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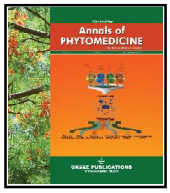

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Investigating the potential of underutilized brassica seed meals as a source of natural antioxidants

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Abstract

The seed meals of two *Brassica* species, yellow seeded *Brassica rapa* (L.) and brown seeded *Brassica juncea* (L.) Czern. & Coss. were examined for antioxidant potential. Four different solvents were used to extract defatted cakes in order to investigate how the extraction solvent and *Brassica* species had an impact on the antioxidant activity of the resulting extracts.When compared *B. juncea* to *B. rapa*, the total phenolic content was found to be substantially greater in *B. juncea* (ranging from 14.99 to 18.00 mg CE g-1 DW) than *B. rapa* (11.01 to 14.45 mg CE g-1 DW). In terms of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, metal chelating activity and ferric ion reduction antioxidant power, *B. rapa* had a much better antioxidant potential. When compared to alternative solvents, the 80 per cent acetone extracts had the highest total phenol content and the strongest *in vitro* antioxidant activity among both species. Furthermore, total phenolic content was found to have a substantial positive relationship (p<0.05) with total flavonoids and total antioxidant activity, but a significant (p<0.05) negative relationship with FRAP and ABTS. According to these findings, various *Brassica* seed meal extracts could be used as high-valued bioactive materials for a range of antioxidant applications across various industries.

1. Introduction

Oilseed crops are important crops that are grown all over the world due to their high economic value. Edible oil is an important component of human nutrition, and oilseed crops are the third most important predictor of agricultural output, behind cereals and legumes. Brassica oilseed species are the third most significant source of vegetable oils, out of nine different oilseed crops (Singh *et al*., 2020; Singh *et al*., 2021). Yellow mustard (*B. rapa*) and brown mustard (*B. juncea*) are the most commonly produced species in India, with seed oil content ranging from 40 to 45 per cent (Swati *et al*., 2015). Oil from *Brassica* oilseed crops, a significant source of essential fatty acids and a staple of our diet, is the main product of these crops (Mirpoor *et al*., 2021). Approximately, 60% of the original seed weight is made up of the by-product of oil extraction known as seed oil cakes, which is another important product made from Brassica oilseeds (Basili and Rossi, 2018). *Brassica* seed meal has a good amino acid profile, contains about 40% protein, and has relatively high levels of crucial amino acids like methionine and cysteine. In addition, it contains vitamins B4 and E and is rich in minerals, particularly calcium, magnesium, and potassium (Punetha *et al*., 2018). Several studies have been published that discuss the nutritional value and antioxidant potential of *Brassica* seed meal (Kumari *et al*., 2016; Kumari *et al*., 2017a, 2017b; Sharma *et al*., 2019). In India, however, oil extraction from Brassica seeds results in the production of around 5.7 million metric tonne (MT) seed meal per year, of which only 0.4 MT is exported and nearly 2.7 MT is squandered (Swati *et al*., 2015).

For oilseed crops to be more economically viable, there needs to be a suitable and profitable way to use seed meal. As a result of their high fibre, protein, and secondary metabolite content, *Brassica* seed cakes are frequently used as animal feed supplements, plant fertiliser, and soil fertiliser. *Brassica* seed meals contain a large number of secondary metabolites, with phenolics ranking as one of the most significant bioactive compounds present. *Brassica* seed meal flours contain 10-30 times more phenolics than seed meal from other oleaginous seeds. The *Brassica* seed meal contains up to five times as much phenolics as soybean meal does overall (Rezaeizad *et al*., 2011). Plants produce polyphenols, which are product of secondary metabolism. They have atleast one aromatic ring whose hydroxyl group is substituted. Polyphenols can lower lipid peroxidation and have a variety of physiological antioxidant actions, according to Metsämuuronen and Sirén's research from 2019. These substances aid plants in protecting themselves from various biotic and abiotic stimuli. Numerous pharmacological and nutraceutical properties of phenolic compounds have been reported (Joana Gil-Chávez *et al*., 2013; Bueno *et al*., 2012; Costa *et al*., 2015; Albuquerque *et al*., 2021; Lourenço *et al*., 2019), but antioxidant and antimicrobial effects

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have recently drawn more attention. Their ability to trap or suppress reactive oxygen/nitrogen species, give free radicals electrons, activate antioxidant enzymes, and lessen oxidative stress and inflammation are what primarily causes their biological effects (Pisoschi *et al*., 2021). Furthermore, synthetic antioxidants are being gradually phased out of the food industry due to their probable carcinogenicity. Plant phenolics can also be used to supplement synthetic antioxidants (Kumar *et al*., 2015). In this regard, the public's interest in dietary phenolic compounds is growing, as is the scientific community's interest in correctly identifying and quantifying these chemicals in plants (Nicácio *et al*., 2021).

The culinary, pharmaceutical, and agricultural industries may find brassica seed meal to be a promising source of polyphenols for natural antioxidants. Different amounts of total phenolic composition from seed meal of different Brassicaceae species/cultivars have already been recorded, with anthocyanins, flavonoids, tannins, and phenolic acids being the most common (Wang *et al*., 2018). Owing to the above facts, optimization of extraction process for gaining maximum yield of polyphenols is necessary. Solvent extraction is the approach most often used to extract antioxidants from plant-based materials. Further extraction yield depends upon the method of extraction used, extraction time, type of solvent and temperature (Cheng *et al*., 2012; Das *et al*., 2019; Hamid *et al*., 2020; Gezici *et al*., 2020). However, there is a dearth of literature comparing the effects of various solvents on polyphenolic extraction from *Brassica* seed meal.

2. Materials and Methods

2.1 Plant materials and sample preparation

The main objective of this study was to examine the antioxidant activity of seed meal extracts from *B. juncea* and *B. rapa*. Oilseeds Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar provided seeds of *Brassica juncea* cultivar RH 725 and *Brassica rapa* cultivar YSH 0401. With a mortar and pestle, we pulverised oven-dried seeds (at 50°C). In Soxhlet's equipment, one gram of powdered material was put in a filter paper thimble and defatted with petroleum ether for 6 h. Polyphenols were extracted from brown and yellow seeded mustard seed meal using four different solvents, *i.e.*, 80% methanol, 80% acetone, 80% ethanol and 80% isopropanol by the method described by Swain and Hillis (1959).

2.2 Chemicals

All the chemicals used during the present course of investigation were of analytical grade and purchased from Sigma Aldrich Chemicals Pvt Ltd, Delhi.

2.3 Estimation of secondary metabolites

The total phenolic and flavonoid content was determined using the procedure described by Swain and Hillis (1959) and Kalita *et al*. (2013), respectively.

2.4 Antioxidant potential

The antioxidant activity of various mustard seed meal extracts was tested *via* performing DPPH free radical scavenging activity (Yen and Duh, 1994), total antioxidant activity (Prieto *et al*., 1999), FRAP assay (Do *et al*., 2014), metal chelating activity (Hsu *et al*., 2013) and ABTS free radical cation scavenging activity (Re *et al*., 1999).

2.5 Statistical analysis

Two-way analysis of variance (ANOVA), followed by Duncan Multiple Range Test (DMRT) was done to compare the significance of treatments ($p \leq 0.05$). The association between total phenolics and other antioxidant characteristics was investigated using Pearson's product moment correlation analysis. The Statistical Tool for Agricultural Research (STAR) version 2.0.1 statistical package created by the International Rice Research Institute (IRRI), Manila, Philippines, was used for all statistical analyses. Microsoft Excel 2013 was used to create the graphs.

3. Results

According to the analysis of variance, variety and solvent had a highly significant impact on the amount of total phenolics (TPC), flavonoids (TFC), DPPH and ABTS scavenging activity, total antioxidant activity (TAA), and ferric ion-reducing antioxidant power (FRAP). However, only the ferric ion reducing antioxidant power was significantly impacted by the interaction of variety and solvent (Table 1).

Table 1: Two-way analysis of variance (ANOVA) for different antioxidant parameters performed in mustard seed meal

		Mean squares						
SV	df	TPC	TFC	DPPH	TAA	FRAP	MCA	ABTS
Species	1	104.33**	0.18**	2273.14**	45.10**	46.12**	8.91	2287.57**
Solvent	3	11.41**	0.31**	510.82**	13.38**	4.83**	40.46**	182.09**
Species × Solvent	3	0.50	0.02	2.90	0.54	1.58**	0.45	3.38
Error	16	0.38	0.01	17.68	1.81	0.20	4.04	9.38

*Significant at $p \leq 0.05$ and **Significant at $p \leq 0.01$; FRAP- Ferric ion reducing antioxidant power;TPC-Total phenolics content; ABTS-2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity;TFC-Total flavonoids content; MCA- Metal chelating activity; TAA-Total antioxidant activity; DPPH-2, 2-diphenyl-1-picrylhydrazyl scavenging activity.

3.1 Total phenolic content and total flavonoid content

Mean comparisons of total phenolic content and total flavonoid content of brown and yellow mustard seed meal extracted with different solvents showed that brown mustard seed meal exhibited higher phenolics (14.99 ± 0.26 to 18.00 ± 0.30 mg CE/g DW) as well as flavonoids content (0.46 ± 0.012 to 0.65 ± 0.015 mg QE/g DW) than yellow mustard seed meal (phenolics content: 11.01 ± 0.04 to 14.45 ± 0.11 mg CE/g DW; flavonoids content: 0.30 ± 0.015to 0.46 ± 0.018 mg QE/g DW) (Figures 1 a and b). Our findings are consistent with prior research by Kumar *et al.* (2018) and Bala *et al.* (2011).

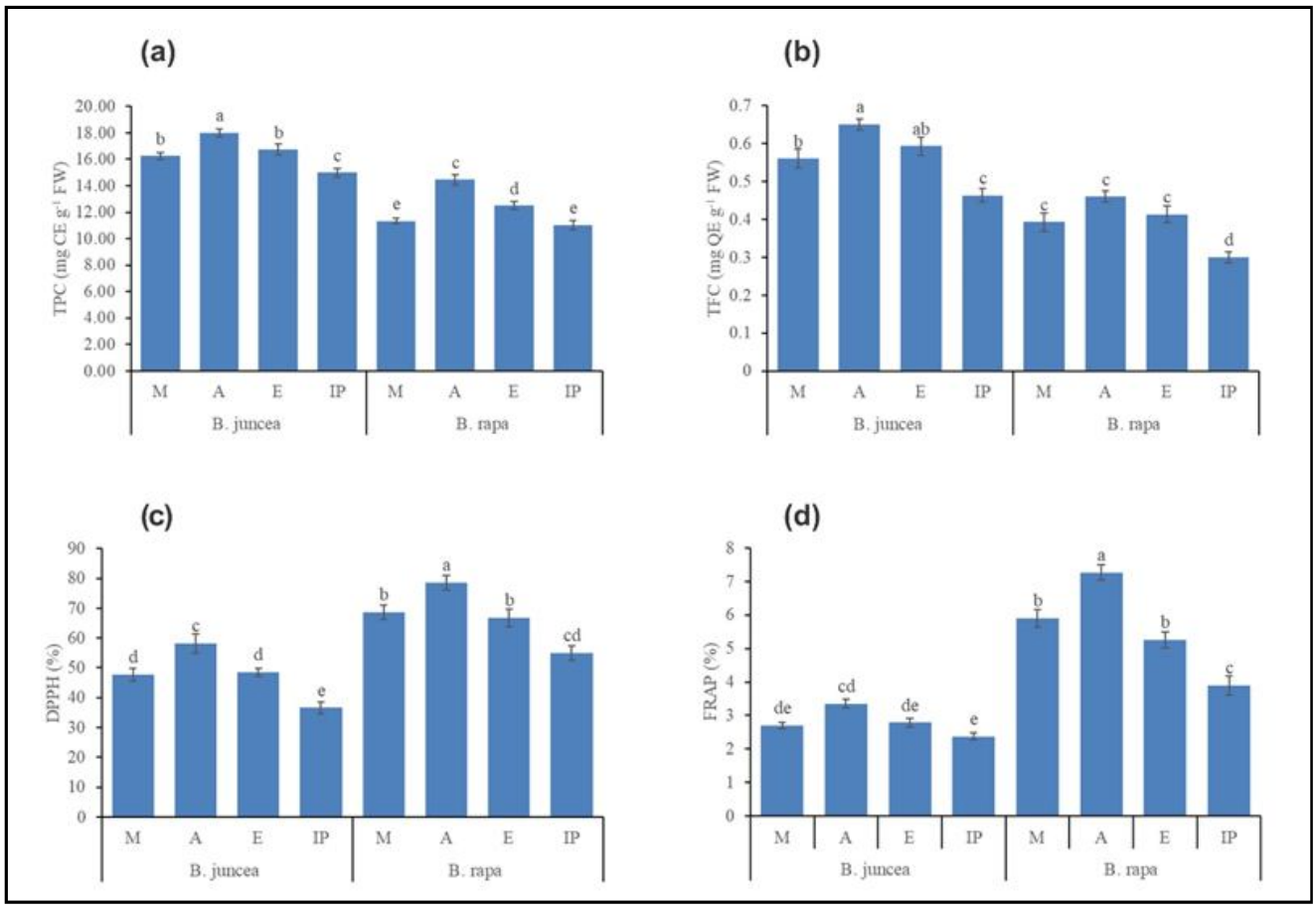

Figure 1: Comparisons of total phenol content (TPC) (a), total flavonoid content (TFC) (b), 2,2'-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (c), ferric-ion reducing antioxidant power (FRAP) activity (d) and extracted from seed meal of brown seeded *B. juncea* and yellow seeded *B. rapa* using different extraction solvents (M-80% methanol, A-80% acetone, E-80% ethanol and IP-80% iso-propanol). A column and bar are used to illustrate the mean and standard error. Different letters on distinct bars denote significant differences ($p<0.05$) based on Duncan Multiple Range Test (DMRT).

3.2 Antioxidant potential of mustard seed meal extracts

The current study also sought to assess the antioxidant efficacy of *Brassica* seed meal extracts prepared using various polarity solvents. The quantity of hydroxyl groups, their arrangements, the type of substituents, and their placement on the ring structure are all aspects that affect the antioxidant properties of phenolic compounds. The radical produced after the neutralisation process is more stable if the hydroxyl group is in the ortho position of the ring (Cuvelier *et al*., 1992). The concentration, chemical composition, and degree of polymerization of a sample affect its antioxidant activity (Gil *et al*., 2000).The DPPH scavenging activity differs considerably according to solvent and variety of mustard seed meal ranging from 36.65 to 78.56% (Figure 1c). The yellow mustard seed meal exhibited higher scavenging activity than brown mustard seed meal despite having low phenolics. As previously stated, a high sample concentration does not automatically imply that it would have a high proportion of DPPH scavenging action. This negative association might be due to the fact that the extracts include various chemicals other than polyphenols, some of which have an antagonistic impact on the polyphenols with antioxidant potential. When the concentration of these chemicals reaches the minimal effective concentration, they have an antagonistic action. So, in the case of *B. juncea* seed meal, the concentration of these antagonistic chemicals may have reached the effective concentration, resulting in a reduction in its DPPH scavenging ability (Ruslan *et al.*, 2018). This research also implies that, depending on their concentration, these polyphenols may behave as pro-oxidants in various test systems (Kilicgun and Altner, 2010). Our results showed that yellow mustard seed meal had more reducing power than brown mustard seed meal. In yellow mustard, it ranges from 3.89 to 7.27 mg AAE/g DW with maximum reducing power in 80% acetone, followed by 80% methanol, 80% ethanol and 80% isopropanol. Reducing power of brown mustard ranges from 2.37 to 3.36 mg AAE/g DW with solvent pattern: 80% acetone > 80% ethanol > 80% methanol > 80% isopropanol (Figure 1d). Further, there is significant difference in the antioxidant activity of the two mustard varieties. Higher antioxidant activity is possessed by brown mustard seed meal (11.53 to 15.44 mg AAE/g DW) while lesser by yellow mustard seed meal (8.73 to 12.02 mg AAE/g DW) (Figure 2b). Pearson's correlation analysis revealedthat total antioxidant activity is positively correlated with total phenolic content. It indicates that polyphenols might be important contributor towards total antioxidant activity.

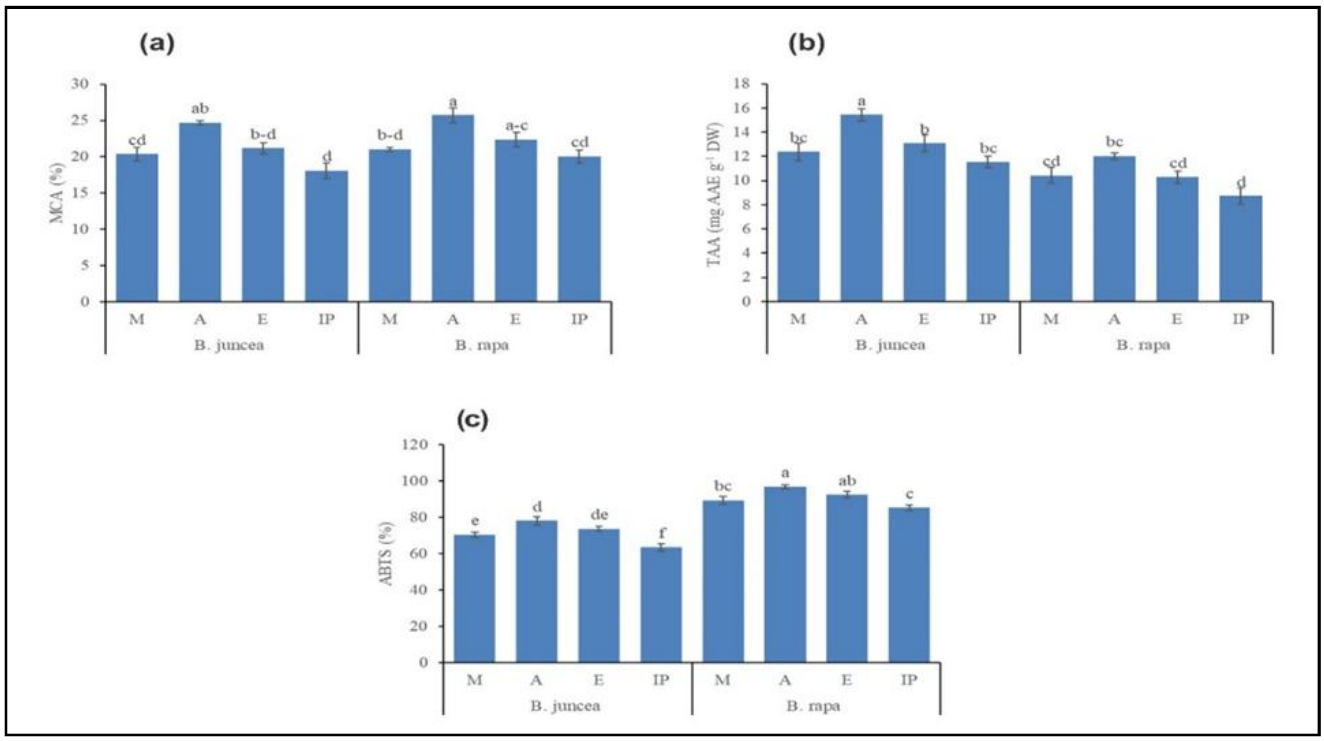

Figure 2: Comparisons of metal chelating activity (MCA) (a), total antioxidant activity (TAA) (b), 2,2ý-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (c) and extracted from seed meal of brown seeded *B. juncea* and yellow seeded *B. rapa* using different extraction solvents (M-80% methanol, A-80% acetone, E-80% ethanol and IP-80% iso-propanol). A column and bar are used to illustrate the mean and standard error. Different letters on distinct bars denote significant differences ($p<0.05$) based on Duncan Multiple Range Test (DMRT).

Further, yellow mustard seed meal had higher $ABTS^{\bullet+}$ scavenging activity than brown mustard seed meal. The $ABTS^{\bullet+}$ radical scavenging activity of yellow mustard ranges from 85.33% to 96.92% while of brown mustard varies from 63.56% to 78.16% (Figure 2c). Furthermore, although having a high concentration of phenolics, *B. juncea* has poor ABTS scavenging activity and FRAP values, which is possibly due to the fact that the quantity of phenolics does not necessarily predict the antioxidant potential; the quality of the phenolics also counts (Huang *et al.*, 2005). The negative association of these parameters with total phenolic content suggests that TPC may not be a significant contributor to the antioxidant capability of *Brassica* seed meal, confirming the Folin-ciocalteu method's non-specificity. According to Magalhaes *et al.* (2006), several nonphenolic substances (D-glucose, citric acid, ferrous sulfate) react with the Folin-ciocalteu reagent but are ineffective as free radical scavengers. Additionally, despite having low phenolic content, the antioxidant activity of *B. rapa* may be enhanced by polysaccharides, sodium sulphite, ascorbic acid, vitamin E and carotenoids (Terpinc *et al.*, 2012). The potential reactivity of the reaction product formed after interaction with DPPH, phosphomolybdenum, and ABTS affects the ability of polyphenols to act as antioxidants. It is not necessary for phenolic compounds to also have DPPH scavenging activity if they have ABTS scavenging activity. As a result, it is challenging to describe the results brought about by the three factors.

Our results showed insignificant difference in the metal chelating activity of both the mustard varieties. It ranged from 18.07 to 25.72% (Figure 2a). However, it was higher in yellow mustard seed meal than brown mustard seed meal. According to Zhou *et al.* (2006), polyphenols with just one hydroxyl group do not have any chelating activity for transition metals. Additionally,according to Andjelkovic *et al.* (2006), ferulic, vanillic, and syringic acid lacks catechol or galloyl moiety, which prevents these acids from having any iron chelating activity. Our findings support earlier research by Granato *et al.* (2015) and Wang *et al.* (2018) on various dietary and herbal matrices, which came to the conclusion that phenolic compounds appear to have very little impact on iron chelating activity. Furthermore, the metal chelating activity is determined by more than just the phytochemicals contained in the extract. The transition metal's binding ability is also affected by the matrix's redox potential and pH (Sunda and Huntsman, 2003).

The association between TPC and antioxidant activity of the extracts was investigated using Pearson's correlation analysis. Total phenolic content exhibited highly significant ($p \leq .01$) and positive correlation with TFC (r = 0.928) and TAA (r = 0.802), while significant ($p \leq 0.05$) and negative association with FRAP (r = -0.470) and ABTS (r = -0.518). TFC exhibited a similar pattern of correlation with various antioxidant parameter. Furthermore, DPPH, FRAP, ICA, and ABTS all revealed a highly significant ($p \leq 0.01$) positive correlation with each other. Total antioxidant activity also had a substantial ($p \leq 0.05$) positive connection with FRAP, but only a minor relationship with MCA and ABTS (Table 2).

4. Discussion

From the results, it has been observed that the solvent pattern is same in both the varieties and in all the biochemical parameters (80%

acetone > 80% ethanol > 80% methanol > 80% isopropanol) except FRAP. The extraction of phenolics from various samples depends on their solubility in the solvents usedand genetic makeup of plants (Xu and Chang, 2007; Ali *et al*., 2011; Cheng *et al*., 2012; Kchaou *et al*., 2013). Therefore, choosing an appropriate solvent to extract phenolic compounds from all samples is challenging. It has been found that 80% acetone was most efficient among all the solvents used. This means that 80% acetone has the highest TPC, TFC, and antioxidant potential, implying that the bulk of antioxidant chemicals in brown and yellow-seeded *Brassica* have a strong affinity for this solvent. Several earlier studies examined how different solvents had an impact on polyphenolic extractionas well as on their antioxidant capacity (Siddhuraju and Becker, 2003; Vuong *et al*., 2013; Abugri and McElhenney, 2013; Dirar *et al*., 2019). Rasera *et al*. (2019) reported the efficacy of 50 per cent acetone in extracting phenolics and exhibiting higher antioxidant activity. The study reported by Naczk *et al*. (2005) explained the high potential of aqueous acetone to extract phenolics from canola hulls. Boulekbache-Makhlouf *et al*. (2013) extracted polyphenols from an eggplant by-product and reported the higher capability of 70% acetone over 70% methanol for polyphenolic extraction. In the present study, aqueous mixtures of organic solvents have been used, as pure organic solvents are inefficient in extracting polyphenols because these compounds are also associated with other biomolecules (Rajbhar *et al*., 2015). Polyphenols are poorly soluble in pure organic solvents because of the strong hydrogen bond between polyphenols and proteins, further on addition of water this hydrogen bond becomes weak and the solubility increases (Sripad *et al*., 1982). According to several studies, aqueous solvents produce more polar conditions, which aid in the polyphenolic extraction (Do *et al*., 2014; Meneses *et al*., 2013). Proanthocyanidins and tannins are best extracted with acetone, flavonoids and their glycosides, catechin, and tannins are best extracted with ethanol, and catechin and phenolic acids are best extracted with methanol (Lasano *et al*., 2019). Thus, compared to the other polyphenols present, proanthocyanidins and tannins may make up a greater portion of *Brassica* seed meal. Therefore, it was found that extraction worked best with 80% acetone. However, it has been found that the extraction of polyphenols is not primarily reliant on the solvent's polarity. The factors that affect extraction include solid-liquid ratio, temperature, particle size, time of extraction and pH (Rajbhar *et al*., 2015; Yadav *et al*., 2020; Nguyen, 2020). Overall, the large difference in the findings highlights the necessity of the research to discover the optimal solvent combination (organic solvent: water combination) for extracting phenolics. Furthermore, selecting the best solvent for both species is one of the most critical aspects for obtaining higher antioxidants.

Table 2: Pearson's product moment correlation coefficients between different total phenol and flavonoids content with different antioxidant parameters assayed from *Brassica* seed meal extracts

	TPC	TFC	DPPH	TAA	FRAP	MCA	ABTS
TPC	1.000	0.928**	-0.349	0.802**	-0.470*	0.171	-0.518**
TFC		1.000	-0.244	0.812**	-0.404*	0.208	-0.444*
DPPH			1.000	-0.139	0.895**	0.655**	0.901**
TAA				1.000	0.248**	0.358	-0.346
FRAP					1.000	0.525**	0.874**
MCA						1.000	0.503**
ABTS							1.000

*Significant at $p \leq 0.05$ and **Significant at $p \leq 0.01$; activity; FRAP- Ferric-ion reducing antioxidant power; TPC-Total phenolics content; ABTS-2, 2a-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity; TFC-Total flavonoids content; MCA- Metal chelating activity; TAA-Total antioxidant activity; DPPH-2, 2-diphenyl-1-picrylhydrazyl scavenging activity.

5. Conclusion

The current study comes to the conclusion that the type of extraction solvent used and the species of *Brassica* had an effect on polyphenolic extraction and antioxidant activity from seed meal. When compared to all other solvents, 80 per cent acetone is most effcient solvent for extracting polyphenols from mustard seed meal.Yellow mustard (*B. rapa*) seed meal possessed higher antioxidant potential. On the other hand, brown mustard (*B. juncea*) exhibited higher total antioxidant activity. According to the findings, a variety of antioxidant applications in the food and other industries can be achieved by using *Brassica* seed meal.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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