

Chemical constituents of Brassica juncea seed oil and its larvicidal activity against the malaria vector of Anopheles stephensi (Diptera: Culicidae)

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ABSTRACT

Chemical constituents of *Brassica juncea* (Mustard) seed oil and larvicidal activity against the malarial vector of *Anopheles stephensi* (Diptera: Culicidae) was studied. The essential oil was extracted by using clevenger apparatus and analyzed for chemical constituents by GC-MS and FTIR. Larvicidal activity against *An. stephensi* was recorded 24 hours after exposure. The LC₅₀ and LC₉₀ values for mosquito larvae were calculated by profit analysis. The GC-MS analysis showed that the essential oil contained 6 different components, which were identified as 2,3-methyl-tetracosanoic acid (12.47%), 2,4,6-cycloheptatrien-1-one, 3,5... (12.75%), octasiloxane, 1,1,3,3,5,7,7,9 (8.34%), Indole-2-one, 2,3-dihydro-N-hyde... (13.54%), silane, trimethyl [5-methyl-2-(1-... (20.99%), and 1,5-methyl-2-phenylindolizine(10.52%). The resonance assignment, intensity and wave number of the main peak were obtained from the absorption spectrum. Various functional groups such as alcohol, ester, carboxylic acid, halo compounds, alkenes etc. were identified by FTIR. The essential oil obtained from the seeds of *B. juncea* indicates good toxicity to *An. stephensi* with LC₅₀ and LC₉₀ values of 66.97 ppm and 78. 60 ppm, respectively. It also showed significant larvicidal activity against *An. stephensi*. It is helpful in creating practical, affordable, eco-friendly, and region-specific methods to control the malaria vector of *An. stephensi*.

Keywords: *Anopheles stephensi*, *Brassica juncea*, GC-MS, FTIR, Larvicidal activity and Mustard seed oil extract

1. INTRODUCTION

Mosquitoes are the cause of several human health issues that lead to disease and death in both adults and children worldwide. In tropical and subtropical countries, they spread a variety of diseases, such as chikungunya, dengue, Japanese encephalitis, malaria, and west Nile virus infection (Baranitharan et al., 2022). Malaria is a life-threatening disease around 3.3 billion people worldwide. In 2013, there were around 198 million malaria infections and 0.58 million fatalities worldwide (Dhanasekaran et al., 2022a). *An. stephensi* is the primary mosquito vector that spreads malaria in Indian cities out of the six species that carry the disease (Prabakaran et al., 2024). Effective management of these illnesses would need a two-pronged approach: (i) timely administration of potent medications and (ii) preventative measures based on vector control. In locations where malaria is endemic, the malarial parasite has developed resistance as a result of the careless use of medications, especially antimalarial ones (Baranitharan et al., 2017). Therefore, mosquito management provides an effective and practical option to control the diseases they cause. Mosquito repellents are good for temporarily reducing mosquito populations, but they are not a permanent solution. Mosquito larvae because they are light weighted, reduced mobility, and most concentrated in their natural habitat; provide a simpler and most efficient point of intervention and control (Baranitharan et al., 2015). However, there are reports of adult mosquitoes and larvae developing resistance and behavioral changes to these chemicals (WHO 2006). In addition, they negatively impact the environment, causing soil, air and water pollution and harming beneficial non-target organisms (Baranitharan et al., 2019). Plant extracts, including pesticides or essential oils derived from plant origin, are attractive options because they contain large quantities of a variety of biologically active compounds, many of which are selective and non-target organisms and have little or no harmful effects on the environment (Gokulakrishnan et al., 2016, Baranitharan et al., 2020, Jebanesan et al., 2020).

Essential oils are naturally occurring volatile substances found in many plants. Essential oils isolated from plants are usually mixtures of several components, mainly biologically active monoterpenes (Deepa et al., 2015). Traditionally, they have been used to enhance the taste of food and in perfumery, pharmaceutical and confectionery industries as flavouring agents, medicines, etc. (Dhanasekaran et al., 2022b). Recently, they have received great attention as human and eco - friendly biopesticides. There is a lot of information available on the larvicidal activity of essential oils obtained from neem, basil, citronella, lemon, eucalyptus, cypress and others (Baranitharan et al., 2016, Krishnappa et al., 2020, Irrusappan et al., 2022). There have been no reports of resistance to plant-derived pesticides so far (Kannathasan et al., 2011). Additionally, Essential oils with multiple active ingredients may act differently on insects or have the potential to target different areas and reduce mosquito populations (Ganesan et al., 2023). The essential oil of this plant can reduce mosquito populations and serve as an organic alternative or complement to conventional insecticides. The insecticidal effects of essential oils and their ability to boost insecticide efficacy are documented (O'Neal et al., 2019). Mustard seeds (*Brassicaceae*) of commercial value include brown (*Brassica juncea*), black (*Brassica nigra*), and yellow/white (*Brassica alba*) (Mejia-Caribay et al., 2015). *Brassica* extracts and seed meal act as eco-friendly repellents and larvicides (Flor-Weyler et al., 2023).

2. MATERIALS AND METHODS

Study area and collection of oil extract

The study was conducted from March 2022 to July 2022 at the Postgraduate and Research Department of Zoology, Raja Doraisingam Government Arts College, Sivagangai, Tamil Nadu, India. *B. juncea* (Mustard) seed oil extract was obtained from a government approved oil store in Madurai, Tamilnadu, India. GC-MS and FTIR analysis of biologically active substances was carried out at the Instrumental Centre of Anja College, Sivakasi, Virudhunagar, Tamil Nadu, India.

Gas chromatography mass spectroscopy

The GC-MS analysis of the sample was carried out on Agilent chromatography GC (Model 7820A series) fitted with detector VL-MSD (Model 5977E). The carrier gas Helium was flown at constant 2 ml/min; the GC oven temperature started at 100°C for 1 min then increased at 10°C/min to 270°C held for 30 min. 1.0 µl of the sample was automatically injected into the column (DB-5) with the injector temperature at 270°C. The injections were performed in split-less mode. The compounds were identified based on the comparison of their retention indices (RI), retention time (RT), mass spectra of WILEY, NIST library data of the GC-MS system, and literature data.

Fourier Transform Infrared Spectrophotometer

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Seed oil extract of *B. juncea* was used for FTIR analysis. 10 mg of the seed oil extract was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The seed oil sample was loaded in FTIR spectroscope (Shimadzu, 8400S), with a scan range from 500 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹.

Mosquito Rearing

The colony of *An. stephensi* mosquitoes was reared in the insectary at the Raja Doraisingam Govt. Arts College, Sivagangai using the standard procedures described by Manh et al., 2019; 2020) The insectary was kept at 27 ± 3 °C, 70%–80% relative humidity with a photoperiod of 12 h light and 12 h dark. Larvae were placed in plastic trays and provided with cat food (Wiskcat), whereas adult mosquitoes were kept in breeding cages (30 cm × 30 cm × 30 cm) and maintained on a 10% sucrose solution. The female mosquitoes were fed with blood of live mice for mosquito reproduction. These studies were conducted following the guide for the care and use of laboratory animals of Raja Doraisingam Govt. Arts College, Sivagangai.

Larvicidal activity

Standard WHO protocol with slight modifications was adopted for the study (WHO, 1996). The 250 ml of plastic cups containing 200 ml of water with different concentrations of extract (50, 100, 150, 200 & 250 ppm). Early fourth instar larvae were introduced in each concentration. Extract from the stock solution, the different concentrations of (50, 100, 150, 200 & 250 ppm) were prepared. Early 20 fourth instar larvae were introduced in 250 ml plastic cups containing 200 ml of water with each concentration. A control was prepared by the addition of acetone to water. Mortality was recorded after 24 hours. For each experiment, four replicates were maintained at a time. The observed percentage mortality was corrected by Abbott's Formula (Abbott, 1925).

$$\% \text{ Mortality in Treated} - \% \text{ Mortality in control}$$

$$\text{Percent Mortality} = \frac{\% \text{ Mortality in Treated} - \% \text{ Mortality in control}}{100 - \% \text{ Mortality in control}} \times 100$$

Statistical Analysis

Mortality was recorded after 24 hours of exposure. Obtained values were subjected to logging probit regression analysis and to obtain LC₅₀ and LC₉₅ values with a 95 % confidence limit (Finney, 1971).

3. RESULTS

The GC-MS characterization of seed oil extract of *B. juncea* was identified and presented in Table 1 and Figure 1. Totally, six major chemical compounds were identified, such as 23-Methyl-tetracosanoic acid (12.47 %), 2,4,6-Cycloheptatrien-1-one, 3,5... (12.75 %), Octasiloxane, 1,1,3,3,5,5,7,7,9 (8.34 %), Indole-2-one, 2,3-dihydro-N-hydr... (13.54 %), Silane, trimethyl[5-methyl-2-(1-... (20.99 %), and 1 5-Methyl-2-phenylindolizine (10.52 %) were present in the seed oil extract of *B. juncea* (Figure 2). The identity of phytochemical compounds was confirmed based on retention time, peak area, molecular formula, and molecular weight.

Table 1. GC-MS analysis of seed oil of *Brassica juncea*

Sl.No	Retention Time	Area	Compounds	Formula	Molecular weight
1	2.27	12.10	Unknown	-----	-----
2	2.31	6.90	Unknown	-----	-----
3	2.35	2.38	Unknown	-----	-----
4	2.99	12.47	23-Methyl-tetracosanoic acid	C ₂₅ H ₅₀ O ₂	382.70
5	16.37	12.75	2,4,6-Cycloheptatrien-1-one, 3,5...	C ₁₃ H ₂₂ Osi ₂	250.48
6	16.43	8.34	Octasiloxane, 1,1,3,3,5,5,7,7,9	C ₁₆ H ₅₀ O ₇ Si ₈	579.248
7	17.17	13.54	Indole-2-one, 2,3-dihydro-N-hydr...	C ₁₁ H ₁₃ NO ₃	207.23
8	17.88	20.99	Silane, trimethyl[5-methyl-2-(1-...	C ₁₃ H ₂₂ OSi	222.40
9	20.00	10.51	5-Methyl-2-phenylindolizine	C ₁₅ H ₁₃ N	207.27

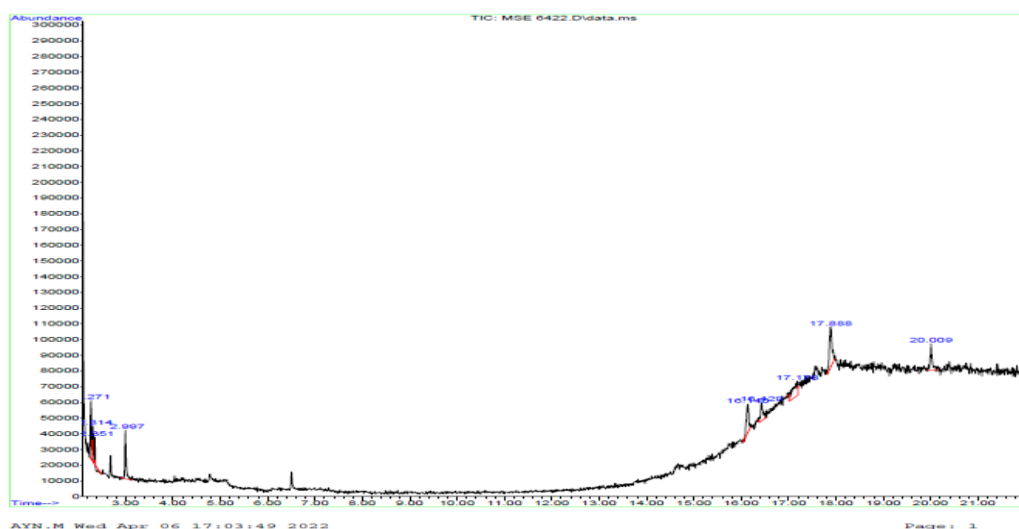


Figure 1. GC-MS analysis of seed oil extract of mustard seed oil

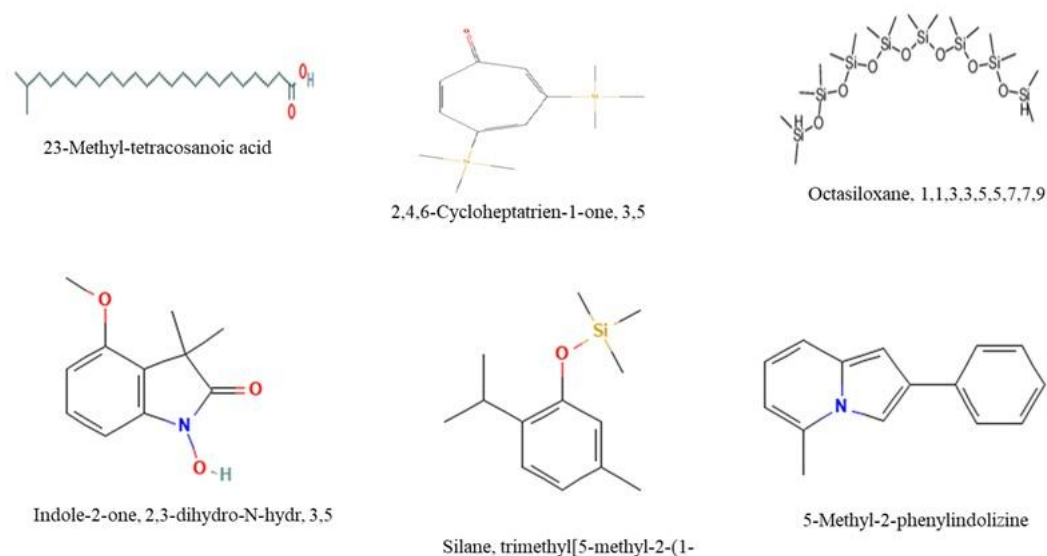


Figure 2. GC-MS analysis of major bioactive compounds in mustard seed oil extract

FTIR analysis of seed oil extract of *Brassica juncea* was carried out and presented in Figure 3. The compounds indicated show that the band at 3652.93, 3102.29, 3009.71, 2925.81, 2083.94, 1746.42, 1651.92, 1605.63, 1463.87, 1376.12, 1240.14, 1162.03, 721.33 and 581.5 cm^{-1} . The broad band at 3652.93 cm^{-1} O-H stretching in alcohol groups. The presence of peaks at 3102.29 cm^{-1} and 2925.81 cm^{-1} corresponding to the carboxylic acid and C-H stretch of alkane groups. The band at 2083.94 cm^{-1} and 1746.42 cm^{-1} to assign the N=C=S stretch isothiocyanate and C=O stretch in esters groups. The peaks at 1651.92 cm^{-1} and 1605.63 cm^{-1} corresponding to the C=N stretch imine and N=H of amine groups. The peak at 1463.87 cm^{-1} , 1376.12 cm^{-1} and 1240.14 cm^{-1} in C=H bend alkane, O=H bending alcohol and C-O stretch alkyl aryl ether groups. The broad band at 1097.42 cm^{-1} C-O stretch in secondary alcohol groups. The peak at 721.76 cm^{-1} and 581.5 cm^{-1} C=C bend alkenes and C-Br stretch in halo compounds.

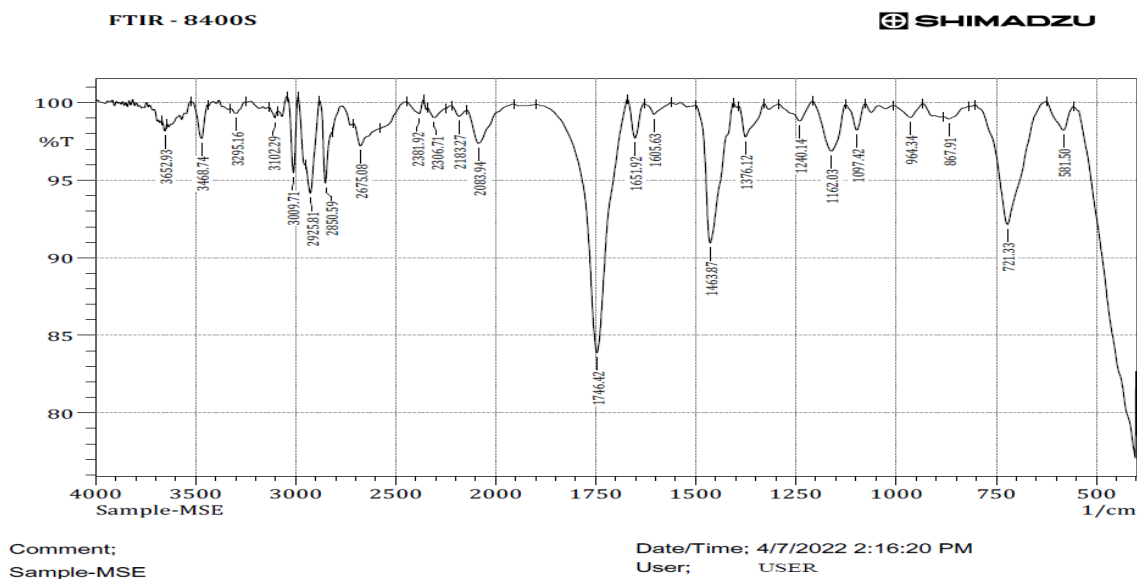


Figure 3. Functional groups of the components of mustard seed oil extract of by FTIR

Larvicidal activity of *B. juncea* against fourth instar larvae of *A. stephensi* was estimated. The larval mortality of the fourth instar larvae of *A. stephensi* found to be increased with increasing concentrations of essential oil extract of *B. juncea* LC₅₀ and LC₉₀ values of 66.97 ppm (39.97 – 87.20), and 78.60 ppm (50.25 – 98.72) was respectively (Table 2).

Table 2. Functional groups of the components of mustard seed oil extract of by FTIR

Sl.No	Absorption (Cm ⁻¹)	Functional Groups	Compounds	Intensity
1	581.5	C-Br stretching	Halo compound	Strong
2	721.33	C=C bending	Alkene	Strong
3	1097.42	C-O stretching	Secondary alcohol	Strong
4	1240.14	C-O stretching	Alkyl aryl ether	Strong
5	1376.12	O-H bending	Alcohol	Medium
6	1463.87	C-H bending	Alkane	Medium
7	1605.63	N-H bending	Amine	Medium
8	1651.92	C=N stretching	Imine	Strong
9	1746.22	C=O stretching	Esters	Strong
10	2083.94	N=C=S stretching	Isothiocyanate	Strong
11	2925.81	C-H stretching	Alkane	Medium
12	3102.29	O-H stretching	Carboxylic acid	Strong
13	3652.93	O-H stretching	Alcohol	Strong

Table 3. Larvicidal activity of *B. juncea* essential oil against *A. stephensi* larvae after 24 h exposure

Concentration (ppm)	% Mortality	LC ₅₀	LCL-UCL	LC ₉₀	LCL-UCL	χ^2
Control	0.0	66.97	39.22-87.20	78.60	50.25-98.72	6.28
50	5.0					
100	15.0					
150	40.0					
200	55.0					
250	95.0					

LC₅₀= Lethal Concentration brings out 50% mortality and LC₉₀= Lethal Concentration brings out 90% mortality. LCL= Lower Confidence Limit, UCL= Upper Confidence Limit, χ^2 =Chi-square

4. DISCUSSION

Essential oils (EOs) are highly promising metabolic products that find applications in various human endeavors, including plant defense and protecting human and animal health from numerous pathogens (Gabasse et al., 2022). Many studies have reported different compositions of essential oils obtained from different plant species (Liu et al., 2012 and Senthilkumar *et al.* 2013, Baranitharan et al., 2021). Plants show significant variation in terms of the number and percentage composition of different components in the essential oil, and this appears to be a characteristic of a particular plant. Thus, the natural diversity of essential oils in native plants provides a good opportunity to develop cost-effective, environmentally friendly, region-specific and practical strategies to control mosquito vectors, which can be used independently or as part of an integrated vector management strategy. Although there are no reports of insecticide/larvicide resistance in the study area, its presence is well documented against all four approved insecticide classes (organochlorines, pyrethroids, carbamates and organophosphates) for *Anopheles* species in African countries. (Kristen et al., 2003).

Additionally, according to our GC-MS analysis of six major biological compounds, 23-Methyl-tetracosanoic acid (12.47 %), 4,6-Cycloheptatrien-1-one, 3,5... (12.75 %), Octasiloxane, 1,1,3,3,5,5,7,7,9 (8.34 %), Indole-2-one, 2,3-dihydro-N-hydr... (13.54 %), Silane, trimethyl [5-methyl-2-(1-... (20.99 %), and 1 5-Methyl-2-phenylindolizine (10.52 %) are all naturally occurring fatty acids (Mandake et al., 2020). Tao et al., (2018) reported that it is difficult to precisely characterize the combined activity of the major components, Tetracosanoic acid at this stage. Tetracosane is an alkane hydrocarbon and the use of hydrocarbons as insecticidal agents has been reported (Siddique et al., 2004). Similarly, there is evidence indicating larvicidal and antimicrobial activities of the other major component, eicosane (Manas et al., 2014).

The FTIR spectra of bulk mustard seeds were quite similar and suggest that the seed oil did not alter the molecular structure of the biocomponents providing chemical stability to the mustard seed oil. Sharma et al., 2016 reported that the larvicidal activity of *B. juncea* essential oil on *An. stephensi* malarial vector may be due to the main components; this study is a step towards demonstrating the larvicidal potential of essential oil derived from a locally available plant against common mosquito species. Chemical analysis shows that the essential oil has a distinct composition of major and minor components, which can be further exploited in studies already reported. The FTIR results clearly indicated the presence of carboxylic acid, alcohol (OH), Esters (C=O), Amine (N=H) and Halo compound (C-Br) groups in mustard seeds, which has been documented in previous literature (Khatami et al., 2016).

The larvicidal activity of *B. juncea* essential oil on *An. stephensi* malarial vector may be due to the main components; this study is a step towards demonstrating the larvicidal potential of essential oil derived from a locally available plant against common mosquito species. Chemical analysis shows that the essential oil has a distinct composition of major and minor components, which can be further exploited in studies already reported. Lafarga et al., 2018 reported that glucosinolates are sensitive to degradation by exposures to high temperature. The heat treatment was also expected to denature the myrosinase enzyme in the seed meal and prevent hydrolysis of the glucosinolates to form the active isothiocyanate (Flor-Weiler et al., 2023).

5. CONCLUSION

The results of this study open new opportunities by showing that defatted Brassica seed meal, a by-product of seed oil extraction, may act as a promising larvicide for mosquito control. This information may help in further exploring plant-based biocontrol agents that should be developed as affordable, practical strategies, cost-effective and environmentally friendly to control mosquito-borne diseases.

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