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# Phytochemical profiling of antimicrobial and potential antioxidant plant: *Nepeta cataria*

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Traditional and phytochemical studies have confirmed the richness and diversity of medicinal plants such as *Nepeta cataria* (*N. cataria*), but more studies are needed to complete its metabolite profiling. The objective of this research was to enhance the metabolomic picture and bioactivity of *N. cataria* for better evaluation. Phytochemical analysis was performed by bio-guided protocols and gas chromatography-mass spectrometry (GC/MS). For this, solvents such as methanol, ethanol, water, acetone, and hexane were used to extract a wide number of chemicals. Antibacterial analysis was performed using the 96-well plate test, Kirby Bauer's disk diffusion method, and the resazurin microdilution test. Antioxidant activity was determined by the DPPH assay and radical scavenging capacity was evaluated by the oxygen radical absorbance capacity (ORAC) assay. GC/MS analysis revealed a total of 247 identified and 127 novel metabolites from all extracts of *N. cataria*. Water and acetone extracts had the highest identified metabolites ( $n = 79$ ), whereas methanol extract was the highest in unidentified metabolites ( $n = 48$ ). The most abundant phytochemicals in methanol extract were 1-isopropylcyclohex-1-ene (concentration = 27.376) and bicyclo [2.2.1] heptan-2-one (concentration = 20.437), whereas in ethanol extract, it was 9,12,15-octadecatrienoic acid (concentration = 27.308) and 1-isopropylcyclohex-1-ene (concentration = 25.854). An abundance of 2 methyl indoles, conhydrin, and coumarin was found in water extracts; a good concentration of eucalyptol was found in acetone extract; and 7,9-di-tert-butyl-1-oxaspiro is the most abundant phytochemicals in hexane extracts. The highest concentration of flavonoids and phenols were identified in hexane and methanol extracts, respectively. The highest antioxidant potential (DPPH assay) was observed in acetone extract. The ethanolic extract exhibited a two-fold higher ORAC than the methanol extract. This examination demonstrated the inhibitory effect against a set of microbes and the presence of polar and non-polar constituents of *N. cataria*.

The results of this study provide a safe resource for the development of food, agriculture, pharmaceutical, and other industrial products upon further research validation.

#### KEYWORDS

*Nepeta cataria*, gas chromatograph/mass spectrometry (GC/MS), antibacterial susceptibility testing (AST), antioxidants, phytochemicals

## Introduction

The *Nepeta* genus belongs to the family Lamiaceae, which is rich in bioactive secondary metabolites. The word *Cataria* was derived from the Latin word for cat, “*Cathus*.” *N. cataria* is a perennial herb that grows to a height of 50–100 cm (Scott, 2003). It has been found predominately in the regions of southern and eastern Europe, the Middle East, Central Asia, and China. Bioactive compounds of *N. cataria* have prehistorically been used and have a wide range of biological activities, including analgesic, anti-asthmatic, anti-cancer, anti-inflammatory, and antimicrobial properties. *Nepeta cataria* essential oil and metabolites have important applications in the pharmaceutical, agrochemical, and food industries (Sharma et al., 2021). Researchers found them to be antifungal, antibacterial (Bandh and Kamili, 2011; Sharma et al., 2019), antioxidant (Adiguzel et al., 2009), insecticidal, anti-inflammatory, anti-nociceptive, and potentially spasmolytic (Pargaïen et al., 2020; Giarratana et al., 2017). Essential oils, flavonoids, phenolic acid, steroids, terpenoids, and terpenoid hydrocarbons have all been found in this plant.

*Nepeta cataria* has widely been used to treat diarrhea because of spasmolytic and myorelaxant metabolites in its extracts (Gilani et al., 2009). Essential oils of *N. cataria* have a promising impact on raw materials of industrial food importance (Frolova et al., 2020). Studies established the presence of nepetalactones in catnip essential oil by TLC and GC–MS analysis. Using GC/MS analysis, three populations of *N. crassifolia* and four populations of *N. nuda* were studied (Sharma et al., 2021).

Essential oils and flavonoids have typically been linked to the therapeutic benefits of *Nepeta* species. Prior investigations on the essential oils of *N. cataria* identified nepetalactone as a major constituent (Mamadaliyeva et al., 2017; Sharma et al., 2019). In a recent study, water-based extracts of *N. cataria* significantly inhibited herpes virus replication in humans (Hinkov et al., 2020). Previously, *N. cataria* has been used to alleviate symptoms of bronchial asthma, bronchitis, and bronchial congestion. The traditional herbal medicine derived from these along with other medicinal plants may have multiple applications, including symptom relief for people with COVID-19 and the development of effective antiviral medicines. During the severe acute respiratory syndrome coronavirus (SARS-CoV-2)

pandemic, also termed COVID-19, leaves of *N. cataria* were used to alleviate symptoms of the disease (Khan et al., 2021). Essential oils from *Nepeta* species that naturally produce nepetalactones can be synthesized in other regions and then be distilled to serve as a natural source of efficient *Aedes aegypti* repellent for effective dengue prevention (Reichert et al., 2019). Previous studies demonstrate that *N. cataria* essential oils effectively reduced liver damage caused by acetaminophen and enhanced mRNA expression of uridine diphosphate glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) and decreased CYP2E1 activity (Tan et al., 2019). It has been shown that *N. cataria* and its derivatives have been used to treat gastrointestinal and respiratory disorders. They have also been reported for their effective antibacterial, antiviral, and antioxidant activities (Sharma et al., 2019). Porcine reproductive and respiratory syndrome virus (PPRSV) affects pigs and causes reproductive failure in developing pigs. According to the findings of a study, the load of PRRSV could be greatly reduced by using *N. cataria* hydrosol. It is strongly recommended that further research be conducted into the antiviral processes and characteristics of these plant hydrosols, both *in vitro* and *in vivo* (Kaewprom et al., 2017).

Recent research has been focused on the essential oils and antibacterial properties of plants, as they have been utilized to increase the shelf life of foods and in traditional medicine (Ergün, 2021; Özkan et al., 2021). Numerous studies demonstrate that the antibacterial and antifungal properties of *N. cataria* are mostly attributable to the essential oil constituents. Surprisingly, less is known about the antimicrobial activity of catnip essential oil. In these investigations, the antimicrobial activity of catnip essential oil was investigated on a limited number of bacteria or fungi (Angelini et al., 2006; Suschke et al., 2007; Bourrel et al., 2011).

In the past two decades, the antioxidant effect of the essential oils and/or extracts of medicinal and aromatic plants has received considerable attention. Therefore, these extracts can be employed as safe and effective synthetic preservative replacements. Natural antioxidants have been investigated extensively for their ability to protect organisms and cells against oxidative stress-induced damage, which is believed to be a cause of aging, degenerative illnesses, and cancer (Sharma et al., 2019). It has been known for some time that plant extracts and/or

essential oils possess antioxidant properties. However, less is known about the antioxidant activity of the essential oil or extract of *N. cataria*.

In another study, aromatic and medicinal plants from Turkey have been characterized and reported on the antibacterial and antioxidant activities of *N. cataria*'s essential oil, methanol extract, and its essential oil composition. They also highlighted essential oil to contain 4 $\alpha$  $\beta$ , 7 $\alpha$ , 7 $\beta$ -nepetalactone, 4 $\alpha\alpha$ , 7 $\alpha$ , 7 $\beta$ -nepetalactone, 1,8-cineole, and elemol as major oil constituents in *N. crassifolia* (Dabiri and Sefidkon, 2003), while 7 $\beta$ -nepetalactone, 4 $\alpha\alpha$ , 7 $\alpha$ , 7 $\beta$ -nepetalactone, pulegone, and piperitenone oxide were identified in *N. nuda* (Narimani et al., 2017). Research studies focused mainly on essential oil extracts of *N. cataria*, which indicated a need to study its metabolites in polar and nonpolar solvents. Our team was motivated to explore the constituents of *N. cataria*, based on polarity, via minor adjustments to already established lab protocols.

## Materials and methods (experimental)

### Plant collection

*Nepeta cataria* was collected from Swat (Himalayas), Khyber Pakhtunkhwa, Pakistan (35°22'59.99" N, 72°10'60.00" E), locally named as catnip mint/catmint (in northern Pakistan) and Badranj boya (in central Pakistan). Species verification and identification were done at the National Herbarium, and they confirmed and identified it as *N. cataria*. Furthermore, it was cleaned, rinsed, dried, and preserved at the Antimicrobial Biological Laboratory (AMBL), International Islamic University Islamabad, Islamabad, Pakistan.

### Plant extraction and filtration

*Nepeta cataria*'s stem and leaves were rinsed, dried, and grounded in a fine powder by a lab grinder carefully. Fine powder was soaked separately in methanol, ethanol, water, acetone, and hexane using 1:10 ratio for 24–48 h at room temperature, to increase the maximum solubility. Filtrations and extraction were done using Whatman's # 41 and rota-evaporator at Stockbridge Medicinal and Aromatic Lab, University of Massachusetts Amherst, USA. Extracts were labeled and aliquoted in glass vials at 4°C until further use.

## Phytochemical analysis

### Qualitative analysis

Saponins and phenolic compounds, water-soluble and insoluble phenols, alkaloid flavonoids, poly-steroids, terpenoids,

cardiac glycosides, free and combined anthraquinones, tannins, and alkaloids were chemically identified in all plant extracts (Prabhavathi et al., 2016).

### Quantitative analysis—Phenols and flavonoids

Concentrations of phenols and flavonoids were identified in all extracts of *N. cataria* via established protocols previously explained in Nadeem et al. (2021).

### GC/MS analysis of *N. cataria* extracts

The GC/MS is the widely adopted technique for the detection of biologically active compounds, i.e., metabolites. A set of extracts, methanol, ethanol, water, acetone, and hexane were subjected to GC/MS analysis to detect bioactive phytochemicals. Phytochemical compounds were identified and presented with their compound names, molecular formulas, molecular weight, and retention times (RT) using NIST Library 17.

Metabolic profiling of *N. cataria* extracts was conducted via GC/MS (Bruker Scion 456 GC, EVOQ triple quadrupole GC-MS/MS). A column of 15 m was used with a diameter and film thickness of 0.25 mm. The flow rate of helium as a carrier gas was 1.5 ml/min. For gas chromatography, temperature conditions were 45°C for 3 min, 250°C at 8°C/min for 10 min. Injection volume was 1  $\mu$ l [varying split ratio (5:1/15:1/20:1), range (45–350 m/z)]. Automated Mass Spectral Deconvolution and Identification System (AMDIS) Software MSWS 8 for GC/MS and NIST library were used for compilation of all results.

### Antibacterial activity

Bacterial cultures (Table 1) were grown on a tryptic soy broth (TSB) medium (Thermo Fisher Scientific, USA) (Nadeem et al., 2021). To evaluate antibacterial susceptibility testing (AST) of *N. cataria* extracts, three different methods were used, i.e., 96-well test, Kirby-Bauer disk diffusion, and resazurin-based well plate microdilution method.

### The 96-well plate method

In each well of a 96-well microtiter plate, 100  $\mu$ l of plant extract and TSB media were used. Each plant extract was checked at five bacterial concentrations (i.e., 1,000, 500, 250, 125, and 62.5  $\mu$ g) for optimum antimicrobial potential. Only TSB medium was added to negative control well to ensure sterility of media. A single negative control lacked plant extract to observe normal bacterial growth. Microtiter plates were incubated for 24 h before reading at 570 nm. Chloramphenicol as standard was used to evaluate the results. Bacterial inhibition was calculated

TABLE 1 Microbial profile of bacterial ingredients used in the antimicrobial analysis.

Microorganism	Accession number	Strain
<i>Escherichia coli</i>	ATCC_25922	Gram negative
<i>Klebsiella oxytoca</i>	ATCC_43863	
<i>Salmonella enterica</i>	ATCC_14028	
<i>Shigella sonnei</i>	ATCC_25931	
<i>Citrobacter ferundii</i>	ATCC_8090	
<i>Bacillus subtilis</i>	ATCC_6051	Gram positive
<i>Lactococcus lactis</i>	ATCC_LMO230	
<i>Listeria monocytogenes</i>	ATCC_LM21	
<i>Micrococcus luteus</i>	ATCC_4698	
<i>Staphylococcus aureus</i>	ATCC_25923	

via the following formula:

$$\text{Bacterial inhibition} = \frac{\text{OD in control} - \text{OD in treatment}}{\text{OD in control}} \times 100$$

### Kirby-Bauer disk diffusion method

Solidified agar plates were used to analyze the antimicrobial potential of *N. cataria* extracts. Paper disks of 10 mm were soaked in 20  $\mu$ l extracts, then placed on prepared culture plates and incubated for 24 h at a 25–35°C temperature. Paper disks (10 mm) were soaked in 20  $\mu$ l of distilled water as a negative control to avoid any influence on bacterial growth (Sarin and Bafna, 2012). Aseptic conditions were maintained via working in a laminar flow. All extracts were tested in biological triplicates, and results were represented as average values of inhibition zones in mm  $\pm$  standard deviation.

### Resazurin-based well plate microdilution method

Resazurin solution was prepared (121.5 mg resazurin powder in 18 ml of ddH<sub>2</sub>O) and mixed for 1 h (pH = 7.4). TSB liquid medium and *N. cataria* extracts (100  $\mu$ l each) were added to each well. Plant extract was added in serial dilution to separate wells. Each well was supplied with 106 CFU/ml of bacterial inoculum. Double negative control well was supplied with TSB media only. Single negative control well was supplied with TSB media and bacterial culture. Plates were incubated overnight and then 20  $\mu$ l of resazurin was added to each well and incubated for another 4 h. Absorbance at 550–590 nm was read via spectrophotometer (SPECTRA MAX M2e plate reader) (Packialakshmi and Naziya, 2014).

### DPPH antioxidant assay

The Bersuder (Edewor and Usman, 2011) method was used for antioxidant determination via DPPH radical scavenging assay. All solvent extracts were mixed with DMSO addition and DPPH-ethanol reagent was made separately. Plant-DMSO mix was saturated with DPPH-ethanol reagent for 6 h. Negative control was prepared by dissolving ascorbic acid in DMSO (50–500  $\mu$ mol/L), which was used to generate calibration curve with 517 nm absorbance read via SPECTRA MAX M2e plate reader (Packialakshmi and Naziya, 2014).

### Oxygen radical absorbance capacity assay protocol

Various dilutions of methanolic and extracted samples were mixed with buffered saline (10 mM, pH 7.6). Decaying of fluorescein induced by AAPH was compared to Trolox (positive control) over 120 min to evaluate the antioxidant activity via the SPECTRAMAX M2e Plate reader. Results were presented as  $\mu$ M Trolox Equivalent/100  $\mu$ l of plant extract.

### Statistical analysis

The results of all the experiments were analyzed under a complete randomized design (CRD) with three replications for each treatment. Results were statistically analyzed using GraphPad Prism and Microsoft Office Excel 2016 version. Means were calculated, and one-way analysis of variance (ANOVA) test was performed for multiple comparisons of all the mean values. Mean differences were calculated by least significant difference (LSD) at 0.05 probability.

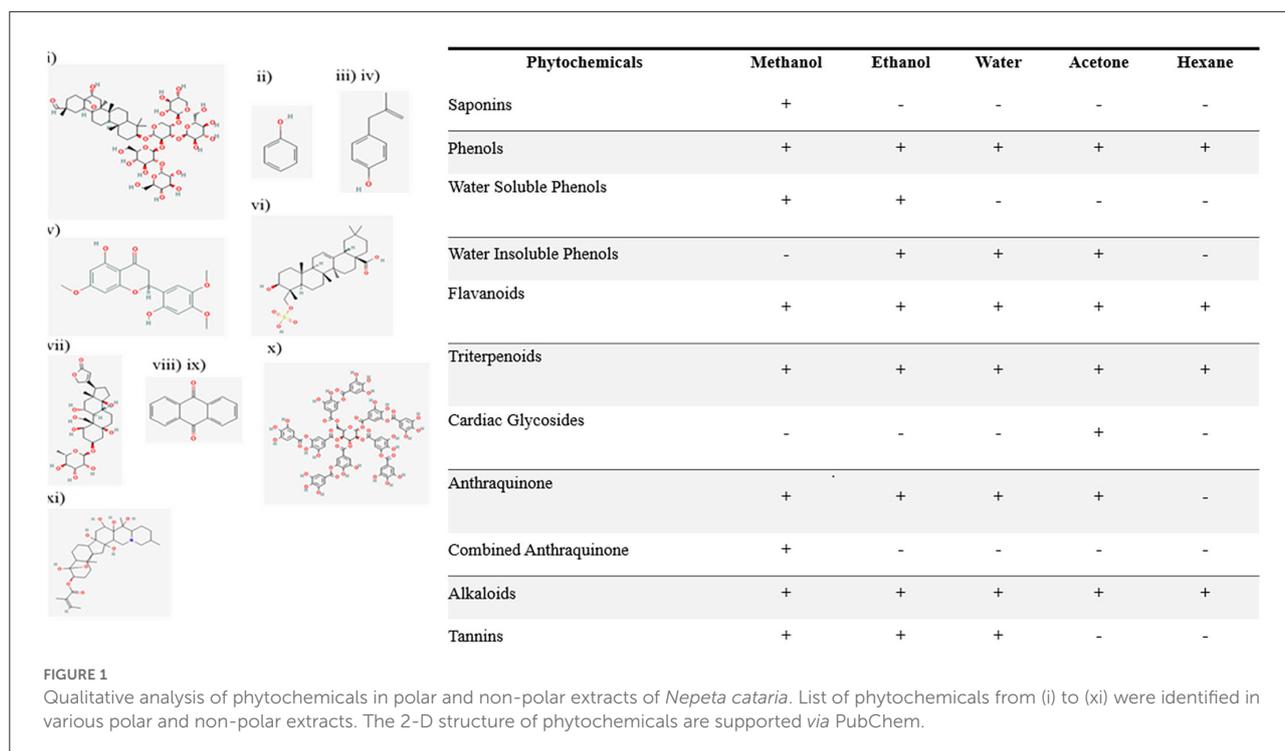
## Results

*Nepeta cataria* contains medicinally important phytochemicals along with many unknown metabolites that need further studies (Elshikh et al., 2016; Mir et al., 2016). High antioxidant activity was exhibited in acetone extract of *N. cataria*. Moreover, high flavonoid content was found in water and hexane extracts, and methanol extracts were specifically rich in phenols.

### Preliminary phytochemical analysis

#### Qualitative phytochemical analysis of *N. cataria*

Saponins were found in the methanol-based extracts of *N. cataria*. Phenols were positive in all extracts and showed



high  $\mu\text{g/ml}$  concentration in methanol. Water-soluble phenols were present in all the polar solvents only. Water insoluble phenols were identified in the ethanol, acetone, and hexane-based extracts. A qualitative test for flavonoids was carried out, and the development of intense yellow color indicates presence of flavonoids (Figure 1). A qualitative test for terpenoids was conducted by observing a reddish-brown coloration development, which confirms the positive test results in all extracts. Cardiac glycosides were indicated via development of green-blue color. Acetone-based extracts were positive only. Free anthraquinones were present in all extracts of *N. cataria* except hexane-based extract. Combined anthraquinones were only present in methanol-based extract of *N. cataria*. Qualitative tests for tannins were found positive only in extraction of polar solvents. Alkaloids were present in all the extracts of *N. cataria*.

## DPPH antioxidant activity

presence of antioxidants was determined in *N. cataria* extracts in a set of different extractions and was measured spectrophotometrically, results were drawn as  $\mu\text{mol}$  of ascorbic acid equivalents/L, and the results are given in Figure 2A. The presence of antioxidants was found in the following order: acetone extracts > water extracts > ethanol extracts > methanol extracts > hexane extracts.

## Total flavonoid and phenol content

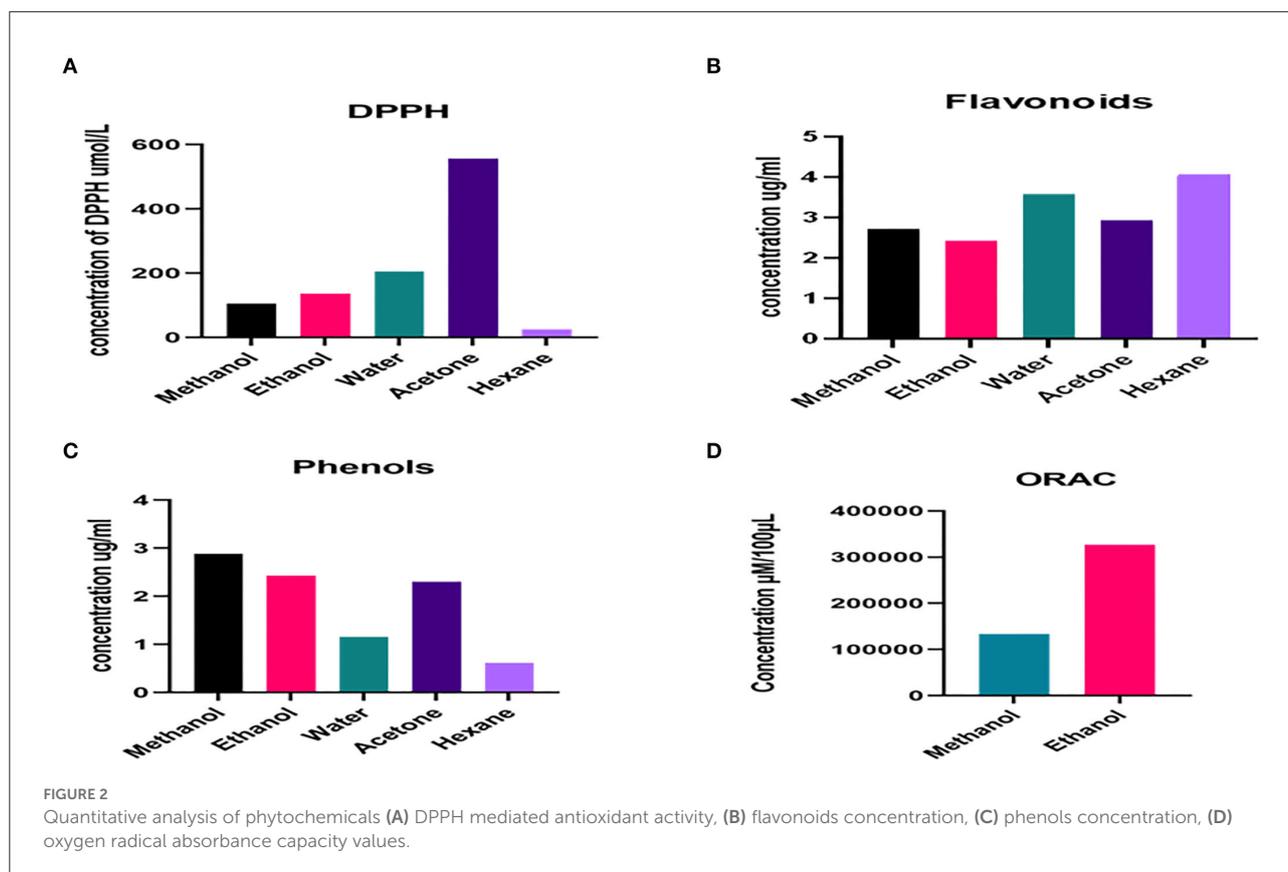
The flavonoids in polar and non-polar extracts of *N. cataria* were quantified in terms of  $\mu\text{g}$  of catechin equivalents/ml. Hexane and water-based extracts showed high levels of flavonoids as compared to acetone, methanol, and ethanol-based extracts. Flavonoid results are summarized in Figure 2B. Several other studies prove the presence of flavonoids in *N. cataria* extract and indicate therapeutic potential for lung cancer because of its flavonoid content (Naguib et al., 2012; Yang et al., 2020).

The methanol, ethanol, water, acetone, and hexane extracts of *N. cataria* were examined in terms of  $\mu\text{g}$  of gallic acid equivalents per ml to quantify levels of total phenols. Methanol, acetone, and ethanol-based extracts showed the maximum presence of phenols as compared to water and hexane-based extracts. The order of phenolics (Figure 2C) presence in the sample was found as follows:

Methanol extracts > Ethanol extracts > Acetone extracts  
> Water extracts > Hexane extracts.

## ORAC assay on *N. cataria* extracts

Oxygen radical absorbance capacity was performed to study the antiradical activity in methanol and ethanol extract of *N. cataria*. Results showed two-fold higher ORAC in ethanolic



extracts than methanol extract (Figure 2D), signifying our results of DPPH, free radical scavenging activity (Lucas-Abellán et al., 2008).

## Determination of antibacterial activity

### Percentage growth inhibition by 96-well method

Percentage growth inhibition of each tested bacteria, viz., *Shigella sonnei*, *Bacillus subtilis*, *Klebsiella oxytoca*, *Escherichia coli*, *Salmonella enterica*, *Micrococcus luteus*, and *Staphylococcus aureus* (*S. Lactococcus lactis*, *Listeria monocytogenes*, and *Citrobacter freundii*). Percentage growth inhibition of bacterial isolates is given in Figure 3.

### Kirby-Bauer disk diffusion method

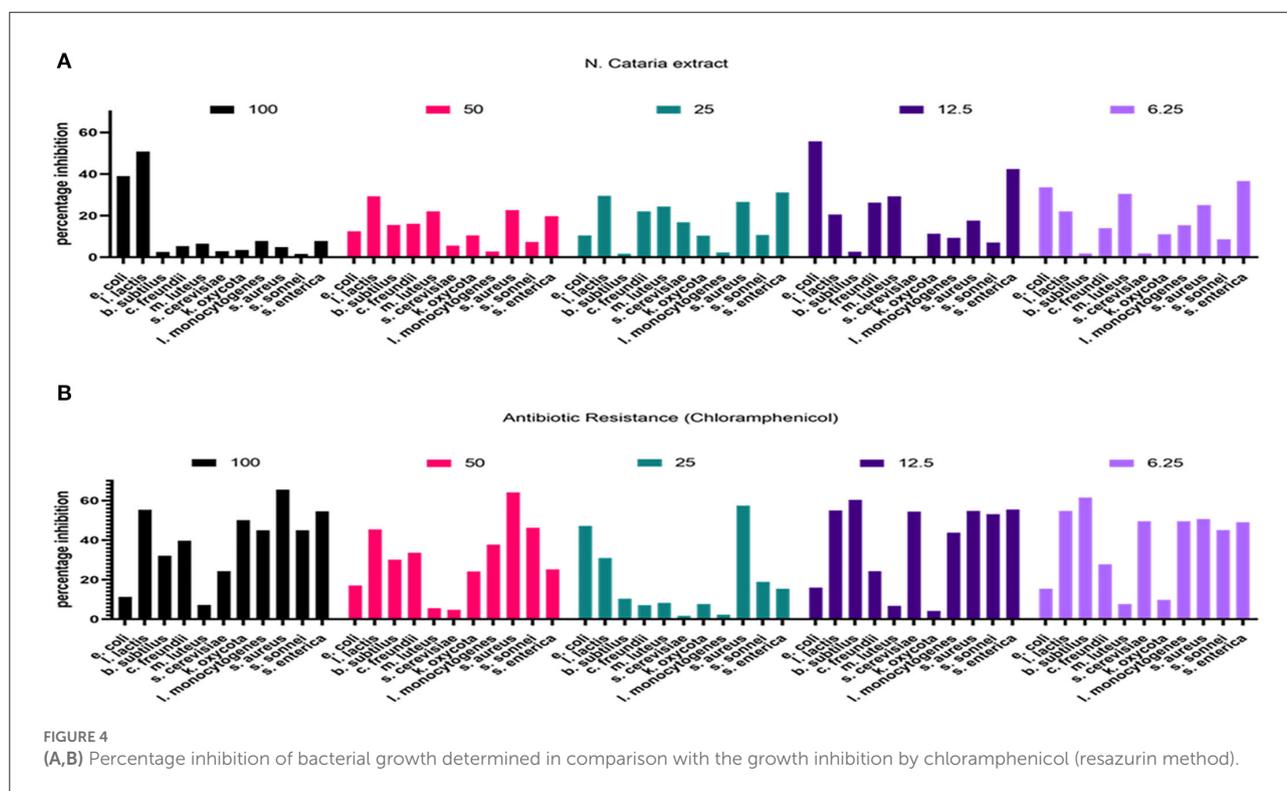
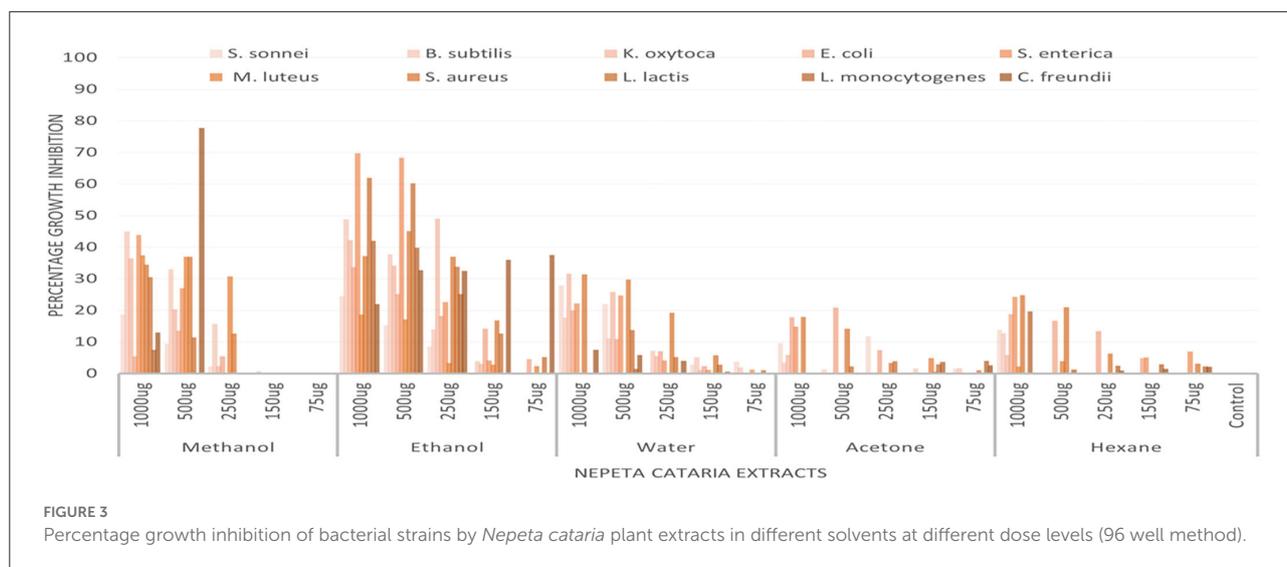
Kirby disk diffusion method was followed to measure the antimicrobial efficacy of plant extracts by the zone of inhibition (mm) *in vitro* conditions on solidifying agar media. Chloramphenicol was used as a standard and zone of inhibition was >25 mm for all strains according to CLSI guidelines (Humphries et al., 2018).

## Resazurin-based well plate microdilution method

The resazurin method was used to check the antimicrobial efficacy of each prepared plant extract against tested bacterial agents. Chloramphenicol was used as a positive control at 6.25–100 µl/ml dose levels, and data on percentage bacterial growth inhibition was recorded. Plant extract of *N. cataria* showed a varied efficacy against all the tested bacterial isolates compared to the positive and negative control, and results are presented in Figure 4.

## GC/MS analysis of *N. cataria*

The GC/MS analysis of a methanolic extract of *N. cataria* showed (68 identified phytochemicals + 48 unmatched) chemicals (Table 2). Analysis of ethanol-based extracts confirmed the existence of 79 known phytochemical constituents, while 31 unmatched chemicals were detected (Table 3). Water-based extracts of *N. cataria* contain 28 known phytochemicals, while 11 unmatched chemicals were also detected (Table 4). Acetone-based extract confirmed the existence of 13 known compounds' extract, while 9 chemical



constituents were unmatched (Table 5). Analysis of hexane-based extracts confirmed the presence of 9 known chemical constituents, while 8 unmatched chemicals were detected, as given in Table 6. GC/MS spectral chromatograms of all the solvent-based extracts are given in Figure 5 along with the most abundant metabolite in each extract. In methanol, water, and acetone extract, 1-isopropylcyclohex-1-ene was the most abundant phytochemical. The most abundant metabolite in ethanol extract is 9,12,15-octadecatrienoic acid,

and the most abundant phytochemical in hexane extract is 7,9-di-tert-butyl-1-oxaspiro (Figure 5).

## Discussion

One of the most well-known species in the genus *Nepeta* is *N. cataria*. Several studies have performed qualitative identification of phytochemical constituents from leaves and flowers of *N.*

TABLE 2 GC/MS analysis of a methanol extract of *N. cataria* using NIST 17 Library showed (68 identified phytochemicals + 48 unmatched) chemicals, arranged according to concentration present.

Compound	Mol. formula	Amount/Conc. %	Mol. weight (g/mol)	RT (Min)	Extract
1-Isopropylcyclohex-1-ene	C <sub>9</sub> H <sub>16</sub>	27.376	124.22	12.402	Methanol
Bicyclo [2.2.1] heptan-2-one,	C <sub>7</sub> H <sub>10</sub> O	20.437	110.15	7.728	Methanol
gamma. -Sitosterol	C <sub>29</sub> H <sub>50</sub> O	8.626	414.7	33.566	Methanol
Eucalyptol	C <sub>10</sub> H <sub>18</sub> O	8.505	154.249	5.112	Methanol
n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	7.973	256.4241	20.364	Methanol
No match	-	6.419	-	6.933	Methanol
9,12,15-Octadecatrienoic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	6.401	278.43	22.304	Methanol
1-Isopropylcyclohex-1-ene	C <sub>9</sub> H <sub>16</sub>	6.144	124.22	13.699	Methanol
1,6-Octadien-3-ol, 3,7-dimet	C <sub>10</sub> H <sub>18</sub> O	5.855	154.25	9.981	Methanol
Ethyl 2-5-methyl-5-vinylnet	C <sub>13</sub> H <sub>22</sub> O <sub>4</sub>	5.845	242.3114	6.551	Methanol
Beta-Sitosterol	C <sub>29</sub> H <sub>50</sub> O	5.461	414.71	32.541	Methanol
No match	-	4.148	-	13.303	Methanol
No match	-	3.893	-	22.205	Methanol
Pentane, 1-chloro-5- methyl	C <sub>5</sub> H <sub>11</sub> Cl	3.739	106.594	10.696	Methanol
No match	-	3.063	-	12.903	Methanol
No match	-	3.008	-	13.718	Methanol
Bicyclo [3.1.0] hexane-2-undec	C <sub>6</sub> H <sub>10</sub>	2.974	82.14	13.804	Methanol
No match	-	2.786	-	26.376	Methanol
Alpha-Amyrin	C <sub>30</sub> H <sub>50</sub> O	2.691	426.729	33.062	Methanol
Pregnan-18-ol, 20-methyl-20-	C <sub>22</sub> H <sub>39</sub> NO	2.64	333.6	13.916	Methanol
No match	-	2.619	-	11.726	Methanol
No match	-	2.43	-	14.296	Methanol
No match	-	2.074	-	21.141	Methanol
No match	-	2.021	-	14.919	Methanol
No match	-	1.975	-	25.235	Methanol
Caryophyllene oxide	C <sub>15</sub> H <sub>24</sub> O	1.916	220.35	15.129	Methanol
No match	-	1.807	-	11.016	Methanol
No match	-	1.659	-	34.964	Methanol
No match	-	1.498	-	16.925	Methanol
No match	-	1.447	-	14.094	Methanol
No match	-	1.436	-	27.233	Methanol
No match	-	1.43	-	35.912	Methanol
2H-1-Benzopyran-2-one, 7-met	C <sub>13</sub> H <sub>15</sub> NO <sub>2</sub>	1.381	217.26	18.071	Methanol
Uvaol	C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>	1.365	442.7	36.319	Methanol
No match	-	1.326	-	35.143	Methanol
Trans-Z-alpha-Bisabolene	C <sub>15</sub> H <sub>24</sub>	1.312	204.35	16.216	Methanol
Ursolic aldehyde	C <sub>30</sub> H <sub>48</sub> O <sub>2</sub>	1.302	440.7	34.718	Methanol
No match	-	1.279	-	7.678	Methanol
No match	-	1.245	-	17.965	Methanol
Methyl 8,11,14-heptadecatrie	C <sub>21</sub> H <sub>36</sub> O <sub>2</sub>	1.22	320.5093	22.864	Methanol
No match	-	1.179	-	12.826	Methanol
Phytol	C <sub>20</sub> H <sub>40</sub> O	1.179	128.1705	21.998	Methanol
No match	-	1.148	-	13.285	Methanol
No match	-	1.08	-	13.897	Methanol
No match	-	1.013	-	26.209	Methanol
No match	-	0.997	-	35.231	Methanol

(Continued)

TABLE 2 (Continued)

Compound	Mol. formula	Amount/Conc.%	Mol. weight (g/mol)	RT (Min)	Extract
Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	0.97	284.48	22.623	Methanol
Hexadecanoic acid, methyl es	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	0.954	270.5	19.887	Methanol
No match	-	0.937	-	12.007	Methanol
Methyl 8,11,14-heptadecatrie	C <sub>21</sub> H <sub>36</sub> O <sub>2</sub>	0.92	320.5093	21.853	Methanol
Betulin	C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>	0.91	442.72	35.472	Methanol
1,1,4a-Trimethyl-5,6-dimethyl	C <sub>15</sub> H <sub>24</sub>	0.891	204.35	33.896	Methanol
Coumarin	C <sub>9</sub> H <sub>6</sub> O <sub>2</sub>	0.878	146.1427	13.867	Methanol
No match	-	0.875	-	12.736	Methanol
2H-1-Benzopyran-2-one, 7-met	C <sub>13</sub> H <sub>15</sub> NO <sub>2</sub>	0.826	217.26	17.04	Methanol
1-Chlorosulfonyl-3-methyl-1-	C <sub>9</sub> H <sub>14</sub> ClNO <sub>3</sub> S	0.823	251.73	16.173	Methanol
Beta-Amyrin	C <sub>30</sub> H <sub>50</sub> O	0.763	426.729	33.739	Methanol
Methyl 2-hydroxy-octadeca-9,	C <sub>19</sub> H <sub>32</sub> O <sub>3</sub>	0.754	308.5	28.775	Methanol
Hexadecanoic acid, 2-hydroxy	C <sub>16</sub> H <sub>32</sub> O <sub>3</sub>	0.744	272.42	26.101	Methanol
No match	-	0.717	-	13.206	Methanol
(1R,7S, E)-7-Isopropyl-4,10-d	C <sub>15</sub> H <sub>24</sub> O	0.702	220.3505	17.243	Methanol
No match	-	0.688	-	35.27	Methanol
Campesterol	C <sub>28</sub> H <sub>48</sub> O	0.657	400.68	32.877	Methanol
Urs-12-en-28-al	C <sub>30</sub> H <sub>48</sub> O	0.654	424.7	35.305	Methanol
2-Butyl-5-methyl-3-2-methyl	C <sub>15</sub> H <sub>26</sub> O	0.645	222.37	14.281	Methanol
Caryophylla-4(12),8(13)-dien	C <sub>15</sub> H <sub>24</sub> O	0.632	220.3505	16.429	Methanol
endo-Borneol	C <sub>10</sub> H <sub>18</sub> O	0.623	154.25	8.246	Methanol
1-Methyl-2-methylenecyclohex	C <sub>8</sub> H <sub>14</sub>	0.622	110.197	14.461	Methanol
No match	-	0.616	-	27.717	Methanol
Caryophylla-4(12),8(13)-dien	C <sub>15</sub> H <sub>24</sub> O	0.603	220.3505	17.45	Methanol
Stigmasterol	C <sub>29</sub> H <sub>48</sub> O	0.595	412.69	33.091	Methanol
No match	-	0.585	-	14.134	Methanol
No match	-	0.579	-	13.446	Methanol
Tritetracontane	C <sub>43</sub> H <sub>88</sub>	0.574	605.2	27.798	Methanol
No match	-	0.566	-	15.531	Methanol
(3S,3aS,6R,7R,9aS)-1,1,7-Tri	C <sub>15</sub> H <sub>24</sub>	0.562	204.3511	19.087	Methanol
Megastigmatrienone	C <sub>13</sub> H <sub>18</sub> O	0.56	190.28	16.78	Methanol
No match	-	0.553	-	12.88	Methanol
No match	-	0.549	-	11.886	Methanol
Urs-12-en-28-oic acid, 3-hyd	C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>	0.546	456.7	35.636	Methanol
No match	-	0.545	-	22.421	Methanol
No match	-	0.543	-	12.559	Methanol
3,5-Dimethylcyclohex-1-ene-4	C <sub>8</sub> H <sub>14</sub>	0.542	110.2	14.226	Methanol
Eicosanoic acid	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	0.515	312.5304	25.775	Methanol
No match	-	0.486	-	13.019	Methanol
Olean-12-en-3-ol, acetate,	C <sub>32</sub> H <sub>52</sub> O <sub>2</sub>	0.486	468.8	32.724	Methanol
Alpha-Tocospiro A	C <sub>29</sub> H <sub>50</sub> O <sub>4</sub>	0.484	462.7	30.208	Methanol
Cyclohexene,1-propyl-	C <sub>9</sub> H <sub>16</sub>	0.483	124.22	11.611	Methanol
Alpha-Tocospiro B	C <sub>29</sub> H <sub>50</sub> O <sub>4</sub>	0.463	462.7049	30.023	Methanol
No match	-	0.447	-	11.436	Methanol
No match	-	0.447	-	12.434	Methanol
Phenol, 2,4-bis 1-methyl-1-p	C <sub>24</sub> H <sub>26</sub> O	0.445	330.5	26.725	Methanol

(Continued)

TABLE 2 (Continued)

Compound	Mol. formula	Amount/Conc.%	Mol. weight (g/mol)	RT (Min)	Extract
11,11-Dimethyl-4,8-dimethyl	C <sub>15</sub> H <sub>24</sub> O	0.429	220.35	16.954	Methanol
No match	–	0.419	–	26.522	Methanol
Tricyclo [20.8.0.07,16] tria	C <sub>30</sub> H <sub>52</sub> O <sub>2</sub>	0.413	444.7	18.261	Methanol
No match	–	0.394	–	17.818	Methanol
1,5,7-Octatrien-3-ol, 3,7-di	C <sub>10</sub> H <sub>16</sub> O	0.39	152.2334	8.782	Methanol
2-Pentadecanone, 6,10,14-tri	C <sub>18</sub> H <sub>36</sub> O	0.386	268.4778	18.826	Methanol
11,14-Octadecadienoic acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	0.364	280.4	22.811	Methanol
No match	–	0.363	–	15.481	Methanol
Caryophylla-4(12),8(13)-dien	C <sub>15</sub> H <sub>24</sub> O	0.358	220.3505	15.937	Methanol
No match	–	0.356	–	34.665	Methanol
5-Cholestene-3-ol, 24-methyl	C <sub>28</sub> H <sub>48</sub> O	0.344	400.7	31.863	Methanol
No match	–	0.325	–	14.381	Methanol
No match	–	0.323	–	11.703	Methanol
No match	–	0.322	–	21.365	Methanol
Neophytadiene	C <sub>20</sub> H <sub>38</sub>	0.313	278.5	18.782	Methanol
No match	–	0.304	–	17.223	Methanol
2-Furanmethanol, 5-ethenylte	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	0.287	170.2487	6.078	Methanol
9,12-Hexadecadienoic acid, m	C <sub>16</sub> H <sub>28</sub> O <sub>2</sub>	0.273	252.39	21.796	Methanol
Beta-Guaiene	C <sub>15</sub> H <sub>24</sub>	0.271	204.351	32.882	Methanol
6-Hydroxy-4,4,7a-trimethyl-5	C <sub>11</sub> H <sub>16</sub> O <sub>3</sub>	0.258	196.24	17.648	Methanol
Bicyclo [2.2.1] heptane, 7,7-d	C <sub>9</sub> H <sub>16</sub>	0.24	124.22	9.955	Methanol
2-Cyclohexen-1-one, 3-methyl	C <sub>7</sub> H <sub>10</sub> O	0.23	110.15	11.529	Methanol
Hentriacontane	C <sub>31</sub> H <sub>64</sub>	0.188	436.85	28.969	Methanol
Methyl octadec-6,9-dien-12-y	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	0.149	280.4	15.763	Methanol

*cataria* extract as well as oils from the plant (Edewor and Usman, 2011; Reichert et al., 2018; Azizian et al., 2021). The antibacterial of *N. cataria* from previous research likewise demonstrated sufficient antibacterial activity against *S. aureus*, *K. pneumoniae* and *S. typhi* (Mukhtar and Singh, 2019). The results from our studies corroborate the results exhibited in previous studies. In addition to *N. cataria*, other species of the *Nepeta* genus have also been studied extensively for their phytochemical analysis, and among all species, *N. cataria* is the most promising of all species (Azizian et al., 2021).

Several studies corroborate our findings and indicate high DPPH activity in acetone extracts while others exhibit versatile results (Dienaite et al., 2018). Some studies presented more efficient DPPH activity in methanol, 70% ethanol and others in aqueous extract of *N. cataria* (Kraujalis et al., 2011; Mihaylova et al., 2013; Dienaite et al., 2018). Modernized extraction protocols, i.e., ultrasound-based microextraction, are being used to maximize output of phenolic compounds from methanol extract of *N. cataria*, which corroborates with our study (Hajmohammadi et al., 2021). Several other studies also indicate rosmarinic acid as a prominent phenolic compound in *N. cataria* extracts (Hadi et al., 2017).

Water extracts of *N. cataria* exhibit reasonable ORAC activity as per different studies (Dienaite et al., 2018;

Baranauskiene et al., 2019). Another study showed excellent radical scavenging properties of *N. cataria* via FRAP assay, which improves the confidence in this plant (Duda et al., 2015).

Among all the treatments, ethanol-based extracts of *N. cataria* showed maximum percentage inhibition of all the tested bacteria at 1,000–250 µg/ml concentration, followed by methanolic extracts at 1,000 and 500 µg/ml dose levels and water-based extracts at 1,000 and 500 µg/ml dose levels. In contrast, acetone and hexane-based extracts of *N. cataria* did not significantly inhibit all the tested bacterial isolates compared to control treatments. Many studies provide insights for the use of *N. cataria* extract in inhibition of *S. aureus* and *B. subtilis* and its oil as a topical treatment of respiratory tract infections (Suschke et al., 2007; Bandh and Kamili, 2011). MIC values indicated that the ethanol-based extract of all *N. cataria* extracts showed maximum inhibition of *B. subtilis*, followed by *C. freundii* and *M. luteus*. At the same time, methanol-based extracts also showed maximum efficacy against *S. sonnei*, *E. coli*, *M. luteus*, and *C. freundii*. Water, acetone, and hexane-based extracts were almost equally effective against tested bacterial isolates, as given in Table 7. Studies indicate promising effect of *N. cataria* extract as antibacterial agent against *S. aureus*, *K. pneumoniae*, and *Salmonella typhi* (Edewor and Usman, 2011). Considering resazurin methodology, by

TABLE 3 GC/MS analysis of ethanol extract of *N. cataria* using NIST 17 Library showed (79 identified phytochemicals + 31 unmatched) chemicals, arranged according to concentration present.

Compound	Mol. formula	Amount/Conc. %	Mol. weight (g/mol)	RT (Min)	Extract
No match	-	57.084	-	2.058	Ethanol
No match	-	42.916	-	2.039	Ethanol
9,12,15-Octadecatrienoic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	27.308	278.43	17.266	Ethanol
1-Isopropylcyclohex-1-ene	C <sub>9</sub> H <sub>16</sub>	25.854	124.22	11.456	Ethanol
1-Isopropylcyclohex-1-ene	C <sub>9</sub> H <sub>16</sub>	14.94	124.22	9.585	Ethanol
1-Isopropylcyclohex-1-ene	C <sub>9</sub> H <sub>16</sub>	13.741	124.22	9.33	Ethanol
Beta-Sitosterol	C <sub>29</sub> H <sub>50</sub> O	13.312	414.71	24.939	Ethanol
n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	10.3	256.424	19.386	Ethanol
Alpha-Amyrin	C <sub>30</sub> H <sub>50</sub> O	6.667	426.729	25.504	Ethanol
No match	-	4.606	-	16.278	Ethanol
Urs-12-en-28-ol	C <sub>30</sub> H <sub>50</sub> O	4.295	426.7	23.833	Ethanol
Methyl 13,14-octadecadienoate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	3.793	294.472	13.689	Ethanol
Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	3.62	284.48	17.464	Ethanol
Hexadecanoic acid, ethyl est	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	3.361	284.477	15.865	Ethanol
Ethyl 9,12,15-octadecatrieno	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	3.315	306.5	21.626	Ethanol
Phytol	C <sub>20</sub> H <sub>40</sub> O	3.068	128.1705	16.907	Ethanol
Coumarin	C <sub>9</sub> H <sub>6</sub> O <sub>2</sub>	2.94	146.1427	9.646	Ethanol
1-Chlorosulfonyl-3-methyl-1-	C <sub>9</sub> H <sub>14</sub> ClNO <sub>3</sub> S	2.175	251.73	15.242	Ethanol
Ursolic aldehyde	C <sub>30</sub> H <sub>48</sub> O <sub>2</sub>	2.109	440.7	33.113	Ethanol
Ethyl 9.cis., 11.trans.-octad	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	2.045	310.515	17.352	Ethanol
No match	-	1.95	-	11.165	Ethanol
No match	-	1.756	-	15.716	Ethanol
No match	-	1.66	-	9.685	Ethanol
4,4,8-Trimethyltricyclo [6.3].	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	1.458	238.366	18.101	Ethanol
No match	-	1.456	-	15.943	Ethanol
2H-1-Benzopyran-2-one, 7-met	C <sub>13</sub> H <sub>15</sub> NO <sub>2</sub>	1.381	217.26	16.049	Ethanol
No match	-	1.199	-	9.727	Ethanol
Hentriacontane	C <sub>31</sub> H <sub>64</sub>	1.197	436.85	20.74	Ethanol
Tetracontane, 3,5,24-trimeth	C <sub>43</sub> H <sub>88</sub>	1.194	605.2	20.201	Ethanol
6-Octadecynoic acid, methyl	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	1.149	296.488	24.253	Ethanol
Eicosanoic acid	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	1.138	312.5304	19.118	Ethanol
Sulfurous acid, butyl tetrad	C <sub>21</sub> H <sub>44</sub> O <sub>3</sub> S	1.134	376.6	23.243	Ethanol
Uvaol	C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>	1.125	442.7	24.513	Ethanol
Bicyclo [3.1.0] hexane-2-undec	C <sub>6</sub> H <sub>10</sub>	1.108	82.14	12.837	Ethanol
Tetracosamethyl-cyclododecas	C <sub>16</sub> H <sub>32</sub>	1	224.425	27.703	Ethanol
No match	-	0.984	-	12.821	Ethanol
Octadecanoic acid, 17-methyl	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	0.982	312.5	17.68	Ethanol
No match	-	0.939	-	12.875	Ethanol
Methyl 2-hydroxy-octadeca-9,	C <sub>19</sub> H <sub>32</sub> O <sub>3</sub>	0.895	308.5	21.548	Ethanol
2H-1-Benzopyran-2-one, 7-met	C <sub>13</sub> H <sub>15</sub> NO <sub>2</sub>	0.893	217.26	12.956	Ethanol
No match	-	0.884	-	11.045	Ethanol
No match	-	0.865	-	13.906	Ethanol
No match	-	0.836	-	15.628	Ethanol
[1,1'-Bicyclopropyl]-2-octan	C <sub>21</sub> H <sub>38</sub> O <sub>2</sub>	0.823	322.5	16.857	Ethanol
11,14-Octadecadienoic acid,	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	0.819	280.4	21.561	Ethanol
Betulin	C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>	0.802	442.72	33.839	Ethanol

(Continued)

TABLE 3 (Continued)

Compound	Mol. formula	Amount/Conc. %	Mol. weight (g/mol)	RT (Min)	Extract
No match	-	0.772	-	19.585	Ethanol
5-Hydroxymethylfurfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	0.768	126.11	6.985	Ethanol
No match	-	0.754	-	12.601	Ethanol
No match	-	0.742	-	11.881	Ethanol
No match	-	0.727	-	12.675	Ethanol
Urs-12-en-28-oic acid, 3-hyd	C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>	0.722	456.7	23.776	Ethanol
Sulfurous acid, butyl tetrad	C <sub>21</sub> H <sub>44</sub> O <sub>3</sub> S	0.667	376.6	22.185	Ethanol
No match	-	0.665	-	11.947	Ethanol
Alpha-Tocospiro A	C <sub>29</sub> H <sub>50</sub> O <sub>4</sub>	0.654	462.7	22.498	Ethanol
Oleic Acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	0.653	282.47	16.515	Ethanol
Tricyclo [20.8.0.07,16] tria	C <sub>18</sub> H <sub>24</sub> O <sub>4</sub>	0.647	304.38	25.158	Ethanol
No match	-	0.643	-	11.217	Ethanol
Stigmasterol	C <sub>29</sub> H <sub>48</sub> O	0.63	412.69	24.507	Ethanol
Methyl 10,11-tetradecadienoa	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	0.573	238.366	10.069	Ethanol
Sulfurous acid, butyl tridec	C <sub>17</sub> H <sub>36</sub> O <sub>3</sub> S	0.572	320.5	22.897	Ethanol
No match	-	0.562	-	12.242	Ethanol
24-Noroleana-3,12-diene	C <sub>29</sub> H <sub>46</sub>	0.537	394.676	31.418	Ethanol
No match	-	0.534	-	9.47	Ethanol
No match	-	0.517	-	22.775	Ethanol
Cholestan-3-ol, 2-methylene-	C <sub>28</sub> H <sub>48</sub> O	0.515	400.7	15.446	Ethanol
Tetracontane, 3,5,24-trimeth	C <sub>43</sub> H <sub>88</sub>	0.506	605.2	25.112	Ethanol
No match	-	0.501	-	26.914	Ethanol
2-Methylindoline	C <sub>9</sub> H <sub>11</sub> N	0.49	133.19	6.58	Ethanol
No match	-	0.481	-	16.473	Ethanol
No match	-	0.457	-	8.924	Ethanol
3,7,11,15-Tetramethyl-2-Hexa	C <sub>20</sub> H <sub>40</sub> O	0.444	296.5	23.191	Ethanol
No match	-	0.435	-	10.634	Ethanol
1-Heptatriacotanol	C <sub>37</sub> H <sub>76</sub> O	0.432	537	13.943	Ethanol
No match	-	0.424	-	13.117	Ethanol
1R,4S,7S,11R-2,2,4,8-Tetrame	C <sub>15</sub> H <sub>26</sub> O	0.419	222.366	31.553	Ethanol
No match	-	0.406	-	10.249	Ethanol
Sulfurous acid, butyl tridec	C <sub>17</sub> H <sub>36</sub> O <sub>3</sub> S	0.399	320.5	24.233	Ethanol
No match	-	0.395	-	16.248	Ethanol
No match	-	0.375	-	25.618	Ethanol
No match	-	0.369	-	23.972	Ethanol
6-Hydroxy-4,4,7a-trimethyl-5	C <sub>11</sub> H <sub>16</sub> O <sub>3</sub>	0.367	196.24	16.663	Ethanol
Ethyl 9.cis.,11. trans.-octad	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	0.34	310.515	17.345	Ethanol
Tau-Cadinol	C <sub>15</sub> H <sub>26</sub> O	0.335	222.37	12.143	Ethanol
24(H)-Benzofuranone, 5,6,7,7	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	0.319	180.244	10.757	Ethanol
Glycine, N-[3alpha, 5beta]	C <sub>30</sub> H <sub>53</sub> NO <sub>4</sub> Si	0.313	519.8	24.109	Ethanol
Tetracontane, 3,5,24-trimeth	C <sub>43</sub> H <sub>88</sub>	0.304	605.2	20.193	Ethanol
2-Pentadecanone, 6,10,14-tri	C <sub>18</sub> H <sub>36</sub> O	0.298	268.478	17.839	Ethanol
Neophytadiene	C <sub>20</sub> H <sub>38</sub>	0.294	278.5	25.337	Ethanol
2-Pentadecanone, 6,10,14-tri	C <sub>18</sub> H <sub>36</sub> O	0.286	268.478	14.346	Ethanol
2,4-Dihydroxy-2,5-dimethyl-3	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	0.284	144.12	3.404	Ethanol
n-Propyl 9,12-hexadecadienoa	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	0.262	294.5	11.116	Ethanol
Tetradecanoic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	0.25	228.3709	13.552	Ethanol

(Continued)

TABLE 3 (Continued)

Compound	Mol. formula	Amount/Conc.%	Mol. weight (g/mol)	RT (Min)	Extract
10,10-Dimethyl-2,6-dimethyle	C <sub>15</sub> H <sub>24</sub>	0.199	204.351	12.067	Ethanol
Ergost-5-en-3-ol (3beta)-	C <sub>28</sub> H <sub>48</sub> O	0.18	400.7	24.141	Ethanol
Fumaric acid, ethyl 2-methyl	C <sub>10</sub> H <sub>14</sub> O <sub>4</sub>	0.179	198.22	11.356	Ethanol
Tritetracontane	C <sub>43</sub> H <sub>88</sub>	0.177	605.2	22.18	Ethanol
Azulene, 1,2,3,3a,4,5,6,7-oc	C <sub>15</sub> H <sub>24</sub>	0.17	204.351	15.056	Ethanol
(4aS,7S,7aR)-4,7-Dimethyl-2	C <sub>10</sub> H <sub>14</sub> O <sub>2</sub>	0.169	166.217	10.885	Ethanol
cis-5,8,11,14,17-Eicosapenta	C <sub>20</sub> H <sub>30</sub> O <sub>2</sub>	0.148	302.5	13.276	Ethanol
Carbamic acid, N-[1,1-bis tr]	C <sub>12</sub> H <sub>24</sub> N <sub>2</sub> O <sub>4</sub>	0.124	260.33	13.319	Ethanol
Bicyclo [4.4.0] dec-1-ene, 2-i	C <sub>15</sub> H <sub>24</sub>	0.116	204.35	11.54	Ethanol
2-Cyclohexen-1-one, 4,5-dime	C <sub>8</sub> H <sub>12</sub> O	0.113	124.18	10.585	Ethanol
12-Methyl-E, E-2,13-octadecad	C <sub>19</sub> H <sub>36</sub> O	0.113	280.489	11.164	Ethanol
Stigmasterol	C <sub>29</sub> H <sub>48</sub> O	0.102	412.69	24.241	Ethanol
2-Cyclohexen-1-one, 3-methyl	C <sub>7</sub> H <sub>10</sub> O	0.083	110.15	8.592	Ethanol
Megastigmatrienone	C <sub>13</sub> H <sub>18</sub> O	0.081	190.28	11.924	Ethanol
2,4-Dihydroxy-2,5-dimethyl-3	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	0.032	144.12	3.23	Ethanol
Cyclopentanecarboxylic acid	C <sub>6</sub> H <sub>10</sub> O <sub>2</sub>	0.008	114.14	9.434	Ethanol
2-Methylindoline	C <sub>9</sub> H <sub>11</sub> N		133.19	8.12	Ethanol

using combined extractions of all solvents in DMSO, *N. cataria* plant extract at the dose level of 12.5 µl/ml showed maximum inhibition of all the bacterial strains, followed by 6.25 µl/ml. The antibacterial screening of the *N. cataria* from other studies also exhibited sufficient evidence of antibacterial activity against *S. aureus*, *K. pneumoniae*, and *S. typhi* (Morombaye et al., 2018).

GC/MS analysis of methanol and ethanol revealed the presence of betulin extracts, which is a promising antitumorogenic candidate and escalates the importance of *N. cataria* in cancer treatment (Liu et al., 2009). Arachidic acid (eicosanoic acid) is used to produce detergents, photographic materials, and lubricants. Caryophyllene oxide is a potential preservative used in food, drugs, and cosmetics. It also displays anti-inflammatory and anti-carcinogenic properties (Salaria et al., 2020). Uvaol also displays anti-inflammatory properties and antioxidant effects (Botelho et al., 2019). Campesterols found in methanol extracts is phytosterol, used in growth induction in animals, commonly abused anabolic steroid in sports can also reduce the absorption of cholesterol in intestine by targeting transporter protein, minimizing the effect of cardiovascular disease (Choudhary and Tran, 2011). Phytol in ethanol has been investigated for its potential anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects (Islam et al., 2018). Phytol is likely the most abundant acyclic isoprenoid compound present in the biosphere and its degradation products have been used as biogeochemical tracers in aquatic

environments (Rontani and Volkman, 2003). Phytol is used in the fragrance industry and is used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents (McGinty et al., 2010). Coumarin (2*H*-1-benzopyran-2-one) in methanol and ethanol is famous for pharmacological properties such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antitubercular, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant, and neuroprotective properties (Venugopala et al., 2013). Similarly in water extracts, 2-methylindole is used as an intermediate to synthesize dyes, pigments, and pharmaceuticals. Conhydrin is a poisonous alkaloid, when ingested interruption with the central nervous system, paralyzing respiratory muscles and causing failure (Hotti and Rischer, 2017). Likewise, extracts of hexane contain eucalyptol, an active ingredient as a cough suppressant as it controls mucus secretion from airway and asthma via anti-inflammatory cytokines (Juergens, 2014). Hexane soluble constituents conformed to identification of 7, 9-di-tert-butyl-1-oxaspiro which is used against skin diseases, gonorrhoea, migraine, intestinal parasites, and warts (Sharif et al., 2015), and dibutyl phthalate is used in making flexible plastics. In addition to this, several other studies indicate presence of nepetalactone and other terpenoids as essential components of oil extracts of *N. cataria* (Handjieva et al., 2011; Sharma et al., 2019).

This study gave a thorough brief of antibacterial and antioxidant activity and its constituents. Present methodology can be beneficial in devising and exploring different bioactive

TABLE 4 GC/MS analysis of water extract of *N. cataria* using NIST 17 Library showed (79 identified phytochemicals + 31 unmatched) chemicals, arranged according to concentration present.

Compound	Mol. formula	Amount/Conc. %	Mol. weight (g/mol)	RT (Min)	Extract
1-Isopropylcyclohex-1-ene	C <sub>9</sub> H <sub>16</sub>	22.387	124.22	10.657	Water
7-Methylhexahydrocyclopenta	C <sub>9</sub> H <sub>14</sub> O <sub>2</sub>	5.399	154.21	11.265	Water
2H-1-Benzopyran-2-one, 7-met	C <sub>13</sub> H <sub>15</sub> NO <sub>2</sub>	5.336	217.26	14.95	Water
(R)-(-)-14-Methyl-8-hexadecy	C <sub>17</sub> H <sub>34</sub> O	5.106	254.4513	10.79	Water
No match	-	4.917	-	15.825	Water
Benzofuran, 2,3-dihydro-	C <sub>8</sub> H <sub>8</sub> O	4.002	120.15	8.48	Water
Hydro coumarin	C <sub>9</sub> H <sub>8</sub> O <sub>2</sub>	3.699	148.1586	10.843	Water
Bicyclo [3.1.0] hexane-2-undec	C <sub>6</sub> H <sub>10</sub>	3.1	82.14	12.831	Water
No match	-	2.942	-	13.861	Water
Coumarin	C <sub>9</sub> H <sub>6</sub> O <sub>2</sub>	2.265	146.1427	11.545	Water
Cyclopentane carboxylic acid,	C <sub>6</sub> H <sub>10</sub> O <sub>2</sub>	2.165	114.14	10.486	Water
13-Tetradec-11-yn-1-ol	C <sub>14</sub> H <sub>24</sub> O	2.146	208.34	11.581	Water
No match	-	1.738	-	11.098	Water
No match	-	1.63	-	14.825	Water
No match	-	1.472	-	15.575	Water
(S-2-1R,4R)-4-Methyl-2-oxo	C <sub>4</sub> H <sub>6</sub> O <sub>3</sub>	1.274	102.0886	12.723	Water
(4R,4aR,7S,7aR)-4,7-Dimethyl	C <sub>10</sub> H <sub>18</sub> O	1.17	154.25	11.326	Water
Homovanillyl alcohol	C <sub>9</sub> H <sub>12</sub> O <sub>3</sub>	1.118	168.19	12.621	Water
2-Cyclohexene-1-one, 4-3-hyd	C <sub>13</sub> H <sub>20</sub> O <sub>2</sub>	0.997	208.2967	13.984	Water
No match	-	0.932	-	13.036	Water
2-Methylindoline	C <sub>9</sub> H <sub>11</sub> N	0.825	133.19	8.353	Water
(E)-2,6-Dimethylocta-3,7-die	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	0.67	170.25	8.078	Water
No match	-	0.562	-	11.045	Water
Ethanone, 1-2-hydroxyphenyl	C <sub>8</sub> H <sub>8</sub> O <sub>2</sub>	0.559	136.15	11.463	Water
2-Methoxy-4-vinyl phenol		0.53		9.845	Water
6-Hydroxy-4,4,7a-trimethyl-5	C <sub>11</sub> H <sub>16</sub> O <sub>3</sub>	0.496	196.24	15.394	Water
No match	-	0.469	-	3.652	Water
No match	-	0.437	-	5.652	Water
No match	-	0.404	-	16.262	Water
3-Acetylthymine	C <sub>5</sub> H <sub>6</sub> N <sub>2</sub> O <sub>2</sub>	0.402	126.1133	13.283	Water
3-Oxo-4-phenylbutyronitrile	C <sub>10</sub> H <sub>9</sub> NO	0.371	159.18	8.825	Water
No match	-	0.337	-	4.368	Water
7-Oxabicyclo [4.1.0] heptan-3-	C <sub>6</sub> H <sub>10</sub> O <sub>2</sub>	0.295	114.14	16.821	Water
n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	0.263	256.4241	17.288	Water
1H-Pyrrole-2,5-dione, 3-ethy	-	0.25	-	8.69	Water
1,7-Octadiene-3,6-diol, 2,6-	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	0.238	170.25	9.271	Water
Conhydrin	C <sub>8</sub> H <sub>17</sub> NO	0.212	143.23	7.847	Water
Methyl 7,8-octadecadienoate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	0.206	294.4721	12.898	Water
1H-Indene, 1-ethylideneoctah	C <sub>11</sub> H <sub>10</sub>	0.07	142.2	14.737	Water

compounds that can be exploited for the constructing novel antimicrobial agents for alternative therapeutic intervention against several bacterial and viral infections after processing. It may also help to treat different antibiotic-resistant pathogens. Its chemicals if used in pharmacology industries can serve as indigenous, cheaper, and readily available source.

## Conclusion

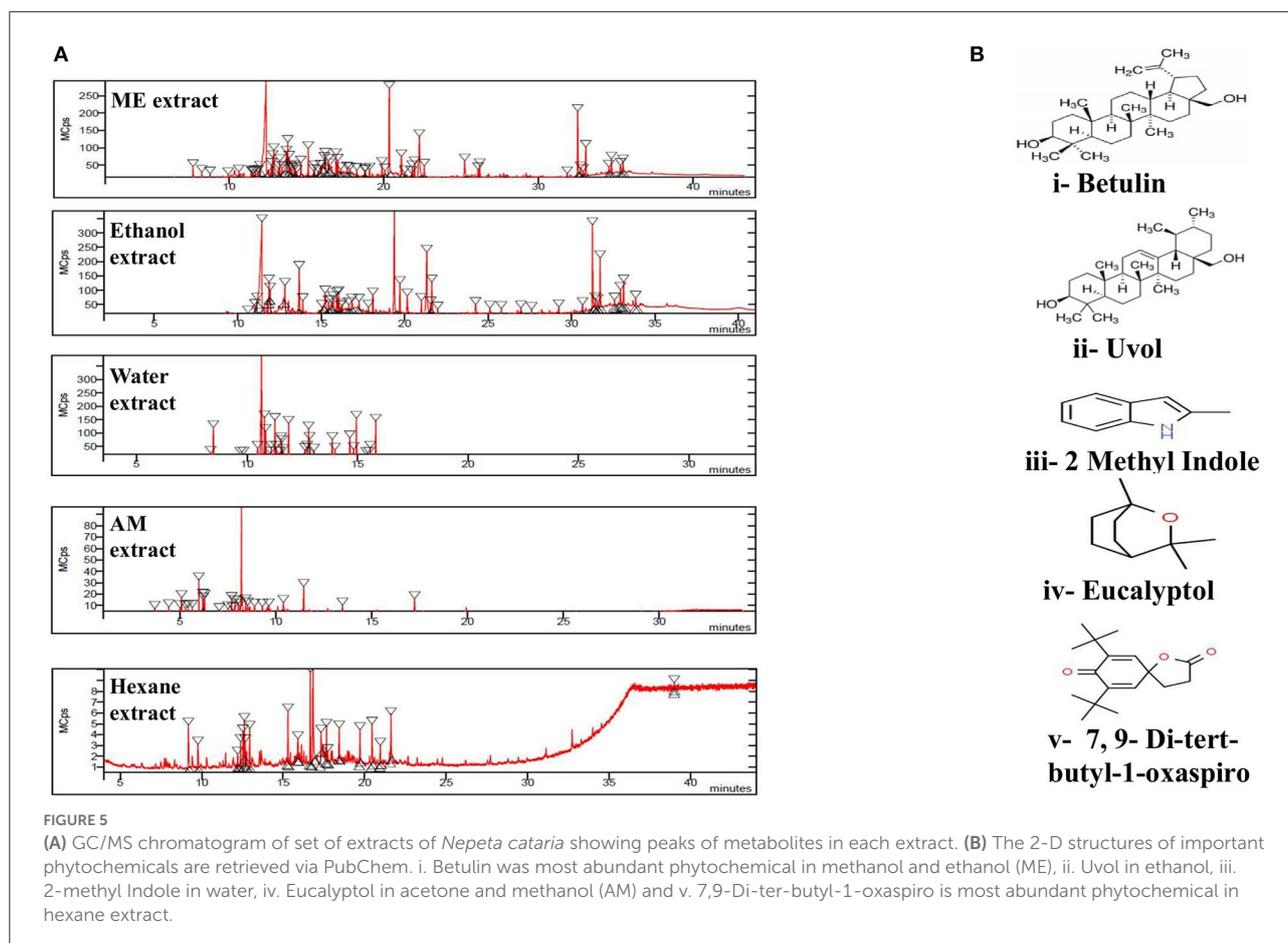
Many aspects of plants were studied, but complete metabolomic profiling and identification of unmatched chemicals remain a question mark. MS-MS analysis of plant metabolites should be considered for knowing the medicinal

TABLE 5 GC/MS analysis of an acetone-based extract of *N. cataria* using NIST 17 Library showed (12 identified phytochemicals + 9 unmatched) chemicals, arranged according to concentration present.

Compound	Mol. formula	Amount/conc. %	Mol. weight (g/mol)	RT (Min)	Extract
Oxime-, methoxy-phenyl-	C <sub>8</sub> H <sub>9</sub> NO <sub>2</sub>	2.849	151.16	3.685	Acetone
1-Isopropylcyclohex-1-ene	C <sub>9</sub> H <sub>16</sub>	29.552	124.22	8.206	Acetone
Caryophyllene oxide	C <sub>15</sub> H <sub>24</sub> O	6.868	220.35	11.452	Acetone
(+)-2-Bornanone	C <sub>10</sub> H <sub>16</sub> O	6.365	152.233	5.984	Acetone
n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	5.337	256.424	17.237	Acetone
No match	-	3.443	-	10.391	Acetone
Endo-Borneol	C <sub>10</sub> H <sub>18</sub> O	3.083	154.25	6.191	Acetone
Hotrienol	C <sub>10</sub> H <sub>16</sub> O	2.947	152.23	7.692	Acetone
No match	-	2.573	-	13.475	Acetone
(E)-2,6-Dimethylocta-3,7-die	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	2.572	170.25	6.217	Acetone
No match	-	2.57	-	8.885	Acetone
Cyclopentanecarboxylic acid	C <sub>6</sub> H <sub>10</sub> O <sub>2</sub>	2.496	114.14	8.04	Acetone
No match	-	2.289	-	9.631	Acetone
No match	-	2.038	-	8.424	Acetone
No match	-	2.007	-	8.53	Acetone
No match	-	1.947	-	8.127	Acetone
No match	-	1.844	-	9.297	Acetone
Eucalyptol	C <sub>10</sub> H <sub>18</sub> O	1.513	154.249	5.004	Acetone
No match	-	1.196	-	7.749	Acetone
Cyclohexane, 1-propyl-	C <sub>9</sub> H <sub>16</sub>	1.093	124.22	7.507	Acetone
alpha-methyl- alpha-[4-methyl]	C <sub>6</sub> H <sub>11</sub> NO <sub>2</sub>	1.026	129.16	5.292	Acetone
1,7-Octadiene-3,6-diol, 2,6-dimethyl	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	0.819	170.25	7.049	Acetone

TABLE 6 GC/MS analysis of a hexane-based extract of *N. cataria* using NIST 17 Library showed (9 identified phytochemicals + 8 unmatched) chemicals, arranged according to concentration present.

Compound	Mol. formula	Amount/Conc. %	Mol. weight (g/mol)	RT (Min)	Extract
(+)-2-Bornanone	C <sub>10</sub> H <sub>16</sub> O	6.809	152.233	9.187	Hexane
Methyl 6,9,12,15,18-heneicos	-	11.008	-	16.663	Hexane
1,2-Benzenedicarboxylic acid	C <sub>8</sub> H <sub>6</sub> O <sub>4</sub>	8.551	166.14	10.181	Hexane
Dibutyl phthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	5.877	278.34	21.611	Hexane
No match	-	5.199	-	12.947	Hexane
No match	-	4.075	-	12.537	Hexane
No match	-	3.969	-	19.713	Hexane
endo-Borneol	C <sub>10</sub> H <sub>18</sub> O	3.719	154.25	9.774	Hexane
Benzophenone	C <sub>13</sub> H <sub>10</sub> O	3.591	182.217	17.321	Hexane
No match	-	3.472	-	18.437	Hexane
No match	-	3.172	-	12.675	Hexane
Tetracontane, 3,5,24-trimeth	C <sub>43</sub> H <sub>88</sub>	2.939	605.2	8.975	Hexane
No match	-	2.756	-	12.391	Hexane
Benzoic acid, 4-ethoxy-, eth	C <sub>11</sub> H <sub>14</sub> O <sub>3</sub>	2.535	194.23	15.905	Hexane
7,9-Di-tert-butyl-1-oxaspiro	C <sub>17</sub> H <sub>24</sub> O <sub>3</sub>	1.956	276.4	20.957	Hexane
No match	-	1.421	-	12.21	Hexane
No match	-	1.176	-	17.73	Hexane



**TABLE 7** Antimicrobial efficacy of *N. cataria* extracts against a set of gram-negative and gram-positive bacterial strains.

Bacterial pathogens		Zone of inhibition (mm)					
		Methanol	Ethanol	Water	Acetone	Hexane	Chloramphenicol
Gram negative	<i>E. coli</i>	15 ± 0.1	14 ± 0.1	12 ± 0.1	0	14 ± 0.1	25 ± 0.2
	<i>K. oxytoca</i>	14 ± 0.2	14 ± 0.1	16 ± 0.1	14 ± 0.2	13 ± 0.3	26 ± 0.1
	<i>S. enterica</i>	13 ± 0.1	14 ± 0.1	0	0	0	25 ± 0.1
	<i>S. sonnei</i>	15 ± 0.2	15 ± 0.1	0	16 ± 0.2	14 ± 0.1	26 ± 0.2
	<i>C. ferundii</i>	15 ± 0.2	22 ± 0.4	12 ± 0.1	12 ± 0.2	11 ± 0.1	25 ± 0.2
Gram positive	<i>B. subtilis</i>	14 ± 0.1	21 ± 0.5	0	0	14 ± 0.2	31 ± 0.1
	<i>L. lactis</i>	0	0	0	13 ± 0.1	0	25 ± 0.2
	<i>L. monocytogenes</i>	13 ± 0.1	14 ± 0.2	13 ± 0.1	13 ± 0.1	0	25 ± 0.2
	<i>M. luteus</i>	15 ± 0.2	16 ± 0.2	16 ± 0.1	16 ± 0.2	0	26 ± 0.1
	<i>S. aureus</i>	13 ± 0.1	13 ± 0.1	0	0	0	20 ± 0.1

potential of unknown and novel plant metabolites. Data compilation and individual chemical studies need a larger scale with a set of skills to combat emerging diseases. Yet, to the best of our knowledge, the concluded information, reported results, and this research is comprehensive to the best of our scale, our team tried to achieve.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## Author contributions

Practical performance and data compilation were performed solely by AN. Experimental assistance for GC/MS, and antibacterial analysis was given by BA. Data analysis was performed by HS. Manuscript drafting and proofreading were conducted by HS, in assistance with MW and AT. All authors contributed to the study design and implementation. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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