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Cestrum nocturnum essential oil: Examining the Chemical, Antioxidant, and Antibacterial Dimensions for Herbal Toothpaste Formulation

Suryakant Pradhan[a], Nishtha Paul [a]*, Narayan Singh[b]*, Manish Kumar[c], Manas Pal[d]*and Deepak Rohilla[e]

[a] Department of Biosciences, University Institute of Biotechnology, Chandigarh University, Gharuan, Mohali, Punjab, India.

[b] Department of Botany, Sai School of Biosciences, Sri Sai University, Palampur, Himachal Pradesh, India.

[c]Department of Chemistry, Kurukshetra University, Kurukshetra, Haryana,136119

[d] Department of Chemistry, School of Science, Indrashil University, Rajpur, Mehsana 382715, Gujarat, India.

[e] Department of Chemistry, Vaish College Bhiwani, Haryana 127021

* Corresponding authors

Email: p.nish8@gmail.com (N. Paul), narayan.thakur90@gmail.com (N. Singh)

manas_chem@yahoo.com (M. Pal)

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Abstract: Herein, we have demonstrated the extraction, analysis, and medicinal use of the essential oil available in the *Cestrum nocturnum* plant. Gas Chromatography-Mass Spectrometry (GC-MS) report of the extracted oil reveals 18 different chemical constituents such as Diethylphthalate (31.88%), *α*-terpineol (31.52%), benzyl acetate (11.61%) and γ-terpineol (9.78%) as major products along with several other compounds. 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay is used to measure the antioxidant yields (IC_{50} value of 15.8±0.51µg/ml) of the extracted oil. Furthermore, the oil (6.25-100µg/ml) was assessed for its antibacterial activity against human pathogens such as *Proteus mirabilis, Klebsiella pneumonia, Shigella flexneri,* and *Clostridium botulinum* The oil revealed the highest inhibition in *C. botulinum* (ZOI= 38.1±0.65 mm), followed by *K. pneumoniae*, *S. flexneri*, and *P. mirabilis*. Further the oil was used to formulate herbal toothpaste. Various aspects of toothpaste, such as pH, stability, moisture content, homogeneity, foaming power and spread-ability, among others, were assessed and found to be quite promising. The toothpaste was also checked for its activity against *Porphyromonasgingivalis*, a bacterium associated with gingivitis. The formulation displayed substantial effects (ZOI=40.5±1.2 mm) with a MIC of 27 µg/mL, surpassing both the Dabur toothpaste (36±0.7 mm; MIC= 24µg/mL) and amoxicillin (38±1.2 mm). In conclusion, this study suggests that the herbal toothpaste derived from *C. nocturnum* seems very competitive for commercialization to prevent gingivitis.

Keywords: *Cestrum nocturnum*• Essential oil • Anti-bacterial activity • Herbal toothpaste • *Porphyromonas gingivalis*

Introduction

The richest source of medicine is found in plants, which have long been valued for their healing properties. The plant-based medicines are novel, and natural, which have very few aftermaths' negative effects on health and the environment. The healing properties of the plants are owed to the existence of secondary metabolites such as phenols, flavonoids, polyketides, and terpenes (Ramawat et al. 2009; Kaur and Ahmed 2021). These metabolites exhibited various biological properties such as antioxidant, antimicrobial, anti-inflammatory, insecticidal, pesticidal, hepatoprotective, anti-cancerous, antiviral, analgesic, sedative, antipyretic and antimutagenic, and others (Pei et al. 2016; Haro-González et al. 2021; Kaushik et al. 2021). The essential oils are the metabolites present in aromatic plants, which are made up mainly of terpenes and their derivatives (Singh et al. 2020). These volatile liquids showed a wide range of bioactivities against various pathogens of mammals without harming their growth and development. In addition, these metabolites are biodegradable and therefore, non-harmful to the ecosystem and can be used without any fear of side effects (Jampílek and Kráľová 2022).

More than 150 species make up the *Cestrum* genus, the majority of which are indigenous to warm subtropical and tropical regions (Monro 2012). The night-blooming jasmine, or *Cestrum nocturnum* (Solanaceae), is an evergreen shrub having smooth, simple, glossy leaves; vine-like branches, and tubular blooms of greenish-white creamy color (Nishtha *et al.* 2017). The plant has a wide presence in tropical and subtropical parts of the globe including India (Rashed et al. 2018). Previously, the plant has been found to have antibacterial, antioxidative, anti-inflammatory, antifungal, antidiabetic, hepatoprotective, and neuroprotective properties (Keshari et al. 2020; Ahmad et al. 2023). Traditionally, *C. nocturnum* has been used for its analgesic, cardiac arrhythmic, anesthetic effect, and inhibitory effect on the central nervous system (CNS), and also used against burn and swelling (Khan et al. 2011). In addition, various bioactive components from its extracts have been separated and identified for their biological properties (Rashed et al. 2018; Valencia-Mejia et al. 2022). The oil of the plant also exhibited immense potential for having various biological activities (Al-Reza et al. 2010).

Oral asepsis is one of the most vital characteristics of human well-being. Healthy teeth lead to better nutrition and quality of life (Rosli et al. 2019). It directly affects oral functions and social interactions. Tooth decay due to the bacteria *Porphyromonas gingivalis* is a major cause of tooth loss due to chronic periodontitis at any stage of life that leads to a complex lifestyle (How et al. 2016). To maintain oral hygiene, different products have been used by humans for ages. In the modern world, synthetic products are constantly replacing natural products and as a consequence, various mouthwash and toothpastes are available in the market (Jardim et al. 2009). Nevertheless, the problem of tooth decay remains the same due to our bad food habits (Sheiham 2001), and sometimes may be due to the resistance of bacteria against the products (Rams et al. 2023). This leads to the continuous search for new products especially natural to fulfil the demand of the market. *C. nocturnum* plant has

already grown in households for its various aesthetic and medicinal uses. Therefore, it is relevant to use its essential oil for its antimicrobial activities against *P. gingivalis*. Thus, the contemporary study is designed to explore the chemical components of essential oil obtained from *C. nocturnum* and to check its antioxidant and anti-bacterial potential. We also prepared formulations of herbal toothpaste using plant extract and essential oil to check for its commercial viability.

Results and Discussion

Chemical Composition

The hydro-distillation of *Cestrum nocturnum* leaves yields a yellow-colored essential oil rich in monoterpenes (49.92%). The detailed chemical composition of this essential oil was established through GC-MS analyses (Figure 1).

In total, 18 components are identified, among which Diethylphthalate (31.88%), *α*-terpineol (31.52%), Benzyl acetate (11.61%), and *γ*-Terpineol (9.78%), were found in the majority (Table 1). There are very few reports available on the composition of *C. nocturnum* essential oil. Previous reports of *C. nocturnum* essential oil (flowers) composition showed the presence of *β*-phellandren, *α*-phellandren and *(E)-β*-ocimene as the major monoterpenes present which is different from the current study (Nickavar et al. 2009). In the current investigation, the oil consisted largely of oxygenated monoterpenes followed by other chemical groups similar to the previous report (Nickavar et al. 2009). Also, one study reported alcohols (~48%), hydrocarbons (~13%), and phenols (~8%) as major constituents of the oil extracted from *C. nocturnum* flowers and monoterpenes constituted only ~2% (Al-Reza et al. 2009). The change in the composition of oil may be due to several reasons such as geographical and climatic conditions, seasonal changes, and variant plant parts taken for extracting oil (Figueiredo et al. 2008; Sampaio et al. 2016).

Antioxidant Activity

In the case of *in vitro* DPPH assay, the percentage free radical scavenging activity of oil showed a dose-dependent increase (Figure 2). The percentage inhibition was observed with in the range of 44.07±1.04 to 89.19±0.27. The IC_{50} value of *C. nocturnum* oil was 15.78±0.51 µl/ml which was comparable to the positive control *i.e.* Ascorbic acid (IC_{50} = 5.58±0.76 µg/ml). This result showed similarity as reported previously by Al-Reza et al. (2010) in which they reported strong antioxidant activities with an IC_{50} value of 24.45±1.7 µg/ml.

The high antioxidant activities of the essential oil may be due to the presence of its major component α-terpineol, which was reported to have antioxidative activities in previous studies (Gouveia et al. 2018; Sales et al. 2020). Besides essential oil, different extracts of the plants also displayed antioxidative properties (Keshari et al. 2020; Ahmad et al. 2023). The statistical analysis of anti-bacterial activity was done using one-way ANOVA which was followed by a comparison of means using post hoc Tukey's test and expressed by a comparison of means using post hoc Tukey's test and expressed as Mean± SEM using the SPSS program.

Antibacterial Activity

By evaluating the diameter of growth inhibition zones at various concentrations (6.25-100µg/ml) of essential oil, the disc diffusion method was used to determine the *in vitro* antibacterial potential of essential oil (Figure 3). This oil showed significant antibacterial activity against four different test organisms (Figure 4). Among the four test pathogens, *Clostridium botulinum* was found to be most sensitive against the oil treatment, followed by *Shigella flexneri* & *Klebsiella pneumoniae*. *Proteus mirabilis* was most resistant to the essential oil as shown in Figure 4.

The range of inhibition zones against the oil treatments was observed to be *P. mirabilis* (5.2±0.35-24.3±0.50 mm), *S. flexneri* (6.9±0.20-30.8±0.75 mm), *K. pneumoniae* (7.2±0.30-

33.9±0.85 mm), and *C. botulinum* (9.5±0.35-38.1±0.65 mm). For *K. pneumoniae*, the essential oil (100µg/ml) demonstrated a higher zone of inhibition than the positive control (30µg/ml). The previous research on the oil's antibacterial activity against the food-borne pathogens also established the antibacterial nature of *C. nocturnum* oil (Al-Reza et al. 2009). The essential oil showed no difference in sensitivity against gram-positive and gram-negative strains, which was similar to the previous studies (Al-Reza et al. 2009).

Owing to the hydrophilic cell wall structure made up mainly of a lipo-polysaccharide that blocks the infiltration of hydrophobic oil and evades the build-up of essential oils in the target cell membrane (Bezić et al. 2003), gram-negative bacteria are considered resilient to the plant-originated oils. The activity against the bacteria can be attributed to the presence of monoterpenes (Trombetta et al. 2005). As *α*-terpineol was reported to have antibacterial activity (Li et al. 2014; Yang et al. 2023), which is one of the major components of *C. nocturnum* essential oil, it may induce antibacterial activity in the oil. Besides oil, various extract of the test plant was reported to have antibacterial activities against various strains of bacteria (Chatterjee et al. 2007; Rashed et al. 2018). Silver nanoparticles of *C. nocturnum'*s leaf extract were reported to have significant antibacterial activity against *Citrobacter*, *Enterococcus faecalis, Escherichia coli, Proteus vulgaris, Salmonella typhi*, and *Vibrio cholera* (Keshari et al. 2020).

Stability and Antibacterial Activity of Toothpaste

From the calculated results, it was established that the essential oil of *Cestrum nocturnum* had significant biological activities and therefore, can be explored for the preparation of herbal toothpaste. Herbal toothpaste has proven to have a beneficial effect on dental cavities and oral hygiene (He et al. 2019; Janakiram et al. 2020), and many of them are already available in the market (e.g. Dabur, Colgate, Patanjali *etc.*). The herbal toothpaste was formulated by using the essential oil and methanolic extract of *Cestrum nocturnum* (Figure

5). This Herbal Toothpaste was tested for its quality and usability. The results showed that the formulation was of high quality and passed all evaluation criteria (Table 2 and Table 3). The toothpaste was avocado green at room temperature with zero microbial load and rough in texture. The pH of the mixture was optimum *i.e.* 7.22 and moisture content of 96%. The paste was uniform and has good spreadability and foamability. It was easy to extrude from the tube.

In the cleaning ability test, the toothpaste showed a positive result on eggshells by removing the food color that was stuck onto it (Figure 6).

After this, an antibacterial activity assay was performed against *Porphyromonas gingivalis* (major cause of oral problems) to check its use against gingivitis. The formulation demonstrated an exceptional zone of inhibition of 40.5±1.20 mm at a MIC of 27 µg/ml, whereas amoxicillin showed 38.0±1.15 mm ZOI at a MIC of 6.25 µg/ml, and market herbal dabur toothpaste exhibited 36.0±0.70 mm ZOI at a MIC of 24 µg/ml. The MIC was comparable in both the toothpaste (*i.e.* our product and Dabur toothpaste). It was the first study on the use of essential oil and methanolic extract of *Cestrum nocturnum* for the preparation of herbal toothpaste.α-Terpineol was reported to have antibacterial activity against *P. gingivalis* (Park et al. 2012), therefore high activity of the toothpaste can be attributed to its presence as a major component of the oil. People today require non-harmful treatment for a variety of dental issues. Herbal components paved the trail for the conception of cosmetics with no negative side effects. The innocuous nature of herbal cosmetics, the evasion of allergic responses, and the time-tested efficacy of numerous components are all advantages.

Conclusion

The current study displayed the antioxidant and antibacterial activity of the essential oil of *Cestrum nocturnum*. The Diethyl phthalate and *α*-terpineol containing oil demonstrated good antioxidative activity in the DPPH assay. Thus, the oil can be used for the preparation of natural antioxidants. The oil also showed significant antibacterial activity against various human pathogens. This provides a good alternative to the synthetic antibiotics available in the market. The study also displayed the potential of *Cestrum nocturnum* plant to be used in herbal formulations against gingivitis. As it is purely laboratory tests, more clinical and field trials are needed before the actual commercialization of the product. This can lead to the formation of new high-value product for oral care.

Experimental Section

Collection and Identification of the Plant

The identification process for *Cestrum nocturnum* involved harvesting the aboveground parts of the plant from Rourkela region of Odisha (a state in India) during November 2022. To identify the species, comparisons were made with genuine herbarium specimens. This identification was further authenticated using diagnostic keys and morphological descriptions from various floras (Nishtha et al. 2017). In contrast to accession number PAN#22617 for *C. nocturnum*, the plant sample was confirmed by depositing a specimen at Panjab University, Chandigarh, India.

Extraction of Essential Oil

The above ground parts of *Cestrum nocturnum* were hydro distilled by adding 1 litre of deionized water in 100 g fresh material and allowed to distill for 4-5 hours in a Clevenger's apparatus fitted with condensation unit. The oil collected in burette is then mixed with n-Hexane solvent in a separating funnel, and after vigorous shaking; the organic layer

containing the oil was collected in a flask. The solvent was then evaporated at 40 °C to extract the essential oil and stored in a glass container at 4 °C for further use.

GC-MS Analysis

The quantitative and qualitative studies of the oil were executed *via* GC-MS (Gas Chromatography-Mass Spectrometry). GC-MS data was recorded on a Shimadzu QP 2010 (Kyoto, Japan) system having an auto-injector (AOC-5000) using a ZB-5MS column (Phenomenex, USA; Film thickness 30 m × 0.25 mm × 0.25 μm). Helium was the carrier gas (flow rate= 1.05 mL/min). The oven temperature was fixed to 70 °C for 5 min, then amplified constantly to 220 °C @ 4 °C/min and held for 5 min; MS data was attained at 70 eV with a mass range of 40-800 *m/z*. The temperature of the injector and interface was set at 240 °C, and 250 °C, respectively. The samples of extracted oil were diluted in dichloromethane (5 mg/2 mL) and 2 μL were injected into the system. Injection mode was applied as a split ratio of (10:1). Under the matching conditions, a standard mixture of *n*-alkane (C9-C24) was introduced into the GC-MS to evaluate the Kovats Retention Index (RI). The components were identified by matching their mass spectrum and comparing RI values given in the libraries (NIST 02, Wiley 7) for the same running conditions and columns.

Antioxidant Assay

The antioxidant perspective of the essential oil was estimated by using a sterilized DPPH-containing purple-colored methanol solution. First of all 1 mM DPPH solution was made using methanol as solvent. Then 3 mL of the essential oil (at concentrations ranging from10-50μl/ml) was added into 1 ml of DPPH solution and kept in the dark for 30 mins followed by measuring the absorbance at 517 nm (Chu et al. 2000). Ascorbic acid was used as positive control.

Free radicals scavenging activity was estimated as follows:

Percentage of radical scavenging activity (%) $= \frac{A0-A1}{A0} \times 100$

Where, A_0 = absorbance of control and A_1= absorbance of sample.

Antibacterial Activity

Antibacterial potential of the essential oil was evaluated against some common disease-causing bacteria obtained from excreta of humans and beet skin and identified for further study. Four distinct bacteria, namely *Proteus mirabilis* (Urinary tract infection), *Shigella flexneri* (Shigellosis), *Klebsiella pneumonia* (Pneumonia), and *Clostridium botulinum* (Botulism), were used to assess the antimicrobial activity of *Cestrum nocturnum*'s oil.

Isolation and evaluation of microbes (Raj et al. 2016)

Proteus mirabilis strain was obtained from human urine and cultured for motility on nutrient agar media. On the agar plate, it manifested as singly arranged, paired, and cluster-like colonies. It is characterized as a Gram-negative, elongated swarmer cell, facultatively anaerobic, chemoorganotrophic, rod-shaped, hyper-flagellated, motile bacterium that does not form spores. The biochemical assays confirmed these characteristics, validated by negative Voges Proskauer and positive results for citrate, urease, catalase, methyl red, H_2S, and nitrate reduction tests.

Shigella flexneri was isolated from fresh human stool. Cultured on blood agar media, it formed translucent colonies. Gram staining revealed it to be a Gram-negative, rod-shaped, non-flagellated, and non-sporing bacterium. Biochemical assays, including negative results for oxidase, urease, citrate, H_2S, and Voges Proskauer, along with positive catalase, methyl red, and nitrate reduction tests, confirmed its phenotype.

Klebsiella pneumoniae was isolated from a pneumonia-affected patient's urine and cultured on blood agar media. The colonies appeared pinkish-grey, mucoid, arranged in pairs and short chains, and were non-haemolytic. This bacterium is Gram-negative, non-spore forming,

non-motile, and is facultative anaerobic. It passed biochemical tests, showing positive results for catalase, citrate, nitrate reduction, urease, and Voges Proskauer, while yielding negative results for H_2S, methyl red, and oxidase.

Clostridium botulinum isolated from beet skins and was grown on a Nutrient agar medium. This anaerobic, rod-shaped, Gram-positive bacillus underwent beta-hemolytic testing to confirm its identity after biochemical assays. The beta-hemolytic test verifies *C. botulinum* after the biochemical assays.

Antibacterial assay

By using the traditional disc diffusion approach (Bauer et al. 1966), the activity was assessed against the isolated bacterial strains. Various concentrations of *Cestrum nocturnum* oil (6.25-100µg/ml *via* diluting in Methanol) were used against the bacterial cultures spread on an agar plate. For positive control, Antibiotic, Gentamycin (30µg/ml) was used and for negative control, Methanol was taken. The plate was placed in an incubator at the temperature of 37°C±1 for 18–24 hours. By measuring the diameter of the inhibited zone, the inhibition zones were calculated after incubation.

Preparation and Assessment of Herbal Toothpaste

Preparation of the Plant Extract

Leaves from the plants were collected in the early hours from the same region for essential oil extraction and transferred to the lab. The leaves were sun-dried for 18 days before being crushed into a coarse powder with the help of a mortar and pestle. The methanolic plant extract was prepared by using a vortex shaker. For this, 5gm powder was added to 20 ml methanol and vortexed for 15 minutes (Rokade et al. 2018).

Preparation of Toothpaste

To formulate the toothpaste, 20 ml of flax seed gel and 20 ml of edible gum were thoroughly combined in a beaker. To this mixture, 7 ml of glycerine was added. For herbal Properties, 3 ml of *Cestrum nocturnum* essential oil and a methanolic extract of leaves (5ml) were added. All components were carefully combined until reaching a consistent mixture. Subsequently, 0.1 g of sodium lauryl sulphate and 5 g of calcium carbonate were added. A few drops of Blueberry emulsion (0.2 ml) were then added as a flavoring agent. The entire blend was stored at room temperature in a plastic utility squeeze tube (Narayanasamy et al. 2023).

Evaluation of Formulated Herbal Toothpaste Sample

pH Determination

Using a digital pH meter, pH of the formulated toothpaste was determined. About 1 gram of toothpaste was dissolved in 5ml of water and its pH was checked. The pH meter was calibrated before using it (Deshmukh et al. 2017).

Moisture Content

To check the moisture content of the herbal formulation, an empty crucible was taken and pre-weighed. It was then kept in the oven for 20 minutes at 105°C to remove any moisture. After drying, it was cooled, and then 3g of the sample was put in it and placed in an oven at 135°C for another 2 h. After complete drying of the sample, it was taken out and ventilated in a desiccator for 10 minutes and the crucible was weighed again (Gautam et al. 2020). The moisture content was measured using the following equation:

$$\textit{Percentage of moisture content (\%)} = \left(\frac{Weight of dry sample}{Fresh\ weight of sample}\right) \times 100$$

Stability of Formulation

Three crucibles were taken, and samples were kept in all three, first one was placed at 4°C, the other one at 25-30°C, and the last one at 65°C. They were kept for 10 days and the most suitable temperature was found for storage (Narayanasamy et al. 2023).

Spreadability

A glass plate was taken and wiped with ethanol. A toothpaste sample was placed in the center of the plate and another glass plate was placed above it. A weight was placed on it and was left for 30 minutes. The load was removed and then the diameter of the spread was measured (Gautam et al. 2020).

Sharp edge and abrasive particle test

On butter paper, the toothpaste sample was spread 10 times continuously. This test was done to test whether the sample contained any sharp, inappropriate materials other than toothpaste components (Oluwasina et al. 2023).

Homogeneity test

A homogeneity test was done to find the amount of toothpaste that oozes out whenever it is squeezed. This quantity must be similar in every squeeze. Three times the toothpaste was squeezed out and the quantity was noted (Deshmukh et al. 2017).

Extrudability test

It is the power required to push that is required to remove the sample from the tube. The product was compressed until the structure of the product was disrupted and it was extruded through the sample tube. It was important to determine the changes in a product's consistency throughout its shelf life (Oluwasina et al. 2023).

Cleaning ability test

In a beaker containing distilled water, food color was added as was boiled. Now to this glacial acetic acid was added and to it, a raw egg was kept into it. This egg was kept in the beaker for about 15 mins. After 15 mins, the egg was taken out, and the sample was brushed over the eggshell with the help of a toothbrush. It was used to check the amount of food color present on the eggshell that was washed out with the help of a sample (Gautam et al. 2020).

Foaming Index

A glass beaker with 100 ml distilled water was taken and is boiled, then 1g of sample was taken and heated at 90°C on the hot plate for 30 minutes followed by continuous stirring. After that, it is cooled and filtered. Now test tubes were taken and different concentrations of the filtrate and distilled water is added, in every test tube, the volume is kept 10ml. The test tubes were shaken for 15 seconds and then a resting phase of 15 minutes was given after which foam was measured (Narayanasamy et al. 2023).

Acquisition and evaluation of microbial isolates for herbal toothpaste

Microbial strain of *Porphyromonasgingivalis* was collected from the pathological lab, PGI Hospital, Chandigarh, India that was used for carrying out further experiments. The microorganisms were nurtured on blood agar until they were isolated from clinical specimens. Using the gram staining method bacteria morphological structure was figured out. It was observed to be black-pigmented, gram-negative, anaerobic, non-motile, rod-shaped bacteria (Coats et al. 2009). The phenotype exhibited by the microbial colonies was subsequently identified by subjecting them to a biochemical test. The presence of *P. gingivalis* was established by catalase, oxidase, and indole test (Gölz et al. 2014).

Antibacterial assay

To check the antibacterial activity of herbal formulation against *Porphyromonasgingivalis,* NAM media was prepared and spread thebacteria onto the agar plate. Put a disc dipped in the toothpaste, antibiotic (Amoxicillin), and market toothpaste, which were kept on the plate. After incubating the Petri dish at 37±1ºC, the zone of inhibition and minimum inhibitory concentration values were calculated and the average was obtained (Tollefson and Miller 2000).

Statistical Analysis

All the tests were scrutinized in randomized design in triplicates. The data collected from all the experiments were analyzed using SPSS 16.0 (SPSS Inc., Chicago). The regression analysis was done using Sigma Plot 8.0.

CRediT authorship contribution statement

Suryakant Pradhan: Formal analysis, Data curation, and writing original draft; **Nishtha Paul**: Conceived the idea, Methodology, Resources, Review, and Supervision; **Narayan Singh:** Investigation, Data curation; Resources, Conceptualization, Supervision; **Deepak Rohilla**: Data analysis and review; **Manas Pal**: Review and editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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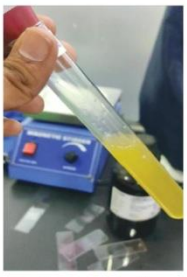
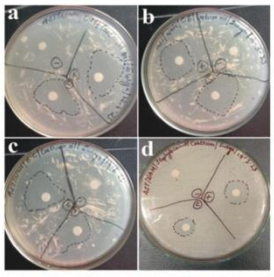
Entry for the Graphical Illustration

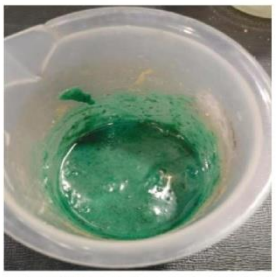
Cestrum nocturnum Plant



Extracted oil

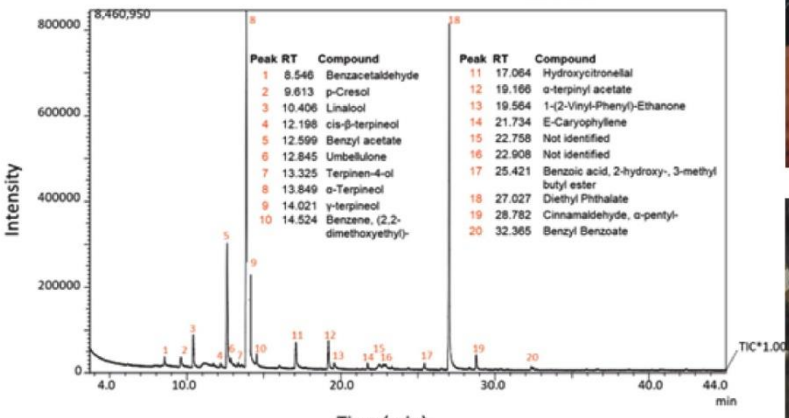


Antibacterial activity against
a. *L. monocytogenes*
b. *C. botulinum*
c. *S. aureus*
d. *E. coli*



Formulated herbal toothpaste

Cleaning test on egg shell



Peak	RT	Compound
1	8.546	Benzacetaldehyde
2	9.613	p-Cresol
3	10.406	Linalool
4	12.198	cis-β-terpineol
5	12.599	Benzyl acetate
6	12.845	Umbellulone
7	13.325	Terpinen-4-ol
8	13.849	α-Terpineol
9	14.021	γ-terpineol
10	14.524	Benzene, (2,2-dimethoxyethyl)-
11	17.064	Hydroxycitronellal
12	19.166	α-terpinyl acetate
13	19.564	1-(2-Vinyl-Phenyl)-Ethanone
14	21.734	E-Caryophyllene
15	22.758	Not identified
16	22.908	Not identified
17	25.421	Benzoic acid, 2-hydroxy-, 3-methyl butyl ester
18	27.027	Diethyl Phthalate
19	28.782	Cinnamaldehyde, α-pentyl-
20	32.365	Benzyl Benzoate

GC-MS Profile of the essential oil