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ANTIOXIDANT EFFICIENCY OF VARIOUS EXTRACTS OF SEED COAT AND COTYLEDON OF *CAJANUS CAJAN* (L.) MILLSP.

H. Pratima [*1] and Pratima Mathad [2]

Department of Post-Graduate Studies and Research in Botany [1], Karnataka State Women's University, Vijayapur - 568101, Karnataka, India.
Department of Post-Graduate Studies and Research in Botany [2], Gulbarga University, Kalaburgi - 585106, Karnataka, India.

Keywords:

Antioxidant activity, Cotyledon, DPPH, FTC, Hydroxyl radical, Seed coat

Correspondence to Author:
Dr. H. Pratima

Guest Lecturer,
Department of Post-Graduate Studies and Research in Botany,
Akkamahadevi Women's University
Vijayapur - 568101, Karnataka, India.

E-mail: pratimakalsanki@gmail.com

ABSTRACT: *Cajanus cajan* L. (Pigeonpea) is an important pulse crop of India. The aim of this study was to determine and compare the antioxidant activity and bioactive compounds of seed coat and cotyledon of *C. cajan*. The antioxidant properties of pet ether, chloroform, ethanol and aqueous extracts of the seed coat and cotyledon of *C. cajan* were examined by a ferric thiocynate (FTC) assay, DPPH radical scavenging assay, hydroxyl radical scavenging assay and ferric reducing power were analyzed separately for its inhibition percentage at different concentration (20 µg, 40 µg, 60 µg, 80 µg and 100 µg/ml). Both the samples possessed antioxidant activity however the seed coat ethanolic extract possessed prominent activity by FTC (68.2 ± 0.07%), DPPH (90.1 ± 0.17%) and hydroxyl radical scavenging assay (75.2 ± 0.05%) at 100 µg/ml compared to other extracts. It was interesting to note that ferric reducing power of ethanolic extract of seed coat (1.590 ± 0.002%) was significantly ($P<0.5$) higher than that of standard BHA (1.575 ± 0.015%) at 100 µg/ml. The cotyledon extracts showed very minimal antioxidant activity compared to seed coat. Total contents of phenols (45.35±0.045 mg/g gallic acid equivalent), flavonoids (32.38 ± 0.021 mg/g catechin equivalent) and tannins (2.38 ± 0.021 mg/g tannic acid equivalent) were superior in ethanolic extract of seed coat compared to cotyledon. These experimental findings suggest that the ethanolic extract of seed coat as a potential extract for the natural antioxidant present in *C. cajan*.

INRODUCTION: Leguminous seeds are an important source of nutrient compounds such as protein, starch, dietary fibre and minerals [1], particularly in third-world countries. Incorporation of leguminous seeds into the human diet in developing countries offer protective effects against chronic diseases [2]. Legumes contain a number of bioactive substances including phenolics that can diminish protein digestibility and mineral bioavailability [3, 4].

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On the other hand, phenolic compounds such as flavonoids, phenolic acids, lignins and tannins have antioxidant properties and these are very important from nutritional and technological points of view.

In India, *C. cajan* seeds are commonly processed by dehulling or milling to improve its cooking and nutritional properties. The cotyledon also known as 'dhal' obtained after dehulling of these legumes is main reserve for protein and starch and is consumed in a diversity of forms. The by-products of dehulling such as seed coat has comparatively low value, because they are used as feed material in livestock production farms. The seed coat has the potential to be used as ingredients in the preparation of speciality products for human consumption [5], in manufacture of biscuits [6] and

also as an anti-microbial agent [7]. Generally the seed coats of legumes, which act as protective barriers for the cotyledon, possess highest concentration of phenolic compounds. Abundant phenolic compounds in the seed coats of legumes are believed to work synergistically to promote human health through a variety of different mechanisms, such as enhancing antioxidant activity, impacting cellular processes associated with apoptosis, platelet aggregation blood vessel dilation and enzyme activities associated with starch, protein and lipid digestion, carcinogen activation and detoxification [8].

Recently, researchers have explored and identified anti-oxidant and anti-hyperglycaemic activities in number of Indian food materials [9, 10, 11, 12]. The edible beans seed coat had highest total phenolics and antioxidant capacity assessed by the DPPH method than in cotyledons [13]. *C. cajan* leaves have been well explored for their anti-oxidant properties [14] and are reported to be the rich source of flavonoids, isoflavonoids and stilbenes [15]. *C. cajan* seed coat soluble crude protein fractions had potent antioxidant property [16]. So the aim of this study is to compare antioxidant potential from various extracts of seed coat and cotyledon of *C. cajan*.

MATERIALS AND METHODS:

Plant Collection and Extract Preparation: The seeds of *Cajanus cajan* var. Maruti (ICP-8863) were collected from Agriculture research station Kalaburgi, Karnataka, India. The variety Maruti ICP-8863 (PN-ABR-333) was authenticated by plant material description no. 44, International crops research institute for the semi-arid tropics, Patancheru, Andhra Pradesh, India [17]. Seeds were moistened for 1h and then dried in oven at 55 ºC overnight. The hull or seed coat was removed mechanically means by using hand grinder. The separated seed coat and cotyledon were made into fine powder using grinder. Five hundred grams of the powder was subjected to the Soxhlet successive extraction method (60-80 ºC) using 2.5 litter of pet ether (PE), chloroform (CHCl3), ethanol (Et-OH) and water (Aq) solvent for a period of 18 h. The extracts obtained were dried at 40 ºC.

Antioxidant Activity by Ferric Thiocynate (FTC) Method: The antioxidant activities of extracts were determined by ferric thiocynate method 18. The sample mixture contains 0.5 ml of extract (at various concentration 20, 40, 60, 80 and 100µg/ml), 2.5 ml of linoleic acid emulsion and 2 ml phosphate buffer (0.05M pH 7.0) in a test tube and incubated in darkness at 37 ºC. The amount of peroxide was determined by reading absorbance at 500 nm after colouring with $FeCl_2$ and thiocynate at intervals during incubation. BHA (butylated hydroxyl anisole) was used as standard antioxidant.

Free Radical Scavenging Activity by DPPH Method: The potential antioxidant activity of extract was determined on the basis of scavenging activity of the stable DPPH free radicals by the method of Blois (1958) [19]. 0.1 ml of sample at various concentrations was mixed with 2.9 ml of methanolic DPPH (60 mM) solution. The mixture was left in the dark for 30 min and absorbance was measured at 517 nm.

Hydroxyl Radical Scavenging Activity: The hydroxyl radical scavenging activity was determined according to the method of Klein *et al*., (1991) [20]. The extracts (100 mg) were dissolved in ethanol and taken in different test tubes than evaporated to dryness. The 1 ml of iron EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 ml of EDTA (0.018%) and 1 ml of DMSO (0.85%) in 0.1M phosphate buffer (pH 7.4) were added to these tubes and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. Test tubes were incubated at 80 - 90 ºC for 15 min on a water bath after the reaction was terminated by the addition of 1 ml of ice-cold trichloro acetic acid (17.5% w/v). 3 ml of Nash reagent was added to all of the tubes and left at room temperature for 15 min for colour development. The intensity of the yellow colour formed was measured spectrophoto-metrically at 412 nm against reagent blank. The percentage of hydroxyl radical scavenging activity was calculated by the following formula;

$$\%\ \text{hydroxyl radical} = \frac{1 - \text{Difference in absorbance of sample}}{\text{Difference scavenging activity in absorbance of blank}} \times 100$$

Determination of Ferric Reducing Power: The reducing power of the prepared extracts was determined using ferricyanide trichloroacetic acid method according to Oyaizu (1986) [21]. Each sample extract was mixed with 200 mM phosphate buffer (pH 6.6) and with 1% potassium ferricyanide in the

ratio of 1:1 (v/v) and the mixture was incubated at 50 ºC for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) was added, the mixture was centrifuged at 659 rpm for 10 min. The upper layer was mixed equally with deionised water and 1 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm. A higher absorbance indicates a higher reducing power. Mean values from the independent samples were calculated for each extract.

Determination of Total Phenols: The phenols content of crude extracts was estimated according to the Folin-Ciocalteau method [22]. The aliquots (0.1ml) of the extracts were taken in a test tube and their volumes adjusted up to 1 ml with distilled water. Then 0.5 ml of Folin-Coicalteau reagent was added. After 3 min of incubation, 2 ml of 20% Na_2CO_3 was added and placed the test tube in boiling water bath for 1 min. Cooled and measured the absorbance at 650 nm against the reagent blank. The content of total phenols was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions (covering the concentration range between 1 and 10 µg/ml) and expressed as gallic acid equivalent in mg/g of extract.

Determination of Total Flavonoids: The flavonoids content of crude extracts was estimated according to the method of Jia *et al*., (1999) [23]. The aliquots (0.1 ml) of the extracts were taken in a test tube and their volumes adjusted up to 1 ml with distilled water followed by addition of 75 µl of a 5% $NaNO_2$ solution. After 6 min 150 µl of a 10% $AlCl_3$ solution was added and allowed to stand for another 5 min before 0.5 ml of 1ml NaOH was added. The mixture was brought to 2.5 ml with distilled water and mixed well. The absorbance was measured immediately against the blank at 510 nm using a spectrophotometer. The content of total flavonoid was calculated as a catechin equivalent from the calibration curve of catechin standard solutions (covering the concen-tration range between 1 and 10 µg/ml) and expressed as catechin equivalent in mg/g of extract.

Determination of Total Tannins: The tannins content of crude extracts was estimated according to the Folin Denis method [24]. The aliquots (0.1 ml) of the extracts were taken in a flask and their volumes adjusted up to 1 ml with distilled water. The flask was heated gently and boiled for 30 min. Centrifuged at 2,000 rpm for 20 min. and collected the supernatant in 100 ml volumetric flask and made up the volume. 1 ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml of water. Added 5 ml of Folin-Denis reagent, 10 ml of sodium carbonate solution and diluted to 100 ml with distilled water and shaken well. The blue colour intensity was measured in a spectrophotometer, read the absorbance at 700 nm after 30 min. The content of total tannin was calculated as a tannic acid equivalent from the calibration curve of tannic acid standard solutions (covering the concentration range between 1 and 10 µg/ml) and expressed as tannic acid equivalent in mg/g of extract.

Statistical Analysis: The data of all measurements are means from three replications. Data and statistical significance of difference were evaluated with analysis of variance (ANOVA) using SPSS 10.0 package.

RESULTS:

Total Antioxidant Activity: The *in-vitro* total antioxidant assay of the crude extracts shows appreciable antioxidant potential compared with the standard BHA, as described with the FTC method. The data presented in **Table 1** shows the dose-response curve of antioxidant activity of seed coat and cotyledon extracts of *C. Cajan*. The antioxidant activity was superior at 100 µg/ml of ethanolic (68.2 ± 0.07%) and aqueous extract (65.4 ± 0.11%) of seed coat, whereas cotyledon extracts shows moderate antioxidant activity, among ethanolic extract (55.2 ± 0.14%) was superior compared to other extracts.

DPPH Radical Scavenging Activity: The DPPH (1, 1-diphenyl-2-picrylhydrazyl) method allows a direct investigation of the ability for the extract or antioxidant to donate hydrogen and/or electrons to quench the DPPH radical.

The percentage of scavenging effect of DPPH on different extracts of seed coat and cotyledon were found dose dependant inhibitory antioxidant potential and compared with standard BHA as shown in **Table 2**. The highest percentage of inhibition was observed at 100 µg/ml of pet ether,

chloroform, ethanol, aqueous extract of seed coat and cotyledon were found to be 35.36 ± 0.04%, 24.92 ± 0.04%, 90.1 ± 0.17%, 70.36 ± 0.14% and 30.58 ± 0.05%, 22.98 ± 0.02%, 43.70 ± 0.12%, 50.3 ± 0.07% respectively. The percentage of DPPH scavenging activity of seed coat extracts of ethanol (90.1 ± 0.17%) was most nearer to standard BHA (95.8 ± 0.05%) at 100 µg/ml. The cotyledon extracts shows moderate antioxidant activity, among aqueous extract (50.3 ± 0.07%) show high activity and chloroform extract (22.98 ± 0.02%) show lower activity at 100 µg/ml.

TABLE 1: ANTIOXIDANT ACTIVITY OF *C. CAJAN* SEED COAT AND COTYLEDON BY FTC METHOD

Plant parts	Extracts	% of inhibition				
		20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
Seed coat	Pet ether	15.1 ± 0.05	17.2 ± 0.02	20.6 ± 0.04	28.3 ± 0.09	33.5 ± 0.02
	Chloroform	12.3 ± 0.03	15.6 ± 0.01	18.2 ± 0.08	24.5 ± 0.04	28.6 ± 0.05
	Ethanol	25.4 ± 0.12	33.1 ± 0.05	49.8 ± 0.02	60.0 ± 0.06	68.2 ± 0.07
	Aqueous	19.3 ± 0.04	20.4 ± 0.02	27.5 ± 0.05	50.4 ± 0.03	65.4 ± 0.11
Cotyledon	Pet ether	8.3 ± 0.05	9.4 ± 0.03	15.3 ± 0.04	18.4 ± 0.01	16.6 ± 0.06
	Chloroform	6.8 ± 0.04	8.5 ± 0.04	10.3 ± 0.02	12.5 ± 0.05	12.8 ± 0.03
	Ethanol	15.2 ± 0.02	16.3 ± 0.12	20.4 ± 0.05	34.5 ± 0.02	55.2 ± 0.14
	Aqueous	14.2 ± 0.03	18.4 ± 0.05	19.4 ± 0.02	22.6 ± 0.08	23.2 ± 0.12
Standard	BHA	66.65 ± 0.11	68.28 ± 0.02	72.44 ± 0.04	73.5 ± 0.06	80.06 ± 0.05

Each value is expressed as mean ± S.D. (n=3) and statistically significant at P<0.5

TABLE 2: DPPH SCAVENGING ACTIVITY OF *C. CAJAN* SEEDCOAT AND COTYLEDON

Plant parts	Extracts	% of inhibition				
		20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
Seed coat	Pet ether	7.42 ± 0.03	10.68 ± 0.08	24.36 ± 0.12	30.28 ± 0.05	35.36 ± 0.04
	Chloroform	5.36 ± 0.05	8.98 ± 0.04	15.45 ± 0.07	18.80 ± 0.03	24.92 ± 0.04
	Ethanol	10.36 ± 0.02	25.23 ± 0.08	50.45 ± 0.03	75.6 ± 0.02	90.1 ± 0.17
	Aqueous	8.34 ± 0.12	10.15 ± 0.05	23.18 ± 0.02	51.23 ± 0.05	70.36 ± 0.14
Cotyledon	Pet ether	12.15 ± 0.10	13.22 ± 0.04	19.36 ± 0.06	23.42 ± 0.08	30.58 ± 0.05
	Chloroform	10.11 ± 0.05	15.25 ± 0.08	18.42 ± 0.07	20.56 ± 0.12	22.98 ± 0.02
	Ethanol	18.21 ± 0.04	22.28 ± 0.13	24.55 ± 0.10	38.63 ± 0.08	43.70 ± 0.12
	Aqueous	20.38 ± 0.02	24.81 ± 0.12	35.36 ± 0.15	41.21 ± 0.03	50.3 ± 0.07
Standard	BHA	52.73 ± 0.04	68.91 ± 0.14	87.01 ± 0.06	90.2 ± 0.02	95.8 ± 0.05

Each value is expressed as mean ± S.D. (n=3) and statistically significant at P<0.5

Hydroxyl Radical Scavenging Activity: In this study, different concentrations (20 µg, 40 µg, 60 µg, 80 µg and 100 µg/ml) of pet. ether, chloroform, ethanol and aqueous extract of seed coat and cotyledon had followed strong hydroxyl radical scavenging activity in dose dependent manner. The ability of the above mentioned extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to be a good scavenger of active oxygen species, thus reducing the rate of the chain reaction. The highest percentage of inhibition was observed at 100 µg/ml of pet. ether, chloroform, ethanol and aqueous extract of seed coat and cotyledon were found to be 44.64 ± 0.03%, 41.24 ± 0.05%, 75.2 ± 0.05%, 36.14 ± 0.04% and 20.8 ± 0.02%, 12.8 ± 0.05%, 52.6 ± 0.07%, 23.8 ± 0.05% respectively **Table 3**.

This result indicated that seed coat ethanol extract (75.2 ± 0.05%) had strongly significant (P<0.05) power than other extracts and correlated with standard BHA (80.3 ± 0.07 %) at 100 µg/ml, whereas lower activity observed in aqueous extract (36.14 ± 0.04%). All the cotyledon extract shows moderate antioxidant activity, among ethanolic extract (52.6 ± 0.07%) was superior compared to other extract at 100 µg/ml.

Ferric Reducing Power: The reducing power is to measure the reductive ability of antioxidant and is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of the sample extract. The results of ferric reducing power from pet. ether, chloroform, ethanol and aqueous extracts of seed coat and cotyledon of *C. cajan* are shown in **Table 4**. The reducing power of seed coat ethanolic extract (1.590 ± 0.002%) was significantly (P<0.5) higher ability than standard BHA (1.575 ± 0.015%), however the aqueous (0.775 ± 0.014%), pet. ether (0.418 ± 0.032%) and chloroform (0.250 ± 0.041%) extracts showed lower reducing ability than BHA

at 100 µg/ml. All the cotyledon extracts showed less reducing power than seed coat, among ethanol extract (1.248 ± 0.023%) was higher than the other extracts at 100 µg/ml.

TABLE 3: HYDROXYL RADICAL SCAVENGING ACTIVITY OF *C. CAJAN* SEED COAT AND COTYLEDON

Plant parts	Extracts	% of inhibition				
		20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
Seed coat	Pet ether	15.26 ± 0.09	24.94 ± 0.04	33.30 ± 0.08	36.29 ± 0.07	44.64 ± 0.03
	Chloroform	13.50 ± 0.03	21.41 ± 0.12	27.80 ± 0.04	30.32 ± 0.02	41.24 ± 0.05
	Ethanol	37.60 ± 0.02	39.16 ± 0.08	52.13 ± 0.11	66.58 ± 0.07	75.2 ± 0.05
	Aqueous	10.30 ± 0.04	16.92 ± 0.12	21.95 ± 0.05	23.59 ± 0.16	36.14 ± 0.04
Cotyledon	Pet ether	11.8 ± 0.05	15.3 ± 0.05	17.6 ± 0.04	18.5 ± 0.08	20.8 ± 0.02
	Chloroform	8.5 ± 0.04	9.4 ± 0.08	10.8 ± 0.03	12.0 ± 0.04	12.8 ± 0.05
	Ethanol	12.6 ± 0.02	15.2 ± 0.04	28.3 ± 0.05	44.3 ± 0.02	52.6 ± 0.07
	Aqueous	10.20 ± 0.01	11.3 ± 0.05	17.3 ± 0.06	20.4 ± 0.08	23.8 ± 0.05
Standard	BHA	40.3 ± 0.07	52.7 ± 0.12	61.3 ± 0.04	72.4 ± 0.05	80.3 ± 0.07

Each value is expressed as mean ± S.D. (n=3) and statistically significant at P<0.5

TABLE 4: REDUCING POWER OF *C. CAJAN* SEED COAT AND COTYLEDON

Plant parts	Extracts	% of inhibition				
		20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
Seed coat	Pet ether	0.134 ± 0.005	0.245 ± 0.021	0.268 ± 0.005	0.290 ± 0.013	0.418 ± 0.032
	Chloroform	0.110 ± 0.002	0.134 ± 0.009	0.190 ± 0.032	0.200 ± 0.012	0.250 ± 0.041
	Ethanol	0.210 ± 0.003	0.445 ± 0.007	0.610 ± 0.043	1.152 ± 0.054	1.590 ± 0.002
	Aqueous	0.120 ± 0.008	0.221 ± 0.013	0.480 ± 0.045	0.511 ± 0.012	0.775 ± 0.014
Cotyledon	Pet ether	0.121 ± 0.005	0.188 ± 0.019	0.220 ± 0.005	0.228 ± 0.014	0.432 ± 0.021
	Chloroform	0.111 ± 0.003	0.145 ± 0.005	0.190 ± 0.012	0.212 ± 0.012	0.320 ± 0.034
	Ethanol	0.340 ± 0.012	0.391 ± 0.004	0.532 ± 0.013	0.745 ± 0.009	1.248 ± 0.023
	Aqueous	0.200 ± 0.018	0.283 ± 0.023	0.545 ± 0.015	0.601 ± 0.008	0.812 ± 0.012
Standard	BHA	0.510 ± 0.023	0.732 ± 0.026	1.110 ± 0.016	1.418 ± 0.043	1.575 ± 0.015

Each value is expressed as mean ± S.D. (n=3) and statistically significant at P<0.5

Total Contents of Phenols, Flavonoids and Tannins in Crude Extracts: The total phenol, flavonoids and tannins of seed coat extracts were 8.09 ± 0.012 to 45.35 ± 0.045 mg/g gallic acid equivalent, 0.134 ± 0.006 to 32.38 ± 0.021 mg/g catechin equivalent and 0.156 ± 0.005 to 2.38 ± 0.021 mg/g tannic acid equivalent could be ranked from high to low *i.e.,* ethanol > aqueous > chloroform > pet. ether extracts. Similarly total phenols, flavonoids and tannins of cotyledon extracts were 0.011 ± 0.021 to 0.030 ± 0.008 mg/g gallic acid equivalent, 0.018 ± 0.003 to 1.030 ± 0.002 mg/g catechin equivalent and 0.18 ± 0.003 to 1.043 ± 0.002 mg/g tannic acid equivalent could be ranked from high to low *i.e.,* ethanol > aqueous > chloroform > pet. ether extracts **Table 5**.

TABLE 5: TOTAL CONTENTS OF PHENOLS, FLAVONOIDS AND TANNINS IN VARIOUS EXTRACTS OF SEED COAT AND COTYLEDON OF *C. CAJAN*

Plant parts	Extracts	Total Phenols (mg/g gallic acid equivalent)	Total flavonoids (mg/g catechin equivalent)	Total tannins (mg/g tannic acid equivalent)
		0.134 ± 0.005	0.245 ± 0.021	0.268 ± 0.005
	Chloroform	0.110 ± 0.002	0.134 ± 0.009	0.190 ± 0.032
	Ethanol	0.210 ± 0.003	0.445 ± 0.007	0.610 ± 0.043
	Aqueous	0.120 ± 0.008	0.221 ± 0.013	0.480 ± 0.045
Cotyledon	Pet ether	0.121 ± 0.005	0.188 ± 0.019	0.220 ± 0.005
	Chloroform	0.111 ± 0.003	0.145 ± 0.005	0.190 ± 0.012
	Ethanol	0.340 ± 0.012	0.391 ± 0.004	0.532 ± 0.013
	Aqueous	0.200 ± 0.018	0.283 ± 0.023	0.545 ± 0.015
Standard	BHA	0.510 ± 0.023	0.732 ± 0.026	1.110 ± 0.016

Each value is expressed as mean ± S.D. (n=3) and statistically significant at P<0.5

The results suggest that the ethanolic extract of seed coat had higher level of phenolic, flavonoid and tannin contents as compared to other extracts. Whereas cotyledon extract had very less amount of phenols, flavonoids and tannins content.

DISCUSSION: Antioxidants have the ability to quench free radicals before they attack cells, maintain the initial cellular and systemic health. In the present study ethanolic extract of seed coat and cotyledon of *C. cajan* which effectively scavenged DPPH free radical, hydroxyl radical and possess more reducing power**.** A result shows that seed coat extract had maximum antioxidant activity along with maximum phenolic, flavonoid and tannin contents compared to cotyledon extracts. This maximum antioxidant activity was positively correlated with maximum total phenolic contents observed in seed coat [25].

Total antioxidant assay using FTC, which indicates the ability of the phytomedicines to minimize oxidative damage to vital organs and tissues *in-vivo.* The results of total antioxidant activity by FTC method revealed that ethanolic extract of seed coat was higher than the other extract. Similarly, the researcher reported that ethanolic extract of *C. cajan* seed had highest antioxidant activity compared to leaf and root [26]. Chiung-Tsun Kuo *et al.,* [27] have observed that ethanolic-water extract of Chinese olive (*Canarium album* L.) fruit exhibited strong activity indicating that polyphenols or flavonoids may play an important role in the antioxidant activities. The result of the DPPH scavenging activity in this study indicates that the extracts were potently active. These results revealed that ethanolic extract (90.1 ± 0.17%) of seed coat of *C. cajan* shows admirable DPPH scavenging activity compared to standard BHA (95.8 ± 0.05%). Similarly, the researchers have reported the ethanolic extract of the aerial part (flower) of *Bougainvillea xbuttiana* showed promising antioxidant activity [28].

The aqueous ethanol seed coat extract of *Vigna mungo* shows better antioxidant activity than methanol extracts of seed coat [29]. The extract has also been shown to possess free-radical scavenging activity, which can be attributed to the phenolic and flavonoid contents of the plant [30, 31]. The results of the hydroxyl radical scavenging activity of ethanol extract of seed coat (75.2 ± 0.05%) were not remarkably different then reference compound BHA (80.3 ± 0.07%) at 100µg/ml. The scavenging ability of the seed coat ethanolic extract might be due to the active hydrogen donor ability of hydroxyl substitution. Hydrogen peroxide can be toxic to cells when active. Therefore, removing H_2O_2 and $O_2^{\cdot-}$ is very important for antioxidant defense in cell or food systems [32].

Results obtained in the present study revealed that ferric reducing power of ethanolic extract of seed coat have higher ability than standard BHA at 100 µg/ml to reduce Fe^{3+} to Fe^{2+}. This may be attributed from hydrogen donation from phenolic compounds related to the presence of reduced agent. Previous reports have shown a direct correlation between antioxidant activity and reducing power of pea seed coats [33].

Total phenolic, flavonoid and tannin contents were measured from the respective extracts and correlated with their antioxidant values. The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties. Researchers have studied tannin and polyphenolic constituents having a potential to medicinal or nutraceutical properties including antioxidant activities [34]. Therefore, the study of the importance and role of nonnutrient compounds, particularly phenolic acids, flavonoids and high molecular tannin as natural antioxidants has greatly increased [35].

In our study it was revealed that the seed coat ethanolic extracts are very promising sources of phenolic contents 45.35 ± 0.045 mg of gallic acid equivalent, flavonoid contents 32.38 ± 0.021 mg of catechin equivalent and tannin content 2.38 ± 0.021 mg/g of tannic acid equivalent compared to cotyledon than those reported by Savita Rani *et al.*, in methanolic extract of seed coat (phenols of 23.25 ± 4.8 mgGAE/g and flavonoids of 20.9 ± .8mg CAE/g) compared to dehusked dal and boiled dal of *C. cajan* [25].

CONCLUSION: The present study revealed that all extracts vary in their ability to scavenge anti-radicals, among ethanolic extract of seed coat being the most potent and cotyledon the least potent when compared with reference compound BHA. The results obtained in this study clearly indicate that the ethanolic extract of seed coat of *C. cajan* may be used as a new potential source of natural nutritional supplement in food or pharmaceutical industries due to rich source of phenolic, flavonoid and tannin contents as well as antioxidant property. Our research findings thus pave way to molecular

pharmaceutics to search for compounds and their mode of action at molecular level.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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