IJPSR (2022), Volume 13, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 February 2022; received in revised form, 26 April 2022; accepted, 29 April 2022; published 01 October 2022

EFFECT OF GERMINATION ON POLYPHENOL CONTENTS AND ANTIOXIDANT ACTIVITY IN MUSTARD SEEDS (*BRASSICA NIGRA*) GROWN IN ETHIOPIA

M. Efrem [1], R. Negussie [2], B. Sekwati-Monang [2], R. I. Kobue-Lekalake [3], T. Selebatso [3], M. R. Setlalekgomo [3] and G. D. Haki [*3]

College of Natural and Computational Science [1], Dire Dawa University, Ethiopia.
College of Natural Sciences [2], Addis Ababa University, Ethiopia
Botswana University of Agriculture and Natural Resources [3], Gaborone, Botswana.

Keywords:

Germination, *Brassica nigra*, Polyphenol contents, Antioxidant activity

Correspondence to Author:
G. D. Haki

Professor,
Botswana University of Agriculture and Natural Resources, Gaborone.

E-mail: hgulelat@buan.ac.bw

ABSTRACT: Mustard seeds, in some countries, are used as a traditional medicine for the treatment of snakebite, toothache, and epilepsy, as well as to treat skin diseases because of their high sulphur content. The aim of this study was to investigate the effect of three germination periods on the polyphenol contents and antioxidant activity of mustard seeds. Phenolic compounds were determined by the Folin-Ciocalteau method, while antioxidant activity was determined using standard methods. The results indicated that the total phenol and flavonoid contents of the one, two, and three days germinated mustard seed were increased by 16%, 17%, 24% and 500%, 647%,747%, respectively. Antioxidant activity determined by reducing power assay (for one, two and three days of germinated mustard seed) also increased by 10%, 34%, 51%, respectively. Germination is thus a better technique to enhance the nutritional and pharmaceutical potential of mustard seed and can be used in the development of functional diets.

INTRODUCTION: The mustard groups are formed by three species of the genus *Brassica* of the *Cruciferae* family: *Brassica nigra*, *Brassica juncea* and *Brassica carinata.* The use of the seeds in the food and beverage industry is immensely growing due to their nutritional and functional properties as they serve as a source for isothiocyanates which are responsible for the specific flavor of mustard [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(10).4087-92

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(10).4087-92

Ethanolic extract of *B. nigra* exhibited anti-proliferative activity in A549 and H1299 human lung cancer cells, probably through the induction of apoptosis and control of cell cycle through replication stress, resulting in fork-collapse DNA lesions [2]. Reports indicated the antimetastatic potential of *B. nigra* extract as it showed anti-migratory and anti-invasive activities, perhaps through down regulation of MMP2, MMP9, Snail, and E-cadherin [2].

It contains unsaturated fatty acids (oleic, linoleic, and erucic acid), and its consumption may prevent memory loss caused by β-amyloid [3]. The viscous liquid present in the seeds of *B. nigra* contains 25%–30% fat, sinapine, and singrin named glycoside and myrosin [4].

The Methanolic crude Extract (MCE) of *B. nigra* revealed the occurrence of alkaloids, flavonoids, tannin, diterpenes, glycosides, carbohydrates, phenols, fixed oils and fat. The fractions confirmed its anthelminthic and anxiolytic activity comparable to the standard drug diazepam 5 and its antidiarrheal potential was associated with flavonoid and tannin contents [6]. Mustard seed is a good source of protein, fiber, minerals, vitamins, antioxidants, and phytonutrients, thus exhibiting several health benefits, including antimicrobial, antibacterial, anti-diabetic, and antimalarial [7].

In Ethiopia, the mustard seed *brassica nigra* grows in many parts of the country. The paste of grounded seeds of *brassica nigra* is commonly used as a traditional pungent spice for consuming raw and roasted beef. Most people in the country favour this spice for its sharp pungent taste. Mustard seeds do not have the pungent flavour naturally but is developed when the seeds are ground and steeped in water. Mustard leaf extracts possess antioxidant properties as they scavenge free radicals. This was demonstrated by a DPPH assay and can be attributed to the bioactive isothiocyanates present in mustard [8].

Leaves, fruits, and roots of Ethiopian *Brassica nigra* are used orally to treat wounds [9]. Seeds collected from the northern part of Ethiopia showed good chemosuppressive and moderate chemo-prophylactic antimalarial activities against *Plasmodium berghei* infection in mice suggesting its potential for preventing malaria [10].

Germination of seeds is a process that starts with dry seeds imbibition of water and completes when the seeds embryonic axis gets longer. During this process, the seeds have manipulated the reserves within the storage tissues of the seed to support seedling growth and develop protective responses through the synthesis of phenolic and other compounds [11]. Germination enhanced several bioactive compounds such as γ-amino butyric acid, polyphenols, and vitamins, leading to greater bioactivity such as anti-diabetic, anti-bacteria, and anti-cancer effects while consuming the seeds [12]. It is also used for the reduction of antinutritive compounds such as tannins and phytates [11]. Germination also affects various legumes, cereals, and some vegetables [13-18].

MATERIALS AND METHODS:

Materials: The mustard seeds were purchased from *Merkato* market in Addis Ababa city, Ethiopia. The plant species used were selected according to documented traditional use acquired through personal interviews with the indigenous communities. Chemicals and Reagents used were ethanol, methanol, sodium hypochlorite, potassium ferricyanide, ascorbic acid, trichloroacetic acid, ferric chloride, Trolox (6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid), DPPH (2,2-diphenyl-1-icrylhydrazyl), ABTS **(**2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) and Folin–Ciocalteu reagent and all were of analytical grade.

Germination and Powder Preparation of the Mustard Seeds: The mustard seeds were first disinfected based on the procedures used by Cevallos-Casals *et al.* [11]. Briefly, 500g mustard seeds were sterilized with 70% ethanol for 2.5m, followed by the addition of 2.5% sodium hypochlorite for 15m. Ethanol and sodium hypochlorite were removed by rinsing with sterile water. After disinfection, mustard seeds were allowed to imbibe water for 24h at room temperature.

Then water was removed, and seeds were dark-germinated in sterile plates with humidified cotton at 28^{o}C. The cotton was kept moist by spraying with sterile water as needed. After one, two and three days of germination periods the germinated mustard seeds were dried at 40^{o}C in an oven for 48 h. The dried germinated mustard seeds were milled and the flour were passed through a sieve of 0.5mm. The powder of germinated mustard seeds was kept in a dark brown glass bottle and stored in a refrigerator until extraction.

Extraction of the Powder of Mustard Seeds: The powder of raw and germinated mustard seeds was extracted following the procedure used by Zakia *et al.* [19]. Briefly, 5g of mustard seeds powder was extracted by stirring with 50 mL of methanol/water (4:1) at 25°C for 24 h using a temperature shaker incubator (ZHWY-103B) and then filtered through Whatman No. 4 paper. The residue was then extracted with two additional 50 mL portions of methanol/water. The combined methanol/water extracts were evaporated at 40°C to dryness using a rotary evaporator (Stuart R3300) and re-dissolved

in methanol/water at the 10 mg/mL concentration and stored in a dark brown glass bottle at 4°C for further use.

Analysis of the Antioxidant Activity:

Determination of DPPH Free Radical Scavenging Activity: The effect of methanol/water (4:1) extracts on DPPH radical was estimated according to Kirby and Schmidt [20]. A 0.004% solution of DPPH radical solution in methanol was prepared and then 4 mL of this solution was mixed with 1mL of the various concentrations (12 – 416 µg/mL) of the extracts in methanol/water. Finally, the samples were incubated for 30m in the dark at room temperature. Scavenging capacity was read in a spectrophotometer (Perkin Elmer Lamda 950 UV/Vis/NIR) by monitoring the decrease in absorbance at 517nm. A standard curve was prepared with ascorbic acids. Results were given as mg ascorbic acids equivalents per g dry weight (mg AE/g). All tests were performed in triplicate.

Determination of ABTS Free Radical Scavenging Activity: The ABTS free radical scavenging activity was determined based on the method developed by Re *et al* [21]. A stable stock solution of $ABTS^{\bullet+}$ was produced by reacting a 7 mM/L aqueous solution of ABTS with 2.45 mM/L potassium per sulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. $ABTS^{\bullet+}$ solution (3.9 mL) was added to 0.1 ml of the test samples and mixed thoroughly. The blank was 3.9 mL of $ABTS^{\bullet+}$ solution with 0.1mL of 80% ethanol. The reactive mixture was allowed to stand at room temperature for 6m and the absorbance was immediately read with a spectrophotometer (Perkin Elmer Lamda 950 UV/Vis/NIR) at 734 nm. A standard curve was prepared with Trolox. Final results were given as mM Trolox equivalents per g dry weight (mM TE/g). All tests were performed in triplicate.

Determination of Total Reducing Power: Total reducing power was carried out according to the method established by Oyaizu [22]. One milliliter of different concentrations (31.25 – 500 µg/mL) of mustard seeds extract was mixed in a test tube with a 2.5 mL of 0.2M phosphate buffer (2.5 mL), pH 6.6 and 2.5 ml of 1% potassium ferric cyanide. The mixture was incubated at 50°C for 20 min to reduce ferric cyanide into Ferrocyanide. Then 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 6,000 rpm for 10 m. The upper layer (2.5 mL) was transferred into another tube, mixed with 2.5 mL deionized water and 0.5mL ferric chloride (0.1%), and left to react for 10 m. Finally, the absorbance of the reaction mixture was measured at 700 nm using a UV-VIS spectrophotometer (Perkin Elmer Lamda 950 UV/Vis/NIR). A standard curve was prepared with ascorbic acids. Final results were given as mg ascorbic acids equivalents per g dry weight (mg AE/g). All tests were performed in triplicate.

Analysis of the Polyphenol Contents:

Determination of Total Phenolic Content: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method used by Afolayan *et al.* [23]. The extract (1mL) was mixed with 2.5mLFolin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 2mL (75 g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30m at 40°C for colour development. The absorbance was measured at 765 nm using a UV-VIS spectrophotometer (Perkin Elmer Lamda 950 UV/Vis/NIR). Total phenolic content was expressed as mg Gallic acid equivalent per g dry weight (mg GAE/g) using the equation obtained from the calibration curve prepared by Gallic acid.

Determination of Total Flavonoid Content: The Total flavonoid content was determined using a spectrophotometric method based on a flavonoid–aluminium complex formation [24]. One milliliter of the extract was placed in a 10mL volumetric flask. Five milliliters of bi-distilled and 0.3mL of sodium nitrite were added and mixed. About 0.6 mL of 10% $A1Cl_3$. $6H_2O$ was added after 5m. Vigorously added two milliliters of NaOH (1M) 5m later in the solution mixture. The absorbance was immediately read with a spectrophotometer (Perkin Elmer Lamda 950 UV/Vis/NIR) at 510 nm. Total flavonoid content was expressed as mg catechin equivalent per g dry weight (mg CE/g) using the equation obtained from the standard calibration curve prepared from catechin.

Statistical Analysis: The results were given as means and SD of three measurements. The data were tested by one-way ANOVA using SPSS 20.0

(SPSS 2006) statistical software package, followed by the Tukey post hoc test. Differences of $p<0.05$ were considered significant.

RESULTS:

Polyphenol Content and Antioxidant Activity: The total phenol, total flavonoid, and antioxidant activity of a three, two, and one day germinated mustard seeds were significantly higher ($p<0.05$) than the raw mustard **Table 1** and **2**. The total phenol content of the first, second, and third days of germinated mustard seed was increased by 16%, 17%, and 24%, and the total flavonoid contents were drastically increased by 500%, 647%, and 747%, respectively. Among the three antioxidant activity assays, the reducing power assay exhibited the highest increment, 10%, 34%, and 51% in 1, 2, and 3days of germination periods, respectively. In contrast, antioxidant activity increments by TEAC and DPPH assays were 8% and 5% after 3days of germination, respectively **Table 2**. In general, the accumulation of total phenol and flavonoid contents as well as the order of antioxidant activity of all assays on the germinated seeds revealed a consistent trend as shown in **Tables 1** and **2**, where 3 days germinated > 2days germinated > 1day germinated > raw mustard seeds.

Even though the effects of germination on different types of seeds have been investigated, there have been no or few reports, especially on the effect of germination on the polyphenol contents and antioxidant activity *Brassica nigra*from Ethiopia. Therefore, this work aimed to report the effect of germination on the polyphenol contents and antioxidant activity of mustard seeds (*Brassica nigra)* grown in Ethiopia.

TABLE 1: THE EFFECT OF GERMINATION ON TOTAL PHENOL AND TOTAL FLAVONOID CONTENTS

	Germination day			
	0	1	2	3
Total phenol (mg GAE/g)	10.03 ± 0.52^{a}	11.62 ± 0.06^{b}	11.68 ± 0.21^{b}	12.39 ± 0.91^{b}
Total flavonoid (mg CE/g)	0.36 ± 0.26^{a}	2.16 ± 0.50^{b}	2.69 ± 0.17^{b}	3.05 ± 1.10^{b}

Different superscripts in the same column mean significant difference ($P \leq 0.05$).

TABLE 2: THE EFFECT OF GERMINATION ON ANTIOXIDANT ACTIVITIES

Germination (day)	DPPH (mg AE/g)	TEAC/ ABTS (mM TE/g)	Reducing power (mg AE/g)
0	1.72 ± 0.01^{a}	2.09 ± 0.04^{a}	10.02 ± 0.57^{a}
1	1.77 ± 0.00^{b}	2.17 ± 0.01^{b}	12.15 ± 0.63^{b}
2	1.78 ± 0.00^{b}	2.21 ± 0.02^{bc}	13.42 ± 0.25^{b}
3	1.80 ± 0.00^{b}	2.26 ± 0.02^{c}	15.12 ± 0.60^{c}

Different superscripts in the same column mean significant difference ($P \leq 0.05$).

DISCUSSION:

Polyphenol Content and Antioxidant Activity: The polyphenol content and antioxidant activity of germinated mustard seeds in this study revealed that they were synthesized during the germination periods, and the polyphenols generated were responsible for th enhanced antioxidant activity. Reports elsewhere have indicated that structural properties of substances belonging to the subgroups of phenolic acids, flavonols, flavanones, dihydrochalcones and flavanols influence antioxidant activities [25]. Moreover, higher antioxidant activity was observed on germinated seeds during water imbibition. Other antioxidant compounds reported in mustard seeds include 3,4-dihydroxybenzoic acid, ferulic acid, sinapic acid, and rutin in black mustard seeds [26]. Extraction of phenolic compounds from mustard seeds is best achieved when a mixture of water and acetone in equal proportions is utilized [26]. The rise in polyphenols and antioxidant activity after germination revealed in this work agrees with previous findings. A strong correlation between antioxidant activity with total phenolic content was reported [27, 28]. Cevallos-Casals *et al.* [11] testified a rise in total phenols by 435% and antioxidant activity by 566% in germinated *Brassica juncea.* In addition, Fernandez-Orozco *et al.* [29] have stated an increment of total phenolic content by 55% and antioxidant capacity (TEAC) by 23% for germinated chickpeas. El-Mergawi and Taie [30] studied germinated *Faba* bean cultivars. They reported an increase in total phenols by 24.3%, total flavonoid by 76.1%, and antioxidant activity (DPPH) by 65%, while the antioxidant activity of lentils by hexane extract increased by 480% and

total phenolic content by 77.4% [31]. Similarly, Suryanti *et al.* [32] showed that for 4 days of germinated lead tree seeds, the antioxidant activity increased by178% while total phenolic content by 630%. Furthermore, Tarzi*a et al* [18] reported that total phenolic compounds of methanol extracts of germinated chickpeas were increased by 92.8% and antioxidant activity by 493% as compared to raw seeds. In the present study, a drastic rise in the total flavonoid contents of germinated mustard seeds was observed compared to polyphenols. A similar trend was shown in the work of Herchia *et al.* [15] who stated that the total flavonoids of germinated flaxseed were increased by 32.5%, though the total phenolic acid content and antioxidant activity decreased as compared to raw seeds. In germinated *faba* be cultivars, the four flavonoid constituents, myricetin, daidzein, apigenin and quercetin, were increased by 228%, 527%, 366%, and 538%, respectively [30]. These results indicated that the germination process is one of the effective methods for the accumulation of flavonoids in seeds and hence higher antioxidant activity. The flavonoid is one of the most widespread and diverse groups of polyphenols in *Brassica* species [33]. The antioxidant ability of flavonoids is related to the number and position of hydroxyl groups in the molecule. Compounds with three hydroxyl groups on the B ring of flavonoids have a high antioxidant activity [33]. The three-day germinated mustard seeds exhibited the strongest antioxidant activity, which is due to the higher polyphenols content. This conclusion is further validated by computing the correlation coefficient between polyphenol contents and antioxidant activity. A positive linear correlation was observed where the correlation coefficients were in the order of $R^2 = 0.9$ **Table 3**. Moreover, the correlation coefficients between total phenol and total flavonoid and between the three antioxidant analysis methods (DPPH, TEAC, and reducing power) were in $R^2 = 0.9$.

TABLE 3: THE CORRELATION COEFFICIENT BETWEEN POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY

	DPPH	TEAC	Reducing power
Total phenol	$R^2 = 0.9909$	$R^2 = 0.9417$	$R^2 = 0.961$
Total flavonoid	$R^2 = 0.9859$	$R^2 = 0.9447$	$R^2 = 0.9117$

CONCLUSION: The phytochemicals of Ethiopian mustard, *B. nigra* seeds changed after germination, and its polyphenol profile and antioxidant activities increased with an increase in germination days. Germination can thus enhance the bioactive compounds in mustard seeds allowing the plant for efficient food and nutraceutical uses.

CONTRIBUTION OF AUTHORS: All authors contributed equally to this work.

ACKNOWLEDGEMENT: The laboratory of aroma research for the food industry in The Katholieke Universiteit Leuven (KU Leuven), technology campus, Ghent, Belgium, is acknowledged.

CONFLICTS OF INTEREST: All authors declare that there is no conflict of interest for the manuscript.

REFERENCES:

1. Lietzow J: Biologically active compounds in mustard seeds: A toxicological perspective. Foods 2021; 10(9): 2089. doi: 10.3390/foods10092089.
2. Ahmed AG, Hussein UK, Ahmed AE, Kim KM, Mahmoud HM, Hammouda O, Jang KY and Bishayee A: mustard seed (*Brassica nigra*) extract exhibits antiproliferative effect against human lung cancer cells through differential regulation of apoptosis, cell cycle, migration, and invasion. Molecules 2020; 25: 2069. https:// doi.org/ 10.3390/ molecules 25092069.
3. Elahe N, Mahnaz K, Leili A, Mohammad Sh, Mahdi V , Mina S, Mehdi S and Seyede N: Beneficial effect of Brassica nigra fixed oil on the changes in memory caused by β-amyloid in an animal model. Pharmaceutical Sciences 2020; 26(3): 261-269.doi: 10.34172/PS.2020.19.
4. Fatma K: Botanical characteristics, potential uses, and cultivation possibilities of mustards in Turkey: a review. Turkish Journal of Botany 2020; 44: 101-127. doi:10.3906/bot-1909-30.
5. Mohammad S, Shalahuddin M, Mohammad S, Saddam H, Giash Uddin, Shafayet A and Mahmuda F: Exploration of in vitro thrombolytic, anthelminthic, cytotoxic and **in-vivo** anxiolytic potentials with phytochemical screening of flowers of *Brassica nigra*. Future Journal of Pharmaceutical Sciences 2020; 6:73. es (2020) 6:73. https://doi.org/ 10.1186/s43094-020-00099-x.
6. Uddin M, Huda MN, Mosharraf S and Millat MS: assessment of antidiarrheal action of the methanolic extract of *Brassica nigra* flower in Swiss albino mice. Discovery Phytomedicine 2019; 6(2): 56-60. DOI: 10.15562/ phytomedicine.2019.84.
7. Mishra N: (Ed.): Ethnopharmacological investigation of Indian spices. IGI Global 2020; https://doi.org/10.4018/978-1-7998-2524-1.

8. Malabed RS, Noel MG, Aton, BI and Toribio E: Cooking affects glucosinolate concentration in mustard leaves and seeds. KIMIKA 2019; 30(2): 39-48. https:// doi.org/10.26534/kimika.v30i2.39-48.
9. Helen B, Haftom G, Kald B and Mariamawit Y: Ethiopian medicinal plants traditionally used for wound treatment: A systematic review. Ethiop J Health Dev 2019; 33(2): 103-127.
10. Muluye AB, Melese E and Adinew GM: Antimalarial activity of 80 % methanolic extract of Brassica nigra (L.) Koch. (Brassicaceae) seeds against Plasmodium berghei infection in mice. BMC Complement Altern Med 2015; 15: 367. https:// doi.org/ 10.1186/s12906-015-0893-z.
11. Cevallos-Casals B and Cisneros-Zevallos L: Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. Food Chem 2010; 119: 1485-90.
12. Anthony T, Oladipupo O, Ademola E and Solomon I: Germination: an alternative source to promote phytonutrients in edible seeds, Food Quality and Safety 2020; 4(3): 129–133. https://doi.org/10.1093/fqsafe/fyz043.
13. Oskaybaş-Emlek B, Özbey A and Kahraman K: Effects of germination on the physicochemical and nutritional characteristics of lentil and its utilization potential in cookie-making. Food Measure 2021; 15: 4245–4255. https://doi.org/10.1007/s11694-021-00958-y.
14. Duong T, Tran M, Phan T and Ha Thanh Toan: Time and temperature dependence of antioxidant activity from soybean seeds (*Glycine max* L. Merr.) during germination. International J of Food Sci and Nutr 2016; 1(50): 22-27.
15. Živilė T, Akvilė V, Honorata D, Pavelas D, Aurelija P and Marek G: Effects of germination time on the antioxidant properties of edible seeds, CyTA - Journal of Food 2019; 17: 1. 447-454, DOI: 10.1080/19476337.2018.1553895.
16. Shakirah O, Chiemela E, Stella O, Ukamaka R, Ayodamola F, Aisha A, Sewuese S, Nahemiah D and Oluwafemi Ay: Impact of germination alone or in combination with solid-state fermentation on the physicochemical, antioxidant, *in-vitro* digestibility, functional and thermal properties of brown finger millet flours 2022; 154: 112734. https://doi.org/10.1016/ j.lwt. 2021. 112734.
17. Eduardo B, Tzayhri G, Anaberta C, Deyanira M, Guillermo O, Jorge C and Cristian Jiménez M: Phenolic compounds profile and antioxidant activity of pea (*Pisum sativum* L.) and black bean (*Phaseolus vulgaris* L.) sprouts. Food Sci. Technol Campinas 2022; 42, 45920. DOI: https://doi.org/10.1590/fst.45920.
18. Haileslassie HA, Henry CJ and Tyler RT: Impact of pre-treatment (soaking or germination) on nutrient and anti-nutrient contents, cooking time and acceptability of cooked red dry bean (*Phaseolus vulgaris* L.) and chickpea (*Cicer arietinum* L.) grown in Ethiopia. International J of Food Science & Technology 2019; 54(8): 2540-2552.
19. Zakia N, Hakmaouib A, Ouatmanea A, Hasiba A and Fernández J: Bioactive components and antioxidant activity of moroccan paprika (*Capsicum annuum* L.) at different period of harvesting and processing. Journal of Biology, Agriculture and Healthcare 2013; 3: 1-8.
20. Kirby A and Schmidt R: The antioxidant activity of Chinese herbs for eczema and of placebo herbs. Journal of Ethno pharmacology 1997; 56: 103-108.
21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C: antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 1999; 26: 1231–1237.
22. Oyaizu M: Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition 1986; 44: 307-315.
23. Afolayan A, Aboyade M and Sofidiya O: Total phenol content and free radical scavenging activity of *Malva parviflora* L. (Malvaceae). Journal of Biological Science 2008; 8: 945-949.
24. Zhishen J, Mengcheng T and Jianming W: Research on antioxidant activity of flavonoids from natural materials. Food Chemistry 1999; 64: 555-559.
25. Platzer M, Kiese S, Tybussek T, Herfellner T, Schneider F, Schweiggert-Weisz U and Eisner P: Radical scavenging mechanisms of phenolic compounds: a quantitative structure-property relationship (QSPR) study. Front. Nutr 2022; 9: 882458. doi: 10.3389/fnut.2022.882458.
26. Anna Grygier: Mustard seeds as a bioactive component of food. Food Reviews International 2022; DOI: 10.1080/87559129.2021.2015774.
27. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R and Koirala N: Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants 2019; 8(4): 96. doi:10.3390/plants8040096.
28. Gabriela Boscariol Rasera, Marina Hermenegildo Hilkner, Severino Matias de Alencar and Ruann Janser Soares de Castro: Biologically active compounds from white and black mustard grains: An optimization study for recovery and identification of phenolic antioxidants. Industrial Crops and Products 2019; 135: 294-300. https:// doi.org/ 10.1016/j.indcrop.2019.04.059.
29. Fernandez-Orozco R, Frias J, Zielinski H, Munoz M, Piskula M, Kozlowska H and Vidal-Valverde C: Evaluation of bioprocesses to improve the antioxidant properties of chickpeas. Food Res Technol 2009; 42: 885-92.
30. El-Mergawi R and Taie H: phenolic composition and antioxidant activity of raw seeds, green seeds and sprouts of ten *Faba bean* (*Vicia faba*) Cultivars Consumed in Egypt. International Journal of Pharma and Bio Sciences 2014; 5: 609-617.
31. Gharachorloo M, Tarzi1 B, Baharinia M and Amir H: Antioxidant activity and phenolic content of germinated lentil (*Lens culinaris*). Journal of Medicinal Plants Research 2012; 6:4562-4566.
32. Suryanti V, Marliyana S and Putri H: Effect of germination on antioxidant activity, total phenolics, β-carotene, ascorbic acid and α-tocopherol contents of lead tree sprouts (*Leucaena leucocephala* (lmk.) de Wit). International Food Research Journal 2016; 23: 167-172.
33. Cartea M, Francisco M, Soengas P and Velasco P: Phenolic Compounds in Brassica Vegetables. Molecules 2011; 16: 251-280.

How to cite this article:
Efrem M, Negussie R, Sekwati-Monang B, Kobue-Lekalake RI, Selebatso T, Setlalekgomo MR and Haki GD: Effect of germination on polyphenol contents and antioxidant activity in mustard seeds (*Brassica nigra*) grown in Ethiopia. Int J Pharm Sci & Res 2022; 13(10): 4087-92. doi: 10.13040/IJPSR.0975-8232.13(10).4087-92.