Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2024i1.1242
*Biol. Clin. Sci. Res. J., Volume, **2024**: 1242*
Orginal Research Article

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VOLATILE COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *CEIBA INSIGNIS* FLOWERS

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(Received, 15nd August 2024, Revised 26th October 2024, Published 29th October 2024)

Abstract *This study reports the volatile oil composition and antibacterial activity of the flower of Ceiba insignis. Oil extraction was carried out by microwave-assisted distillation where a yield of 0.11% was obtained. A total of 17 compounds were successfully identified by gas chromatography mass spectrometry out of which the most abundant constituents were furfural (20.18%), heptadecan-2-one (13.81%), pentacosane (10.82%), methyl-5-furfural (8.27%), heptacosane (6.09%) and phytol (5.88%). The antibacterial potential of this oil was evaluated by using the agar disc diffusion technique against 4 different strains. The oil exhibited the highest activity against K. pneumoniae (12.7mm), followed by S. aureus (12mm), E. coli. (11mm) and P. aeruginosa (10.7mm).*

Keywords: *Ceiba insignis; flowers; volatile oil; GCMS; antibacterial activity*

Introduction

Ceiba insignis is a deciduous, perennial tree found mostly in tropical and subtropical regions. It belongs to the Bombacaceae family. It is distributed in Argentina and South America(Gibbs and Semir, 2002). It is distinguished by its thorny bark and swollen stout trunk. The seeds of *C. insignis* are a quite rich source of proteins(Alfy et al., 2012; Ullah et al., 2023). Its thorns are used to relieve breathing issues alongside toothaches. It is reported to have medicinal properties such as antioxidant, anti-inflammatory as well as notable hepatoprotective activities (El-Alfy et al., 2010). It is highly beneficial for treating diabetes mellitus and liver infections(El-Manawaty and Gohar, 2018). The constituents of its oil have high antimicrobial activities against various bacteria(Awan et al., 2024; Bhatti et al., 2023; Din et al., 2023; Moghimi et al., 2017). *Ceiba insignis* is sometimes confused with another species of the Bombacaceae family, Ceiba speciosa. Both of them have swollen stout trunks covered with thorny spines. The leaves of both these species comprise of 5 to 7 oblong leaflets and their flowers possess 5 petals. Silky floss is released from seeds of both these plants when their green pear-shaped fruits are dried and split open. The only striking difference in their appearance is the color of their flowers. *Ceiba insignis* flowers have golden centers with white tips while *Ceiba speciosa* flowers are pink in color with white throats. Various recent studies have proved the efficacy of volatile oil against resistant and non-resistant strains of bacteria(Aziz et al., 2020; Bano et al., 2020; Hassan et al., 2023; Ishtiaq et al., 2019; Kausar et al., 2020; Tahir et al., 2020). Hence, the pivotal aim of this study was to examine the oil composition of *Ceiba insignis* flowers and its antibacterial activity against different microbes due to the reason that previously, there has hardly been a study on its volatile oil composition.

Materials and Methods

Volatile oil extraction of Ceiba insignis flowers.

The sample flowers of *Ceiba insignis* were taken from Jinnah Garden Lahore, Pakistan. The identification of these flowers was done by Dr. Abdul Rehman Khan Niazi and its voucher specimen (LAH# 24621A) was then deposited to the herbarium in the Department of Botany, University of the Punjab, Lahore. The oil was extracted from 100 grams of fresh flowers of *Ceiba insignis* by microwave-assisted extraction technique in 35 minutes at 60% power range by using a custom-modified domestic microwave from Orient (model OM46SS). The extractions were performed three times. Dichloromethane (DCM) was utilized as the

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collecting solvent and this distillate was shifted from a separating funnel to a sealed vial. The vial was then stored at almost -10 °C. This distillate was later used for GCMS analysis while the DCM was cautiously evaporated on a hot plate by regulating the temperature between 37°C to 40°C for the antibacterial analysis.

Antibacterial assay.

The bacterial strains were generously provided by Sheikh Zayed Hospital, Lahore. These strains included gram-negative *E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* along with gram-positive *Staphylococcus aureus* bacterial strains. The antibacterial assay was performed by disc diffusion method. LB agar was used for bacterial culture and the sample dilutions were prepared in dimethyl sulfoxide (DMSO). Three experiments were carried out for each strain and the mean was calculated.

Results and Discussion

Composition of volatile oil

This bright yellow volatile oil of Ceiba insignis flowers had a yield of 0.11%. The relative abundance of the oil came out to be 88.9% and a total of 17 constituents were identified. The most abundant compounds were furfural (20.18%), heptadecan-2-one (13.81%), pentacosane (10.82%), methyl-5-furfural (8.27%), heptacosane (6.09%) and phytol (5.88%) as presented in table 1. The major compound in this oil, furfural, has a sweet taste and hence is found in various baked items. It has notable fungicidal properties(Hoydonckx et al., 2000). Furfural proved to be a strong nematicidal agent and also exhibited pesticidal and herbicidal activities in combination with sodium(Rodriguez-Kabana, 2006). The various commercially used chemicals, additives, as well as fuels, are manufactured by furfural (Abbas et al., 2021; AMIN et al., 2023; Mariscal et al., 2016). 5-methyl furfural is also highly active against microbes with an odor similar to that of almonds. Phytol has high medicinal importance with antioxidant, and anti-inflammatory potential alongside anti-microbial activities and is used in perfume industries because of its floral smell(Islam et al., 2018). Another constituent compound, eucalyptol, having a spicy taste and fresh odor is reported to exhibit very high antifungal activities(Morcia et al., 2012). The total ion chromatogram is shown in Figure 1, mass spectra of major compounds are shown in Figure 2 and all identified constituents of this volatile oil are provided in Table 1.

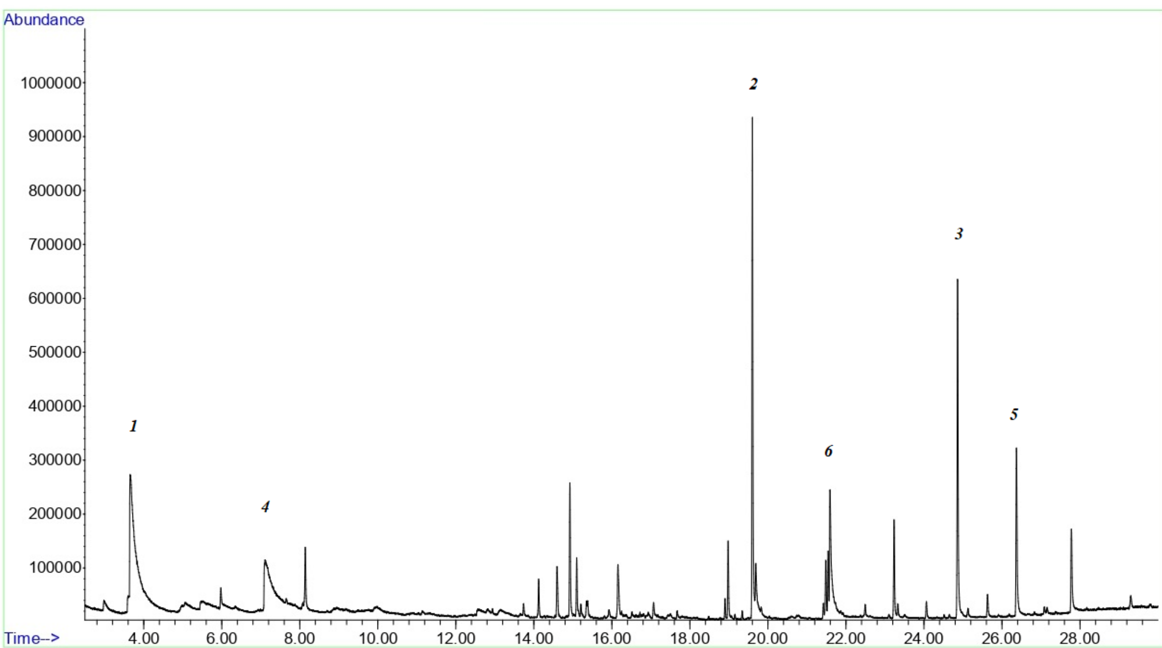

Fig 1. Total ion chromatogram of *Ceiba insignis* flower oil. Number represents the major compounds (1. Furfural (20.18%), 2. Heptadecan-2-one (13.81%), 3. Pentacosane (10.82%), 4.Methyl-5-furfural (8.27%), 5. Heptacosane (6.09%) and 6. Phytol (5.88%).

Table 1. Components of volatile oil of *Ceiba insignis* flowers

Sr. No.	RT (min)	Name of compounds	RI (cal)	RI(lit)	Relative abundance (%)	Match Quality (%)
1	3.65	Furfural	839	839	20.18	94

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2	7.11	Methyl-5-furfural	981	980	8.27	95
3	8.14	Eucalyptol	1031	1031	1.97	95
4	14.12	Caryophyllene	1421	1421	1.10	91
5	14.59	Humulene	1458	1458	1.74	97
6	14.92	Germacrene D	1484	1484	3.93	93
7	15.09	Ledene	1498	1498	1.77	78
8	16.15	Spathulenol	1586	1585	2.49	94
9	18.98	Phytone	1843	1843	2.20	91
10	19.60	Heptadecan-2-one	1904	1906	13.81	95
11	19.69	2-Heptadecanol	1913	1909	2.45	91
12	21.48	Methyl oleate	2100	2104	1.62	98
13	21.54	2-Nonadecanone	2106	2106	1.57	95
14	21.59	Phytol	2112	2112	5.88	95
15	23.23	Tricosane	2299	2300	3.01	91
16	24.85	Pentacosane	2499	2500	10.82	94
17	26.37	Heptacosane	2698	2700	6.09	97

RT= Retention time, RI(cal) = Retention indices calculated relative to authentic standards C_7-C_{30} analyzed at same conditions as that of volatile oil, RI(lit) = Retention indices from literature i.e obtained from Adams and online NIST database.

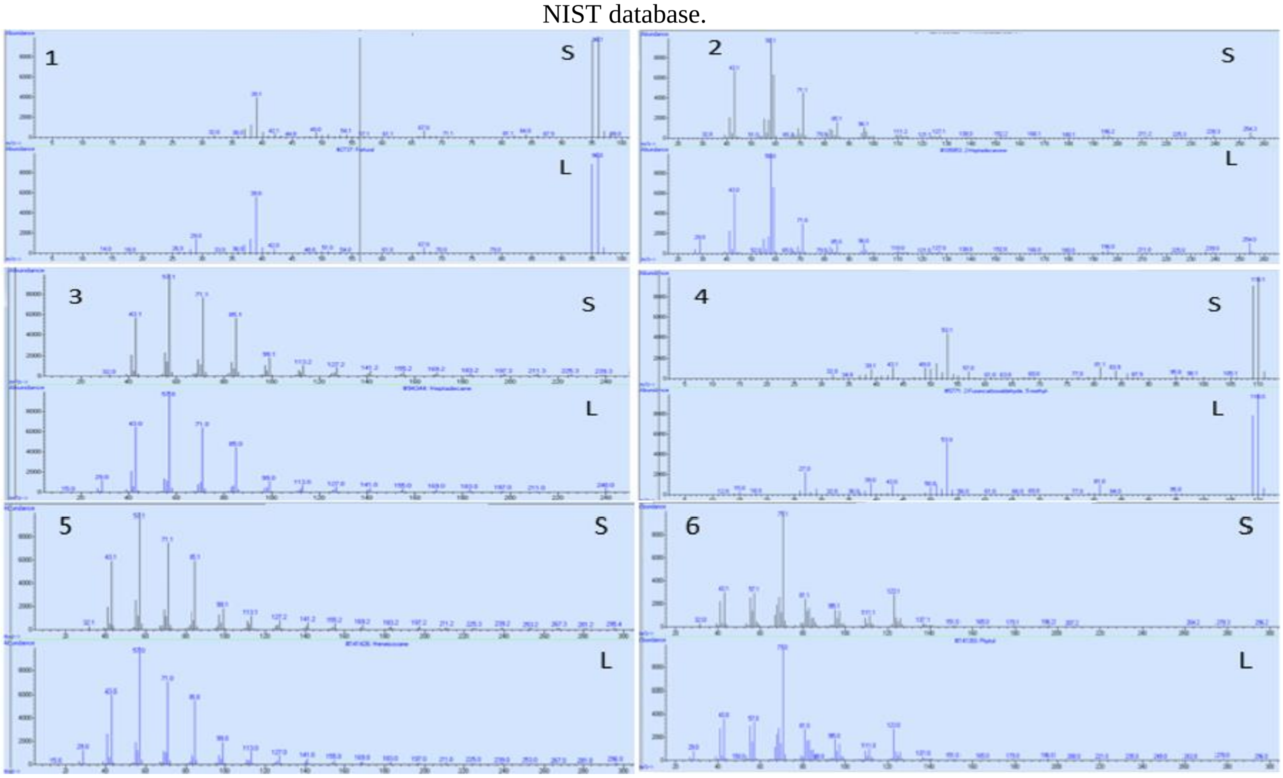

Fig. 2. Six mass spectra of major components from volatile oil of *Chorisia insignis* flower with matching similarity relative to NIST-2011 standards 1. Furfural, 2. Heptadecan-2-one, 3. Pentacosane, 4. Methyl-5-furfural, 5. Heptacosane and 6. Phytol. (Where S is spectra of present components in the oil sample while L is the matched spectra from NIST-2011 library.)

Table 2. Classification of oil components

Classification of components	Serial numbers	Percentages(%)
Terpenoids	3, 14	7.85
Sesquiterpenes	4, 5, 6	6.77
Sesquiterpenoids	7, 8	4.26
Ketones	9, 10, 13	17.58
Aldehydes	1, 2	28.45
Others	11, 12, 15, 16, 17	23.99
		Total: 88.9

Table 2 shows the classification of *C. insignis* volatile oil components. Aldehydes (28.45%) are the most abundant class including furfural and methyl-5-furfural with notable antimicrobial potential(Chai et

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al., 2013). Ketones are also dominating with an abundance of 17.58% (containing active constituents phytone, 2-Heptadecanone and 2-Nonadecanone). Terpenoids in this oil (7.85%) including eucalyptol exhibits antimicrobial properties (Moghimi et al., 2017). However, previously a total of 25 compounds were identified in the light yellow oil of *C. speciosa* leaves constituting 0.21% yield. On the other hand, important bioactive sesquiterpenes such as caryophyllene, humulene, germacrene D, etc. were also present in a considerable quantity. Caryophyllene humulene and germacrene D have been found in *Chorisia speciosa* and are commonly known antibacterial compounds (Kausar et al., 2020).

Antibacterial activity

The antibacterial potential of *Ceiba insignis* flowers was examined against 4 bacterial strains by agar disc diffusion technique that showed good activity as shown in Table 2. The order of the antibacterial activity came out to be: *K. pneumonia*> *S. aureus*>*E. coli*. > *P. aeruginosa.* The oil exhibited high activity against *K. pneumoniae* (12.7mm), followed by *S. aureus* (12mm), *E. coli*. (11mm) and *P. aeruginosa* (10.7mm) as shown in table 3. The antibacterial activity of this oil is obviously because of its active compounds especially those that are present in higher abundance such as furfural which has previously shown significant antimicrobial activities against both *B. subtilis* and *Salmonella* along with high anti-tyrosinase activity (Chai et al., 2013). Another major constituent in this volatile oil, phytol exhibited promising antibacterial activities against *P. aeruginosa* when it was exposed to oxidative stress (Lee et al., 2016). Moreover, phytol in *E. sonchifolia* showed promising biopesticidal activity by inhibiting *C. lunatus* growth at a particular concentration of 72mg per mL according to the most recent report(Ilondu, 2020). Besides, other compounds present in the *Ceiba insignis* oil also had great significance like caryophyllene which is a strong antimicrobial agent alongside exhibiting antioxidant and anticancer properties(Dahham et al., 2015). Caryophyllene and germacrene D showed quite high antibacterial potential *against S. aureus* with a 30.33 mg/mL MIC range of the later (Rather et al., 2012). Another study revealed that germacrene D is a strong antimicrobial agent against *P. aeruginosa, S. aureus* and *E. coli* as compared to the antibiotic streptomycin (Hsouna et al., 2013). Another minor but highly active compound in this oil, spathulenol, showed notable antimicrobial effects against various strains including *E. coli* and *B. subtilis* (with 20 and 15.6 mg/mL MIC concentrations) (Rahman et al., 2016). A recent study also showed the high activity of spathulenol against many bacteria, specifically *M. tuberculosis* (with a 15.3 selective index and 6.5 mg/mL MIC range) (Dzul-Beh et al., 2020). In a report, eucalyptol in the *Rosmarinus officinalis* oil showed high antibacterial activity when tested against various foodborne pathogenic strains (with <0.5µL per mL MBC and MIC concentrations (Jordán et al., 2013). 1,8-cineol or eucalyptol found in *Thymus vulgaris* oil proved to be highly active against various foodborne and pathogenic bacterial species in a report (Hussain et al., 2011). Another study revealed that eucalyptol has notable antibacterial activity against various bacteria i.e., *E. coli*, *P. aeruginosa* and *S. aureus* (with 1,1 and 2 mg/mL MIC) (Moghimi et al., 2017). At 3.6mg concentration of leaf oil of *Ceiba speciosa*, the zones of inhibition for *S. aureus, E. coli* and *S. typhi* came out to be 25 mm, 15 mm and 9 mm respectively with maximum inhibitory activity against *S. aureus*(Kausar et al., 2020). However, previously the ethanol extract of *Ceiba pentandra* leaf oil showed the highest antimicrobial potential against *E. coli* and *S. aureus*(Bhavani et al., 2016). Thus, it can be inferred that the antimicrobial nature of *Ceiba insignis* flower oil is because of its major and minor antibacterial constituents along with the probale synergistic effect of these components.

Bacterial Strains	**Concentration of dissolved oil 20 µL (2.1 mg)**		
	Zone of inhibition (mm)		
Escherichia coli	10	11	12
Klebsiella pneumoniae	14	13	11
Staphylococcus aureus	13	11	12
Pseudomonas aeruginosa	12.1	10	10

Conclusions

The chemical composition and antibacterial analysis of *Ceiba insignis* flowers led to the successful identification of a total of 17 compounds containing furfural (20.18%), heptadecan-2-one (13.81%), pentacosane (10.82%), methyl-5-furfural (8.27%), heptacosane (6.09%) and phytol (5.88%)in dominance. The most abundant class of the constituent components of oil was found to be aldehydes (28.45%). This oil exhibited maximum activity against *K. pneumoniae* and *S. aureus*among all tested strains which shows the potential of this oil.

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Declaration

Acknowledgement

Authors are grateful to University of the Punjab, Lahore for literature support regarding this study.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

The study was approved by authors.

Funding Statement

Not applicable

Authors' Contribution

All authors contributed equally.

Conflict of interest

There is no conflict of interest among the authors of the manuscript.

[Citation: Amjad, M., Intisar, A., Sattar, T., Hussain, T., Batool, N., Ahmad, S., Mutahir, Z. Chattha, M.B. (2024). Volatile composition and antibacterial activity of *Ceiba insignis* flowers. *Biol. Clin. Sci. Res. J.*, **2024***: 1242*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1242]