporator and dried in vacuum. Stock solutions of 1 mg/ml concentration were prepared by dissolving the extracts in methanol. 0.5 ml of stock solutions was taken in 25 ml volumetric flask, added 10 ml of distilled water, 1.5 ml of FCR and allowed to stand for 5 min. 4 ml of 20 % sodium carbonate solution was then added and made up the volume to 25 ml with distilled water. The prepared mixtures were kept in dark for 30 min. The stock solution of gallic acid of 0.1 mg/ml concentration was prepared as standard. Total phenolic content was calculated by using gallic acid standard curve with the measurement of absorbance at 765 nm through UV spectrophotometer. The equation: $y=115.9x+0.113$, correlation coefficient (r^2)=0.999 was used according to calibration curve (y=Absorbance, x=Gallic Acid Equivalent (GAE))[33].

Total flavonoids:

Total flavonoid content was estimated by extracting 50 g of powdered materials with 250 ml methanol. The stock solutions of methanolic extracts at 1 mg/ml concentration were prepared. In 1 ml sample, added 1 ml of 2 % methanolic aluminium chloride solution, made the volume to 10 ml using methanol and allowed to stand the mixtures for 1 h. Similar process was repeated for preparation of quercetin stock solution of 0.1 mg/ml concentration as standard and prepared the dilutions using 0.2, 0.4, 0.6, 0.8 and 1 of stock solution. Total flavonoid content was calculated through quercetin standard curve with the help of absorbance recorded at 420 nm using UV spectrophotometer. The equation $74.61x+0.058$, r^2=0.998 was used for the calculation of

total flavonoid content (y=Absorbance, x=Quercetin Equivalent (QE) in mg/ml)[34].

Antioxidant activity:

The samples were prepared by extracting the materials in methanol. The stock solutions of methanolic extract of samples and 0.135 mM methanolic solution of DPPH were prepared in 1 mg/ml concentration. Different dilutions were prepared by taking 0.02, 0.04, 0.06, 0.08 and 0.1 ml methanolic stock solution of samples, added 1 ml DPPH solution and made the volume up to 2 ml with methanol. The mixtures were allowed to stand for 30 min in the dark. The similar process was repeated to prepare the dilutions of BHA of 1 mg/ml concentration as antioxidant standard. The absorbance was measured at 517 nm through UV spectrophotometer[35].

Statistical analysis:

The statistical analysis of data was carried out by performing the experiments in triplicate. The data was represented by mean±Standard Deviation (SD). The linear correlation coefficient was observed using MS Office Excel 2007. Regression equation was used for phenolics, flavonoids concentrations and Half-Maximal Inhibitory Concentration (IC_{50}) values in DPPH radical scavenging assay calculation. The p values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The organoleptic properties and solubility of seed, cotyledon and endosperm powders were determined for the quality and acceptability of powdered materials (Table 1 and Table 2). The qualitative study showed that phytochemicals were majorly found in alcohol and water extracts of the seed, cotyledon and endosperm (Table 3).

Quantitative estimation revealed that total sugar was 4.43 % in seed, 4.73 % in cotyledon and 0.56 % in endosperm. Cotyledon part was found to have 17.83 % carbohydrate which was higher than in the seed i.e. 9.66 % and in endosperm i.e. 3.16 %. Total protein was estimated up to 30.45 % in the cotyledon part which was significantly higher than in seed i.e. 1.23 % and in endosperm i.e. 0.99 %. The cotyledon was also rich in oil i.e. 11.78 % while seed had 7.16 % and endosperm part had only 0.18 %. Starch was estimated in similar ranges i.e. 2.86 % in the seed and endosperm part and 2.30 % in the cotyledon. Total tannin varied as 1 % in cotyledon, 0.7 % in seed and 0.46 % in the endosperm respectively (fig. 1).

TABLE 1: ORGANOLEPTIC PROPERTIES OF *A. nilotica* SEED AND THEIR PARTS

Sample	Color	Odour	Taste	Texture
Seed	Creamish brown	Characteristic	Neutral	Amorphous
Cotyledon	Cream	Characteristic	Nutty with slight bitterness	Amorphous
Endosperm	Brown	Characteristic	Nutty with slight bitterness	Amorphous

TABLE 2: SOLUBILITY OF *A. nilotica* SEED AND THEIR PARTS

Sample	Hexane	Chloroform	Acetone	Ethanol	Water	Boiling water
Seed	-	-	-	-	+	+
Cotyledon	-	-	-	-	+	+
Endosperm	-	-	-	-	+	+

Note: (+): Soluble and (-): Insoluble

TABLE 3: PHYTOCHEMICAL SCREENING OF *A. nilotica* SEED AND THEIR PARTS

Phytochemical groups	Seed					Cotyledon					Endosperm				
	H	Chl	Ac	Al	W	H	Chl	Ac	Al	W	H	Chl	Ac	Al	W
Carbohydrate	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
Protein	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
Alkaloid	-	+	+	+	+	-	+	-	+	+	-	-	-	+	-
Phenolic	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-
Flavonoid	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-
Tannin	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
Saponin	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+
Glycoside	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tri-terpenoid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sterols	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: H: n-hexane; Chl: Chloroform; Ac: Acetone; Al: Alcohol; W: Water; (+): Present and (-): Absent

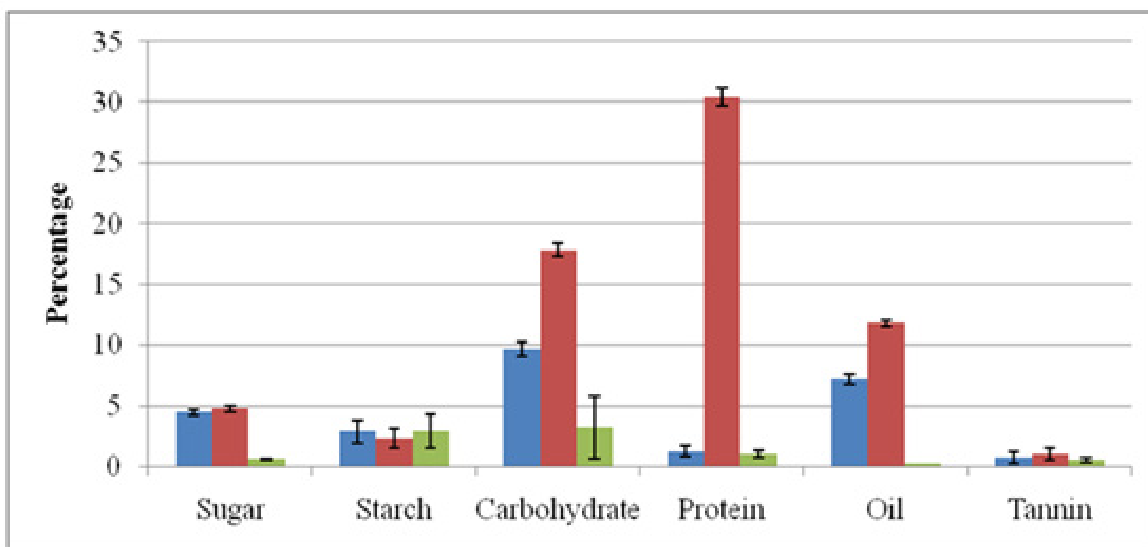

Fig. 1: Phytochemicals percentage in seed, cotyledon and endosperm
Note: Comparative phytochemicals (%) estimated in *A. nilotica* seed and their parts, ( ) Seed; ( ) Cotyledon and ( ) Endosperm

The quantification of phenolic and flavonoid content were determined through calibration curves of gallic acid and quercetin (fig. 2 and fig. 3). The phenolic content was 2.66 mg/g GAE in endosperm part which was significantly higher than 1.80 mg/g and 1.26 mg/g GAE in seed and cotyledon respectively. Endosperm part was also rich in flavonoid content with 7.66 mg/g QE which was noteworthy and more than in seed 4.0 mg/g QE and cotyledon 1.33 mg/g QE (Table 4).

The DPPH radical scavenging assay of *A. nilotica* seed, cotyledon and endosperm in comparison to standard BHA showed that percentage (%) inhibition of free radicals in BHA was 82.88 % and in seed, cotyledon and endosperm was found to be 49.02 %, 15.69 % and 74.07 % respectively (fig. 4). *A. nilotica* endosperm was comparable to standard and exhibit more potentiality for free radical reducing ability than the seed and cotyledon. The inhibitory concentration (IC_{50}) value and % inhibition for antioxidant activity are inversely proportional to each other; % inhibition increases and IC_{50} value decreases. % inhibition of BHA was higher with lower IC_{50} value, i.e. 10.61 µg/ml. Endosperm showed most significant antioxidant activity with IC_{50} value, i.e. 25.84 µg/ml, lower than that of seed and cotyledon, i.e. 55 and 191.10 µg/ml respectively as compared to BHA (Table 5). Thus, endosperm was identified as most potential inhibitor of DPPH radicals. Further, the graph (fig. 5) of reducing power capacity of seed, cotyledon and endosperm in methanol extracts as compared to standard BHA showed that as the concentration of the extracts increased, inhibition of DPPH radicals also increased.

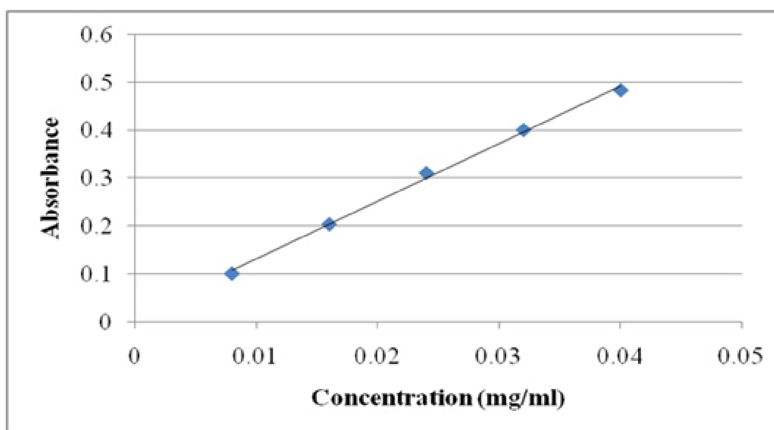

Fig. 2: Calibration curve of gallic acid
Note: Absorbance at different concentrations of gallic acid showed linear curve for which y=12.03x+0.011 and r^2=0.997 and ( ) Absorbance

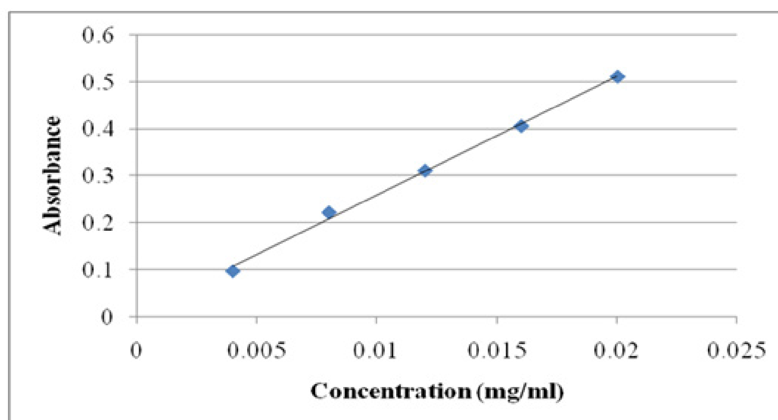

Fig. 3: Calibration curve of quercetin
Note: Absorbance at different concentrations of quercetin showed linear curve for which y=25.3x+0.006 and r^2=0.996 and ( ) Absorbance

TABLE 4: TOTAL PHENOLIC AND FLAVONOID CONTENT IN *A. nilotica* SEED AND THEIR PARTS

Sample	Total phenolic content [GAE (mg/g)]±SD (n=3)	Total flavonoid content [QE (mg/g)]±SD (n=3)
Seed	1.80±0.40	4.0±1.0
Cotyledon	1.26±0.11	1.33±0.57
Endosperm	2.66±0.18	7.66±1.52

Note: GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; ±SD: Standard Deviation in values, n=3. Values were performed in triplicates and taken as mean which are significant (p<0.05)

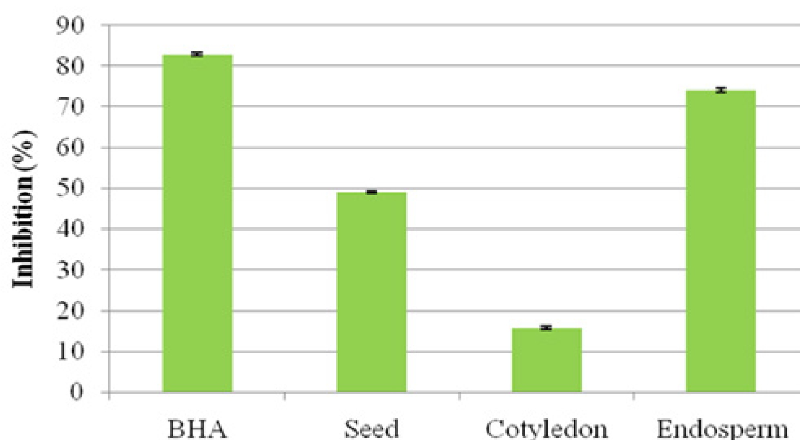

Fig. 4: DPPH radical scavenging activity of standard (BHA) and samples
Note: Data were taken in triplicate and means were calculated (mean±SD) i.e. significant (p<0.05); DPPH: 2,2-Diphenyl-1-Picryl-hydrazyl and BHA: Butylated Hydroxylanisole

TABLE 5: IC_{50} VALUES IN DPPH RADICAL SCAVENGING MODEL IN *A. nilotica* SEED AND THEIR PARTS

Sample	IC_{50} value (μg/ml)±SD (N=3)
BHA	10.61±0.63
Seed	55±0.78
Cotyledon	191.10±18.79
Endosperm	25.84±0.21

Note: Values were represented in triplicate (mean±SD). Mean values were statistically significant (p<0.05) and BHA: Butylated Hydroxylanisole

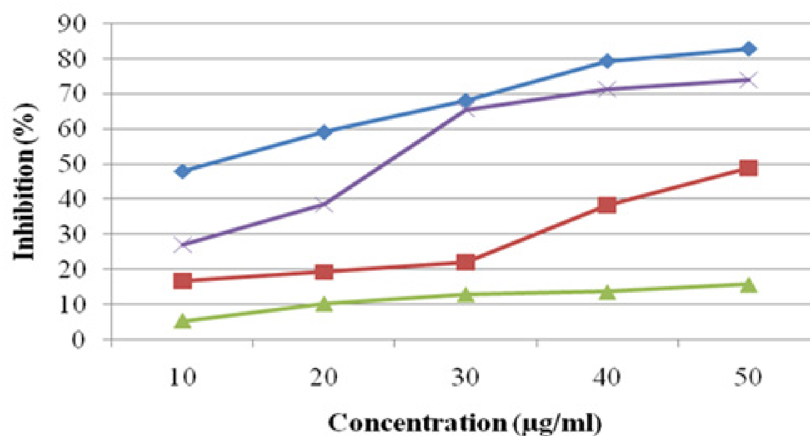

Fig. 5: % Inhibition of DPPH against different concentrations
Note: DPPH radical scavenging activity was increased with increase in concentration of BHA and samples, (♦) BHA; (■) Seed; (▲) Cotyledon and (x) Endosperm

The results revealed that the *A. nilotica* seed contained nutritionally and medicinally important phytochemicals. The part specific study of seed explored the utilization of seed parts for specific purpose. The cotyledon part was rich in sugar, carbohydrate, protein and oil for nutritional applications, while the endosperm part was useful for antioxidant property due to phenolics and flavonoids. The antioxidants reduce the level of Reactive Oxygen Species (ROS). ROS is a major cause of oxidative stress diseases such as pain, inflammation, tissue damage or injury, neurodegenerative disorder, cardiovascular problem and cancer. Thus, the study prospect for plant based better source materials to develop safe and eco-friendly healthcare industrial products.

Acknowledgements:

Authors are thankful to the Director, CSIR-National Botanical Research Institute, Lucknow, for providing facilities and support. Geetendra Kumar is also thankful to University Grant Commission for financial support.

Conflict of interests:

The authors declared no conflict of interest.

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