Annals of Biology **39** (2) : 321-325, 2023

Impact Score : 0.32 (Scopus)

GC-MS Analysis and Antimicrobial Potential of Seed Oil Derived from *Brassica nigra*

VIJAY PAL*, KANTA RANI[1], RINKLE[1] AND AARTI CHAUDHARY[2]

Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur-300 202 (Rajasthan), India
**(e-mail: Gujjarvijay66@gmail.com; Mobile: 94669 31266)*

(Received: March 11, 2023; Accepted: April 18, 2023)

ABSTRACT

The purpose of this investigation was to examine the antibacterial activity and fatty acid analysis of oil derived from *Brassica nigra*. The seed oil was analyzed using GC-MS/FID, and the results showed that it contained a variety of fatty acids, such aserucic acid (46.40), linoleic acid (16.34), alpha linolenic acid (11.32), oleic acid (9.12), ecosenoic acid (5.42) and palmitic acid (2.63). Research was also done to investigate the antibacterial activity of the compound against a variety of bacterial and fungal species, when employed in concentrations of 1 µl, the oil showed minimum inhibition zone of 5 mm against the bacteria, *Escherichia coli* and 3 mm *Serratia marcenscens*. Similarly, utilized in concentrations of 1 µl for antifungal action 5 mm of minimum inhibition zone against *Aspergillus niger* and 2 mm against *Penicillium digitatum*. These findings impled that *Brassica nigra* seed oil had the potential to be used in the food and pharmaceutical industries, and the oil generated from *Brassica nigra* may be a promising natural antibacterial agent.

Key words: *Brassica nigra*, seed oil, fatty acid analysis, antibacterial, antifungal

INTRODUCTION

The seed oil obtained from certain plants is rich in fatty acids. Along with these fatty acids, the presence of many non-polar substances has also been recorded. The fatty acids, their derivatives and other active compounds have been explored for therapeutic purposes, especially against microbes, including fungi and bacteria, about 80% of the world population uses traditional medicines (Pal *et al.*, 2022). Brassicaceae family includes 340 genera and 3350 species mainly distributed in north temperate zone and has many economical edible and ornamental plants (Singh, 2019). It is commonly known as mustard family due to presence of sulfur compound glucosinolate, phenols and tocopherols (Avato and Argentieri, 2015). *Brassica nigra* is a very important crop plant known for its seed oil, medicinal value, nutritional value and seed oilcake. The seed primarily contains oligosaccharides, amino acids, fatty acid, vitamins, minerals and some anti-nutritional factors, antidiabetic, anticonvulsant, antithrombotic, antifungal, antibacterial and antioxidant activity (Okunade *et al.*, 2015; Krishnaveni and Suranya, 2016).

The seed oil of *B. nigra* shows antioxidant and antimicrobial property against *Bacillus cereus, Salmonella* and *Staphylococcus aureus* (Amare *et al.*, 2015). *Jatropha curcus* seed oil synergism in combination with commercially available antibiotics like ofloxacin, ciprofloxacin, moxifloxacin, cefotaxime and rifampicin, rifampicin had strong synergistic effects in combination with methanolic extract against *Acinetobacter baumannii, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Enterococcus faecalis* and *Staphylococcus aureus* (Haq *et al.,* 2019). In the present study, the fatty acid analysis of seed oil of *B. nigra* was performed by using Gas chromatography and Mass spectrophotometry (GC-MS) and its antimicrobial properties were studied against *E. coli, S. marcenscens, A. niger* and *P. digitatum.*

MATERIALS AND METHODS

Brassica nigra seeds were collected from field of Gurgaon region, Haryana and identified from GGDSD College Palwal, Haryana. Seed oil was

[1]Department of Botany, GGDSD College, Palwal-121 102 (Haryana), India.
[2]Department of Botany, Raja Balwant Singh College, Dr. Bhimrao Ambedkar University, Agra-282 002 (Uttar Pradesh), India.

extracted by cold press method and stored at room temperature till further studies.

For fatty acid analysis GC FID No Trace 1300 with analytical column ZB FAME 30 × 0.25 m MID×0.24 µm was used. The oil was converted to fatty acid methyl ester (FAME) and 1 µl of solution was injected in inlet column with the help of syringe. Hydrogen gas was used as carrier gas and the flow rate was 1.2 µl/min. The initial temperature of inlet column was 60°. The rise in temperature was 5°/min, final temperature was 260° and FID detector temperature was 280° with 2 min of hold time.

Antimicrobial property of seed oil was studied by CLSI Performance Standards for Antimicrobial Disk Susceptibility against *E. coli, S. marcenscens, A. niger* and *P. digitatum.* Muller Hinton Agar medium was used for antibacterial activity by disk/well diffusion susceptibility method. Cultured bacterial and fungal samplers were mixed with peptone water to make 0.5 McFarland Turbidity standards. Culture sample was swabbed on Muller Hinton agar surface and wells were created with the help of sterile tips. 1 µl of each treatment (oil, methanol and standard antibiotics) was loaded into respective well. The cultures plates were incubated at 37°C for 24 h.

Sabouraud Dextrose Agar medium was used for antifungal activity by well diffusion method. Cultures were mixed with peptone water to make 0.5 McFarland Turbidity standards. Culture was swabbed on Sabouraud Dextrose Agar surface and wells were created with the help of sterile tips. One µl of each test sample (oil, methanol and standard antibiotics) was loaded into respective well and incubated at 37°C for 24 h.

Different phytochemicals were estimated as detiled below. For tannins few drops of 1% lead acetate were added to 5 ml of the oil extract in a test tube, a yellow precipitate indicated the presence of tannins. For saponins: the oil extract was diluted with 2 ml of distilled water and it was agitated in a test tube for about 15 min. The formation of 0.1 cm layer of foam showed the presence of saponins. For flavonoids: few drops of dilute sodium hydroxide were added to 1 ml of the oil extract in a test tube. An intense yellow colour was formed which turned colourless on addition of few drops of dilute acid indicating the presence of flavonoids. For alkaloids: few drops of potassium iodide were mixed in oil extract. Reddish brown precipitate showed positive results. For steroids: 10 ml of chloroform was added to 1 ml of oil extract in a test tube. Equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turned red, whereas the sulphuric acid layer turned yellow with green. This indicated the presence of steroids. For carbohydrate: added a few drops of alcoholic alpha napthol solution to the extract and a few drops of concentrated sulphuric acid along the sides of the test tube. Positive result gave purple or violet coloured ring at the junction. For proteins: bluish violet colour formed when a solution of ninhydrin reagent and extract mixture was heated indicated the presence of proteins.

RESULTS AND DISCUSSION

B. nigra seeds oil GC-MS analysis showed that oil consisted of 63.26% monounsaturated fatty acids, 30.12% polyunsaturated and 6.62% saturated fatty acids. The maximum amount of fatty acid was erucic acid (46.40%) which was followed by linoleic acid (16.34%), alpha linolenic acid (11.32%), oleic acid (9.12%), ecosenoic acid (5.42%), palmitic acid (2.63%) and trace amounts of stearic acid, arachidic acid, behenic acid, lignoceric acid palmitoleic acid, nervonic acid, eicosadienoic acid and docosadienoic acid (Table 1). Seven major fatty acids extracted from *B. nigra* were found to be palmitic, stearic, oleic, linoleic, linolenic, eicosanoic and erucic acids. According to Krishnaveni and Saranya (2016) 12 different type of phytochemicals were reported, highest peak was observed for octadecadienoic acid followed by erucic acid; this difference may be due to method of detection or environmental conditions.

B. nigra seed oil was tested for its antimicrobial properties against *E. coli, S. marcenscens, A. niger* and *P. digitatum* [(Fig. 1 (A, B, C and D)]. *E. coli* and *P. digitatum* showed a maximum inhibitory zone of 5 mm. *S. marcenscens* exhibited a 3 mm of inhibition zone, while *A. niger* exhibited a minimum 2 mm inhibition. The antibiotic amoxicillin potassium clavulanate was used as a positive control in contrast to methanol, which was used as a negative control. Amoxicillin potassium clavulanate exhibited a maximum 10 mm of inhibition, as indicated in Table 2. Similar

Table 1. Fatty acid profile of *Brassica nigra*

S. No.	No. of carbon atom	Fatty acid	% area
1.	C16:0	Palmitic acid	2.63
2.	C18:0	Stearic acid	1.35
3.	C20:0	Arachidic acid	0.95
4.	C22:0	Behenic acid	1.09
5.	C24:0	Lignoceric acid	0.62
6.	C16:1	Palmitoleic acid	0.18
7.	C20:1n9	Eicosenoic acid	5.42
8.	C18:1n9c	Oleic acid	9.12
9.	C22:1n9	Erucic acid	46.40
10.	C24:1n9	Nervonic acid	2.14
11.	C18:2n6c	Linoleic acid	16.34
12.	C20:2	Eicosadienoic acid	0.94
13.	C22:2	Docosadienoic acid	1.51
14.	C18:3n3	Alpha linolenic acid	11.32
15.	Total saturated fatty acid		6.62
16.	Total mono unsaturated fatty acid		63.26
17.	Total poly unsaturated fatty acid		30.12

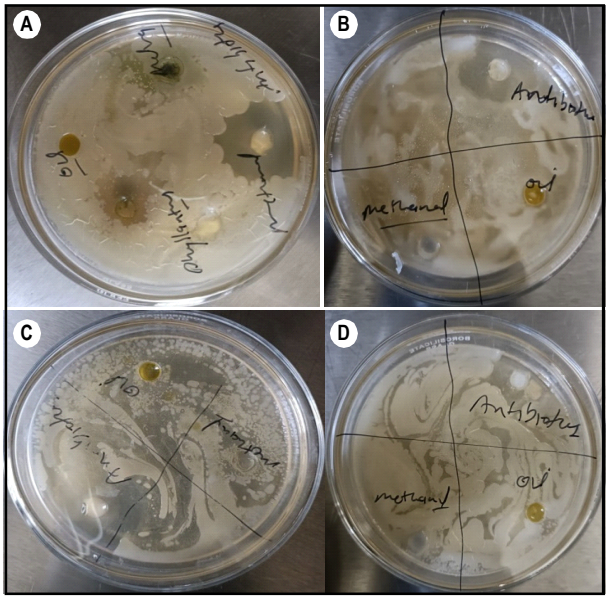

Fig. 1. A and B showing antifungal activity of *Brassica nigra* seed oil against *Aspergillus niger* and *Penicellium digitatum*. C and D showing antibacterial activity against *Serratia marcescens* and *Escherichia coli*. Methanol used as negative and amoxicillin potassium clavulanate used as positive control.

findings were observed by Azeem *et al.* (2021) from the seed oil from *Atropa belladonna* and *Thymus linearis*, which showed antibacterial properties against *Pseudomonas aeruginosa, S. marcescens* and *E. coli.* and *Klebesiella pneumonia. Passiflora edulis* var. Flavicarpa seed oil also showed antimicrobial potential against the bacteria strains Gram-positive *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778) and Gram-negative *E. coli* (ATCC 25922) and *Salmonella enteritidis* (ATCC 13076) as reported by Pereira *et al.* (2019).

In order to manage and cure adrenoleukodystrophy (a rare genetic disorder that produced too much very long fatty acid which destroy the myelin sheath of neuron if accumulate in brain) without causing cardio-toxicity, erucic acid was combined with oleic acid (Kumar and Sharma, 2022). According to Blondeau *et al.* (2015) alpha linolenic acid was a possible nutraceutical that could reduce inflammation and increase neuroplasticity and also protect the brain from stroke. The treatment of demyelinating disorders, prevention of mortality from cardiovascular diseases, and

Table 2. Antimicrobial property of seed oil of *Brassica nigra*

S. No.	Compound	*Escherichia coli* (mm)	*Serratia marcescens* (mm)	*Penicellium digitatum* (mm)	*Aspergillus niger* (mm)
1.	*Brassica nigra* seed oil	5	3	5	2
2.	Amoxicillin and potassium clavulanate (positive control)	10	10	10	10
3.	Methanol (negative control)	00	00	00	00

the diagnosis of chronic renal disease also involved the use of nerveonic acid (Li *et al.*, 2019). According to Ghavam *et al.* (2021) oleic acid had anti-inflammation, anticancer, wound-healing, antifungal and antibacterial capabilities against different harmful microorganisms. According to Shreekumar *et al.* (2014) oleic acid was used as a preservative and a hypocholerolemic, anti-inflammatory, anti-androgenic and anticancer agent. The combination of n-hexadecanoic acid and palmiticacid showed anti-inflammatory function and could be used tropical, indirectly validate rigorous use of medicated oil for treatment of rheumatic symptoms in the traditional medical system of India.

B. nigra seeds contained fatty acids such as palmitic, stearic, oleic, linoleic, linolenic, eicosenoic, erucic acids, vitamins, minerals, glucosinolates and a wide range of phenolic compounds with antidiabetic, anticonvulsant, antithrombotic, antibacterial, antifungal and antioxidant potential (Table 3). It also provided protection against factors leading to gastrointestinal cancer (De Zoysa and Waisundara, 2021). According to Nazari *et al.* (2020) *B. nigra* seed oil slowed down memory degradation in rats by producing Beta amyloids (causing diseases like Alzheimer) in their brains. *B. nigra* seed oil is used as lubricants (hydraulic oil) or in the field of oleo chemistry and has been preferred in biodiesel production in some countries of the World (Aslan and Eryilmaz, 2020).

Table 3. Phytochemical analysis of seed oil of *Brassica nigra*

S. No.	Compounds	Results
1.	Saponins	+
2.	Tannins	+
3.	Alkaloids	+
4.	Flavonoids	+
5.	Steroids	+
6.	Carbohydrates	+
7.	Proteins	+

CONCLUSION

GC-MS analysis of *Brassica nigra* seed oil showed 13 different type of fatty acids, out of which maximum amount was erucic acid followed by linoleic acid, alpha linolenic acid, oleic acid. nerveonic acid which can be used to treat demyelinating disorder, and erucic acid with alpha linolenic acid having nutraceutical properties. Oleic acid may reduce inflammation, anticancer and wound healing potential. Seed oil had potential as an antimicrobial agent and shows minimum inhibition zone of 5 mm against *E. coli* and *P. digitatum;* similarly 3 and 2 mm against *S. marcenscens* and *A. niger,* respectively.

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