The Phytochemical analysis, Metabolic profiling, Anti-bacterial and Anti-oxidant activity of *Nepeta cataria*

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Abstract

**Introduction:** *Nepeta cataria* is a naturally growing plant in the regions of Pakistan, in English name is Catnip mint and the local name is Badranj boya. This plant has immense pharmacological and medical importance. It is used in herbal and traditional medicine as an antipathogenic agent. However detailed phytochemical analyses in a wide spectrum of solvents and their antimicrobial analysis against both Gram-positive and negative limited studied.

**Objectives:** This study aimed to identify phytochemicals, metabolome, antimicrobial potential, and antioxidant analysis of *Nepeta cataria* (Pakistani plant).

**Methods:** Plant collection, identification, cleaning, and grinding were performed followed by extract preparation in five solvents i.e. methanol, ethanol, water, acetone, and hexane. Qualitative phytochemical analysis was performed by general biochemical tests. GC/MS was used to identify metabolic compounds in all extracts. Antibacterial analysis for both gram-positive and negative bacteria was performed by Kirby’s Disc method, 96 well test, and Resazurin test. The antioxidant activity was determined by DPPH assay.

**Results:**

Kirby disk diffusion method results indicated that *Nepeta cataria* extracts in different solvents maximum inhibition percentage against tested bacteria was 250-1000 µg/ml. Resazurin method inhibition against bacterial strains was 6.25-100 µl/ml. The Phytochemical analysis for Phenolic Compounds, Flavonoids, Terpenoids, Cardiac Glycosides, Free
Anthraquinones, Combined Anthraquinones, Tannins, Alkaloids, and DPPH was positive in the different extracts. The ORAC was 133759.021um/100ml in concentration. GC/MS analysis of methanolic extract (71 identified + 48 unknown), ethanol-based extract (80 known + 31 unknown), water-based extract (28 known + 11 unknown), acetone-based extract (13 known + 9 unknown), hexane based extract (11 known + 8 unmatched) phytochemicals were detected.

**Conclusion:** Current study concludes the remarkable antibacterial and antioxidant potential of *Nepeta cataria* extracts.

**Key words:** *Nepeta cataria*, Extremophile, GS/MS
**Introduction**

Extremophile plants grow in harsh environments with a high degree of abiotic stress. The plant growth and development of secondary metabolites are determined by environmental conditions besides the plant genome. These plants required drought, heat, cold, and soil salinity, pH, pressure, and water shortage [1]. Medicinal plants are known as drug candidates [2]. Different plants used in the medicine range is 4 -20% in various regions of the world, 2500 species are being traded [3].

Plant-derived flavonoids and their analogs possess antioxidant, pharmacological, antiviral, anti-carcinogenic, therapeutic, and cytotoxic properties [4], flavonoids are biologically active compounds which explored against Alzheimer’s disease, Parkinson’s disease, and hepatic injury [5,6].

*Nepeta cataria* is an extensively studied medicinal plant, with key importance in pharmacological and various researchers as antifungal, antibacterial, antioxidant, insecticidal, anti-inflammatory, anti-nociceptive, and spasmodylic potential agents. This plant contains essential oils, flavonoids, phenolic acid, steroids, terpenoids, and terpenoid hydrocarbons [7].

In a recent study, water-based extracts of *Nepeta cataria* significantly inhibited the replication of the herpes virus in humans. Moreover, various phenolic compounds and flavonoids were detected by NMR technology [8]. A few other studies have been reported secondary
metabolites[9-11]. However, complete metabolomics profiling of this plant has not reported yet.

[30]. Diarrhea is characterized as increased gastrointestinal motility, decreased fluid absorption, and electrolytes. *Nepeta cataria* is widely used to treat diarrhea [12].

**Aims and Objectives**

*Nepeta cataria* extremophile collection, screening (antimicrobial), Characterization of active metabolites by GC-MS/NMR were the main objectives of this study.

**Method**

**Collection of Plant**

*Nepeta cataria* is collected from the Himalayas. This plant was brought to the AMLB Laboratory, IIU Islamabad, Pakistan.

**Plant Extractions**

Fresh parts (Stem and leaves) of *Nepeta cataria* plant cleaned with tap water and then washed with distilled water, shade dried for 1 hour. After drying cut into small pieces and grinded. Fine powder particles extract prepared by using methanol, ethanol, water, acetone, and hexane. Plant powder separately macerated in each of the solvents at room temperature for 24 to 48 h and then filter( Whatman No. 41 filter paper), all solvent mixed with crude extracts in 1: 10 ratio. The percentage yield of plant extracts in different solvents is given in Table 1.1 (See appendices) [13].
Screening of Nepeta Cataria for Antimicrobial Activities by Minimum Inhibitory Concentration (MIC) 96 Well Plate Method

Plant Extracts Concentrations Preparation

For the Kirby disc diffusion method, plant extract in each solvent was maintained at 67µg/µL and 20µL of each prepared concentration used in each cell of the test plate.

Culture Media and Bacterial Inoculum Preparation

Tryptic Soy Broth (TSB) medium (Thermo Fisher Scientific, USA) used for the cultivation of bacterial agents. The ingredients of the TSB medium are given as under in Table 2 (See Appendices). Bacterial agents used in this study were *Shigella sonnei* (25931), *Micrococcus luteus* (4698), *Bacillus subtilis* (6051), *Staphylococcus aureus* (25923), *Klebsiella oxytoca* (43863), *Lactococcus lactis* (LMO230), *Escherichia coli* (25922), *Citrobacter freundii* (8090), *Salmonella enterica* (14028) and *Listeria monocytogenes* (LM21). These bacterial agents were cultured on LB medium (for mass culturing) (Table 3; See Appendices) containing sterilized tubes, placed on a rotatory shaker for 24 hours. The concentration of each bacterium was maintained at $10^5$ to $10^6$ CFU/ml before inoculating in the well-containing tray.

Kirby-Bauer Disk Diffusion Method
Kirby disk diffusion method was applied for antimicrobial efficacy testing of plant extracts under in vitro conditions [14]. Solidified agar containing plates were swabbed with bacterial inoculum $(10^6 \text{ CFU/mL})$ in standard concentration. Paper discs soaked in plant extract and placed on the solidified medium under aseptic conditions followed by incubation at $26 \pm 2 \, ^{\circ}C$. The inhibition zone was measured in millimeters (mm) after 24 h of inhibition to confirm the susceptibility of bacterial agents against control discs. Kirby's experiment was carried out in the Food Sciences Department of UMASS, Massachusetts USA. The experiment was carried out in three replications for each treatment and obtained results were averaged to get mean values. Standard errors of the mean values were calculated.

**Well Plate Test Procedure**

A 96 well sterile microtiter tray labeled, added 100 µL of TSB medium in each well. 100 µL of each plant extract fraction at 5 dilutions such as $(1000 \mu g, 500 \mu g, 250 \mu g, 125 \mu g, \text{and} 62.5 \mu g)$ were loaded in each well followed by inoculation with 50 µL of each bacterial culture separately at $10^5$ to $10^6 \text{ CFU/ml}$ concentration, control wells contain only TSB medium to check sterility of medium and negative control wells contain only TSB medium and bacterial inoculation. Plates coved with lids and sealed with tape, incubated at room temperature for 24 h. Standard readings for MIC were taken at 570 nm absorbance by using Elx 800 plate reader. Kirby's experiment was carried out in the Food Sciences Department of UMASS, Massachusetts
USA. The following formula was used for the calculation percentage of bacterial inhibition by each plant extract:

\[
\text{Percentage Inhibition} = 100 - 100 \times \frac{OD \text{ of sample}}{OD \text{ of control}}
\]

Resazurin based Well Plate Microdilution Method

Plant Extracts Concentrations and Bacterial Inoculum preparation

Plant extracts of *Nepeta cataria* prepared in different solvents mixed in 5% DMSO to observe the effects of plant extracts against tested microbes. Pure bacterial cultures of *S. sonnei*, *B. subtilis*, *K. oxytoca*, *E. coli*, *S. enterica*, *M. luteus*, *S. aureus*, *L. lactis*, *S. cerevisiae*, and *L. monocytogenes* were maintained (10⁶ CFU/ml) on TSB medium.

Preparation of Resazurin Solution

Resazurin (7-Hydroxy-3H-phe-noxazin-3-one 10-oxide) is a blue color dye which reduced in pink and highly red fluorescent resorufin by an oxidoreductase. Resazurin solution was firstly prepared by adding 121.1 mg of resazurin powder in 18 mL sterile distal water and mixed by a vortex mixer for 1 h. Resazurin solution was prepared in dark and stored in a brown bottle to avoid its light exposure. The pH of the solution was maintained at 7.4 by using PSB buffer.

Resazurin based Well Plate Test Procedure
For this test, 100 µL of TSB liquid medium was dispensed in each well, and 100 µL of plant extract concentrations were added to the wells (five wells/dilution). Bacterial inoculum (10^6 CFU/mL) was added to each well. Un-inoculated wells contain TSB medium confirmed the sterility of the medium while negative control contains only the application of TSB, bacterial agents, and Resazurin dye. After overnight at room temperature, 20 µL of Resazurin dye was added to wells and again incubated for 2 – 4 h for color change. Standard reading was taken at 550-590 nm absorbances on SPECTRA MAX M2e plate reader. The resazurin-based experiment was carried out in the Food Sciences Department of UMASS, Massachusetts USA [15].

**Qualitative Phytochemical Analysis**

**Qualitative Test for Saponin**

For this test, plant extract (1 mL ) mixed in 1 ml of distilled water, shaken vigorously, and left for 15 min for the formation of Persistent frothing. Emulsion formation on adding 3 drops of olive oil showed positive results [16].

**Qualitative test for Phenolic compounds**

The Phenolic compounds in the plant extract sample were tested by adding 2 mL (3 % aqueous Na₂CO₃ ) solution in 200 µL plant extract. In the reaction mixture, 200 µL of Folin Ciocalteu reagent was added and left for 30 mint. The development of blue/grey color indicated the presence of Phenolic compounds in plant extract samples [17].
Qualitative test for Water Soluble Phenol

To indicate the presence of water-soluble phenol in the plant extract sample, 2 drops of 1% ferric chloride solution added in plant extract (500 µL). Red color development confirmed the presence of water-soluble phenol in the test sample [18].

Qualitative test for Water Insoluble Phenol

For the analysis of water-insoluble phenol, 500 µL of plant extract added in 500 µL of CH₂Cl₂, 3 drops of ferric chloride, and 1 drop of Pyridine was added to this solution. The appearance of any color change confirmed the presence of water-insoluble phenols.

Qualitative test for Flavonoids

For this test, 100 µL of aqueous NaOH was added in the plant extract sample (1 mL) and intense yellow color development confirmed the presence of flavonoids in the tested sample [19].

Qualitative test for Poly steroid

Liebermann-Burchard’s test was performed to detect the presence of Poly steroid in plant extract sample, 500 µL of plant extract was added with 3 drops of acetic anhydride and concentrated H₂SO₄ and allowed to stand for 5 min. The development of blue-green color confirmed the positive result [20].

Qualitative test for Terpenoids
Added 1 mL plant extract sample, a mixture of 400 µL Chloroform and 400 µL Concentrated H$_2$SO$_4$ was added. Reddish-brown coloration was noted in positive test results [21].

**Qualitative test for Cardiac Glycosides**

Cardiac glycosides in the test plant samples were confirmed by treating 500 µL plant extract separately with 500 µL of glacial acetic acid and a few drops of 1% aqueous FeCl$_3$ and H$_2$SO$_4$. Green-blue color development confirmed the cardiac glycosides in plant extract [22].

**Qualitative test for Free Anthraquinones**

For this test 1 ml of the extract was added in 20 ml of chloroform followed by heating for 5 min on steam bath then the filtrate was allowed to cool, 20 ml of 10% Ammonia solution was added and shaken. Rose pink color development in the upper layer indicated a positive result [20].

**Qualitative test for Combined Anthraquinones**

Combined anthraquinones in plant extracts were confirmed by treating 450 µL of plant extract of each sample with 500 µL of chloroform and 50 µL of concentrated HCl. The appearance of rose-pink color confirmed the positive test results [20].

**Qualitative test for Tannins**

For this test 2 drops of 1%, ferric chloride (FeCl$_3$) were added to the 500 µL plant extract sample. Development of blue, green, or black color confirmed positive test results [23].
Qualitative test for Alkaloids

Alkaloids were confirmed in the plant extract sample by treating 200 µL of a plant extract with few drops of aqueous HCl followed by treatment with 500 µL of Mayer’s reagent. White precipitation confirmed the alkaloids in plant extract samples [16].

Quantitative Phytochemical Analysis

Quantitative Analysis for Phenols (96 well plate method)

In this assay, 75 µL of double distilled water (DDW) was added in well followed by 25 µL sample or standard was added. Folin C (F–C reagent) 25 µL/well (diluted 1: 1 (v/v) with DDW) added , left for 6 min. After that 100 µL of Na₂CO₃ (75 g/L) was added, mixing, plates were put in dark for 90 mins. Absorbance was measured at 765 nm by using a SPECTRA MAX M2e plate reader. Readings for sample control (sample and DDW) were taken before proceeding further. Gallic acid was taken as a standard at 12.5–400 µg/ml produces a calibration curve. The standard and plant extract solution was analyzed in three replications and averaged before making standard curves. Phenols were determined as µg of gallic acid equivalents / mL; calculated by the formula, $y = 0.6053 x - 0.0567$, where y is the absorbance at 765 nm and x is representing the amount of gallic acid equivalent in µg/Ml[24].

Quantitative Analysis for Flavonoids (96 well plate method)
To quantify flavonoids in plant extract sample, 100 µL of double distilled water (DDW) was dispensed in 96 wells, 10 µL of NaNO$_2$ (50 g/L), and 25 µL of standard or plant extract sample added in wells followed by incubation for 5 mint at room temperature. After this, 15 µL of AlCl$_3$ (100 g/L) was added to the mixture and left for 6 mint. After that, 50 µL of NaOH (1 mol/L) and 50 µL of DDW were added to each well and the plate was shaken for the 30s and absorbance was measurement at 510 nm. Catechin was used as a standard at 5–500 µg/mL to draw a calibration curve. All standards and plant extract samples were analyzed by using SPECTRA MAX M2e plate reader in triplicates and obtained readings were averaged before making standard curves. A standard curve of Catechin obtained and flavonoids of plant sample were expressed in µg of Catechin equivalents / mL, and were calculated by the formula, $y = 0.5377 \times + 0.316$, where $y$ is the absorbance at 510 nm and $x$ is representing the amount of Catechin equivalent in µg/mL[24].

**Quantitative Analysis for DPPH (96 well plate method)**

For this, 200 µL of DPPH (DPPH; Sigma-Aldrich, Germany) solution (150 mmol/L) was added to each well except blank wells. The plant extract, control, or standard solutions were added 25 µL in the wells, mixed and left to react in dark for 6 h, and absorbance measured at 517 nm by using SPECTRA MAX M2e plate reader. Ascorbic acid is used as a standard at 50–500 µmol/L concentrations to draw a calibration curve. All the standards and plant extract samples were
analyzed in triplicates and obtained readings were averaged before making standard curves. An ascorbic acid-based standard curve was obtained and DPPH in plant sample was expressed as µmol of Ascorbic acid equivalents / L and were calculated by the formula, \( y = 0.0319 \times + 0.1007 \), where \( y \) is the absorbance at 517 nm and \( x \) is taken the amount of Ascorbic acid equivalent in µmol / L[24].

**Quantitative Analysis for Alkaloids**

Plant extracts(10 ml), \( \text{H}_2\text{SO}_4 \) (10 %) 1 ml, \( \text{NH}_3 \) (10 %) 2 ml, Chloroform 3 ml. 10 ml of plant extracts separately mixed with 1 mL of \( \text{H}_2\text{SO}_4 \) (10 %), 2 mL of \( \text{NH}_3 \) (10 %) and 3 mL of Chloroform, thoroughly mixed, and passed through filter paper to get residues. These residues were dried and weighted [20].

**Quantitative Analysis for ORAC**

Various dilutions of Trolox (75 mM concentration) and plant extract were made in 10 mM phosphate buffer with pH 7.6. Plant extract sample (20 µL) was added to each well with the pipette. In each working well, 40 µL Fluorescein of 10 nM concentration was added. Phosphate buffer (25 µL) was used as blank. Sealed microplates were incubated in a spectrophotometer at 37 °C for 30 min. Initial fluorescence was measured after every 90 s at a wavelength of 485 nm and an emission wavelength of 520 nm by using a SPECTRA MAX M2e plate reader. After 3 cycles, 140 µL of 2,2'-Azobis(2-amidopropane) dihydrochloride (AAPH) was added to the
sample containing well. After adding AAPH, the test was run again, and readings were taken up to 120 min. The ORAC value for each plant extract was calculated against standard Trolox concentration by constructing a regression equation and standard curve [25].

**Gas Chromatography/Mass Spectrometry (GC/MS) Analysis**

Gas Chromatography / Mass Spectrometry (GC/MS) analysis was performed to analyze the phytochemical constituents based on the spectral output of all the compounds that get separated from plant extract sample [26].

**Plant Extracts Preparation**

Plant extracts of *Nepeta cataria* were prepared in 5 different solvents including Methanol, Ethanol, Water, Acetone, and Hexane. Plant extracts concentrated by sing rotary evaporator and obtained products were considered as crude solvent extracts and 2 µL of the prepared sample solution used in GC/MS.

**GC/MS Spectroscopy**

GC/MS analysis of all the plant extract samples was carried out by using SHAMIDZU GC/MS system, Model (SH-Rxi-5ms), and catalog No. (221-75940-30) (Shimadzu Corporation, Japan). Helium (He) (99.9 %) used as a carrier gas at a flow rate of 1 ml/min, and a volume of 2 µl was injected (split ratio of 10:1, 15:1 or 20:1) by using the split sampling technique, while injection
temperature was kept 250 °C and the oven temperature was set from 60 °C for 5 min, increased at a rate of 20 °C per min to 200°C, then finally to 330 °C at the rate of 10 °C per min to 330 °C for 5 min. The total pressure was maintained up to 66.7 kPa and the total flow was 32.9 mL/min while column flow was 1.12 mL/min with a linear velocity of 38.6 cm/sec. The total GC/MS analysis time was 40 min. The relative percentage of the constituent was found out by comparing its average peak area to the total areas. Mass spectrum results of plant extract fraction were intenerated by using the database of NIST and phytochemicals were characterized and identified.

NMR-based plant metabolomics analysis

Nuclear magnetic resource analysis is a powerful tool used to detect and measure all the metabolites present in test samples both on a qualitative and quantitative basis under certain conditions. NMR is used to detect a variety of secondary metabolites along with primary metabolites. Signals generated in the NMR spectrum are proportional to the molar concentration of the compounds present, and it directly compares the concentrations of all the present compounds without making calibration curves of each compound. This technique is very helpful for structure elucidation. The following procedure is adopted to carry out this analysis.

Harvesting of Plants

Leaves from the plants were carefully harvested and were transferred to the tubes containing liquid nitrogen.
**Freeze Dried sample Preparation**

Frozen leaves were grind in a pre-cooled pestle and mortar in liquid nitrogen and powdered material was transferred into plastic tubes by using a spatula. Before freeze-drying, samples were kept in the deep freezer. After that, samples were put in the freeze-dryer for 24 to 48 h.

**Sample Preparation and NMR Analysis**

The freeze-dried sample was weighed in an Eppendorf tube. 0.75 ml of CH$_3$OH-d$_4$ and 0.75 ml of KH$_2$PO$_4$ buffer in D$_2$O (pH 6.0) containing 0.1% (wt/wt) TSP were added to the sample and vortexed for 1 min at room temperature. The prepared sample was ultrasonicated for 10 – 20 min at room temperature. The clear supernatant was obtained by centrifugation (17,000 g) of the sample for 5 – 10 min at room temperature in a microtube centrifuge. The supernatant was shifted to a 1.5 ml Eppendorf tube and 800 μl of supernatant was shifted to a 5 mm NMR tube. It was then placed into the spectrometer at 25 ºC. Spectrometer frequency was locked to the deuterium resonance arising from the NMR solvents and the most suitable experiment model was used. NMR signals are considered as directly proportional to the molar concentration of the characteristic of a metabolite and the concentration of detected metabolites can be obtained by comparing the peak intensity with an internal standard (TSP).

**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 Statistix 8.1 and Microsoft office excel 2010 version. Means were calculated and a 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

Kirby-Bauer Disk Diffusion Method for Anti-Microbial Activities

Kirby disk diffusion method used for measuring antimicrobial efficacy of plant extracts under in vitro conditions. Chloramphenicol was used as a standard antibiotic. Results indicated that *Nepeta cataria* ethanol-based extract showed maximum inhibition of *B. subtilis* followed by *C. freundii* and *M. luteus* while methanol-based extracts also showed maximum efficacy against *S. sonnei, E. coli, M. luteus,* and *C. freundii.* Water, acetone, and Hexane based extracts were equally effective against tested bacterial isolates as shown in Figure:1.
Figure: 1. Antimicrobial efficacy of *Nepeta cataria* based plant extracts in different solvents against bacterial isolates.

**Determination of Minimum Inhibitory Concentration (MIC) by Well Plate Method**

Percentage growth inhibition of each tested bacteria viz, *Shigella sonnei, Bacillus subtilis, Klebsiella oxytoca, Escherichia coli, Salmonella enterica, Micrococcus luteus, Staphylococcus aureus, Lactococcus lactis, Listeria monocytogenes,* and *Citrobacter freundii* was computed by the formula:

\[
\% \text{ bacterial growth inhibition} = \frac{\text{Optical density in control} - \text{Optical density in treatment}}{\text{Optical density in control}}
\]

Percentage growth inhibition of bacterial isolates is given in Figure 2. Among all, ethanol-based extracts of *Nepeta cataria* showed maximum inhibition percentage of all tested
bacteria at 250-1000 µg/ml concentration followed by methanolic extracts at 500-1000 µg/ml dose levels and water-based extracts at 1000 and 500 µg/ml dose levels, acetone and hexanes-based extracts of *Nepeta cataria* did not show significant inhibition against all bacterial isolates as compared to control treatments Figure: 2.

Figure: 2. *Nepeta cataria* plant extracts a percentage of bacterial strains growth inhibition in different solvents at different dose levels.

**Resazurin based Well Plate Microdilution Method**

The Resazurin method is 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 level of efficacy against all tested bacterial isolates in comparison to positive and negative control. *N. cataria* plant extract at
the dose level of 12.5 µl/ml showed maximum inhibition followed by 6.25 µl/ml. Plant extract at 100 µl/ml showed maximum inhibition against *L. lactis* and *E. coli* Figure 3.

![Figure 3](image)

Figure 3. Resazurin based well plate Microdilution assay of *N. cataria* against bacterial strains.

**Qualitative Phytochemical Analysis of N. cataria**

**Qualitative Test for Saponin**

Water-based extracts of *N. cataria* were positive for saponin.

**Qualitative Test for Phenolic Compounds**

Water-based, methanol, ethanol, and acetone based extracts showed positive results while hexane showed the negative result.

**Qualitative Test for Water Soluble Phenol**
Water-based and methanol extracts showed positive results while ethanol, acetone, and hexane showed negative results.

**Qualitative Test for Water Insoluble Phenol**

Methanol, ethanol, and acetone based extracts showed positive results while hexane and water-based showed a negative result.

**Qualitative Test for Flavonoids**

Test for flavonoids was carried out and the development of intense yellow color is positive test results indicate. Methanol and acetone-based extracts showed positive results while hexane, ethanol, and water-based showed negative result.

**Qualitative Test for Terpenoids**

For the triterpenoids testing, reddish-brown coloration development confirms the positive test results. Water-based, methanol, ethanol, acetone, and hexane-based extracts were positive.

**Qualitative Test for Cardiac Glycosides**
In the cardiac glycosides test, the development of green-blue color is the confirmation of positive results. Acetone-based extracts were positive while water-based, methanol, ethanol, and hexane-based extracts were negative.

**Qualitative Test for Free Anthraquinones**

The water-based extracts, methanol, ethanol, and acetone based extract were positive while hexane-based extracts were negative.

**Qualitative Test for Combined Anthraquinones**

This test was performed to confirm the presence of combined anthraquinone in the test plant sample. water-based extracts extract were positive, while methanol, ethanol, acetone, and hexane-based extracts were negative.

**Qualitative Test for Tannins**

In this test, water-based, methanol, and ethanol extracts were positive, while acetone and hexane-based extracts were negative

**Qualitative Test for Alkaloids**

In this test, water-based, methanol, ethanol, acetone, and hexane-based extracts were positive.
Quantitative Phytochemical Analysis

Quantitative Analysis for Phenols (96 well plate method)

The methanol, ethanol, water, acetone, and hexane extracts of *Nepeta cataria*, were examined in terms of μg of Gallic Acid Equivalents per mL. Methanol, acetone, and ethanol-based extracts showed the maximum presence of phenols as compared to water and hexane-based extracts. The order of phenol presence in the sample was Methanol extracts > ethanol extracts > acetone extracts > water extracts > hexane extracts showed in Figure 4.

![Figure 4 Concentration of phenols in *Nepeta cataria* extracts in different solvents.](image)

**4.5.2 Quantitative Analysis for Flavonoids (96 well plate method)**

The Flavonoids in methanol, ethanol, water, acetone, and hexane extracts of *Nepeta cataria* was quantified in terms of μg of Catechin equivalents/mL. Hexane, acetone, and water-based
Extracts showed a high level of flavonoids which as compared to methanol and ethanol-based extracts. Flavonoids results are summarized in Figure: 5.

![Figure 5. The concentration of Flavonoids in Nepeta cataria extracts in different solvents.](image)

### 4.5.3 Quantitative Analysis for DPPH (96 well plate method)

The presence of 2, 2-diphenyl-1-picrylhydrazyl DPPH was determined in *Nepeta cataria* extracts by using different solvents viz., methanol, ethanol, water, acetone, and hexane which measured by Spectrophotometrically and results are drawn as µmol of ascorbic acid equivalents / L and results are given in Figure 6. Results showed that acetone-based plant extract contained a high concentration of DPPH followed by water-based extracts and results compared with hexane extracts, ethanol, and methanol-based extracts. The presence of DPPH was in order acetone extracts > water extracts > ethanol extracts > methanol extracts > hexane extracts.
Figure 6. The concentration of DPPH in *Nepeta cataria* plant extracts prepared in different solvents.

**Quantitative Analysis for Alkaloids**

The alkaloid percentage method was used to quantify the alkaloids in plants. Alkaloids quantity in tested plant extracts was varied from 0.204 / 0.5 g plant.

**Oxygen Radical Absorbance Capacity (ORAC)**

Oxygen Radical Absorbance Capacity Assay (ORAC) was performed to study the antiradical activity of *Nepeta cataria* prepared in methanol extract. The results of the ORAC test was 133759.021μm/100ml in concentration.

**Gas Chromatography/Mass Spectrometry (GC/MS) ANALYSIS**

Gas Chromatography/Mass Spectrometry (GC/MS) is the widely adopted technique for the detection of biologically active compounds. Plant extracts of *Nepeta cataria* in 5 different solvents viz., 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 time (RT).

GC/MS analysis of a methanolic extract of *Nepeta cataria* showed (71 identified phytochemicals + 48 un-matched ] chemicals (Table 1). Analysis of ethanol-based extracts confirmed the existence of 80 known phytochemical constituents while 31 unmatched chemicals were detected (Table 2). Water-based extracts of *Nepeta cataria* contain 28 known phytochemicals while 11 un-matched chemicals were also detected (Table 3). Acetone-based extract confirmed the existence of 13 known compounds extract while 9 chemical constituents were unmatched (Table 4). Analysis of hexane based extract confirmed the presence of 11 known chemical constituents while 8 unmatched chemicals were detected as given in Table 5. GCMS spectral chromatograms of all the solvent-based extracts are given in Figure; 7-11.
Figure: 7. GCMS Spectral Chromatogram of Methanolic Extract of *Nepeta cataria*

**Table 1** GC-MS Analysis for the Identification of Phytochemicals in the Methanolic Extract of *Nepeta criteria*.

| S. No. | Compound                                      | Mol. Formula | Amount / Con. | Mol. weight | RT     |
|--------|-----------------------------------------------|--------------|---------------|------------|--------|
| 1      | 2-Furanmethanol, 5-ethenylte                  | -            | 0.287         | -          | 6.078  |
| 2      | endo-Borneol                                   | C_{10}H_{16}O | 0.623         | 154.25 g/mol | 8.246  |
| 3      | 1,5,7-Octatrien-3-ol, 3,7-di                  | C_{10}H_{20}O | 0.390         | 152.2334 g/mol | 8.782  |
| 4      | Bicyclo [2.2.1] heptane, 7,7-d               | C_{9}H_{16}  | 0.240         | 124.22 g/mol | 9.955  |
| 5      | 2-Cyclohexen-1-one, 3-methyl                   | C_{9}H_{16}O | 0.230         | 110.15 g/mol | 11.529 |
| 6      | 1-Isopropylcyclohex-1-ene                     | C_{9}H_{16}  | 27.376        | 124.22 g/mol | 12.402 |
| 7      | Bicyclo [3.1.0] hexane-2-undec                | C_{9}H_{18}  | 2.974         | 82.14 g/mol | 13.804 |
| 8      | 3,5-Dimethylcyclohex-1-ene-4                  | C_{9}H_{14}  | 0.542         | 110.2 g/mol | 14.226 |
| 9      | 2-Butyl-5-methyl-3-(2-methyl                  | C_{15}H_{20}O | 0.645         | 222.37 g/mol | 14.281 |
| 10     | Caryophyllene oxide                           | C_{15}H_{20}O | 1.916         | 220.35 g/mol | 15.129 |
| 11     | Methyl octadec-6,9-dien-12-y                  | C_{15}H_{20}O | 0.149         | 280.4 g/mol | 15.763 |
| 12     | Caryophylla-4(12),8(13)-dien                  | C_{15}H_{20}O | 0.358         | 220.3505 g/mol | 15.937 |
| 13     | 1-Chlorosulfonyl-3-methyl-1-                  | C_{9}H_{14}ClNO_{3}S | 0.823 | 251.73 g/mol | 16.173 |
| 14     | trans-Z-, alpha. -Bisabolene e                | -            | 1.312         | -          | 16.216 |
| 15     | Caryophylla-4(12),8(13)-dien                  | C_{15}H_{20}O | 0.632         | 220.3505 g/mol | 16.429 |
| 16     | 2H-1-Benzopyran-2-one, 7-met                  | C_{13}H_{18}NO_{2} | 0.826 | 217.26 g/mol | 17.040 |
| 17     | 6-Hydroxy-4,4,7a-trimethyl-5                  | C_{11}H_{16}O_{3} | 0.258 | 196.24 g/mol | 17.648 |
| 18     | Tricyclo [20.8.0.0(7,16)] tria                | -            | 0.413         | -          | 18.261 |
| 19     | Neophytadiene                                  | C_{20}H_{38} | 0.313         | 278.5 g/mol | 18.782 |
| 20     | 2-Pentadecanone, 6,10,14-tri                  | C_{18}H_{36}O | 0.386         | 268.4778 g/mol | 18.826 |
| 21     | (3S,3aS,6R,7R,9aS)-1,1,7-Tri                 | -            | 0.562         | -          | 19.087 |
| 22     | Hexadecanoic acid, methyl es                  | C_{17}H_{34}O_{2} | 0.954 | 270.5 g/mol | 19.887 |
| 23     | n-Hexadecanoic acid                           | C_{16}H_{32}O_{2} | 7.973 | 256.4241 g/mol | 20.364 |
| 24     | 9,12-Hexadecadienoic acid, m                 | C_{16}H_{32}O_{2} | 0.273 | 252.39 g/mol | 21.796 |
| 25     | Methyl 8,11,14-heptadecatrie                  | -            | 0.920         | -          | 21.853 |
| 26     | Phytol                                        | C_{20}H_{40}O | 1.179         | 128.1705 g/mol | 21.998 |
|   | Name                                                                 | Formula         | Mol. Wt | Mol. % |   |
|---|----------------------------------------------------------------------|-----------------|---------|-------|---|
| 27| 9,12,15-Octadecatrienoic acid                                        | C_{18}H_{30}O_{2} | 278.43  | 6.401 | 22.304 |
| 28| Octadecanoic acid                                                     | C_{18}H_{36}O_{2} | 284.48  | 0.970 | 22.623 |
| 29| Hexadecanoic acid, 2-hydroxy                                          | C_{16}H_{32}O_{3} | 272.42  | 0.744 | 26.101 |
| 30| 5-Cholestene-3-ol, 24-methyl                                        | C_{28}H_{46}O    | 400.7   | 0.344 | 31.863 |
| 31| beta. -Sitosterol                                                     | C_{29}H_{54}O    | 414.71  | 5.461 | 32.541 |
| 32| Olean-12-en-3-ol, acetate, (                                          | C_{32}H_{52}O_{2} | 468.8   | 0.486 | 32.724 |
| 33| beta. -Guaiene                                                        | C_{15}H_{34}     | 204.351 | 0.271 | 32.882 |
| 34| alpha. -Amyrin                                                        | C_{30}H_{56}O    | 426.729 | 2.691 | 33.062 |
| 35| Ursolic aldehyde                                                      | C_{30}H_{58}O_{2} | 440.7   | 1.302 | 34.718 |
| 36| Urs-12-en-28-al                                                      | C_{30}H_{46}O    | 424.7   | 0.654 | 35.305 |
| 37| Betulin                                                              | C_{30}H_{56}O_{2} | 442.72  | 0.910 | 35.472 |
| 38| Eucalyptol                                                           | C_{10}H_{16}O    | 154.249 | 8.505 | 5.112  |
| 39| 2-Furanmethanol, 5-ethenylte                                         | -                | 6.840   | -     | 6.165  |
| 40| Ethyl 2-(5-methyl-5-vinyltet)                                        | -                | 5.845   | -     | 6.551  |
| 41| Bicyclo [2.2.1] heptan-2-one,                                        | C_{7}H_{12}O    | 110.15  | 20.437 | 7.728 |
| 42| 1,6-Octadien-3-ol, 3,7-dimet                                         | C_{10}H_{16}O    | 154.25  | 5.855 | 9.981  |
| 43| Pentane, 1-chloro-5- (methylene)                                      | -                | 3.739   | -     | 10.696 |
| 44| Pregnan-18-ol, 20-methyl-20-                                          | C_{22}H_{50}O   | 333.6   | 2.640 | 13.916 |
| 45| Caryophyllene oxide                                                   | C_{15}H_{25}NO  | 220.35  | 12.423 | 15.140 |
| 46| Cyclohexene,1-propyl-                                                | C_{8}H_{16}     | 124.22  | 0.483 | 11.611 |
| 47| 1-Isopropylcyclohex-1-ene                                            | C_{5}H_{16}     | 124.22  | 6.144 | 13.699 |
| 48| Coumarin                                                             | C_{3}H_{6}O_{2}  | 146.147 | 0.878 | 13.867 |
| 49| 1-Methyl-2-methylene cyclohex                                         | C_{8}H_{14}     | 110.197 | 0.622 | 14.461 |
| 50| Caryophyllene oxide                                                   | C_{15}H_{24}O   | 220.35  | 2.144 | 16.143 |
| 51| Megastigmatrieneone                                                  | C_{13}H_{16}O   | 190.28  | 0.560 | 16.780 |
| 52| 11,11-Dimethyl-4,8-dimethyle                                         | C_{15}H_{26}O   | 220.35  | 0.429 | 16.954 |
| 53| (1R,7S, E)-7-Isopropyl-4,10-d                                       | C_{15}H_{26}O   | 220.35  | 0.702 | 17.243 |
| 54| Caryophylla-4(12),8(13)-dien                                         | C_{15}H_{26}O   | 220.35  | 0.603 | 17.450 |
| 55| 2H-1-Benzopyran-2-one, 7-met                                         | C_{13}H_{16}NO_{2} | 217.26   | 1.381 | 18.071 |
| 56| 11,14-Octadecadienoic acid,                                          | C_{18}H_{34}O_{2} | 280.4   | 0.364 | 22.811 |
| 57| Methyl 8,11,14-heptadecatrie                                         | -                | 1.220   | -     | 22.864 |
| 58| Eicosanoic acid                                                      | C_{20}H_{40}O_{2} | 312.530 | 0.515 | 25.775 |
|   | Name                                                      | Molecular Formula | MW | Density | MP (°C) |
|---|----------------------------------------------------------|-------------------|----|---------|---------|
| 59| Phenol, 2,4-bis (1-methyl-1-pentyloxy)                  | C_{24}H_{36}O     | 330.5 g/mol | 26.725 |
| 60| Tritetracontane                                         | C_{43}H_{88}      | 605.2 g/mol | 27.798 |
| 61| Methyl 2-hydroxy-octadeca-9,10-dimethoxy ether          | C_{19}H_{20}O_{3} | 308.5 g/mol | 28.775 |
| 62| Hentriacontane                                          | C_{31}H_{64}      | 436.85 g/mol | 30.023 |
| 63| alpha-Tocopherol B                                      | C_{29}H_{56}O_{4} | 462.7049 g/mol | 30.777 |
| 64| alpha-Tocopherol A                                      | C_{29}H_{56}O_{4} | 462.7 g/mol | 30.208 |
| 65| Campesterol                                              | C_{27}H_{48}O     | 400.68 g/mol | 32.877 |
| 66| Stigmasterol                                             | C_{29}H_{48}O     | 412.69 g/mol | 33.091 |
| 67| gamma-Sitosterol                                         | C_{29}H_{50}O     | 414.7 g/mol | 33.566 |
| 68| beta-Amyrin                                              | C_{30}H_{50}O     | 426.729 g/mol | 33.739 |
| 69| 1,1,4a-Trimethyl-5,6-dimethy-1,2-dihydrosutchin         | C_{15}H_{24}      | 204.35 g/mol | 33.896 |
| 70| Urs-12-en-28-oic acid, 3-hydroxy-28-oxo                 | C_{30}H_{46}O_{3} | 456.7 g/mol | 35.636 |
| 71| Uvaol                                                    | C_{30}H_{50}O_{2} | 442.7 g/mol | 36.319 |
| 72| No Match                                                 | -                 | 1.279 | 7.678   |
| 73| No Match                                                 | -                 | 0.447 | 11.436  |
| 74| No Match                                                 | -                 | 0.323 | 11.703  |
| 75| No Match                                                 | -                 | 0.549 | 11.886  |
| 76| No Match                                                 | -                 | 0.937 | 12.007  |
| 77| No Match                                                 | -                 | 0.875 | 12.736  |
| 78| No Match                                                 | -                 | 1.179 | 12.826  |
| 79| No Match                                                 | -                 | 3.063 | 12.903  |
| 80| No Match                                                 | -                 | 0.717 | 13.206  |
| 81| No Match                                                 | -                 | 1.148 | 13.285  |
| 82| No Match                                                 | -                 | 0.579 | 13.446  |
| 83| No Match                                                 | -                 | 3.008 | 13.718  |
| 84| No Match                                                 | -                 | 1.080 | 13.897  |
| 85| No Match                                                 | -                 | 0.325 | 14.381  |
| 86| No Match                                                 | -                 | 0.363 | 15.481  |
| 87| No Match                                                 | -                 | 0.566 | 15.531  |
| 88| No Match                                                 | -                 | 1.498 | 16.925  |
| 89| No Match                                                 | -                 | 0.304 | 17.223  |
|   |     | 0.394 | 17.818  |
|---|-----|-------|------|
|   |     | 2.074 | 21.141 |
|   |     | 0.322 | 21.365 |
|   |     | 1.975 | 25.235 |
|   |     | 1.013 | 26.209 |
|   |     | 6.419 | 6.933  |
|   |     | 1.807 | 11.016 |
|   |     | 2.619 | 11.726 |
|   |     | 4.148 | 13.303 |
|   |     | 0.356 | 34.665 |
|   |     | 1.659 | 34.964 |
|   |     | 1.326 | 35.143 |
|   |     | 0.997 | 35.231 |
|   |     | 0.688 | 35.270 |
|   |     | 1.430 | 35.912 |
|   |     | 0.447 | 12.434 |
|   |     | 0.543 | 12.559 |
|   |     | 0.553 | 12.880 |
|   |     | 0.486 | 13.019 |
|   |     | 1.447 | 14.094 |
|   |     | 0.585 | 14.134 |
|   |     | 2.430 | 14.296 |
|   |     | 2.021 | 14.919 |
|   |     | 1.245 | 17.965 |
|   |     | 3.893 | 22.205 |
|   |     | 0.545 | 22.421 |
|   |     | 2.786 | 26.376 |
|   |     | 0.419 | 26.522 |
|   |     | 1.436 | 27.233 |
|   |     | 0.616 | 27.717 |
Figure 8. GCMS Spectral Chromatogram of Ethanolic Extract of *Nepeta cataria*.

Table 2. GC-MS Analysis for the Identification of Phytochemicals in the Ethanolic Extract of *Nepeta cataria* (A1).

| S. No. | Compound                                      | Mol. formula | Amount/Con. | Mol. weight | RT  |
|-------|-----------------------------------------------|--------------|-------------|-------------|-----|
| 1     | 2,4-Dihydroxy-2,5-dimethyl-3                  | C₈H₁₀O₄      | 0.284       | 144.12 g/mol| 3.404|
| 2     | 1-Isopropylcyclohex-1-ene                     | C₉H₁₆        | 14.940      | 124.22 g/mol| 9.585|
| 3     | Coumarin                                      | C₆H₈O₂       | 2.940       | 146.1427 g/mol| 9.646|
| 4     | Hexadecanoic acid, ethyl est                  | C₁₈H₃₂O₂     | 3.361       | 284.4727    | 15.865|
| 5     | Oleic Acid                                    | C₁₈H₃₄O₂     | 0.653       | 282.47 g/mol| 16.515|
| 6     | Phytol                                        | C₂₀H₄₀O₂     | 3.068       | 128.1705 g/mol| 16.907|
| 7     | 9,12,15-Octadecatrienoic acid                 | C₁₈H₃₀O₂     | 27.308      | 278.43 g/mol| 17.266|
| 8     | Ethyl 9.cis.,11. trans.-octadecanoic acid     | C₁₈H₃₂O₂     | 2.045       | -           | -    |
| 9     | Octadecanoic acid                             | C₁₈H₃₂O₂     | 3.620       | 284.48 g/mol| 17.464|
| 10    | Octadecanoic acid, 17-methyl                  | C₂₀H₄₀O₂     | 0.982       | 312.5 g/mol| 17.680|
| 11    | Eicosanoic acid                               | C₂₀H₄₀O₂     | 1.138       | 312.5304 g/mol| 19.118|
| 12    | Tetracontane, 3,5,24-trimeth                   | C₃₁H₆₄       | 1.194       | 605.2 g/mol| 20.201|
| 13    | Hentriacontane                                | C₃₁H₆₄       | 1.197       | 436.85 g/mol| 20.740|
| 14    | Methyl 2-hydroxy-octadeca-9                   | C₁₈H₃₂O₃     | 0.895       | 308.5 g/mol| 21.548|
| 15    | Sulfurous acid, butyl tetrad                  | C₂₁H₄₄O₃S   | 0.667       | 376.6 g/mol| 22.185|
| 16    | alpha.-Tocospiro A                            | C₂₉H₅₀O₄     | 0.654       | 462.7 g/mol| 22.498|
| 17    | Sulfurous acid, butyl tridec                  | C₁₇H₅₀O₃S   | 0.572       | 320.5 g/mol| 22.897|
| No. | Name                                                                 | Formula   | MW/mol        | Molar Mass    |
|-----|----------------------------------------------------------------------|-----------|---------------|---------------|
| 18  | 3,7,11,15-Tetramethyl-2-hexa                                         | C<sub>20</sub>H<sub>46</sub>O | 0.444         | 296.5 g/mol   | 23.191        |
| 19  | Sulfurous acid, butyl tetrad                                           | C<sub>21</sub>H<sub>44</sub>O<sub>3</sub>S | 1.134         | 376.6 g/mol   | 23.243        |
| 20  | Sulfurous acid, butyl tridec                                           | C<sub>17</sub>H<sub>36</sub>O<sub>3</sub>S | 0.399         | 320.5 g/mol   | 24.233        |
| 21  | Stigmasterol                                                           | C<sub>29</sub>H<sub>50</sub>O | 0.630         | 412.69 g/mol  | 24.507        |
| 22  | beta.-Sitosterol                                                       | C<sub>29</sub>H<sub>50</sub>O | 13.312        | 414.71 g/mol  | 24.939        |
| 23  | alpha.-Amyrin                                                          | C<sub>30</sub>H<sub>50</sub>O | 6.667         | 426.729 g/mol | 25.504        |
| 24  | 2-Methylindoline                                                       | C<sub>8</sub>H<sub>13</sub>N        | 133.19 g/mol  | 8.120         |
| 25  | 2-Cyclohexen-1-one, 4,5-dime                                          | -         | 0.113         | -             | 10.585        |
| 26  | 1-Isopropylethylhex-1-ene                                              | C<sub>8</sub>H<sub>16</sub>         | 25.854        | 124.22 g/mol  | 11.456        |
| 27  | Bicyclo[3.1.0] hexane-2-undec                                         | C<sub>8</sub>H<sub>10</sub>         | 1.108         | 82.14 g/mol   | 12.837        |
| 28  | Methyl 13,14-octadecadienoan                                          | C<sub>19</sub>H<sub>34</sub>O<sub>2</sub> | 3.793         | 294.472 g/mol | 13.689        |
| 29  | Azulene, 1,2,3,3a,4,5,6,7-octadecadienoate                             | C<sub>15</sub>H<sub>34</sub>        | 0.170         | 204.3511 g/mol| 15.056        |
| 30  | 1-Chlorosulfonyl-3-methyl-1-tetralan                                  | C<sub>8</sub>H<sub>14</sub>ClNO<sub>3</sub>S | 2.175         | 251.73 g/mol  | 15.242        |
| 31  | Cholestan-3-ol, 2-methylene-                                          | C<sub>20</sub>H<sub>40</sub>O | 0.515         | 400.7 g/mol   | 15.446        |
| 32  | 2H-1-Benzopyran-2-one, 7-methyl                                        | C<sub>12</sub>H<sub>14</sub>NO<sub>2</sub> | 1.381         | 217.26 g/mol  | 16.049        |
| 33  | 6-Hydroxy-4,4,7a-trimethyl-5-7a-octadecadienoate                       | C<sub>11</sub>H<sub>16</sub>O<sub>3</sub> | 0.367         | 196.24 g/mol  | 16.663        |
| 34  | [1,1'-Bicyclopropyl]-2-octan                                          | C<sub>21</sub>H<sub>30</sub>O<sub>2</sub> | 0.823         | 322.5 g/mol   | 16.857        |
| 35  | 2-Pentadecanone, 6,10,14-tri                                          | C<sub>18</sub>H<sub>30</sub>O<sub>2</sub> | 0.298         | 268.4778 g/mol| 17.839        |
| 36  | 4,4,8-Trimethyltricyclo[6.3.3]                                         | C<sub>15</sub>H<sub>26</sub>O<sub>2</sub> | 1.458         | 238.366 g/mol | 18.101        |
| 37  | n-Hexadecanoic acid                                                   | C<sub>16</sub>H<sub>32</sub>O<sub>2</sub> | 10.300        | 256.4241 g/mol| 19.386        |
| 38  | 11,14-Octadecadienoic acid,                                          | C<sub>19</sub>H<sub>32</sub>O<sub>2</sub> | 0.819         | 280.4 g/mol   | 21.561        |
| 39  | Ethyl 9,12,15-octadecatrieno                                          | C<sub>20</sub>H<sub>42</sub>O<sub>2</sub> | 3.315         | 306.5 g/mol   | 21.626        |
| 40  | 6-Octadecanoic acid, methyl                                           | -         | 1.149         | -             | 24.253        |
| 41  | Tetracontane, 3,5,24-trimethyl                                        | C<sub>42</sub>H<sub>88</sub>         | 0.506         | 605.2 g/mol   | 25.112        |
| 42  | 24-Noroleana-3,12-diene                                                | C<sub>29</sub>H<sub>46</sub>         | 0.537         | 394.6755 g/mol| 31.418        |
| 43  | 1R,4S,7S,11R-2,2,4,8-Tetramer                                         | C<sub>15</sub>H<sub>30</sub>O<sub>2</sub> | 0.419         | 222.366 g/mol | 31.553        |
| 44  | Ursolic aldehyde                                                       | C<sub>30</sub>H<sub>50</sub>O<sub>2</sub> | 2.109         | 440.7 g/mol   | 33.113        |
| 45  | Betulin                                                               | C<sub>30</sub>H<sub>50</sub>O<sub>2</sub> | 0.802         | 442.72 g/mol  | 33.839        |
| 46  | 2,4-Dihydroxy-2,5-dimethyl-3                                           | C<sub>6</sub>H<sub>10</sub>O<sub>4</sub> | 0.032         | 144.12 g/mol  | 3.230         |
| 47  | 2-Methylindoline                                                       | C<sub>8</sub>H<sub>13</sub>N         | 0.490         | 133.19 g/mol  | 6.580         |
| 48  | 5-Hydroxymethylfurfural                                               | C<sub>6</sub>H<sub>10</sub>O<sub>3</sub> | 0.768         | 126.11 g/mol  | 6.985         |
| 49  | 2-Cyclohexen-1-one, 3-methyl                                          | C<sub>7</sub>H<sub>10</sub>O<sub>2</sub> | 0.083         | 110.15 g/mol  | 8.592         |
|    | Name                                                                 | Formula  | MW     | Molecular Weight (g/mol) | Molecular Weight (g/mol) |
|----|----------------------------------------------------------------------|----------|--------|--------------------------|--------------------------|
| 50 | 1-Isopropylcyclohex-1-ene                                            | C₈H₁₆    | 128.25 | 142.25                    | 17.33                     |
| 51 | Cyclopentanecarboxylic acid,                                        | C₆H₁₀O₂  | 0.008  | 114.14                    | 9.434                     |
| 52 | Coumarin                                                              | C₈H₆O₂   | 1.727  | 146.1427                  | 9.618                     |
| 53 | Methyl 10,11-tetradecadienoa                                        | -        | 0.573  | -                         | 10.069                    |
| 54 | 2(4H)-Benzofuranone, 5,6,7,7                                          | C₁₁H₁₂O₂ | 0.319  | 180.2435                  | 10.757                    |
| 55 | (4aS,7S,7aR)-4,7-Dimethyl-2,                                          | C₁₀H₁₄O₂ | 0.169  | 166.2170                  | 10.885                    |
| 56 | n-Propyl 9,12-hexadecadienoa                                         | C₁₅H₃₀O₂ | 0.262  | 294.5                     | 11.116                    |
| 57 | 12-Methyl-E, E-2,13-octadecad                                        | C₁₈H₃₂O   | 0.113  | 280.48854                 | 11.164                    |
| 58 | Fumaric acid, ethyl 2-methyl                                         | C₁₀H₁₄O₄ | 0.179  | 198.22                    | 11.356                    |
| 59 | Bicyclo [4.4.0] dec-1-ene, 2-i                                       | C₁₅H₃₄    | 0.116  | 204.35                    | 11.540                    |
| 60 | Megastigmatrienone                                                    | C₁₃H₁₆O   | 0.081  | 190.28                    | 11.924                    |
| 61 | 10,10-Dimethyl-2,6-dimethylene                                       | C₁₅H₃₄    | 0.199  | 204.351                   | 12.067                    |
| 62 | tau. -Cadinol                                                         | C₁₅H₂₀O   | 0.335  | 222.37                    | 12.143                    |
| 63 | 2H-1-Benzopyran-2-one, 7-met                                          | C₁₃H₁₈NO₂ | 0.893  | 217.26                    | 12.956                    |
| 64 | cis-5,8,11,14,17-Eicosapenta                                         | C₂₀H₃₈O₂  | 0.148  | 302.5                     | 13.276                    |
| 65 | Carbamic acid, N- [1,1-bis (tr                                         | -        | 0.124  | -                         | 13.319                    |
| 66 | Tetradecanoic acid                                                    | C₁₄H₃₀O₂  | 0.250  | 228.3709                  | 13.552                    |
| 67 | 1-Heptatriacetonol                                                    | C₇H₁₆O    | 0.432  | 537                      | 13.943                    |
| 68 | 2-Pentadecanone, 6,10,14-tri                                         | C₁₈H₃₀O   | 0.286  | 268.4778                  | 14.346                    |
| 69 | Ethyl 9,cis.,11. trans.-octad                                         | -        | 0.340  | -                         | 17.345                    |
| 70 | Tetracontane, 3,5,24-trimeth                                         | C₄₃H₈₈    | 0.304  | 605.2                    | 20.193                    |
| 71 | Tritetracontane                                                        | C₄₃H₈₈    | 0.177  | 605.2                    | 22.180                    |
| 72 | Urs-12-en-28-oic acid, 3-hyd                                          | C₃₈H₆₄O₃  | 0.722  | 456.7                    | 23.776                    |
| 73 | Urs-12-en-28-ol                                                       | C₃₈H₆₀O   | 4.295  | 426.7                    | 23.833                    |
| 74 | Glycine, N- [3. alpha.,5. beta                                      | C₃₀H₅₃NO₄Si | 0.313  | 519.8                    | 24.109                    |
| 75 | Ergost-5-en-3-ol, (3. beta.)-                                        | C₂₈H₄₆O   | 0.180  | 400.7                    | 24.141                    |
| 76 | Stigmasterol                                                          | C₂₉H₄₆O   | 0.102  | 412.69                   | 24.241                    |
| 77 | Uvaol                                                                | C₃₆H₆₀O₂  | 1.125  | 442.7                    | 24.513                    |
| 78 | Tricyclo [20.8.0.0(7,16)] tria                                        | -        | 0.647  | -                         | 25.158                    |
| 79 | Neophytadiene                                                         | C₂₆H₃₈    | 0.294  | 278.5                    | 25.337                    |
| 80 | Tetracosamethyl-cyclododecas                                          | -        | 1.000  | -                         | 27.703                    |
| 81 | No Match                                                              | -        | 42.916 | -                         | 2.039                     |
|   | Match | Value 1 | Value 2 | Value 3 |
|---|-------|---------|---------|---------|
| 82| No Match | - | 57.084 | - | 2.058 |
| 83| No Match | - | 4.606 | - | 16.278 |
| 84| No Match | - | 0.481 | - | 16.473 |
| 85| No Match | - | 0.369 | - | 23.972 |
| 86| No Match | - | 0.884 | - | 11.045 |
| 87| No Match | - | 1.950 | - | 11.165 |
| 88| No Match | - | 0.742 | - | 11.881 |
| 89| No Match | - | 0.665 | - | 11.947 |
| 90| No Match | - | 0.865 | - | 13.906 |
| 91| No Match | - | 0.836 | - | 15.628 |
| 92| No Match | - | 1.756 | - | 15.716 |
| 93| No Match | - | 1.456 | - | 15.943 |
| 94| No Match | - | 0.395 | - | 16.248 |
| 95| No Match | - | 0.457 | - | 8.924 |
| 96| No Match | - | 0.534 | - | 9.470 |
| 97| No Match | - | 1.660 | - | 9.685 |
| 98| No Match | - | 1.199 | - | 9.727 |
| 99| No Match | - | 0.406 | - | 10.249 |
| 100| No Match | - | 0.435 | - | 10.634 |
| 101| No Match | - | 0.643 | - | 11.217 |
| 102| No Match | - | 0.562 | - | 12.242 |
| 103| No Match | - | 0.754 | - | 12.601 |
| 104| No Match | - | 0.727 | - | 12.675 |
| 105| No Match | - | 0.984 | - | 12.821 |
| 106| No Match | - | 0.939 | - | 12.875 |
| 107| No Match | - | 0.424 | - | 13.117 |
| 108| No Match | - | 0.772 | - | 19.585 |
| 109| No Match | - | 0.517 | - | 22.775 |
| 110| No Match | - | 0.375 | - | 25.618 |
| 111| No Match | - | 0.501 | - | 26.914 |
Figure 9. GCMS Spectral Chromatogram of Water Extract of *Nepeta cataria*.

Table 3  GC-MS Analysis for the Identification of Phytochemicals in the Water Extract of *Nepeta cataria*

| S. No. | Compound                        | Mol. formula | Amount/Con. | Mol. weight      | RT   |
|--------|---------------------------------|--------------|-------------|-----------------|------|
| 1      | Conhydrin                       | C₈H₁₇NO      | 0.212       | 143.23 g/mol     | 7.847|
| 2      | (E)-2,6-Dimethylocta-3,7-die    | C₁₀H₁₈O₂      | 0.670       | 170.25 g/mol     | 8.078|
| 3      | 2-Methylindoline                | C₉H₁₁N       | 0.825       | 133.19 g/mol     | 8.353|
| 4      | Benzofuran, 2,3-dihydro-        | C₈H₈O        | 4.002       | 120.15 g/mol     | 8.480|
| 5      | 1H-Pyrrole-2,5-dione, 3-ethy    | -            | 0.250       | -                | 8.690|
| 6      | 3-Oxo-4-phenylbutyronitrile     | C₁₀H₈NO      | 0.371       | 159.18 g/mol     | 8.825|
| 7      | 1,7-Octadiene-3,6-diol, 2,6-    | C₁₀H₁₄O₂      | 0.238       | 170.25 g/mol     | 9.271|
| 8      | 2-Methoxy-4-vinyl phenol        | -            | 0.530       | -                | 9.845|
| 9      | Cyclopentanecarboxylic acid,    | C₆H₁₀O₂      | 2.165       | 114.14 g/mol     | 10.486|
| 10     | 1-Isopropylcyclohex-1-ene       | C₉H₁₆         | 22.387      | 124.22 g/mol     | 10.657|
| 11     | (R)-(+-)-14-Methyl-8-hexadecy  | -            | 5.106       | -                | 10.790|
| 12     | Hydrocoumarin                   | C₉H₆O₂       | 3.699       | 148.1586 g/mol   | 10.843|
| 13     | 7-Methylhexahydrocyclopenta [1] | C₉H₁₂O₂      | 5.399       | 154.21 g/mol     | 11.265|
| 14     | (4R,4aR,7S,7aR)-4,7-Dimethyl    | C₁₀H₁₈O      | 1.170       | 154.25 g/mol     | 11.326|
| 15     | Ethanone, 1-(2-hydroxyphenyl)   | C₆H₈O₂       | 0.559       | 136.15 g/mol     | 11.463|
| 16     | Coumarin                        | C₉H₆O₂       | 2.265       | 146.1427 g/mol   | 11.545|
|   | Name                                                        | Formula  | MW   | Molar Mass (g/mol) | RF   |
|---|-------------------------------------------------------------|----------|------|-------------------|------|
|17 | 13-Tetradec-11-yn-1-ol                                      | C₁₄H₂₄O  | 2.146| 208.34 g/mol       | 11.581|
|18 | Homovanillyl alcohol                                         | C₉H₁₂O₃  | 1.118| 168.19 g/mol       | 12.621|
|19 | S-(2-((1R,4R)-4-Methyl-2-oxo)                               |          | 1.274|                  | 12.723|
|20 | Bicyclo [3.1.0] hexane-2-undec                             | C₆H₁₀    | 3.100| 82.14 g/mol        | 12.831|
|21 | Methyl 7,8-octadecadienoate                                 |          | 0.206|                  | 12.898|
|22 | 3-Acetylthymine                                             |          | 0.402|                  | 13.283|
|23 | 2-Cyclohexen-1-one, 4-(3-hyd                              |          | 0.997|                  | 13.984|
|24 | 1H-Indene, 1-ethylideneoctah                                | C₁₁H₁₀   | 0.070| 142.2 g/mol        | 14.737|
|25 | 2H-1-Benzopyran-2-one, 7-met                                | C₁₀H₁₅NO₂ | 5.336| 217.26 g/mol       | 14.950|
|26 | 6-Hydroxy-4,4,7a-trimethyl-5                                | C₁₁H₁₆O₃ | 0.496| 196.24 g/mol       | 15.394|
|27 | 7-Oxabicyclo [4.1.0] heptan-3-                            | C₅H₁₀O₂  | 0.295| 114.14 g/mol       | 16.821|
|28 | n-Hexadecanoic acid                                         | C₁₆H₃₂O₂  | 0.263| 256.4241 g/mol     | 17.288|
|29 | No Match                                                    |          | 0.469|                  | 3.652 |
|30 | No Match                                                    |          | 0.337|                  | 4.368 |
|31 | No Match                                                    |          | 0.437|                  | 5.652 |
|32 | No Match                                                    |          | 0.562|                  | 11.045|
|33 | No Match                                                    |          | 1.738|                  | 11.098|
|34 | No Match                                                    |          | 0.932|                  | 13.036|
|35 | No Match                                                    |          | 2.942|                  | 13.861|
|36 | No Match                                                    |          | 1.630|                  | 14.825|
|37 | No Match                                                    |          | 1.472|                  | 15.575|
|38 | No Match                                                    |          | 4.917|                  | 15.825|
|39 | No Match                                                    |          | 0.404|                  | 16.262|

**Figure 10. GCMS Spectral Chromatogram of Acetone Extract of Nepeta cataria**
Table 4. GC-MS Analysis for the Identification of Phytochemicals in the Acetone Extract of *Nepeta cataria* (A1).

| S. No. | Compound                                      | Mol. formula | Amount/Con. | Mol. weight       | RT   |
|--------|-----------------------------------------------|--------------|-------------|-------------------|------|
| 1      | Oxime-, methoxy-phenyl-                      | C₈H₉NO₂      | 2.849       | 151.16 g/mol      | 3.685|
| 2      | Eucalyptol                                    | C₁₀H₁₈O      | 1.513       | 154.249 g/mol     | 5.004|
| 3      | alpha. -Methyl- alpha. - [4-me                | C₈H₁₄NO₂      | 1.026       | 129.16 g/mol      | 5.292|
| 4      | (+)-2-Bornanone                               | C₁₀H₁₆O      | 6.365       | 152.2334 g/mol    | 5.984|
| 5      | endo-Borneol                                  | C₁₀H₁₈O      | 3.083       | 154.25 g/mol      | 6.191|
| 6      | (E)-2,6-Dimethylocta-3,7-die                 | C₁₀H₁₈O₂      | 2.572       | 170.25 g/mol      | 6.217|
| 7      | 1,7-Octadiene-3,6-diol, 2,6-                | C₁₀H₁₈O₂      | 0.819       | 170.25 g/mol      | 7.049|
| 8      | Cyclohexene,1-propyl-                        | C₉H₁₆        | 1.093       | 124.22 g/mol      | 7.507|
| 9      | Hotrienol                                     | C₁₀H₁₆O      | 2.947       | 152.23 g/mol      | 7.692|
| 10     | Cyclopentanecarboxylic acid,                 | C₆H₁₀O₂      | 2.496       | 114.14 g/mol      | 8.040|
| 11     | 1-Isopropylcyclohex-1-ene                    | C₉H₁₆        | 29.552      | 124.22 g/mol      | 8.206|
| 12     | Caryophyllene oxide                          | C₁₅H₂₄O      | 6.868       | 220.35 g/mol      | 11.452|
| 13     | n-Hexadecanoic acid                          | C₁₆H₃₂O₂     | 5.337       | 256.4241 g/mol    | 17.237|
| 14     | No Match                                     | -            | 1.196       | -                 | 7.749|
| 15     | No Match                                     | -            | 1.947       | -                 | 8.127|
| 16     | No Match                                     | -            | 2.038       | -                 | 8.424|
| 17     | No Match                                     | -            | 2.007       | -                 | 8.530|
| 18     | No Match                                     | -            | 2.570       | -                 | 8.885|
| 19     | No Match                                     | -            | 1.844       | -                 | 9.297|
| 20     | No Match                                     | -            | 2.289       | -                 | 9.631|
| 21     | No Match                                     | -            | 3.443       | -                 | 10.391|
| 22     | No Match                                     | -            | 2.573       | -                 | 13.475|

**Figure 11** GCMS Spectral Chromatogram of Hexane Extract of *Nepeta cataria*
Table 5. GC-MS Analysis for the Identification of Phytochemicals in the Hexane Extract of *Nepeta cataria* (A1).

| S. No. | Compound                                      | Mol. formula | Amount/Con. | Mol. weight   | RT  |
|--------|----------------------------------------------|--------------|-------------|---------------|-----|
| 1      | (+)-2-Bornanone                               | C_{10}H_{16}O | 6.809       | 152.2334 g/mol| 9.187 |
| 2      | endo-Borneol                                  | C_{10}H_{18}O | 3.719       | 154.25 g/mol  | 9.774 |
| 3      | Benzoic acid, 4-ethoxy-, eth                 | C_{11}H_{14}O | 2.535       | 194.23 g/mol  | 15.905 |
| 4      | Methyl 6,9,12,15,18-heneicos                  | -            | 11.008      | -             | 16.663 |
| 5      | Diethyl Phthalate                             | C_{13}H_{14}O | 17.465      | 222.24 g/mol  | 16.828 |
| 6      | Benzophenone                                  | C_{13}H_{10}O | 3.591       | 182.217 g/mol | 17.321 |
| 7      | 7,9-Di-tert-butyl-1-oxaspiro                 | C_{17}H_{24}O | 1.956       | 276.4 g/mol   | 20.957 |
| 8      | Dibutyl phthalate                             | C_{18}H_{22}O | 5.877       | 278.34 g/mol  | 21.611 |
| 9      | Tetracotane, 3,5,24-trimeth                   | C_{26}H_{38}  | 2.939       | 605.2 g/mol   | 8.975  |
| 10     | 1,2-Benzenedicarboxylic acid                 | C_{8}H_{16}O  | 8.551       | 166.14 g/mol  | 10.181 |
| 11     | 7,9-Di-tert-butyl-1-oxaspiro                 | C_{17}H_{24}O | 6.924       | 276.4 g/mol   | 10.338 |
| 12     | No Match                                     | -            | 1.421       | -             | 12.210 |
| 13     | No Match                                     | -            | 2.756       | -             | 12.391 |
| 14     | No Match                                     | -            | 4.075       | -             | 12.537 |
| 15     | No Match                                     | -            | 3.172       | -             | 12.675 |
| 16     | No Match                                     | -            | 5.199       | -             | 12.947 |
| 17     | No Match                                     | -            | 1.176       | -             | 17.730 |
| 18     | No Match                                     | -            | 3.472       | -             | 18.437 |
| 19     | No Match                                     | -            | 3.969       | -             | 19.713 |

**DISCUSSION**

*Nepeta cataria* is a naturally growing plant in the regions of Pakistan, known as Catnip mint (English) and the local name is Badranj boya. This plant has an immense pharmacological and medical characteristics. It is historically used in herbal and traditional medicine due to
antipathogenic activity. However detailed phytochemical analysis in a wide spectrum of solvents and their antimicrobial analysis against both Gram-positive and negative bacteria has limited studied yet.

*Nepeta cataria* is reported excellent agent against Gastrointestinal and respiratory hyperactive disorders, antibacterial, antifungal, and analgesic, bronchodilatory related disease [27-29].

Various laboratory procedures were adapted to investigate the antimicrobial efficacy of *Nepeta cataria* extracts against pathogenic bacteria such as *Shigella sonnei, Bacillus subtilis, Klebsiella oxytoca, Escherichia coli, Salmonella enterica, Micrococcus luteus, Staphylococcus aureus, Lactococcus lactis, Saccharomyces cerevisiae, Listeria monocytogenes*. Minimum inhibitory concentration (MIC) of leaf extracts of *Nepeta cataria* was studied. Extracts were prepared in 5 different solvents: methanol, ethanol, water, acetone, and hexane.

These solvent-based extracts of *Nepeta cataria* showed diverse levels of MIC against all tested bacteria ranging from 62.5 -750 µg/mL. Methanol based extracts MIC was 93.7 µg/mL against *B. subtilis* and *E. coli* while ethanolic extracts inhibited the *E. coli, S. enterica, M. luteus,* and *L. lactis* growth at MIC 62.5 µg/mL. Water-based extracts MIC was
62.5 µg/mL against *B. subtilis, S. enterica, and S. cerevisiae* while acetone and hexane extracts showed MIC 62.5 µg/mL against *S. aureus, S. cerevisiae, and L. monocytogenes*.

The phytochemical qualitative Analysis of *N. cataria* shown in water-based extracts, water, methanol, ethanol, and acetone based extracts showed positive results for phenolic compounds, water, and methanol-based extracts showed positive results for water-soluble phenol, methanol, ethanol, and acetone based extracts showed positive results for water-insoluble phenol, methanol, and acetone-based extracts showed positive results for flavonoids, water-based, methanol, ethanol, acetone, and hexane-based extracts were positive for Terpenoids, acetone-based extracts were positive for cardiac glycosides, water-based extracts, methanol, ethanol, and acetone based extract were positive for free Anthraquinones, water-based extracts extract were positive for Combined Anthraquinones, water-based, methanol, and ethanol extracts were positive for Tannins, water-based, methanol, ethanol, acetone, and hexane-based extracts were positive for Alkaloids. The quantitative analysis for phenols showed maximum levels in methanol, acetone, and ethanol-based extracts. The hexane, acetone, and water-based extracts showed a high level of flavonoids. The quantitative analysis for the high concentration of DPPH showed in the acetone-based plant extract. Alkaloids quantity in tested plant extracts was varied from 0.204 / 0.5 g plant. *Nepeta cataria* in methanol extract results for ORAC test was 133759.021um/100ml. Gas Chromatography/Mass Spectrometry (GC/MS) is used for *Nepeta cataria* extract to detect
the bioactive phytochemicals in 5 different solvents viz., methanol, ethanol, water, acetone, and hexane.

GC/MS analysis of a methanolic extract of *Nepeta cataria* showed the presence of 71 identified phytochemicals while 48 un-matched chemicals were detected which needed to be explored further. Analysis of ethanol-based extracts confirmed the existence of 80 known phytochemical constituents while 31 unmatched chemicals were also detected. Water-based extracts of *Nepeta cataria* contain 28 known phytochemicals while 11 un-matched chemicals were also detected. A total of 13 known compounds were detected in acetone-based extract while 9 chemical constituents were unmatched. Analysis of hexane based extract confirmed the presence of 11 known chemical constituents while 8 unmatched chemicals were also detected.

The current study result was remarkable against the antibacterial and antioxidant activity. This plant is a natural source of various pharmacological and organic compounds that can be used against several bacterial infections after processing. It may help out to treat different antibiotic-resistant pathogens. Its chemicals may be used in pharmacology industries as a cheaper and easily available source.

**Conclusion**
The current study concludes remarkable antibacterial and antioxidant potential in *Nepeta cataria* extracts. This plant is a natural source of various pharmacological and organic compounds that can be used against several bacterial infections after processing.

**Conflict of Interest**

The authors declare that they have no conflict of interest in the publication.

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### Appendix

**Table 1.1 Percentage yield of *Nepeta cataria* extracts in different solvents.**

| Plant       | Methanol % | Ethanol % | Distilled Water % | Acetone % | Hexane % |
|-------------|------------|-----------|-------------------|-----------|----------|
| *Nepeta cataria* | 14         | 20.6      | 9.06              | 0.13      | 2.27     |

**Table 1.2 Ingredients of The TSB Medium Used for Bacterial Culturing.**
| Ingredient            | Quantity | Ingredient           | Quantity |
|-----------------------|----------|----------------------|----------|
| Casein Peptone        | 17.0 g   | Dextrose             | 2.5 g    |
| Sodium Chloride       | 5.0 g    | Dipotassium Phosphate| 2.5 g    |
| Soy Peptone           | 3.0 g    | Distilled water      | 1000 ml  |

pH 7.3 ± 0.2 @ 25 ºC.

**Table 1.3 Ingredients of The LB Medium Used for Bacterial Mass Culturing.**

| Ingredient       | Quantity | Ingredient   | Quantity |
|------------------|----------|--------------|----------|
| Tryptone         | 10.0 g   | NaCl         | 5.0 g    |
| Yeast extract    | 5.0 g    | Distilled water | 1000 ml |