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MODULATION OF PHENOLIC CONTENTS OF SOME EDIBLE SEEDS ASA SOURCE OF NATURAL ANTIOXIDANTS IN FUNCTIONAL FOODS VIA DARK GERMINATION

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ABSTRACT

Germination is among common effective methods to enhance the value and quality of legumes. Essential components; e.g. antioxidants, Vitamins, and others might be considered useful and affect the course of germination. Antioxidants activity of many components may be important for protection, and/or prevent rancidity or other flavor deterioration in foods. In this study, germination then extraction of some edible seeds and their sprouts could be employed as a source for natural phenolic compounds potential anti-oxidant activity. The selected edible seeds included lettuce, chickpea, linseed, lentil, dry green pea, lupine, black-eyed pea, radish, fenugreek, fava bean, and turnip. Folin Ciocalteau, $AlCl_3$m and ABTS reagents were used to examine the total phenolic contents, total flavonoids, and antioxidant activity respectively. The results revealed that dark germination under dark condition enhances the antioxidant activity. In spite of the enhancement of the total phenolic contents during germination, the flavonoid content was significantly decreased. There was a significant positive correlation (R= 0.5912) between the polyphenols content of sprouts and anti-oxidant activity. This preliminarily study indicated that either edible seeds sprouts or their extracts might be used as a source of natural antioxidants in functional foods or in the formulation of supplements or medicine in the different pharmaceutical dosage forms.

KEYWORDS

Dark germination, Total phenolic content, Anti-oxidant activity and Sprouts.

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INTRODUCTION

The liver research laboratory (FAB-Lab, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt) presented several approaches for a better utilization of natural products as potential therapeutic agents; Anti-herpes ((Badria *et al*, 2003)[1], immunomodulatory (Mikhaeil *et al*, 2003)[2], schistosomicidal drug (Badria *et al*, 2001)[3],

antimutagens (Badria, 1994)[4], colon cancer therapy (Ibrahim *et al*, 2014)[5].

However, there are number of examples which deal with enzymes as drug targets involved in the designing of enzyme inhibitors from commonly available natural products, such as; potential cataract therapy with differential inhibitory activity on aldose reductase (Elimam *et al*, 2017)[6], tyrosinase inhibitors for hyper pigmentation (Badria, 2001)[7].

Later, modulation of different biological activities via semi-synthesis of commonly available natural products was extensively studied by Badria's group including the followings; potent topoisomerase inhibitors (Abdel Bar *et al*, 2009)[8], LTA4H inhibitory as potential colorectal cancer therapy (El-Naggar *et al*, 2017)[9], breast cancer inhibitors (Abdel Bar *et al*, 2010)[10], chemo-sensitization of cisplatin resistant ovarian cancer by cucurbitacin B (El-Senduny *et al*, 2016)[11], acetyl cholinesterase inhibitors as a selective anti-Alzheimer agent (Abdel Bar *et al*, 2019)[12].

In the last decades, considerable progress has been made concerning the production of secondary metabolites and/or bio-active compounds by using plant tissue culture techniques owing to the advantages of this platform over other production systems (Goncalves and Romano, 2018)[13].

Legumes, one of the most important sources of food having many health benefits and play a vital role in human nutrition in many countries (Prodanov *et al*, 1997)[14]. There are many biotechnological processes such as germination which are considered both simple and economical to enhance the nutritive value of legumes (Fernandez-Orozco *et al*, 2009)[15]. Extensive breakdown of seed-storage compounds and synthesis of structural proteins and other cell components take place during the germination (Kuo *et al*, 2004)[16].

It is known that the germination process generally improves the nutritional value of legumes, not only by reducing the antinutritive compounds, but also by increasing the levels of free amino acids, available carbohydrates, dietary fiber, and other components, and increasing the functionality of the seeds due to the subsequent increase in the bioactive compounds (Kuo *et al*, 2004)[16]. One of these bioactive compounds are polyphenols which are quite suitable for protecting cell membranes against the damage induced by reactive free radicals and are able to reduce the LDL aggregation (Fernandez-Orozco *et al*, 2006)[17]. Phenolic compounds not only effectively prevent the oxidation in foods they also act as protective factors against oxidative damage in the human body. Epidemiological studies show that the consumption of food with high phenolic content is correlated with reduced cardiovascular, inflammation, cancer mortality and some other disease rates (Ardekani *et al*, 2011)[18].

The aim of the present study is the evaluation of the germination process influence on the phenolic content and antioxidant capacity of some edible seeds in order to obtain suitable flour or extract with high nutritive value and antioxidant activity as an ingredient in supplements or medicine formulation.

MATERIAL AND METHODS

Chemicals

Azino-bis-(3-ethyl benzthiazoline-6-sulfonic acid) (ABTS), gallic acid, aluminium chloride, quercetin (Sigma Chemicals, St. Louis, USA), manganese dioxide (MnO_2) (DBL chemicals, Germany), ascorbic acid (Cevarol®) tablets (Memphis Pharmaceutical Co., Cairo, Egypt), Folin-Ciocalteau reagent (Sigma, USA), and sodium carbonate (El-Nasr, Egypt).

Plant material and germination conditions

Seeds presented in Table No.1 were prepared for sprouting. Seeds were rinsed in distilled water and immersed in 5g/L sodium hypochlorite under aeration for 24 h. After pouring off the soaking water, the seeds were spread evenly on trays lined with cotton and irrigated everyday with dist. water with 5g/L sodium hypochlorite. Sprouts were covered with perforated aluminum foil for increasing stem elongation at room temperature in the range of 28±2. Sprouts were collected after 3 days of growth for analysis (Baenas, *et al*, 2014)[19].

Extraction

The ground seeds and sprouts were extracted by shaking with methanol overnight. Extractions were carried out three times and the organic solvents were removed at 50°C using a rotary vacuum evaporator.

Determination of total phenolic content (TPC)

This method was carried out according to (Tega *et al*, 1984). Briefly, one milligram of the extract was dissolved in 1ml of MeOH/ H_2O (6: 4) containing 0.3 % HCl. To 100µl of the extract and 100µl Folin-Ciocalteau regent (10 % v/v), 2ml sodium carbonate (2% w/v) were added and mixed completely. After 30minutes, the absorbance of the solution was measured at 750nm. Quantitation was based on the standard curve of gallic acid (0-50µg/ml), dissolved in methanol/water (6:4) containing 0.3 % HCl. Phenolic content was expressed as µg/ mg extract of gallic acid equivalent (GAE).

Determination of total flavonoid content (TFC)

The flavonoid content was estimated by the $AlCl_3$ method (Lamaison and Carnat, 1990)[20]. Briefly, 1ml of methanolic extract solution (10mg/ml) was added to 1ml of 2% methanolic AlCl3, 6H2O. The absorbance was measured 10 min later at 450nm. The results were expressed in µg quercetin/10mg extract by comparison with standard quercetin treated in the same conditions.

ABTS Antioxidant Assay

Anti-oxidant activity was estimated as described by (Lissi *et al*, 1999)[21]. Briefly, the reaction mixture consisted of 2 ml of ABTS solution (60 µM) and 3 ml of MnO_2 solution (25 mg/ml), all prepared in phosphate buffer (*p*H 7, 0.1M). The mixture was shaken, centrifuged, and decanted. The absorbance ($A_{control}$) of the resulting green-blue solution ($ABTS^+$ radical solution) was recorded at λ_{max} 750 nm. The absorbance (A_{test}) was measured upon the addition of 20µl of 1mg/ml solution of the test sample in spectroscopic grade MeOH/buffer (1:1 v/v) to the ABTS solution. The decrease in absorbance is expressed as % inhibition which is calculated from the equation:

$$\% \text{ inhibition} = \frac{A_{control} - A_{test}}{A_{control}} X\ 100$$

Ascorbic acid 100µl (2mM) solution was used as standard antioxidant (positive control). Blank sample was run using solvent without ABTS.

RESULTS AND DISCUSSION

The aim of this study was to investigate the effect of dark germination on the phenolic acids and flavonoids content, as well as antioxidant activity, in the seeds and sprouts of the selected edible seeds.

Total phenolic contents

Cereals and vegetables (including seed and sprouts) are a good source of phenolic compounds. Germination resulted in significant changes in the phenolic composition, due to activation of endogenous enzymes and the complex biochemical metabolism of seeds during this process (Duenas *et al*, 2009)[22].

Figure No.3 showed total phenolic content (expressed as mg GAE/mg extract) in the analyzed seeds and sprouts. Germination increased the total phenolic content of most seeds in the following order Lettuce >Chickpea >Linseed >Lentil > Dry green Pea > Lupine > Black-eyed pea> Radish>Fenugreek>Fava bean >Turnip, as shown in Table No.2.

(Pasko *et al*, 2008)[23] also reported higher total phenolic content in sprouts compared to seeds, suggesting that synthesis of phenolic antioxidants during germination may occur. It is thought that seeds mainly act as a reservoir for the development of the sprouts (Perez-Balibrea *et al*, 2011)[24].

Total flavonoid content

The total flavonoid content in seeds and sprouts determined as quercetin equivalents. Dark germination decreased the total flavonoids content of most seeds and they are arranged in the following order: Alfalfa > Chickpea > Black-eyed pea >Eruca sativa> Radish > Fenugreek, as shown in Table No.3. These results are in agreement with those published by (Kubasek *et al*, 1992)[25] who reported that the levels of flavonoid genes were very low in seedlings grown in darkness.

Antioxidant activity

An increase in the total phenolic content a long with the seeds` germination may influence their free radical scavenging activity. The methanolic extracts of the seeds and sprouts were analyzed in respect to their antioxidant activity against ABTS. The results are presented in Table No.4. The antioxidant activity of the different extracts can be correlated to their total polyphenol concentration (Yi *et al*, 2006)[26]. Antioxidant activity of seeds was generally increased during germination. The sprouts of Radish, Eruca sativa, Linseed, Turnip and Lettuce demonstrated the highest antioxidant activity, evaluated using the ABTS method (Figure No.4). Alfalfa and Fenugreekseeds exhibited higher antioxidant activity than their sprouts.

There were high and significant linear correlations between total polyphenols content of the seeds and antioxidant activity evaluated using ABTS (Figure No.5A) (R =0.8008). This is a strong positive correlation and these results suggested that phenolic compounds are good predictors of *in vitro* antioxidant activity ((Pasko *et al*, 2008)[23]. There was a weaker but still statistically significant (R= 0.5912) correlation between total phenolic content of the sprout and antioxidant activity (Figure No.5B).

These results may be attributed to the nature of antioxidant compound in the sprout which differs from the compound present in the seed that may be non-phenolic (Antioxidant activity of methanolic extracts, increase by the time in case of sprouts while remains constant in seeds).

Lower, but also statistically significant correlations between total flavonoid content and anti-oxidant activity of the seeds and sprouts (R =0.4838, 0.5784, respectively), (Figure No.6A, 6B).

Table No.1: Selected seeds for germination

S.No	Name of plant	Genus, species	Family
1	Alfalfa seed	Medicago sativa	Fabaceae
2	Chickpea	*Cicer arietinum*	Fabaceae
3	Cowpea(Black-eyed pea)	*Vigna unguiculata*	Fabaceae
4	Dry green peas	*Pisum sativum*	Fabaceae
5	Eruca sativa seed	*Eruca sativa*	Brassicaceae
6	Fava beans	*Vicia faba*	Fabaceae
7	Fenugreek	*Trigonella foenum-graecum*	Fabaceae
8	*Lettuce*	*Lactuca sativa*	Asteraceae
9	Linseed	*Linum usitatissimum*	Linaceae
10	Lupines	*Lupinus termis*	Fabaceae
11	Radish seed	*Raphanus sativus*	Brassicaceae
12	Turnip	*Brassica rapa* L	Brassicaceae
13	Yellow lentils	*Lens culinaris*	Fabaceae

Table No.2: Total phenolic content (µg/1mg extract) of selected seed and sprouts

S.No	Name of plants	Sprout	Seed	% Increase of total phenolic content
1	Turnip	29.17	23.44	24.46
2	Radish	28.73	20.79	38.18
3	Chickpea	8.29	4.02	105.83
4	Lupine	30.54	20.94	45.88
5	Eruca sativa	20.05	28.88	-30.54
6	Fenugreek	9.91	7.26	36.43
7	Fava bean	20.35	16.23	25.36
8	Linseed	9.76	5.94	64.35
9	Lentil	8.09	5.35	51.28
10	Dry green Pea	10.64	7.21	47.55
11	Black-eyed pea	8.88	6.13	44.72
12	Alfalfa	17.77	37.26	-52.28
13	Lettuce	43.44	18.58	133.70

Table No.3: Total flavonoids (µg/10mg extract) of selected seed and sprouts

S.No	Name of plant	Sprout	Seed	% Decrease in total flavonoid content
1	Turnip	43.93	44.76	1.86
2	Radish	37.94	42.18	10.05
3	Chickpea	35.97	44.53	19.22
4	Lupine	44.01	45.14	2.51
5	Eruca sativa	53.70	59.84	10.25
6	Fenugreek	42.18	46.20	8.68
7	Fava bean	38.85	40.82	4.82
8	Linseed	48.62	40.67	-19.55
9	Lentil	39.31	38.55	-1.96
10	Dry green Pea	37.54	36.05	-4.13
11	Black-eyed pea	36.02	41.35	12.88
12	Alfalfa	37.31	91.78	59.34
13	Lettuce	61.03	60.95	-0.12

Table No.4: Antioxidant activity of selected seed and sprout using ABTS assay

S.No	Name of plant	Sprout	Seed	% increase
1	Turnip	92.31	86.92	6.19
2	Radish	96.15	91.54	5.04
3	Chickpea	33.84	15.38	119.99
4	Lupine	53.84	14.61	268.43
5	Eruca sativa	97.69	90.00	8.54
6	Fenugreek	25.38	36.92	-31.25
7	Fava bean	55.38	57.69	-4.00
8	Linseed	93.39	19.81	371.42
9	Lentil	45.28	7.547	500.00
10	Dry green Pea	27.35	20.75	31.81
11	Black-eyed pea	47.16	15.09	212.50
12	Alfalfa	71.69	86.79	-17.39
13	Lettuce	88.67	29.24	203.22

Figure No.1: Photograph showing seeds immersion in 5g/L sodium hypochlorite under aeration for 24 h

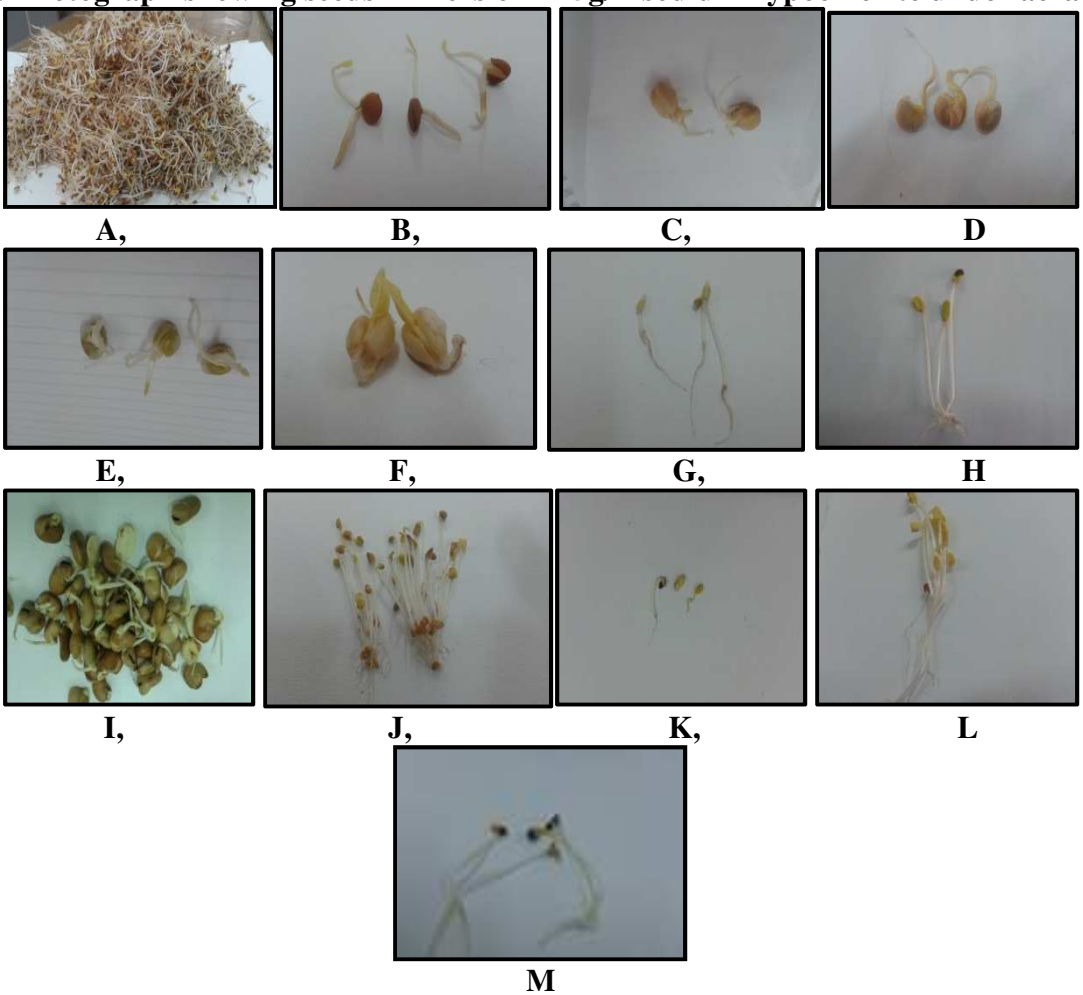

Figure No.2: a) Alfalfa sprout, b) Lentils sprout, c) Chickpea sprout, d) Lupines sprout, e) Dry green peas sprout, f) Black-eyed pea sprout, g) Lettuce sprout, h) Linseed sprout, i) *Fava bean*s sprout, j) *Eruca sativa* sprout, k) Turnip sprout, l) Radish sprout, m) Fenugreek sprout after 3 days of growth

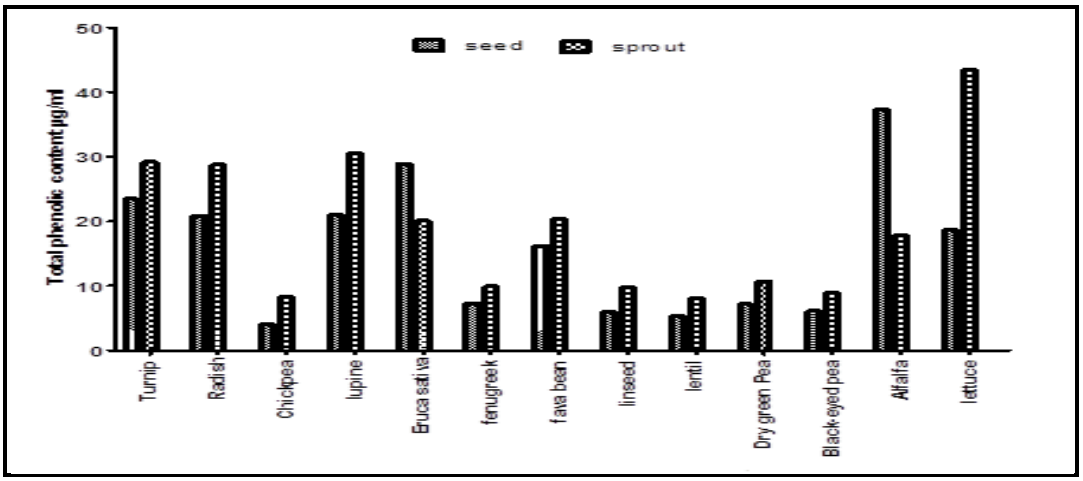

Figure No.3: Total Phenolic Content in seeds and sprouts

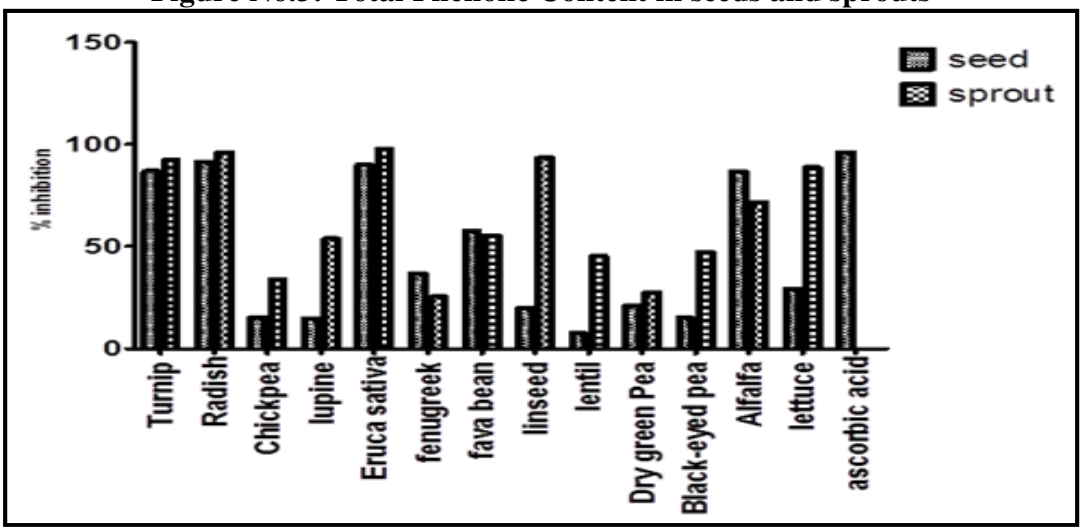

Figure No.4: Antioxidant activity of selected seed and sprouts using ABTS assay

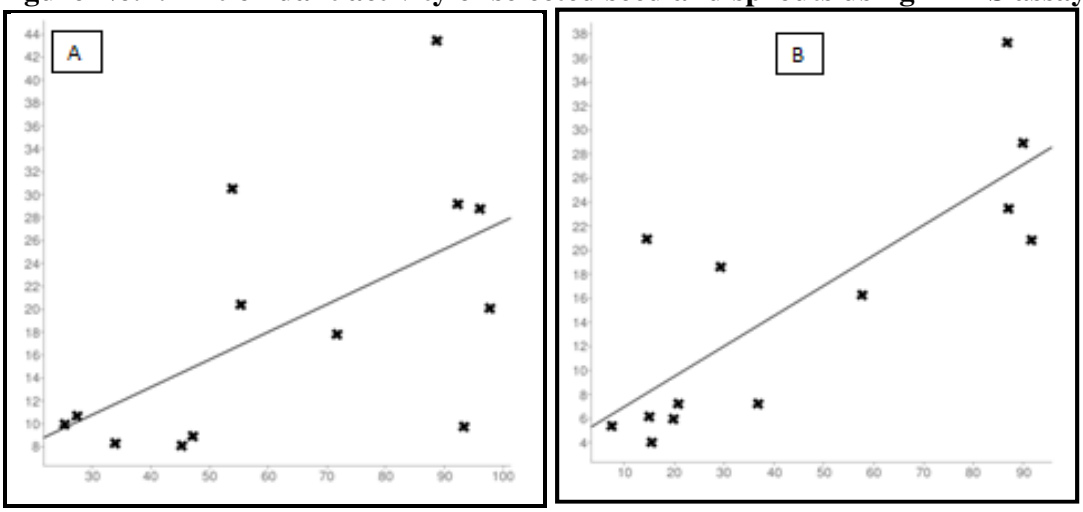

Figure No.5: A) The relation between the ABTS of seeds Vs. T. phenolics (R = 0.8008), B) The relation between the ABTS of sprouts Vs. T. phenolics (R = 0.5912)

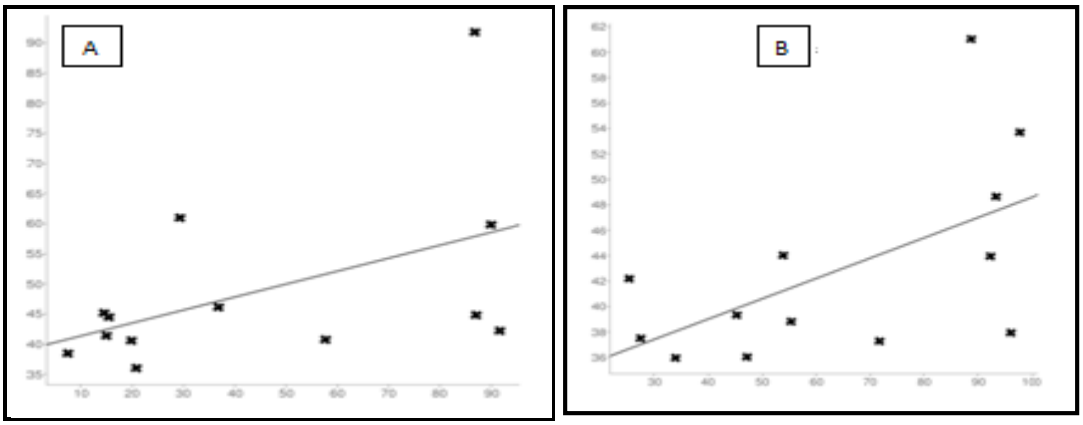

Figure No.6: A) The relation between the ABTS of seeds Vs. its total flavonoids (R = 0.4838), B) The relation between the ABTS of sprouts Vs. its total flavonoids(R = 0.5784).

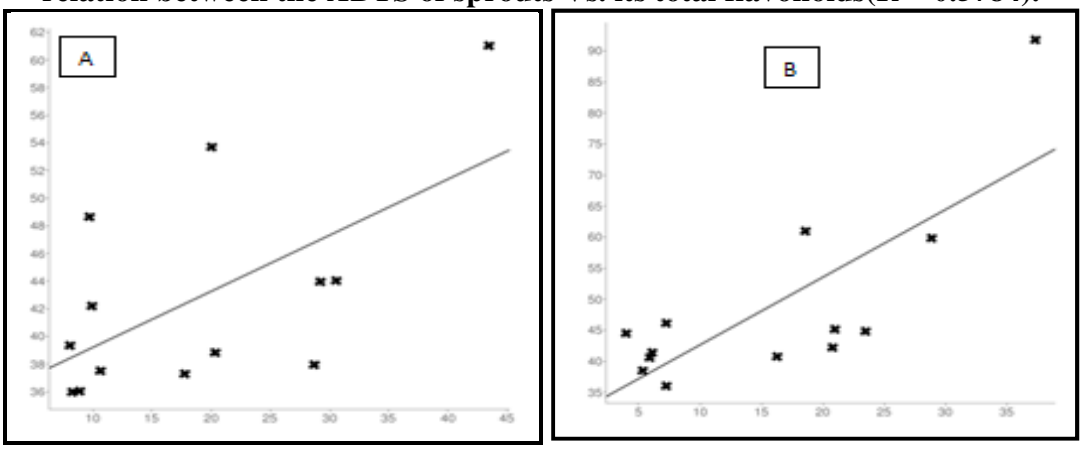

Figure No.7: A) The relation between the total flavonoids Vs. total phenolics of sprouts (R =0.6002), B) The relation between the total flavonoids Vs. total phenolics of seeds (R = 0.7716)

CONCLUSION

Dark germination significantly increases the levels of phenolic acids and their antioxidant activity in edible seeds which could be a very valuable source of natural antioxidants. Also Lyophilized sprouts could be used as beneficial ingredients in functional foods for therapeutic purposes.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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