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

Farid A. Badria* and Sara Abou Zeid

1Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.

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, AlCl₃m and ABTS reagents were used to examine the total phenolic contents, total flavonoids, and anti-oxidant 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.

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), colon cancer therapy (Ibrahim et al., 2014). 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), tyrosinase inhibitors for hyper pigmentation (Badria, 2001).

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), LTA4H inhibitory as potential colorectal cancer therapy (El-Naggar et al., 2017), breast cancer inhibitors (Abdel Bar et al., 2010), chemo-sensitization of cisplatin resistant ovarian cancer by cucurbitacin B (El-Senduny et al., 2016), acetyl cholinesterase inhibitors as a selective anti-Alzheimer agent (Abdel Bar et al., 2019).

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). 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). 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). Extensive breakdown of seed-storage compounds and synthesis of structural proteins and other cell components take place during the germination (Kuo et al., 2004).

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). 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). 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).

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 (MnO2) (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. Sprouts were collected after 3 days of growth for analysis (Baenas, et al., 2014).
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/ H2O (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 AlCl3 method (Lamaison and Carnat, 1990). 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). Briefly, the reaction mixture consisted of 2 ml of ABTS solution (60 µM) and 3 ml of MnO2 solution (25 mg/ml), all prepared in phosphate buffer (pH 7, 0.1M). The mixture was shaken, centrifuged, and decanted. The absorbance (Acontrol) of the resulting green-blue solution (ABTS+ radical solution) was recorded at λmax 750 nm. The absorbance (Atest) 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:

% inhibition = \( \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100 \)

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.

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).

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) 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).

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) 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 along 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). 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 Fenugreek seeds 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). 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).

| S.No | Name of plant  | Genus, species        | Family     |
|------|----------------|-----------------------|------------|
| 1    | Alfafa 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 beans 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)
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.

ACKNOWLEDGEMENT
Authors acknowledge the support Fab-La, Liver Research Lab, and those who maintain it. We do acknowledge and appreciate the diligent efforts of Ms Rowida M. Omar, An assistant Lecturer, Pharmacognosy Department, Faculty of Pharmacy, Delta university, Gamasa, Egypt for her proof reading of the manuscript.

CONFLICT OF INTEREST
We declare that we have no conflict of interest.

BIBLIOGRAPHY
1. Badria F A, Abu-Karam M, Mikhaeil B R, Maatooq G T, Amer M. Anti-Herpes Activity of Isolated Compounds From Frankincense, *Biosciences Biotechnology Research Asia*, 1(1), 2003, 1-10.
2. Mikhaeil B R, Maatooq G T, Badria F A, Amer M M. Chemistry and immunomodulatory activity of frankincense oil, *Zeitschrift für Naturforschung C*, 58(3-4), 2003, 230-238.
3. Badria F A, Abou-Mohamed G, El-Mowafy A, Masoud A, Salama O. Mirazid: A new
schistosomicidal drug, *Pharmaceutical Biology*, 39(2), 2001, 127-131.

4. Badria F A. Is man helpless against cancer? An environmental approach: antimutagenic agents from egyptian food and medicinal preparations, *Cancer Letters*, 84(1), 1994, 1-5.

5. Ibrahim A, Sobeh M, Ismail A, Alaa A, Sheashaa H, Sobh M, Badria F A. Free-B-Ring flavonoids as potential lead compounds for colon cancer therapy, *Molecular and clinical oncology*, 2(4), 2014, 581-585.

6. Elimam D M A, Uddin Ibrahim A S, Liou G I, Farid Abd Elrehim Abd Elaziz Badria. Olive and ginkgo extracts as potential cataract therapy with differential inhibitory activity on aldose reductase, *Drug discoveries and therapeutics*, 11(1), 2017, 41-46.

7. Badria F A. A new type of tyrosinase inhibitors from natural products as potential treatments for hyperpigmentation, *Bollettino chimico farmaceutico*, 140(4), 2001, 267-271.

8. Abdel Bar F M, Khanfar M A, Elnagar A Y, Liu H, Zaghloul A M, Badria F A, Sylvester P W, Ahmad K F, Raisch K P, El Sayed K A. Rational design and semisynthesis of betulinic acid analogues as potent topoisomerase inhibitors, *Journal of natural products*, 72(9), 2009, 1643-1650.

9. El-Naggar M H, Mira A, Bar F M A, Shimizu K, Amer M M, Badria F A. Synthesis, docking, cytotoxicity, and LTA4H inhibitory activity of new gingerol derivatives as potential colorectal cancer therapy, *Bioorganic and medicinal chemistry*, 25(3), 2017, 1277-1285.

10. Abdel Bar F M, Khanfar M A, Elnagar A Y, Badria F A, Zaghloul A M, Ahmad K F, Sylvester P W, El K S. Design and pharmacophore modeling of biaryl methyl eugenol analogs as breast cancer invasion inhibitors, *Bioorganic and medicinal chemistry*, 18(2), 2010, 496-507.

11. El-Senduny F F, Badria F A, El-Waseef A M, Chauhan S C, Halaweish F. Approach for chemosensitization of cisplatin-resistant ovarian cancer by cucurbitacin B, *Tumor Biology*, 37(1), 2016, 685-698.

12. Abdel Bar F M, Elimam D M, Mira A S, El-Senduny F F, Badria F A. Derivatization, molecular docking and in vitro acetylcholinesterase inhibitory activity of glycyrrhizin as a selective anti-Alzheimer agent, *Natural product research*, 33(18), 2019, 2591-2599.

13. Goncalves S, Romano A. Production of plant secondary metabolites by using biotechnological tools, *Secondary Metabolites-Sources and Applications*, 5, 2018, 81-99.

14. Prodanov M, Sierra I, Vidal-Valverde C. Effect of germination on the thiamine, riboflavin and niacin contents in legumes, *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, 205(1), 1997, 48-52.

15. Fernandez-Orozco R, Frias J, Zielinski H, Munoz R, Piskula M K, Kozlowska H, Vidal-Valverde C. Evaluation of bioprocesses to improve the antioxidant properties of chickpeas, *LWT-Food Science and Technology*, 42(4), 2009, 885-892.

16. Yu-Haey Kuo, Rozan P, Lambein F, Frias J, Vidal-Valverde C. Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes, *Food Chemistry*, 86(4), 2004, 537-545.

17. Fernandez-Orozco R, Piskula M K, Zielinski H, Kozlowska H, Frias J, Vidal-Valverde C. Germination as a process to improve the antioxidant capacity of Lupinus angustifolius L. var. Zapaton, *European Food Research and Technology*, 223(4), 2006, 495-502.

18. Ardekani M R S, Hajimahmoodi M, Oveisi M R, Sadeghi N, Jannat B, Ranjbar A M,
Gholam N, Moridi T. Comparative antioxidant activity and total flavonoid content of Persian pomegranate (Punica granatum L.) cultivars, *Iranian journal of pharmaceutical research: IJPR*, 10(3), 2011, 519-524.

19. Nieves Baenas, Garcia-Viguera Cristina, Diego A. Moreno. Biotic Elicitors Effectively Increase the Glucosinolates Content in Brassicaceae Sprouts, *Journal of Agricultural and Food Chemistry*, 62(8), 2014, 1881-1889.

20. Lamaison J, Petitjean-Freytet C, Carnat A. Medicinal Lamiaceae with Antioxidant Properties, a Potential Source of Rosmarinic Acid, *Pharmaceutica Acta Helvetiae*, 66(7), 1990, 185-188.

21. Eduardo A. Lissi, Brenda Modak, Rene Torres, Jorge Escobar. Total antioxidant potential of resinous exudates from Heliotropium species, and a comparison of the ABTS and DPPH methods, *Free Radical Research*, 30(6), 1999, 471-477.

22. Duenas M, Hernandez T, Estrella I, Fernandez D. Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (Lupinus Angustifolius L.), *Food Chemistry*, 117(4), 2009, 599-607.

23. Pasko P, Sajewicz M, Gorinstein S, Zachwieja Z. Analysis of selected phenolic acids and flavonoids in Amaranthus cruentus and Chenopodium quinoa seeds and sprouts by HPLC, *Acta Chromatographica*, 20(4), 2008, 661-672.

24. Perez-Balibrea S, Moreno D A, Garcia-Viguera C. Genotypic effects on the phytochemical quality of seeds and sprouts from commercial broccoli cultivars, *Food Chemistry*, 125(2), 2011, 348-354.

25. Kubasek W L, Shirley B W, Mckillop A, Goodman H M, Briggs W, Ausubel F M. Regulation of flavonoid biosynthetic genes in germinating Arabidopsis seedlings, *The Plant Cell*, 4(10), 1992, 1229-1236.

26. Yi W, Akoh C C, Fischer J, Kremer G. Effects of phenolic compounds in blueberries and muscadine grapes on HepG2 cell viability and apoptosis, *Food research international*, 39(5), 2006, 628-638.

Please cite this article in press as: Farid A. Badria and Sara Abou Zeid. Modulation of phenolic contents of some edible seeds ASA source of natural antioxidants in functional foods VIA Dark germination, *Asian Journal of Phytomedicine and Clinical Research*, 8(1), 2020, 29-38.