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Aman Verma
ICAR-DGR, Junagadh, Gujarat, India

Anubhuti Sharma
ICAR-DRMR, Sewar, Bharatpur, Rajasthan, India

Meghna
ICAR-DRMR, Sewar, Bharatpur, Rajasthan, India

PK Rai
ICAR-DRMR, Sewar, Bharatpur, Rajasthan, India

Correspondence
Aman Verma
ICAR-DGR, Junagadh, Gujarat, India

Impact of soxhlet extraction method on oil yield and antioxidant potential of *Brassica juncea*

Aman Verma, Anubhuti Sharma, Meghna and PK Rai

Abstract
Indian mustard is the preferred source of edible oil and occupies a premier place in the world among oilseed crops. In major states of India, many farmers and small scale industrialists are earning their livelihood due to this crop. To benefit stakeholders, a cost-effective extraction method with high oil percent yield, with optimal antioxidant potential for further application at industrial scale production, is needed. This study aimed to investigate effect of two different extraction solvents (petroleum ether and n-hexane) with soxhlet method on oil yield and antioxidant potential. Petroleum ether resulted in the higher percentage of oil yield (37.01±0.28) in PDZ1 variety than PM21. In addition to it, PDZ1 in which the DPPH activity was higher also showed concomitant increase in TAA. This is clearly evident from the results that genotypic expression plays pivotal role in determining antioxidant potential. In brief, present study says that, soxhlet extraction with petroleum ether in Indian mustard varieties is an economic and effective method for oil extraction in terms of yield and antioxidant potential (DPPH activity and TAA).

Keywords: Indian mustard, double low, single low, oil yield, antioxidant activity, extraction

Introduction
Brassica juncea L. is an annual herb that belongs to the family *Brassicaceae* [1]. It was widely believed to be one of the earliest domesticated plant and condiment, benefiting human race since ages [2, 3]. Oil extracted from Indian mustard has a nutty taste, strong smell, pungent and sulphury odor, and widely used in Indian cooking [4]. It has high amount of monounsaturated fatty acids (MUFA) and a balanced ratio of polyunsaturated fatty acids (PUFA). *B. juncea* is known for its antioxidant potential and various health benefits including anticancerous activity, prevention from asthma, lowers high blood pressure and restores normal sleep pattern in women going through menopause phase and prevent cardiac arrest in patients with atherosclerosis or diabetes [5, 6, 7, 8]. Antioxidant activities of *B. juncea* are due to the presence of many phenolic acids, flavonoids, carotenoids, alkaloids and vitamins [9].
Extraction of oil is the foremost and crucial step in the analysis of oilseed plants, because proper extraction would maintain the desired chemical components in the oil after separation. In general, solvent extraction is the most widely adopted method for oil extraction for seed meal which comprises of equilibrating the solvents with the samples [10]. Range of solvents are tested for solvent extraction depending upon their polarity and boiling point [11, 12] like petroleum ether, hexane, acetone, methanol, ethanol *etc*. Some of them have shown high solvent extraction capacity and does not impact the quality of oil [13]. Keeping in mind the polarity and stability of Indian mustard oil, two extraction solvents namely n-hexane and petroleum ether, were employed in the present study and a relationship was tried to establish between total oil yield percentage and its antioxidant potential.

Materials and Methods
Seed Material
Pure clean seeds of PM21 and PDZ1 varieties of Indian mustard seeds were procured from ICAR-DRMR, Bharatpur, Rajasthan, India and were used in the analysis.

Total lipid extraction
Total lipid extraction was performed according to AOAC (2000) [14]; briefly, dried seeds (5 g) were ground to fine powder using a pestle and mortar and extracted using a thimble. Gravimetrically samples of both the varieties were extracted continuously in organic solvents (petroleum ether and n-hexane) and complete extraction procedure lasted from 6-8 h. The oil extract was dried, weighed and stored at 4 °C.

Analytical Methods

Prior to the analysis, seed meal was defattened by homogenizing seeds and leaving them overnight in n-hexane at room temperature. This was repeated three to four times to ensure complete extraction of oil. The seed meal was dried till n-hexane is completely evaporated and stored at 4 °C. Dried seed meal (0.2 g) was mixed in 2ml of 80% methanol. This homogenate was centrifuged at 3000 rpm for 4 min, after keeping overnight at room temperature. The supernatant was collected after centrifugation and made up to 2 ml with 80 % methanol. Methanolic extract of the samples were used for the estimation of DPPH activity and TAA.

Total antioxidant activity

Total antioxidant activity (TAA) was estimated in defatted and methanolic extract of the samples using the method of Prieto *et al*. (1999) [15]. To 100 µl of the methanol extract of samples, 2.5 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added and the reaction mixture was incubated in boiling water bath for 90 min. After cooling, the absorbance was measured at 695 nm on UV-visible spectrophotometer, Labomed. Inc., UVD3500 and results were expressed as µg g^{-1}. Calibration curve was prepared by series of standard solutions of ascorbic acid (0-50µg/ml).

DPPH radical scavenging assay

The DPPH radical scavenging assay method is based on the reduction of 1,1-Diphenyl-2-picrylhydrazyl (DPPH), a stable free radical [16]. Volumes of 500 µl of 80% methanolic extract of samples as well as standard compound (Ascorbic acid) were taken in screw capped glass vials of amber color and the volume was made uniformly to 1ml using 80% methanol. Each of the samples was then further diluted up to 5 ml with methanol and to each 5 ml DPPH (0.01 M) was added. Absorbance was taken after 30 min incubation in dark at 517 nm using methanol as blank. The IC_{50} values for each compounds as well as standard preparation were calculated. The DPPH free radical scavenging activity was calculated using the following formula:

% Radical scavenging capacity = (Absorbance of control - Absorbance of test sample/Absorbance of control) × 100

Results and Discussions

Indian mustard seeds are the biggest source of edible oil in northern part of India. Thus, oil extraction using different solvents was performed to find a better solvent system for improved oil per cent yield with simultaneous maintenance of its natural, inherited antioxidant potential. Petroleum ether being non-polar and charged can penetrate easily into the matrix of the seed. However, due to its lack of linkage, it has O-H ends that then might interfere with the extraction process [17]. It is used as an alternate extraction solvent because of its cost effectiveness, non-polar and volatile nature for extracting oil from oilseeds. It has high solvent extraction capacity, thus it does not impact the chemical properties of oil [13]. Contrastingly, with a boiling point of 68.95 °C, n-hexane is able to retain its liquid state at all atmospheric conditions other than for extreme climates. Its reasonable volatility aids easy removal from solids and oil, using low energy. A brief comparative information about the physiochemical properties of the solvents has been provided in the table no. 1.

Table 1: Physiochemical Properties of Petroleum ether and n-Hexane

Physiochemical Properties	Petroleum ether	n-Hexane
Melting point	-73 °C	-95 °C
Boiling point	90-100 °C	68.95 °C
Density	0.77 g mL^{-1} at 20 °C	0.659 g mL^{-1} at 25 °C
Vapor density	3 (vs air)	3.5 (vs air)
Vapor pressure	256 mm Hg (37.7 °C)	40 mm Hg (20 °C)
Refractive index	n20/D 1.428	n20/D 1.388
Flash point	-9 °F	-14.8 °F
Polarity index	0.11	0.1
Explosive limit (V)	0.7-6.5%	1.0-8.1%

The major percentage of Indian mustard production goes for oil extraction; hence oil yield from seeds of mustard is one of the most important quantitative traits in oilseed industries. Environmental factor and genotypic characteristics are mainly responsible for variations in its oil content and quality. Improved oil yield has been seen in PDZ1. Extraction using petroleum ether and n-hexane has shown less variation in terms of oil yield (Figure 1A). It is interesting to note here that the time consumed for the separation of oil from the petroleum ether was also less as compared to n-hexane and it holds true for both the varieties. Total oil content of the seeds ranged from 25.15±0.35 for PM21 while 37.01±0.28 for PDZ1 using petroleum ether as extraction solvent. Our results are in line with earlier reports in *Brassica* [18].

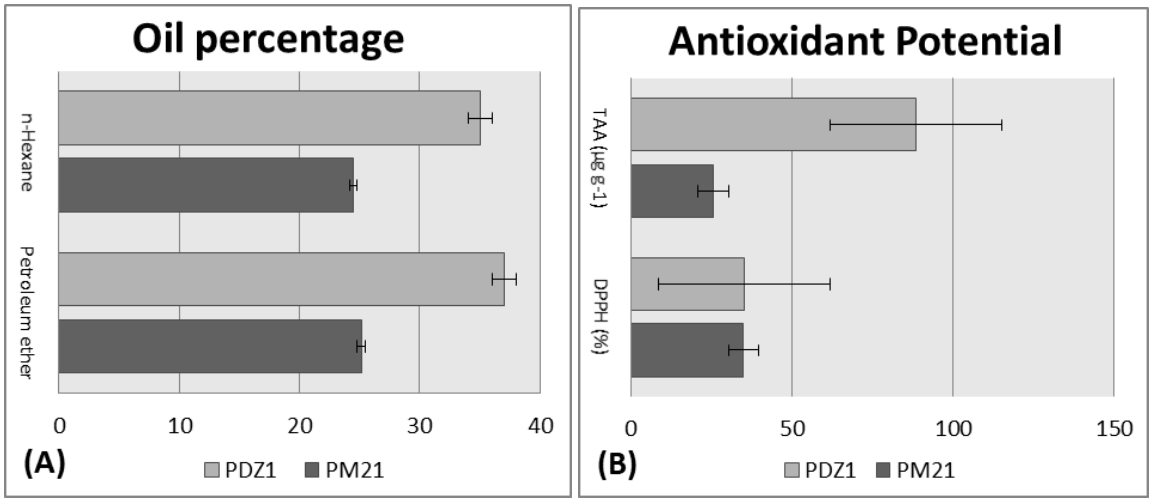

Fig 1: A-Oil percentage of PM21 and PDZ1 variety extracted from n-hexane and petroleum ether; B- Antioxidant potential of two aforesaid varieties measuring TAA (µg g^{-1}) and DPPH activity (%). Each value is represented as the mean ± SE.

DPPH analysis is one of the simple and most commonly employed method for analyzing antioxidant capacity [19]. We have found that solvent type also influences the DPPH scavenging activity in Indian mustard varieties (Figure 1B). Performance of both the varieties was found to be at par in terms of radical scavenging potential. It is clear from the results that PDZ1 have shown a little better DPPH activity (35.18±7.71) as compared to PM21 (35.04±8.19). This variability can be explained by the influence of genetic, environmental, agronomic and extraction procedural factors, which would affect the level of antioxidants [20]. Perhaps, regardless of the variety, the 80% methanol extract exhibited significant DPPH radical scavenging activity though reports are there where researchers have used 70% ethanol, 50% acetone, and absolute ethanol under the same experimental conditions [19]. This indicates that extraction procedures may alter the overall effectiveness of antioxidant capacity.

The effect of extraction solvent on total antioxidant activity was also evaluated. The antioxidants in defatted Indian mustard seed extracts acts as effective electron\H^+ donor and this is responsible for the antioxidant capacity. A marked significant increase in TAA has been observed in PDZ1 (88.43µg g^{-1}) as compared to PM21 (25.60µg g^{-1}). It may be noted that the PM21 in which the DPPH activity was lowered also showed concomitant reduction in TAA (Figure 1B). This is evident that genotypic expression plays pivotal role in determining antioxidant potential. Also, increase in TAA with increasing polarity of extracting solvent also adds to the stability of the bioactive compounds present in the oilseeds [20-22].

Conclusion

In summary, this study revealed the potential methodology and effects of extracting solvent on oil per cent yield and antioxidant activity for Indian mustard varieties. Petroleum ether (v/v) is the promising solvent for oil extraction with concomitant increased antioxidant potential of extracts from double low PDZ1 for routine analytical work. Moreover, a reasonable cost of the soxhlet method suggests the worthiness of this methodology as an applicable procedure which can be implemented in the industries based on oilseeds. Knowing the fact that consumption of double low Indian mustard varieties may reduce risk of chronic diseases and/or promote general human health, this study would be informative for the stakeholders linked to this particular oilseed industry. Thus, double low varieties have good potential for their use in future as a food or nutraceutical supplement formulation. Additional research is required to investigate the influence of extraction solvent on the stability of chemical composition with better shelf life and digestibility of edible oil (in progress).

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