

## Effect of *Bistorta affinis* (D. Don) and *Malcolmia cabulica* (Boiss) Essential Oils on Human and Foodborne Pathogens, Insects and Termites

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### Abstract

This current study investigates the biological efficiency of essential oils extracted from *Bistorta affinis* and *Malcolmia cabulica* on human and foodborne pathogens as well as against insects and termites. The oils were obtained by steam distillation using a Clevenger-type system and analyzed for their constituents using GC-MS. Twenty compounds were identified, wherein carvacrol and thymol were the main constituents in both plants. Essential oils exhibited significant activity against all tested foodborne bacteria, fungi, and other pathogens. In addition, essential oils from both plants have shown promising activity against all tested insects, which is a positive sign of being used as an insect repellent. In contrast, no activity was observed against termites. The bioactivities are mainly due to carvacrol and thymol's levels in the essential oils that known for their effectiveness against foodborne pathogens and pests. The present study constitutes a basis for further investigation and development of bioactive compounds in *Bistorta affinis* and *Malcolmia cabulica*.

**Keywords:** Carvacrol, Thymol, GC-MS, Biological Activities, Insecticidal Activity, Antifungal Activity

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## INTRODUCTION

Essential oil (EO) is a complex phytochemicals in volatile liquids characterized by their intense aroma. EO has rare color and is soluble in both lipids and organic solvents. It is present in different parts of the plant and synthesized in all its organs, like stems, leaves, fruits, flowers, buds, roots, bark or wood, seeds, and twigs. EO is stored in secretaries, cavities, canals, epidermic cells, or glandular trichomes.<sup>1</sup> Medicinal plants contain EO, extracted by the secondary metabolism of a complex mixture of volatile molecules containing 20-60 components.<sup>2</sup> EO has many food technological applications, including phyto-preparations, cosmetics, fragrances, and folk medicine.<sup>3</sup> EO from medicinal plants exhibited bactericidal, virucidal, and fungicidal activities and has several constituents responsible for the growth inhibition of many pathogenic microorganisms like bacteria, viruses, fungi, and protozoa.<sup>4</sup> EO's use is a promising approach to combat the growth of many foodborne pathogens and multiple drug-resistant microorganisms. There are wide applications of EO in food safety and preservation to increase its shelf-life<sup>5</sup> and limit the use of synthetic additives.<sup>6</sup> Besides, the EO obtained from different plant species is known for repellent properties against many insect species, which are considered more environmentally friendly compatible pesticides and exhibit ovicidal and larvicidal activities.<sup>7</sup> The invention of botanical insecticides derived from plants has limited the use of synthetic insecticides, and their demand has increased currently due to their safety.

*Bistorta affinis* is a perennial herb that belongs to the family *Polygonaceae* and is commonly called the Himalayan fleece flower. This family encompasses various medicinal plants known for many bioactivities because of many phytochemical constituents.<sup>8</sup> Several phenolics were identified in the genus *Polygonum*; some of them proved remarkable biological activities.<sup>9</sup> Phytochemical studies on this family revealed many bioactive chemicals like alkaloids, tannins, saponin, flavonoids, unsaturated sterols, and anthraquinones.<sup>10</sup> *B. affinis* has also been used traditionally due to its numerous medicinal values. It is used in the traditional medicine of Tibetan as antipyretic and anti-inflammatory. It treats fever,

tonsillitis, cough, and cold in Nepal's traditional medicine.<sup>11</sup> This plant's rhizome helps prepare tea in Pakistan and treats abdominal pain, flu, back pain, expels worms, lung disorders, and fever.<sup>12</sup>

*Malcolmia cabulica* is an annual or biennial herb that belongs to the family *Brassicaceae* (*Cruciferae*). It is used in the traditional medicine of Lakki Marwat in Pakistan for animal weight loss, carminative, and galactagogue.<sup>13</sup> The family *Brassicaceae* is known for its effectiveness in treating cancer and diabetes. In addition, it has been proven for significant antibacterial, antifungal, and anti-rheumatic properties. Moreover, the family *Brassicaceae* has been reported for its intense insecticidal action.<sup>14</sup> This family also incorporates plants mainly used for edible or ornamental purposes; thus, they are vital economic plants. Owing to the sharp flavor of sulfur metabolites, these plants are often called the "mustard" plant family.

The present study aims to identify EO's volatile constituents from *B. affinis* and *M. cabulica* using GC-MS and evaluate their antibacterial, antifungal, insecticidal, and anti-termite activities.

## MATERIALS AND METHODS

### Collection of plants

*Bistorta affinis* was collected from Deosai, Gilgit Baltistan division, and *Malcolmia cabulica* was harvested from Lakki Marwat district, Khyber Pakhtunkhwa (KPK), Pakistan. The plants were identified by Mr. Ghulam Jelani, Department of Botany, University of Peshawar, KPK, Pakistan.

### Isolation of EO

The EO of air-dried plants was extracted by hydrodistillation with a Clevenger type system for 3 h according to the standard procedure recommended in the British Pharmacopoeia.<sup>15</sup> Then, the EO was dried with the help of anhydrous sodium sulfate and stored at a temperature of 4°C in a sealed vial until analysis.

### Gas Chromatography-mass Spectrometry (GC-MS Analysis)

EO components were identified using Gas Chromatography-Mass Spectrometry (GC-MS).<sup>16,17</sup> The analysis was performed using the Perkin Elmer-Auto XL GC and Perkin Elmer

TurboMass mass spectrometry. PE-5ms (5% phenyl-95% methylpolysiloxane) column (20×0.18 mm Ø with 0.18-µm film thickness) was used with helium at 0.5 mL/min as the carrier gas. GC oven temperature was kept at 45°C for 2 min and programmed to 240°C at a rate of 6°C/min and kept constant at 240°C for 5 min. The split ratio was adjusted to 1:100, while the injection volume was 0.1 µL. Electron impact mass spectrometry (EI-MS) was taken at 70 eV ionization energy. The mass range was from m/z. 35 to 350 amu. The MSD Chem-Station was used as operating software. Retention indices were calculated using retention times of *n*-alkanes (C<sub>8</sub>-C<sub>24</sub>) that were injected after the EO at the same conditions. Library research was performed using NIST and Wiley's GC-MS Library of EO. A computerized integrator calculated the relative percentage amount of separated compounds from an ion chromatogram.

#### Antibacterial Activity

Antibacterial activity of EO was tested against *Salmonella typhi*, *Escherichia coli*, *Klebsella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus mirabilis*, and *Serratia marcescens* according to Nisar et al.<sup>18</sup> Eighteen hours of the test organism's culture was transferred to the nutrient broth and then to sterile nutrient agar plates to make a bacterial lawn. After 30 min, wells were dug in plates using a sterile 6 mm borer. Finally, 100 µL of EO were loaded to their respective labeled wells. Amoxicillin was used as a positive control. The zone of inhibition was measured (mm) compared to positive control.

#### Antifungal Activity

The antifungal activity of EO has been carried out against *Alternaria solanni*, *Aspergillus niger*, *Paecilomyces fulvus*, *Penicillium pallidum*, *Fusarium oxysporum*, *Rhizopus stolonifer*, and *Triticum harzianum*.<sup>19</sup> Sabouraud Dextrose Agar (SDA) media was used to refresh the fungal test isolates in Petri plates. First, 4 mL of SDA media was introduced into the test tubes to make slants. After autoclaving, when the temperature was about 50°C, 66.6 µL of EO were introduced into respective test tubes. Next, the seven-day-old fungal culture was introduced into the labeled test tubes and incubated at 25±1°C for 7 days

in a growth chamber. Tubes supplemented with Miconazole served as a positive control. The results were taken on day 7 by measuring the linear growth on the slanted test tubes compared to the positive control.

#### Insecticidal Activity

The EO was evaluated for possible insecticidal traits against three insect species: *Tribolium castaneum*, *Rhyzopertha dominica*, and *Callosobruchus analis*, according to a standard protocol.<sup>20</sup> One mL of EO was introduced into the *Petri* plates using a micropipette having blotting paper at the base. The next day, 10 active and healthy insects (having the same age and size) were released to each plate. The standard drug (Permethrin, 393.17 µg/cm<sup>2</sup>) served as a positive control. The plates were incubated in the growth chamber at 27°C for 24 h with 50% relative humidity. The number of survived insects was counted for calculation, and the mortality (%) was determined. Results were the mean of three different experiments.

#### Anti-termite Activity

The anti-termite effect was evaluated against test termite *Heterotermes indicola*, according to Salihah et al.<sup>21</sup> The EO at a concentration of 1 mL of both plant species was introduced into the *Petri* plates having blotting paper at the base. Twenty-five healthy termites were released to each *Petri* dish using a clean brush and noticed their percent mortality after 24 h. All the experiments were performed in triplicate, and the average number of termites killed each day was noted.

## RESULTS AND DISCUSSION

#### Chemical Composition of EO

The flowers of *B. affinis* yielded 0.3% (v/w), and *M. cabulica* yielded 0.2 % (v/w) of yellowish oil with an aromatic odor. Twenty different chemical components, comprising 99.1% of the total EO, were identified in the flowers of *B. affinis* and *M. cabulica*. The identified components and their percentage are given in Table 1, wherein the components are listed in order of their elution on the HP-5MS column. The results of the current GC-MS examination of *B. affinis* and *M. cabulica*

**Table 1.** Composition of EO from *B. affinis* and *M. cabulica*

No.	Compound	M.W.	Molecular formula	Retention time (min)	(% concentration)		RI <sub>Exp</sub>	RI <sub>Lit</sub>
					<i>B. affinis</i>	<i>M. cabulica</i>		
1.	3-Octanone	128	C <sub>8</sub> H <sub>16</sub> O	6.940	0.21	0.15	965	984
2.	p-Cymene	134	C <sub>10</sub> H <sub>14</sub>	8.189	2.15	2.92	1007	1015
3.	α-Pinene	136	C <sub>10</sub> H <sub>16</sub>	5.800	3.20	3.72	930	936
4.	β-Pinene	136	C <sub>10</sub> H <sub>16</sub>	7.2 10	1.32	1.28	966	978
5.	Terpinolene	136	C <sub>10</sub> H <sub>16</sub>	7.960	0.43	0.30	1079	1086
6.	α-Terpinene	136	C <sub>10</sub> H <sub>16</sub>	9.389	1.85	1.75	1010	1017
7.	Camphene	136.2	C <sub>10</sub> H <sub>16</sub>	32.710	3.05	3.18	940	950
8.	Limonene	137	C <sub>10</sub> H <sub>17</sub>	32.700	2.38	1.01	1018	1025
9.	Thymol	150	C <sub>10</sub> H <sub>14</sub> O	18.750	28.89	30.56	1258	1267
10.	Carvacrol	150	C <sub>10</sub> H <sub>14</sub> O	30.035	35.54	31.05	1264	1278
11.	Durenol	150	C <sub>10</sub> H <sub>14</sub> O	19.195	3.00	2.89	956	983
12.	Linalool	154	C <sub>10</sub> H <sub>18</sub> O	10.850	4.89	4.15	1083	1086
13.	δ-Terpineol	154	C <sub>10</sub> H <sub>18</sub> O	13.950	0.24	0.23	1148	1164
14.	α-Terpineol	154	C <sub>10</sub> H <sub>18</sub> O	14.525	0.38	0.38	1175	1189
15.	O-Methylthymol	164	C <sub>11</sub> H <sub>16</sub> O	16.699	0.45	0.33	1214	1234
16.	Thymol acetate	192	C <sub>12</sub> H <sub>16</sub> O <sub>2</sub>	22.001	0.78	0.65	1342	1356
17.	Caryophyllene	204	C <sub>15</sub> H <sub>24</sub>	23.892	1.09	1.66	1565	1578
18.	Alloaromadendrene	204	C <sub>15</sub> H <sub>24</sub>	24.673	0.31	0.29	1459	1464
19.	Viridiflorene	204	C <sub>15</sub> H <sub>24</sub>	26.904	0.15	0.11	1492	1494
20.	Isospathulenol	220	C <sub>15</sub> H <sub>24</sub> O	30.240	0.71	0.56	1625	1633
<b>Total</b>					<b>91.02</b>	<b>87.17</b>	<b>23,496</b>	<b>23,717</b>

EO extracted from flowers displayed 20 different chemical components, wherein carvacrol and thymol were the major constituents in both essential oils. Carvacrol (35.5%) and thymol (28.8%) were detected in the EO of *B. affinis*, while carvacrol (31.0%) and thymol (30.5%) were detected in the EO of *M. cabulica*.

Earlier reports mentioned that phenolic constituents like thymol, carvacrol, and eugenol in many medicinal plants' EO showed good antibacterial activity against several foodborne pathogens.<sup>22</sup> Thymol, carvacrol, terpinen-4-ol terpinen were reported earlier as major chemical constituents in the EO obtained from *Origanum* species. Carvacrol has a strong aroma that acts as a fumigant and is known for strong acaricidal and insecticidal effects against medical, agricultural, and stored-product pests. On the other hand, it is highly lethal to the adults of pulse beetles, mites, rice weevils, cigarette beetles, and the nymphs of termites.<sup>23</sup>

The second major component in *B. affinis* and in *M. cabulica* was linalool (4.89%, and 4.15%, respectively). The occurrence of linalool

in the EO has pleasant, fragrant, refreshing, and sweet characteristics known for good antibacterial effect.<sup>24</sup> Linalool found in the EO of both plants suggests that these oils might have a significant antibiotic effect and might also be used in food systems to control the contamination of bacteria.<sup>25</sup> Other workers have also documented the presence of linalool in *Rosa abyssinica* (12.7%),<sup>26</sup> *R. centifolia* (6.9%),<sup>27</sup> and *R. centifolia* (0.22%).<sup>28</sup>

The major monoterpene of hydrocarbon nature is α-pinene, which is known for significant bacteriostatic and bactericidal activities.<sup>29</sup> The presence of α-pinene in both plants' EO might help in the manufacturing of many antibacterial formulations. A few monoterpenes were detected in the EO of both plants, which are widely utilized in food, spices, flavors, condiments, beverages, cosmetics, and pharmaceutical industries.<sup>30</sup> Therefore, it could be concluded that this EO might be essential for good human health. The obtained EO of both plants may be used by industries as flavor and fragrance and may be commercialized for the local people's income generation. The remaining components detected in both plants'

EO were less than 1%, while the components like  $\beta$ -myrcene, germacrene D, and cis-beta-ocimene were not observed in EO.

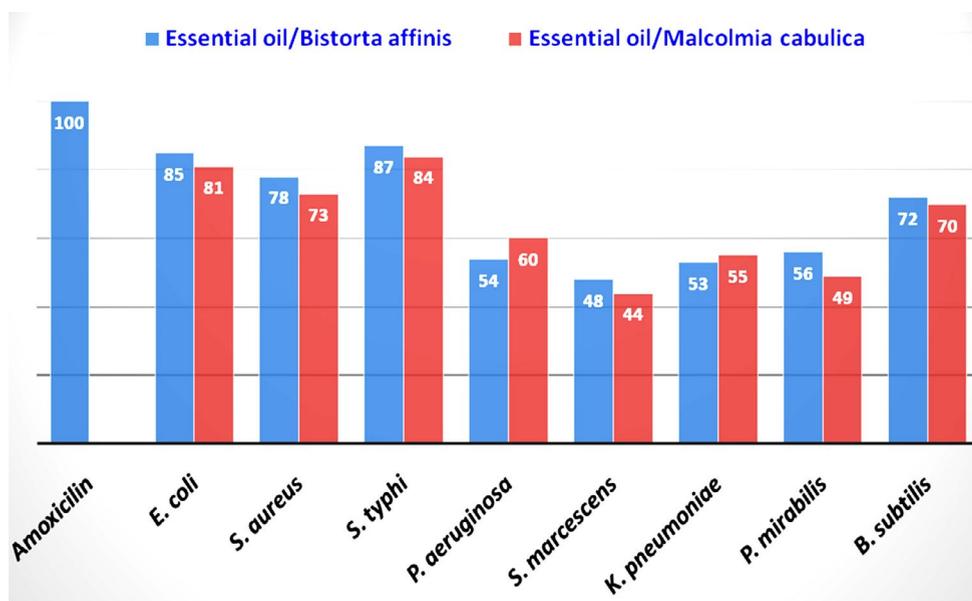
### Antibacterial Activity

The EO derived from plants has been used for thousands of years in pharmaceuticals, natural therapies, food preservation, and alternative medicine.<sup>31,32</sup> There are novel antimicrobial components found in the EO of plants,<sup>33</sup> having a potential effect against many bacterial pathogens. The present investigation of antibacterial studies revealed that both plants' EO inhibited bacterial isolates' growth, but their effectiveness varied. The result of the antibacterial activity is given in Figure 1, and the values of MIC<sub>50</sub> and MBC are given in Table 2. The EO of *B. affinis* showed maximum inhibition against *S. typhi* (85.7% with MIC<sub>50</sub> = 6.4 mg/mL and MBC=13.6 mg/mL), followed by *E. coli* (82.2% with MIC<sub>50</sub> = 3.9 mg/mL and MBC=8.6 mg/mL), *S. aureus* (80% with MIC<sub>50</sub> = 3.7 mg/mL and MBC=7.9 mg/mL), *B. subtilis* (65.5% with MIC<sub>50</sub> = 2.3 mg/mL and MBC=5.0 mg/mL), *P. mirabilis* (48.6% with MIC<sub>50</sub> = 6.9 mg/mL and MBC=2.3 mg/mL), *K. pneumoniae* (48.1% with MIC<sub>50</sub> = 2.0 mg/mL and MBC=4.3 mg/mL), *P. aeruginosa* (47.7% with MIC<sub>50</sub> = 2.3 mg/mL and MBC=5.0 mg/mL), and *S. marcescens* (45.5% with MIC<sub>50</sub> = 4.2 mg/mL and MBC=9.4 mg/mL). The EO of *M. cabulica*

showed the highest inhibition against *S. typhi* (81.4% with MIC<sub>50</sub> = 5.2 mg/mL and MBC=11.1 mg/mL), followed by *S. aureus* (79.1% with MIC<sub>50</sub> = 3.0 mg/mL and MBC=6.2 mg/ml), *K. pneumoniae* (76.1% with MIC<sub>50</sub> = 2.8 mg/mL and MBC=5.9 mg/mL), *P. aeruginosa* (75.7% with MIC<sub>50</sub> = 2.9 mg/mL and MBC=6.4 mg/mL), *E. coli* (70.0% with MIC<sub>50</sub> = 4.3 mg/mL and MBC=8.8 mg/mL), and *S. marcescens* (65.5% with MIC<sub>50</sub> = 2.3 mg/mL and MBC=4.9 mg/mL). EO moderately inhibited the growth of *B. subtilis* (48.3% with MIC<sub>50</sub> = 2.6 mg/mL and MBC=5.7 mg/mL) and *P. mirabilis* (40.6% with MIC<sub>50</sub> = 2.5 mg/mL and MBC=5.6 mg/mL).

**Table 2.** MIC50 and MBC values of EO from *B. affinis* and *M. cabulica* against tested bacteria

Bacteria Names	Essential oil of <i>B. affinis</i>		Essential oil of <i>M. cabulica</i>	
	MIC <sub>50</sub>	MBC	MIC50	MBC
<i>E.coli</i>	3.9	8.6	4.3	8.8
<i>S. aureus</i>	3.7	7.9	3.0	6.2
<i>S. typhi</i>	6.4	13.6	5.2	11.1
<i>P. aeruginosa</i>	2.3	5.0	2.9	6.4
<i>S. marcescens</i>	4.2	9.4	2.3	4.9
<i>K. pneumoniae</i>	2.0	4.3	2.8	5.9
<i>P. mirabilis</i>	3.2	6.9	2.5	5.6
<i>B. subtilis</i>	2.3	5.0	2.6	5.7



**Figure 1.** Antibacterial effect of *B. affinis* and *M. cabulica* EO

### Antifungal Activity

The main bioactive components found in EO have been widely used against moulds. The EO extracted from citrus, basil, lemongrass, fennel, oregano, thyme, and rosemary is known for its extensive antifungal activity against various fungal pathogens.<sup>34</sup> Therefore, the EO of both plants were evaluated for antifungal activity, and their results are depicted in Figure 2. The EO of *B. affinis* showed the maximum inhibitory activity (80%)

against *A. niger* and *R. stolonifer*, good inhibitory activity against *A. solanni* (62%), moderate inhibitory activity against *P. pallidum* (53%) and *F. oxysporum* (50%), low inhibitory activity against *T. harzianum* (42%) and *P. fulvus* (40%). On the other hand, the EO of *M. cabulica* showed the maximum inhibitory activity (79%) against *A. niger* and *R. stolonifer*, good inhibitory activity against *A. solanni* (60%), moderate inhibitory activity against *P. pallidum* (51%), and *F. oxysporum* (50%), and low

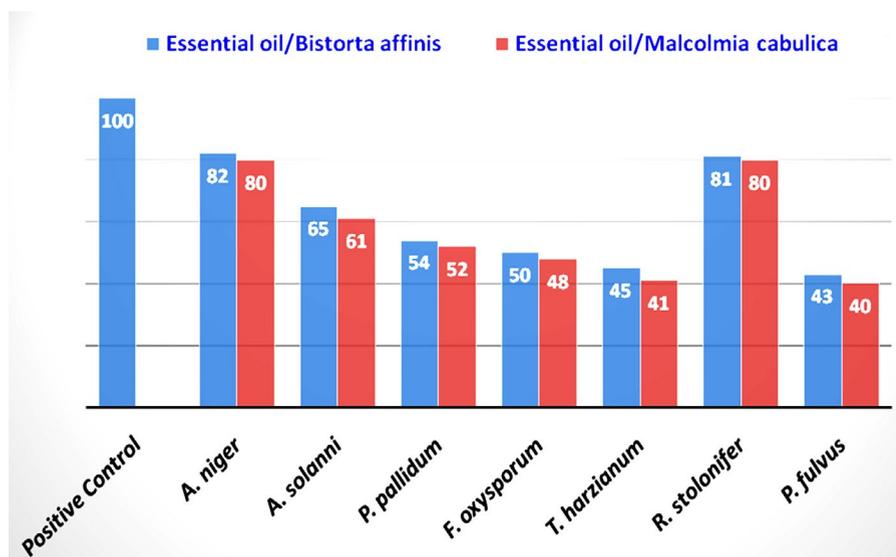


Figure 2. Antifungal effect of *B. affinis* and *M. cabulica* EO

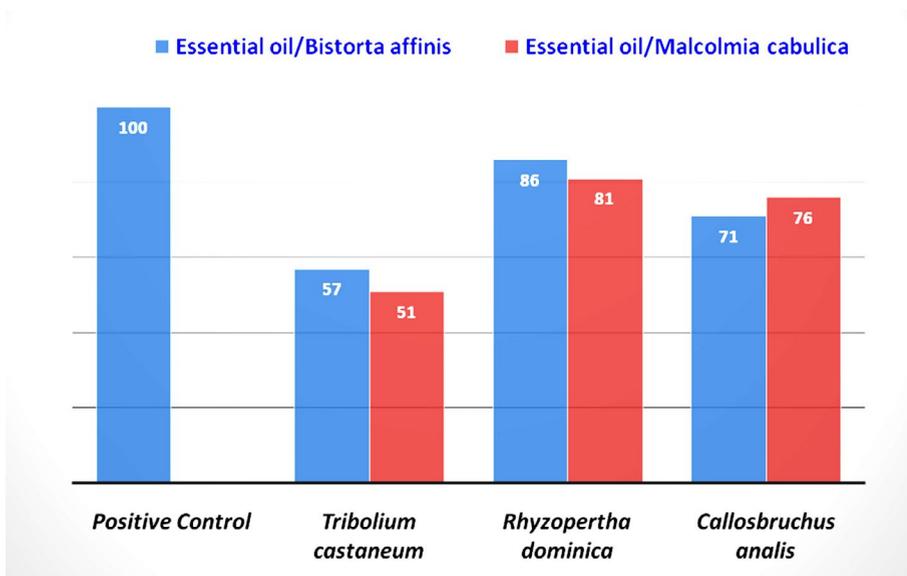
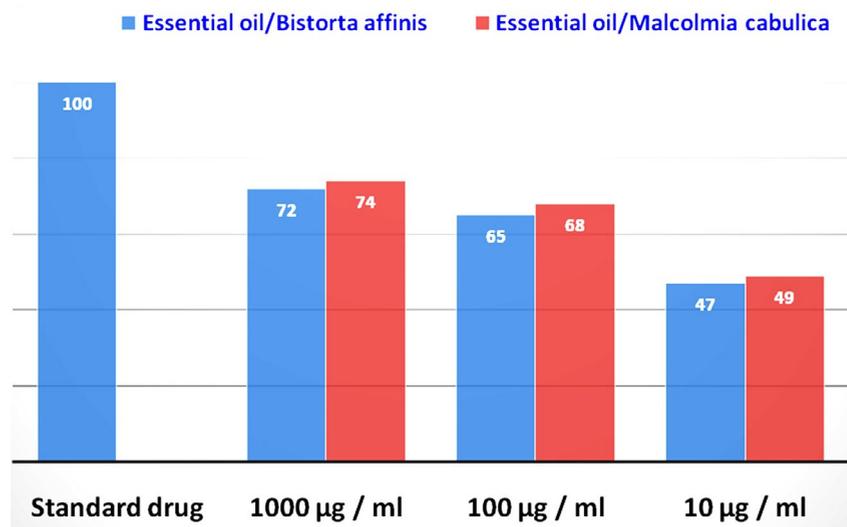


Figure 3. Insecticidal effect of *B. affinis* and *M. cabulica* EO



**Figure 4.** Antitermite effect of *B. affinis* and *M. cabulica* EO

inhibitory activity against *T. harzianum* (40%) and *P. fulvus* (38%).

#### Insecticidal Activity

Synthetic insecticides have a toxic and harmful effect on humans and the environment. To prevail over this toxic effect, plants' natural insecticides should replace these harmful insecticides.<sup>20</sup> The insecticidal effect of EO against test insects *Tribolium castaneum*, *Rhyzopertha dominica*, and *Callosobruchus analis* were evaluated (Figure 3). The EO of *B. affinis* showed maximum activity against *R. dominica* (84.2%), good activity against *C. analis* (71.0%), and low activity showed against *T. castaneum* (48.3%). In the case of *M. cabulica*, significant insecticidal activity showed by the EO against *R. dominica* (80.3%), good activity against *C. analis* (78.0%), and low activity showed against *T. Castaneum* (45.7%).

#### Antitermite Activity

Termites help aerate the soil and act as decomposers that decompose the principal constituent of wood (i.e., cellulose). In another way, they become economic pests causing a tremendous economic loss when they start destroying wood and wooden products of human homes, forests, and other commercial products.<sup>35</sup> The EO from both plant species showed

significant activities against *H. indicola*, and the experiment was extended for three days. Three concentrations, such as 1000, 100, and 10 µg/mL of both plant species EO, were checked in the experiment (Figure 4). The EO of *B. affinis* killed the average percent of termites (73%) at 1000 µg/mL, (63%) at 100 µg/mL, and (48%) at 10 µg/mL, respectively. In *M. cabulica*, high percent mortality (74%) showed by EO at a concentration of 1000 µg/mL, 68% at 100 µg/mL, and 50% at 10 µg/mL, respectively.

#### CONCLUSION

A wide variety of volatile constituents in the EO include aliphatic and aromatic compounds, phenolics, terpenoids, and terpenes that might have bactericidal, fungicidal, and virucidal efficacy. The EO derived from plants directly targets the pathogenic microorganism cell membrane, which causes an increase in permeability, leakage of essential intracellular components, and finally disrupts cellular respiration and enzyme organization of microorganisms. Moreover, the EO also possesses a cytotoxic effect on living cells, depending on their concentration and type. The results of the current study suggested that the EO extracted from *B. affinis* and *M. cabulica* might be used as a natural alternative source due to its significant antimicrobial efficacy and

help in discovering and synthesizing novel drugs. Furthermore, the detected fumigant effect of EO revealed that it contains active biological vapours having potential insect and termite repellent activity. Thus, the prospect of employing these natural fumigants for controlling insects and termites in stored products may be valuable for further exploration.

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#### CONFLICT OF INTEREST

The authors declare that their is no conflict of interest.

#### AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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#### DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

#### ETHICS STATEMENT

Not applicable.

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