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COMPARATIVE STUDY OF PHARMACOGNOSTIC PARAMETERS, ANTIOXIDANT AND ANTICHOLINESTERASE POTENTIAL OF *BRASSICA NAPUS* AND *BRASSICA OLERACEA* VAR. *ACEPHALA*

S. Kaur * and R. Shri

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala - 147002, Punjab, India.

Keywords:

Brassica species, Pharmacognostic characters, Phytochemical profiles, Antioxidant and Antiacetylcholinesterase activities

Correspondence to Author:
Sanimardeep Kaur

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala - 147002, Punjab, India.

E-mail: nijjarsanimar@yahoo.co.in

ABSTRACT: Introduction: Green leafy vegetables are known for their anti-alzheimer potential. There are many leafy vegetables, however, that have not been examined scientifically for this activity. The present study was designed to evaluate antioxidant and antiacetylcholinesterase activities of two *Brassica species* namely *B. napus* and *B. oleracea* var. *acephala*, along with the comparison of their pharmacognostic characters and phytochemical profiles. **Methods:** A comparative evaluation of macroscopy, microscopy, physico-chemical parameters and qualitative phytochemical screening was carried out on leaves of the selected plants as per Indian Pharmacopoeia. Further, a parallel analysis of antioxidant and anti-acetylcholinesterase activities of both species was done using DPPH and Ellman assay, respectively, followed by standardisation of the extracts on the basis of total phenol and total flavonoid content. **Results:** The hydromethanolic extract of *B. oleracea* var. *acephala* showed higher radical scavenging activity than that of *B. napus*. Similar results were obtained in case of total phenol content (*B. oleracea* var. *acephala*: 15.18 ± 1.82% w/w, *B. napus*: 12.69 ± 2.26% w/w). The hydromethanolic extract of *B. napus* showed higher acetylcholinesterase inhibitory activity than *B. oleracea* var. *acephala*. **Conclusion:** The pharmacognostic profiles of the two *Brassica species* generated in the present study could assist in proper identification, collection and investigation of the plant material in future. Both plants have good antioxidant effect but *B. napus* showed significant antiacetylcholinesterase activity and it could be investigated for anti-alzheimer potential.

INTRODUCTION: Alzheimer's disease (AD) is the most common cause of dementia leading to a slow progressive and irretrievable ruination of mental health [1]. Modern treatment strategies comprise of anticholinesterases, antioxidants, α- and β-secretase inhibitors, N-methyl-D-aspartate receptor antagonists, *etc*. [2, 3].

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In light of side effects of current medications, there is an increasing trend to explore plants / phyto-constituents for management of dementia of alzheimer's type.

Literature shows that consumption of green leafy vegetables prevents the onset as well as progression of AD [4]. Hence, in the present study anti-acetylcholinesterase and antioxidant potential of two common leafy vegetables namely *B. napus* and *B. oleracea* var. *acephala* (Family Brassicaceae) was examined. Further standardisation of the extracts was done on the basis of total phenol and total flavonoid contents. In spite of the numerous medicinal uses attributed to these *Brassica species*

[5, 6, 7, 8], there are no records of pharmacognostic standards of these plants that are required for quality control of the crude drug. Hence, in the present study, a comparative evaluation of macroscopy, microscopy, physicochemical parameters and qualitative phytochemical screening was also carried out on leaves of two *Brassica species* with a view to establish standards for their identity, quality, purity and chemical composition.

MATERIALS AND METHODS:

Cultivation and Collection of Plant Material: Seeds of *B. napus* and *B. oleracea* var. *acephala* were procured from Green My Life Nursery (Receipt no: 4216) and sown in Punjabi University Patiala campus in last week of October 2014 and the leaves were collected during the month of January 2015.

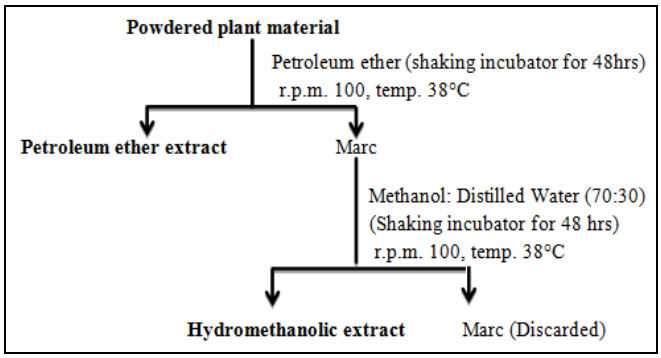
Macroscopic Evaluation: The various macroscopic features of the fresh leaves like size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour, taste *etc*. were evaluated [9, 10].

Microscopic Evaluation: Transverse sections of fresh leaves and ground powders were observed under a microscope to determine the anatomical characteristics. Various tissues were distinguished by differential staining technique. Quantitative leaf microscopy to determine palisade ratio, stomatal number, stomatal index, vein-islet number and veinlet termination number was performed on the epidermal strips [9, 11].

Physicochemical Evaluation: Various parameters *i.e*. foreign organic matter, moisture content, extractive values and ash values were evaluated for identity, purity and strength according to IP, 1997 [12]. All the readings were taken in triplicate.

Chemicals: Acetylthiocholine iodide (ATCI), acetylcholinesterase (AChE) from electric eel (Type VI-S lyophilized powder), 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (Steinheim, Germany). All other chemicals, solvents and buffers were of analytical grade.

Preparation of Extracts: Leaves were dried in shade and reduced to a coarse powder. Two extracts (*viz.* petroleum ether and hydromethanolic) were prepared from dried powdered plant material (150 g) by successive exhaustive extraction. Preparation of different extracts was done according to the given **Scheme 1**.



SCHEME 1: PREPARATION OF EXTRACTS

Each extract was concentrated on rotatory vacuum evaporator. Extracts were weighed and percentage yield was calculated in terms of the air dried weight of the plant material.

Phytochemical Evaluation: Extracts were subjected to various chemical tests to assay for the presence of phytoconstituents such as alkaloids, tannins, flavonoids, triterpenoids, sterols, saponins etc. using standard experimental procedures [10, 13, 14].

Standardisation of Extracts:

Total Polyphenol Content Analysis: Total polyphenolic compounds of the hydromethanolic extracts were determined by Folin-Ciocalteau procedure [15].

Total Flavonoid Content Analysis: Flavonoid contents were determined according to the method of Madaan *et al*. [15]

Antioxidant Activity: The antioxidant potential was evaluated using 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay [16, 17]. The DPPH radical- scavenging activity in terms of percentage was calculated according to the following equation:

DPPH scavenging activity (%) = {1- (Abs sample / Abs DPPH solution)} × 100

***In-vitro* Acetylcholinesterase Inhibitory Activity:** Acetylcholinesterase inhibition by hydromethanolic extracts was determined spectrophotometrically by modified Ellman's method using a 96-well microplate assay [18]. Percentage enzyme inhibition was calculated by the following formula:

Percentage of enzyme inhibition = Absorbance of control – Absorbance of sample / Absorbance of control × 100

Analyses were run in triplicate. IC_{50} values were obtained by plotting the percentage inhibition against the extract concentrations.

Statistics: The results have been expressed as mean ± standard deviation (SD). The test extracts were compared with standard drug by one way analysis of variance (ANOVA) followed by Student Newman Keul's test as post hoc analysis.

RESULTS AND DISCUSSION:

Macroscopic Evaluation: According to World Health Organization (WHO) the first step towards establishing the identity and purity of a medicinal plant is the macroscopic and microscopic evaluation. Organoleptic evaluation is a qualitative technique based on the study of morphological and sensory features of whole drugs [19]. Various organoleptic features of the leaves of *B. napus* and *B. oleracea* var. *acephala* **Fig. 1** and **2** were observed and results are presented below in **Table 1**.

FIG. 1: *B. NAPUS* LEAVES

FIG. 2: *B. OLERACEA* VAR. *ACEPHALA* LEAVES

TABLE 1: ORGANOLEPTIC CHARACTERS OF *B. NAPUS* AND *B. OLERACEA* VAR. *ACEPHALA* LEAVES

S. no.	Character	*B. napus*	*B. oleracea* var. *acephala*
1	Colour	Green	Dark green
2	Odour	Characteristic	Characteristic
3	Taste	Characteristic (cabbage like and bit peppery)	Characteristic (slight bitter tinge)
4	Surface	Smooth	Smooth with a waxy covering
5	Size	Length: 15-30 cm, Width: 4-10 cm	Length: 20-30 cm, Width: 5-11 cm
6	Apex	Obtuse	Obtuse
7	Base	Obtuse	Obtuse
8	Margin	Lobed, lobes are generally separated towards the base of the leaf. The terminal lobe is largest with a rounded tip	Undulate
9	Type	Simple	Simple
10	Venation	Reticulate	Reticulate

The findings are in accordance with the available literature but detailed examination regarding the macroscopic features of leaves of *B. napus* and *B. oleracea* var. *acephala* has not been carried out in the previous studies [20].

Microscopic Evaluation: Microscopic evaluation is one of the simplest and cheapest methods to establish the identity of plant materials. It is mostly used for qualitative evaluation of organized crude drugs in entire and powder forms with help of microscope [11, 19, 21].

The microscopic characters of the leaves of *B. napus* and *B. oleracea* var. *acephala* have not been evaluated to the best of our knowledge.

Transverse Section of Leaves: Free hand sections of fresh leaves were cut and photographs were taken. The results are presented in **Fig. 3** and **4**.

***B. napus*- Transverse Section of Leaf Showed:**

- Single layered epidermis
- Collenchymatous cells
- Vascular bundles
- Spongy parenchyma

***B. oleracea* var. *acephala* - Transverse Section of Leaf Showed:**

- Single layered epidermis
- Vascular bundles containing xylem and phloem
- Palisade cells and spongy tissue

FIG. 3: TRANSVERSE SECTION OF *B. NAPUS* LEAF

FIG. 4: TRANSVERSE SECTION OF *B. OLERACEA* VAR. *ACEPHALA* LEAF

Determination of Leaf Constants: Leaf constants of both the plants were determined and results are presented in **Table 2**.

TABLE 2: LEAF CONSTANTS OF *B. NAPUS* AND *B. OLERACEA* VAR. *ACEPHALA*

S. no.	Parameter	Value per sq. mm (Mean^n)	
		B. napus	***B. oleracea* var. *acephala***
1	Stomatal number	Upper surface: 14 Lower surface: 16	Upper surface:19 Lower surface: 22
2	Stomatal index	Upper surface: 9 Lower surface: 12	Upper surface: 10 Lower surface: 16
3	Palisade ratio	6.7	8.2
4	Vein-islet number	4.5	6.7
5	Veinlet termination number	3.8	4.2

n= 10

Powder Microscopy:

***B. napus*:** Following diagnostic characters of powdered material of *B. napus* were observed **Fig. 5a**, **b**, **c**:

- Colour: Green
- Odour: Characteristic
- Taste: Characteristic
- Stomata: Anisocytic and anomocytic
- Unicellular covering trichomes
- Thin walled fibres
- Irregular shaped calcium oxalate crystals

***B. oleracea* var. *acephala*:** Following diagnostic characters of powdered material of *B. oleracea* var. *acephala* were observed **Fig. 6a**, **b**, **c**:

- Colour: Dark green
- Odour: Specific
- Taste: Characteristic
- Stomata: Anisocytic
- Unicellular covering trichomes
- Spiral vessels
- Leaf fragment with veins and veinlets
- Calcium oxalate crystals

Anisocytic and anomocytic stomata | **Unicellular covering trichomes** | **Calcium oxalate crystals**

FIG. 5: DIAGNOSTIC MISCROSCOPIC FEATURES OF POWDERED *BRASSICA NAPUS* LEAVES

Anisocytic stomata **Unicellular covering trichomes** **Calcium oxalate crystals**

FIG. 6: DIAGNOSTIC MICROSCOPIC FEATURES OF *POWDERED BRASSICA OLERACEA* VAR. *ACEPHALA* LEAVES

Physicochemical Evaluation: The evaluation of physicochemical parameters helps to determine the identity, purity and quality. Extractive values give an idea about the nature of the chemical constituents present in the plant material. Since the water soluble extractive value was found to be higher than ethanol soluble extractives in both the plants, this indicates that the concentration of polar compounds may be high in *B. napus* and *B. oleracea* var. *acephala.* Ash content of a drug provides information regarding various impurities like carbonates, oxalates and silicates present in the plant material [11, 20, 22].

The water soluble ash provides information about the amount of inorganic compounds present in herbal drugs while acid insoluble ash gives an idea about the amount of silica present in the form of earthy matter [23]. The results of this study **Table 3** reveal a high level of ash values, foreign matter and moisture content in case of *B. oleracea* var. *acephala* while *B. napus* has higher values of water soluble and ethanol soluble extractives.

TABLE 3: PHYSICOCHEMICAL PARAMETERS OF *B. NAPUS* AND *B. OLERACEA* VAR. *ACEPHALA*

Parameter	Mean^{n}±S.D. (% w/w air dried plant material)	
	B. napus	***B. oleracea* var. *acephala***
Foreign organic matter	1.42 ± 0.01	1.8 ± 0.06
Loss on drying	18.78 ± 0.67	20.31 ± 0.74
Ethanol soluble extractives	4.79 ± 0.19	4.4 ± 0.34
Water soluble extractives	14.17 ± 0.21	12.19 ± 0.23
Total ash	8.95 ± 0.17	13.18 ± 0.46
Acid insoluble ash	1.30 ± 0.10	2.61 ± 0.05

n=3

Preparation of Extracts: Petroleum ether and hydromethanolic (70:30) extracts of *B. oleracea* var. *acephala* and *B. napus* were prepared. Following yields of extracts were obtained **Table 4**.

Phytochemical Screening: The results of phytochemical screening of the prepared extracts are given in **Table 5**. The results revealed some differences in the constituents of the two species studied. The results are in accordance with the earlier reports [24, 25].

TABLE 4: PERCENTAGE YIELD AND ORGANOLEPTIC PROPERTIES OF EXTRACTS OF *B. NAPUS* AND *B. OLERACEA* VAR. *ACEPHALA*

Parameter	***B. napus***		***B. oleracea* var. *acephala***	
	PE	**HME**	**PE**	**HME**
Yield*	5.50	20.37	2.74	17.85
Colour	Olive green	Reddish brown	Henna green	Greenish black
Odour	Characteristic	Characteristic	Odourless	Mild characteristic
Consistency	Solid	Semisolid	Solid	Semisolid and sticky

*Yield–% w/w, dry weight basis; PE= Petroleum ether extract; HME= Hydromethanolic extract

TABLE 5: PHYTOCHEMICAL SCREENING OF PLANT EXTRACTS

Constituents	***B. napus***		***B. oleracea* var. *acephala***	
	PE	**HME**	**PE**	**HME**
Carbohydrates	-	+	-	+
Proteins	-	+	-	+
Alkaloids	-	+	-	+
Glycosides	-	+	-	+
Saponins	-	-	-	-
Tannins	-	+	-	+
Triterpenoids	+	+	+	+
Steroids	-	-	+	+
Flavonoids	-	+	-	+

PE= Petroleum ether extract; HME= Hydromethanolic extract + Presence of phytoconstituent, - Absence of phytoconstituent

Antioxidant Activity: The free radical scavenging activities of the plant extracts were assessed by DPPH assay **Table 6**. DPPH free radical method is an easy, rapid and sensitive way to explore the antioxidant potential of plant extracts [17]. The hydromethanolic extracts have higher radical scavenging activities than petroleum ether extracts. Hence these were investigated further.

TABLE 6: DPPH FREE RADICAL SCAVENGING ACTIVITIES OF THE TEST EXTRACTS

Plant / Chemical	IC_{50} (µg/ml) (Mean^n ± S.D.)	
	Petroleum ether	Hydromethanolic
B. napus	4044.43 ± 0.61	201.80 ± 0.61
B. oleracea var. *acephala*	3029.61 ± 0.29	171.7 ± 0.97
Ascorbic acid	4.25 ± 0.36	

n=3

Standardisation of Bioactive Extracts:

Estimation of Phenol and Flavonoid Content in Hydromethanolic Extracts: The total phenol and flavonoid content of all the plant extracts are shown in **Table 7**.

TABLE 7: TOTAL PHENOL AND FLAVONOID CONTENT OF HYDROMETHANOLIC EXTRACTS

Plant	Total Phenol Content (% w/w) (Mean^n ± SD)	Flavonoid Content (% w/w) (Mean^n ± SD)
B. napus	12.69 ± 2.26	3.89 ±1.95
B. oleracea var. *acephala*	15.18 ± 1.82	1.96 ± 0.52

n=3

Hydromethanolic extract of *B. oleracea* var. *acephala* has higher total phenolic content in comparison with *B. napus* extract.

***In-vitro* Acetylcholinesterase Inhibitory Activity:** Acetylcholinesterase inhibitory activity of the hydromethanolic extracts of *B. napus* and *B. oleracea* var. *acephala* was analysed by Ellman method using donepezil as a standard **Table 8**.

TABLE 8: ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF HYDROMETHANOLIC EXTRACTS

Plant	IC_{50} value (mg/ml) (Mean^n ± S.D.)
B. napus	257.73 ± 2.21*
B. oleracea var. *acephala*	595.23 ± 2.80*
Donepezil	7.25 ± 0.19 µg/ml

n=3. The data was expressed as Mean ± S.D.; *p<0.05 vs. donepezil; one way ANOVA followed by Student Newman Keul's test.

Amongst the two plants, the hydromethanolic extract of *Brassica napus* showed higher activity (indicated by lower IC_{50} values) than *B. oleracea* var. *acephala*.

CONCLUSION: *B. napus* and *B. oleracea* var. *acephala* have high culinary value and are known to have wide array of pharmacological activities. The results of the pharmacognostic evaluation in the present study could assist in proper identification, collection and investigation of the plant material in future.

The present study shows that hydro-methanolic extracts of both species have antioxidant and acetylcholinesterase inhibitory activity. Of the two species *B. napus* showed marked acetyl-cholinesterase inhibitory activity and it could be investigated for anti-alzheimer potential.

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CONFLICT OF INTEREST: Nil

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