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

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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 + 31unknown), 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 spasmolytic 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 CFU/mL) in standard concentration. Paper discs socked in plant extract and placed on the solidified medium under aseptic conditions followed by incubation at 26 ± 2 ºC. 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 µg, 500 µg, 250 µg, 125 µg, and 62.5 µg) were loaded in each well followed by inoculation with 50 µL of each bacterial culture separately at 10^5 to 10^6 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:

$$Percentage\ Inhibition = 100 - 100\ X\ \frac{OD\ of\ sample}{OD\ of\ control}$$

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 (10^6 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 Na_2CO_3) 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 CH_2Cl_2, 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 H_2SO_4 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_2SO_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_2SO_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 Na_2CO_3 (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, $y = 0.5377 x + 0.316$, 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 x + 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), H_2SO_4 (10 %) 1 ml, NH3 (10 %) 2 ml, Chloroform 3 ml. 10 ml of plant extracts separately mixed with 1 mL of H_2SO_4 (10 %), 2 mL of NH3 (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 ºC 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 mint. 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_3OH-d_4 and 0.75 ml of KH_2PO_4 buffer in D_2O (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 Satistix 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.

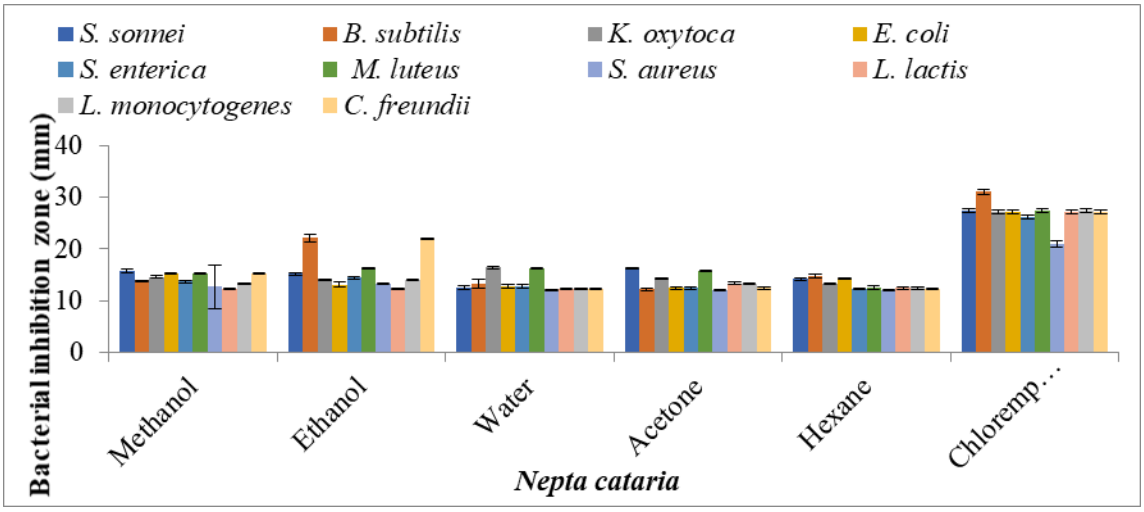

Figure: 1. Antimicrobial efficacy of *Nepeta cataria* based plant extracts in different solvents against bacterial isolates.

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:

% bacterial growth inhibition = Optical density in control - Optical density in treatment / 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.

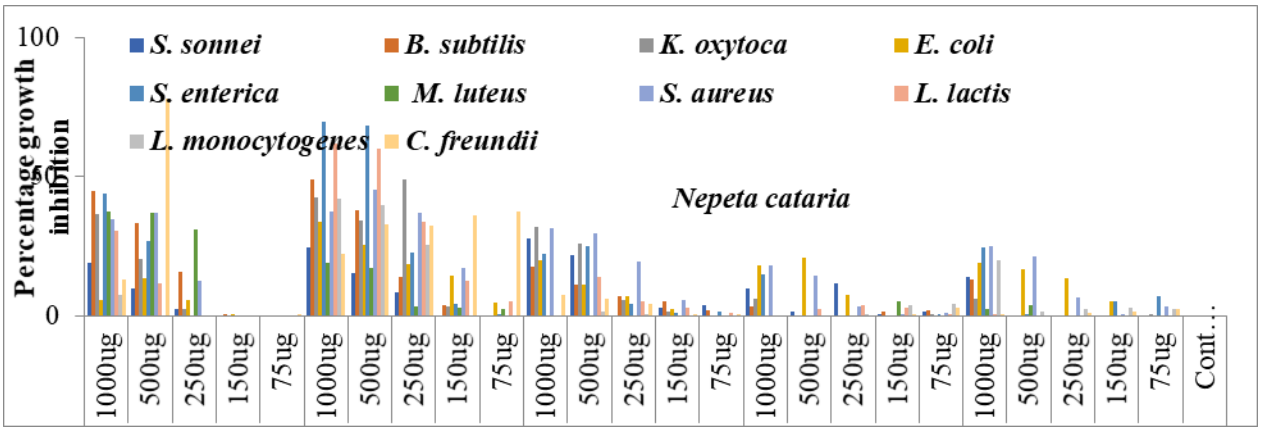

Figure: 2. *Nepeta cataria* plant extracts a percentage of bacterial strains growth inhibition in different solvents at different dose levels.

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.

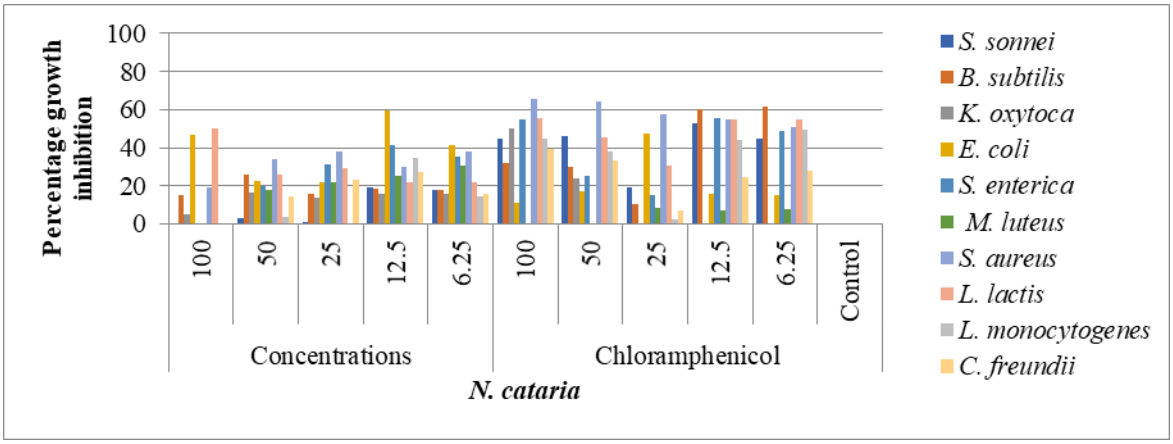

Figure : 3. Resazurin based well plate Microdilution assay of *N. cataria* against bacterial strains.

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.

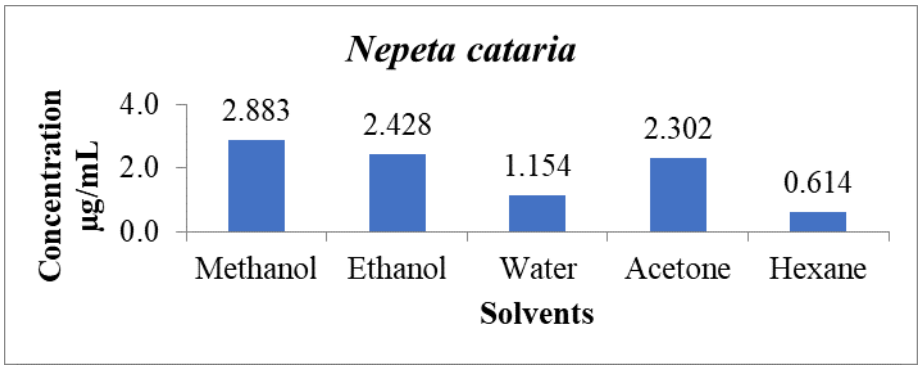

Figure 4 Concentration of phenols in *Nepeta cataria* extracts in different solvents.

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.

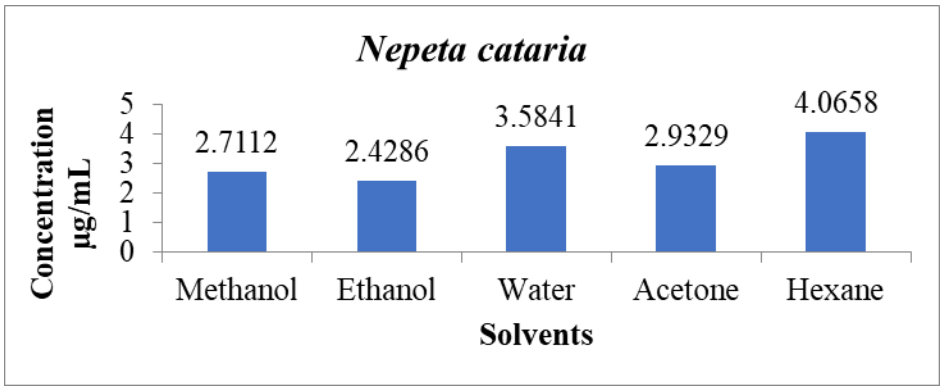

Figure: 5. The concentration of Flavonoids in *Nepeta cataria* extracts in different solvents.

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.

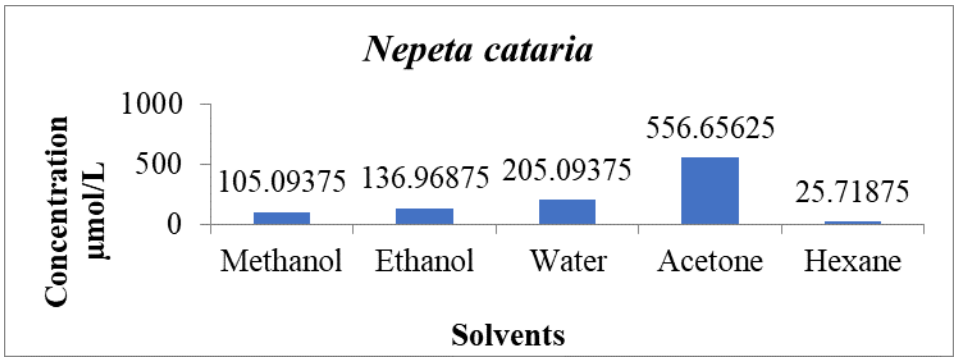

Figure:6. The concentration of DPPH in *Nepeta cataria* plant extracts prepared in different solvents.

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 133759.021um/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.

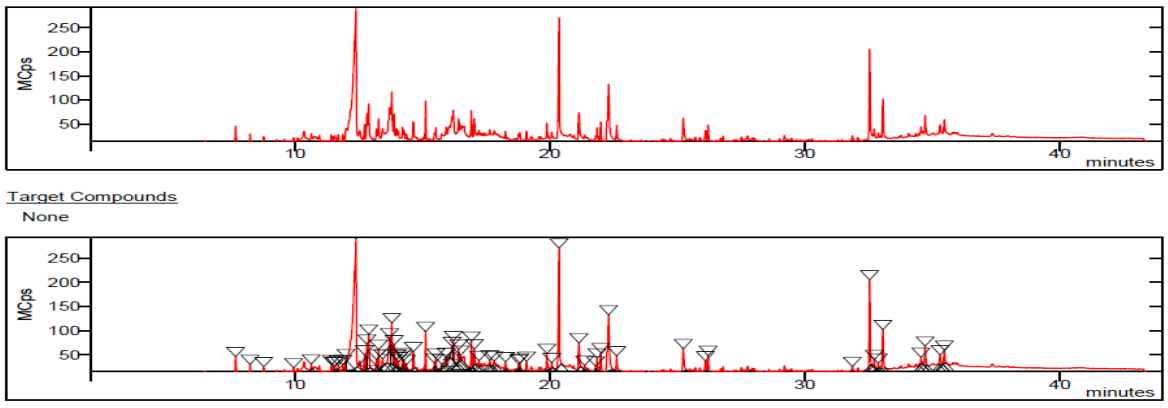

Figure: 7. GCMS Spectral Chromatogram of Methanolic Extract of *Nepeta cataria*

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	$C_{10}H_{18}O$	0.623	154.25 g/mol	8.246
3	1,5,7-Octatrien-3-ol, 3,7-di	$C_{10}H_{16}O$	0.390	152.2334 g/mol	8.782
4	Bicyclo [2.2.1] heptane, 7,7-d	C_9H_{16}	0.240	124.22 g/mol	9.955
5	2-Cyclohexen-1-one, 3-methyl	$C_7H_{10}O$	0.230	110.15 g/mol	11.529
6	1-Isopropylcyclohex-1-ene	C_9H_{16}	27.376	124.22 g/mol	12.402
7	Bicyclo [3.1.0] hexane-2-undec	C_6H_{10}	2.974	82.14 g/mol	13.804
8	3,5-Dimethylcyclohex-1-ene-4	C_8H_{14}	0.542	110.2 g/mol	14.226
9	2-Butyl-5-methyl-3-(2-methyl	$C_{15}H_{26}O$	0.645	222.37 g/mol	14.281
10	Caryophyllene oxide	$C_{15}H_{24}O$	1.916	220.35 g/mol	15.129
11	Methyl octadec-6,9-dien-12-y	$C_{18}H_{32}O_2$	0.149	280.4 g/mol	15.763
12	Caryophylla-4(12),8(13)-dien	$C_{15}H_{24}O$	0.358	220.3505 g/mol	15.937
13	1-Chlorosulfonyl-3-methyl-1-	$C_9H_{14}ClNO_3S$	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	$C_{15}H_{24}O$	0.632	220.3505 g/mol	16.429
16	2H-1-Benzopyran-2-one, 7-met	$C_{13}H_{15}NO_2$	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	$C_{16}H_{28}O_2$	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

27	9,12,15-Octadecatrienoic aci	$C_{18}H_{30}O_2$	6.401	278.43 g/mol	22.304
28	Octadecanoic acid	$C_{18}H_{36}O_2$	0.970	284.48 g/mol	22.623
29	Hexadecanoic acid, 2-hydroxy	$C_{16}H_{32}O_3$	0.744	272.42 g/mol	26.101
30	5-Cholestene-3-ol, 24-methyl	$C_{28}H_{48}O$	0.344	400.7 g/mol	31.863
31	beta. -Sitosterol	$C_{29}H_{50}O$	5.461	414.71 g/mol	32.541
32	Olean-12-en-3-ol, acetate, (	$C_{32}H_{52}O_2$	0.486	468.8 g/mol	32.724
33	beta. -Guaiene	$C_{15}H_{24}$	0.271	204.3511	32.882
34	alpha. -Amyrin	$C_{30}H_{50}O$	2.691	426.729 g/mol	33.062
35	Ursolic aldehyde	$C_{30}H_{48}O_2$	1.302	440.7 g/mol	34.718
36	Urs-12-en-28-al	$C_{30}H_{48}O$	0.654	424.7 g/mol	35.305
37	Betulin	$C_{30}H_{50}O_2$	0.910	442.72 g/mol	35.472
38	Eucalyptol	$C_{10}H_{18}O$	8.505	154.249 g/mol	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,	$C_7H_{10}O$	20.437	110.15 g/mol	7.728
42	1,6-Octadien-3-ol, 3,7-dimet	$C_{10}H_{18}O$	5.855	154.25 g/mol	9.981
43	Pentane, 1-chloro-5- (methyle	-	3.739	-	10.696
44	Pregnan-18-ol, 20-methyl-20-	$C_{22}H_{39}NO$	2.640	333.6 g/mol	13.916
45	Caryophyllene oxide	$C_{15}H_{24}O$	12.423	220.35 g/mol	15.140
46	Cyclohexene,1-propyl-	C_9H_{16}	0.483	124.22 g/mol	11.611
47	1-Isopropylcyclohex-1-ene	C_9H_{16}	6.144	124.22 g/mol	13.699
48	Coumarin	$C_9H_6O_2$	0.878	146.1427 g/mol	13.867
49	1-Methyl-2-methylenecyclohex	C_8H_{14}	0.622	110.197 g/mol	14.461
50	Caryophyllene oxide	$C_{15}H_{24}O$	2.144	220.35 g/mol	16.143
51	Megastigmatrienone	$C_{13}H_{18}O$	0.560	190.28 g/mol	16.780
52	11,11-Dimethyl-4,8-dimethyle	$C_{15}H_{24}O$	0.429	220.35 g/mol	16.954
53	(1R,7S, E)-7-Isopropyl-4,10-d	$C_{15}H_{24}O$	0.702	220.3505 g/mol	17.243
54	Caryophylla-4(12),8(13)-dien	$C_{15}H_{24}O$	0.603	220.3505 g/mol	17.450
55	2H-1-Benzopyran-2-one, 7-met	$C_{13}H_{15}NO_2$	1.381	217.26 g/mol	18.071
56	11,14-Octadecadienoic acid,	$C_{18}H_{32}O_2$	0.364	280.4 g/mol	22.811
57	Methyl 8,11,14-heptadecatrie	-	1.220	-	22.864
58	Eicosanoic acid	$C_{20}H_{40}O_2$	0.515	312.5304 g/mol	25.775

59	Phenol, 2,4-bis (1-methyl-1-p	$C_{24}H_{26}O$	0.445	330.5 g/mol	26.725
60	Tritetracontane	$C_{43}H_{88}$	0.574	605.2 g/mol	27.798
61	Methyl 2-hydroxy-octadeca-9,	$C_{19}H_{32}O_3$	0.754	308.5 g/mol	28.775
62	Hentriacontane	$C_{31}H_{64}$	0.188	436.85 g/mol	28.969
63	alpha. -Tocospiro B	$C_{29}H_{50}O_4$	0.463	462.7049 g/mol	30.023
64	alpha. -Tocospiro A	$C_{29}H_{50}O_4$	0.484	462.7 g/mol	30.208
65	Campesterol	$C_{28}H_{48}O$	0.657	400.68 g/mol	32.877
66	Stigmasterol	$C_{29}H_{48}O$	0.595	412.69 g/mol	33.091
67	gamma. -Sitosterol	$C_{29}H_{50}O$	8.626	414.7 g/mol	33.566
68	beta. -Amyrin	$C_{30}H_{50}O$	0.763	426.729 g/mol	33.739
69	1,1,4a-Trimethyl-5,6-dimethy	$C_{15}H_{24}$	0.891	204.35 g/mol	33.896
70	Urs-12-en-28-oic acid, 3-hyd	$C_{30}H_{48}O_3$	0.546	456.7 g/mol	35.636
71	Uvaol	$C_{30}H_{50}O_2$	1.365	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

90	No Match	-	0.394	-	17.818
91	No Match	-	2.074	-	21.141
92	No Match	-	0.322	-	21.365
93	No Match	-	1.975	-	25.235
94	No Match	-	1.013	-	26.209
95	No Match	-	6.419	-	6.933
96	No Match	-	1.807	-	11.016
97	No Match	-	2.619	-	11.726
98	No Match	-	4.148	-	13.303
99	No Match	-	0.356	-	34.665
100	No Match	-	1.659	-	34.964
101	No Match	-	1.326	-	35.143
102	No Match	-	0.997	-	35.231
103	No Match	-	0.688	-	35.270
104	No Match	-	1.430	-	35.912
105	No Match	-	0.447	-	12.434
106	No Match	-	0.543	-	12.559
107	No Match	-	0.553	-	12.880
108	No Match	-	0.486	-	13.019
109	No Match	-	1.447	-	14.094
110	No Match	-	0.585	-	14.134
111	No Match	-	2.430	-	14.296
112	No Match	-	2.021	-	14.919
113	No Match	-	1.245	-	17.965
114	No Match	-	3.893	-	22.205
115	No Match	-	0.545	-	22.421
116	No Match	-	2.786	-	26.376
117	No Match	-	0.419	-	26.522
118	No Match	-	1.436	-	27.233
119	No Match	-	0.616	-	27.717

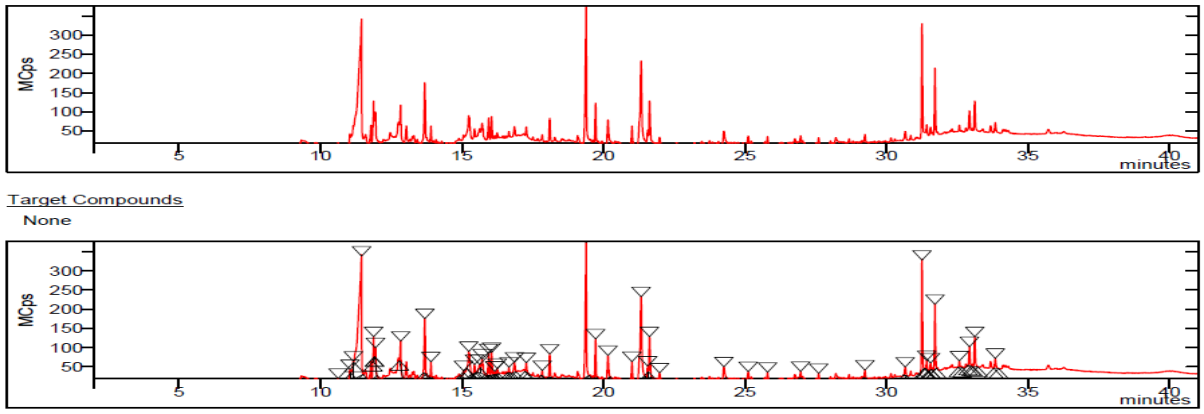

Figure 8. GCMS Spectral Chromatogram of Ethanolic Extract of *Nepeta cataria*.

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	$C_6H_8O_4$	0.284	144.12 g/mol	3.404
2	1-Isopropylcyclohex-1-ene	C_9H_{16}	14.940	124.22 g/mol	9.585
3	Coumarin	$C_9H_6O_2$	2.940	146.1427 g/mol	9.646
4	Hexadecanoic acid, ethyl est	$C_{18}H_{36}O_2$	3.361	284.4772	15.865
5	Oleic Acid	$C_{18}H_{34}O_2$	0.653	282.47 g/mol	16.515
6	Phytol	$C_{20}H_{40}O$	3.068	128.1705 g/mol	16.907
7	9,12,15-Octadecatrienoic aci	$C_{18}H_{30}O_2$	27.308	278.43 g/mol	17.266
8	Ethyl 9.cis.,11. trans.-octad	-	2.045	-	17.352
9	Octadecanoic acid	$C_{18}H_{36}O_2$	3.620	284.48 g/mol	17.464
10	Octadecanoic acid, 17-methyl	$C_{20}H_{40}O_2$	0.982	312.5 g/mol	17.680
11	Eicosanoic acid	$C_{20}H_{40}O_2$	1.138	312.5304 g/mol	19.118
12	Tetracontane, 3,5,24-trimeth	$C_{43}H_{88}$	1.194	605.2 g/mol	20.201
13	Hentriacontane	$C_{31}H_{64}$	1.197	436.85 g/mol	20.740
14	Methyl 2-hydroxy-octadeca-9,	$C_{19}H_{32}O_3$	0.895	308.5 g/mol	21.548
15	Sulfurous acid, butyl tetrad	$C_{21}H_{44}O_3S$	0.667	376.6 g/mol	22.185
16	alpha. -Tocospiro A	$C_{29}H_{50}O_4$	0.654	462.7 g/mol	22.498
17	Sulfurous acid, butyl tridec	$C_{17}H_{36}O_3S$	0.572	320.5 g/mol	22.897

18	3,7,11,15-Tetramethyl-2-hexa	$C_{20}H_{40}O$	0.444	296.5 g/mol	23.191
19	Sulfurous acid, butyl tetrad	$C_{21}H_{44}O_3S$	1.134	376.6 g/mol	23.243
20	Sulfurous acid, butyl tridec	$C_{17}H_{36}O_3S$	0.399	320.5 g/mol	24.233
21	Stigmasterol	$C_{29}H_{48}O$	0.630	412.69 g/mol	24.507
22	beta. -Sitosterol	$C_{29}H_{50}O$	13.312	414.71 g/mol	24.939
23	alpha. -Amyrin	$C_{30}H_{50}O$	6.667	426.729 g/mol	25.504
24	2-Methylindoline	$C_9H_{11}N$		133.19 g/mol	8.120
25	2-Cyclohexen-1-one, 4,5-dime	-	0.113	-	10.585
26	1-Isopropylcyclohex-1-ene	C_9H_{16}	25.854	124.22 g/mol	11.456
27	Bicyclo [3.1.0] hexane-2-undec	C_6H_{10}	1.108	82.14 g/mol	12.837
28	Methyl 13,14-octadecadienoat	$C_{19}H_{34}O_2$	3.793	294.472 g/mol	13.689
29	Azulene, 1,2,3,3a,4,5,6,7-oc	$C_{15}H_{24}$	0.170	204.3511	15.056
30	1-Chlorosulfonyl-3-methyl-1-	$C_9H_{14}ClNO_3S$	2.175	251.73 g/mol	15.242
31	Cholestan-3-ol, 2-methylene-	$C_{28}H_{48}O$	0.515	400.7 g/mol	15.446
32	2H-1-Benzopyran-2-one, 7-met	$C_{13}H_{15}NO_2$	1.381	217.26 g/mol	16.049
33	6-Hydroxy-4,4,7a-trimethyl-5	$C_{11}H_{16}O_3$	0.367	196.24 g/mol	16.663
34	[1,1'-Bicyclopropyl]-2-octan	$C_{21}H_{38}O_2$	0.823	322.5 g/mol	16.857
35	2-Pentadecanone, 6,10,14-tri	$C_{18}H_{36}O$	0.298	268.4778 g/mol	17.839
36	4,4,8-Trimethyltricyclo [6.3.	$C_{15}H_{26}O_2$	1.458	238.366 g/mol	18.101
37	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	10.300	256.4241 g/mol	19.386
38	11,14-Octadecadienoic acid,	$C_{18}H_{32}O_2$	0.819	280.4 g/mol	21.561
39	Ethyl 9,12,15-octadecatrieno	$C_{20}H_{34}O_2$	3.315	306.5 g/mol	21.626
40	6-Octadecynoic acid, methyl	-	1.149	-	24.253
41	Tetracontane, 3,5,24-trimeth	$C_{43}H_{88}$	0.506	605.2 g/mol	25.112
42	24-Noroleana-3,12-diene	$C_{29}H_{46}$	0.537	394.6755 g/mol	31.418
43	1R,4S,7S,11R-2,2,4,8-Tetrame	$C_{15}H_{26}O$	0.419	222.366 g/mol	31.553
44	Ursolic aldehyde	$C_{30}H_{48}O_2$	2.109	440.7 g/mol	33.113
45	Betulin	$C_{30}H_{50}O_2$	0.802	442.72 g/mol	33.839
46	2,4-Dihydroxy-2,5-dimethyl-3	$C_6H_8O_4$	0.032	144.12 g/mol	3.230
47	2-Methylindoline	$C_9H_{11}N$	0.490	133.19 g/mol	6.580
48	5-Hydroxymethylfurfural	$C_6H_6O_3$	0.768	126.11 g/mol	6.985
49	2-Cyclohexen-1-one, 3-methyl	$C_7H_{10}O$	0.083	110.15 g/mol	8.592

50	1-Isopropylcyclohex-1-ene	C_9H_{16}	13.741	124.22 g/mol	9.330
51	Cyclopentanecarboxylic acid,	$C_6H_{10}O_2$	0.008	114.14 g/mol	9.434
52	Coumarin	$C_9H_6O_2$	1.727	146.1427 g/mol	9.618
53	Methyl 10,11-tetradecadienoa	-	0.573	-	10.069
54	2(4H)-Benzofuranone, 5,6,7,7	$C_{11}H_{16}O_2$	0.319	180.2435	10.757
55	(4aS,7S,7aR)-4,7-Dimethyl-2,	$C_{10}H_{14}O_2$	0.169	166.2170 g/mol	10.885
56	n-Propyl 9,12-hexadecadienoa	$C_{19}H_{34}O_2$	0.262	294.5 g/mol	11.116
57	12-Methyl-E, E-2,13-octadecad	$C_{19}H_{36}O$	0.113	280.48854 g/mol	11.164
58	Fumaric acid, ethyl 2-methyl	$C_{10}H_{14}O_4$	0.179	198.22 g/mol	11.356
59	Bicyclo [4.4.0] dec-1-ene, 2-i	$C_{15}H_{24}$	0.116	204.35 g/mol	11.540
60	Megastigmatrienone	$C_{13}H_{18}O$	0.081	190.28 g/mol	11.924
61	10,10-Dimethyl-2,6-dimethyle	$C_{15}H_{24}$	0.199	204.351 g/mol	12.067
62	tau. -Cadinol	$C_{15}H_{26}O$	0.335	222.37 g/mol	12.143
63	2H-1-Benzopyran-2-one, 7-met	$C_{13}H_{15}NO_2$	0.893	217.26 g/mol	12.956
64	cis-5,8,11,14,17-Eicosapenta	$C_{20}H_{30}O_2$	0.148	302.5 g/mol	13.276
65	Carbamic acid, N- [1,1-bis (tr	-	0.124	-	13.319
66	Tetradecanoic acid	$C_{14}H_{28}O_2$	0.250	228.3709 g/mol	13.552
67	1-Heptatriacotanol	$C_{37}H_{76}O$	0.432	537 g/mol	13.943
68	2-Pentadecanone, 6,10,14-tri	$C_{18}H_{36}O$	0.286	268.4778 g/mol	14.346
69	Ethyl 9.cis.,11. trans.-octad	-	0.340	-	17.345
70	Tetracontane, 3,5,24-trimeth	$C_{43}H_{88}$	0.304	605.2 g/mol	20.193
71	Tritetracontane	$C_{43}H_{88}$	0.177	605.2 g/mol	22.180
72	Urs-12-en-28-oic acid, 3-hyd	$C_{30}H_{48}O_3$	0.722	456.7 g/mol	23.776
73	Urs-12-en-28-ol	$C_{30}H_{50}O$	4.295	426.7 g/mol	23.833
74	Glycine, N- [(3. alpha.,5. beta	$C_{30}H_{53}NO_4Si$	0.313	519.8 g/mol	24.109
75	Ergost-5-en-3-ol, (3. beta.)-	$C_{28}H_{48}O$	0.180	400.7 g/mol	24.141
76	Stigmasterol	$C_{29}H_{48}O$	0.102	412.69 g/mol	24.241
77	Uvaol	$C_{30}H_{50}O_2$	1.125	442.7 g/mol	24.513
78	Tricyclo [20.8.0.0(7,16)] tria	-	0.647	-	25.158
79	Neophytadiene	$C_{20}H_{38}$	0.294	278.5 g/mol	25.337
80	Tetracosamethyl-cyclododecas	-	1.000	-	27.703
81	No Match	-	42.916	-	2.039

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

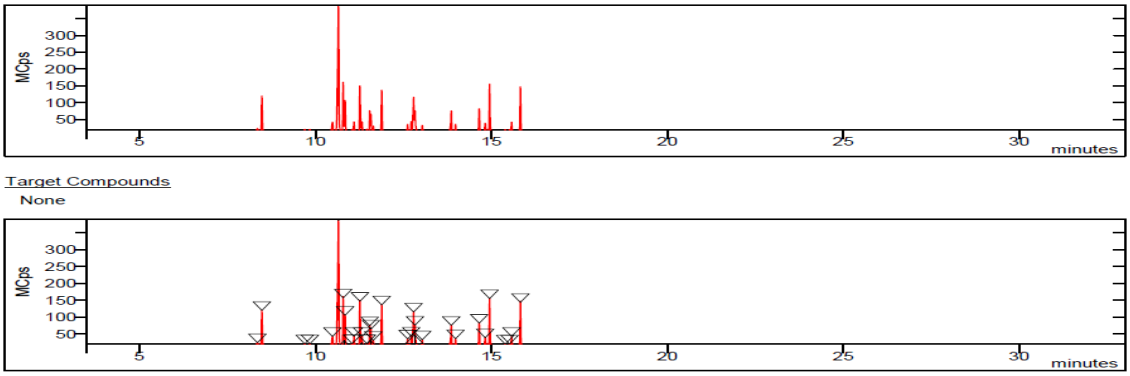

Figure 9. GCMS Spectral Chromatogram of Water Extract of *Nepeta cataria*.

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	$C_8H_{17}NO$	0.212	143.23 g/mol	7.847
2	(E)-2,6-Dimethylocta-3,7-die	$C_{10}H_{18}O_2$	0.670	170.25 g/mol	8.078
3	2-Methylindoline	$C_9H_{11}N$	0.825	133.19 g/mol	8.353
4	Benzofuran, 2,3-dihydro-	C_8H_8O	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	$C_{10}H_9NO$	0.371	159.18 g/mol	8.825
7	1,7-Octadiene-3,6-diol, 2,6-	$C_{10}H_{18}O_2$	0.238	170.25 g/mol	9.271
8	2-Methoxy-4-vinyl phenol		0.530		9.845
9	Cyclopentanecarboxylic acid,	$C_6H_{10}O_2$	2.165	114.14 g/mol	10.486
10	1-Isopropylcyclohex-1-ene	C_9H_{16}	22.387	124.22 g/mol	10.657
11	(R)-(-)-14-Methyl-8-hexadecy	-	5.106	-	10.790
12	Hydrocoumarin	$C_9H_8O_2$	3.699	148.1586 g/mol	10.843
13	7-Methylhexahydrocyclopenta [	$C_9H_{14}O_2$	5.399	154.21 g/mol	11.265
14	(4R,4aR,7S,7aR)-4,7-Dimethyl	$C_{10}H_{18}O$	1.170	154.25 g/mol	11.326
15	Ethanone, 1-(2-hydroxyphenyl	$C_8H_8O_2$	0.559	136.15 g/mol	11.463
16	Coumarin	$C_9H_6O_2$	2.265	146.1427 g/mol	11.545

17	13-Tetradece-11-yn-1-ol	$C_{14}H_{24}O$	2.146	208.34 g/mol	11.581
18	Homovanillyl alcohol	$C_9H_{12}O_3$	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	C_6H_{10}	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	$C_{11}H_{10}$	0.070	142.2 g/mol	14.737
25	2H-1-Benzopyran-2-one, 7-met	$C_{13}H_{15}NO_2$	5.336	217.26 g/mol	14.950
26	6-Hydroxy-4,4,7a-trimethyl-5	$C_{11}H_{16}O_3$	0.496	196.24 g/mol	15.394
27	7-Oxabicyclo [4.1.0] heptan-3-	$C_6H_{10}O_2$	0.295	114.14 g/mol	16.821
28	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	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

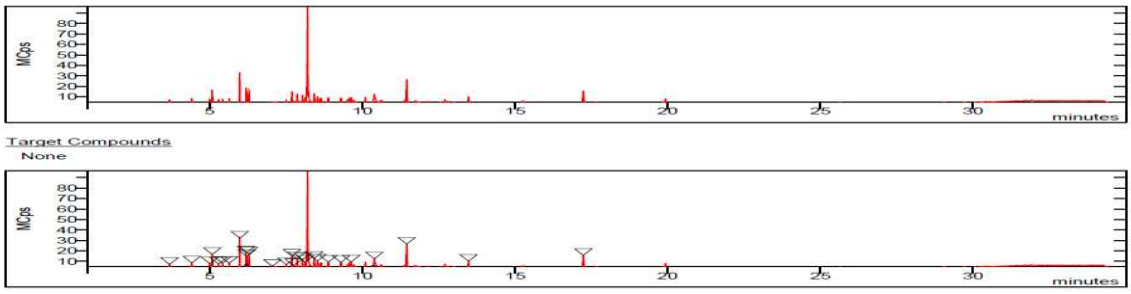

Figure 10. GCMS Spectral Chromatogram of Acetone Extract of *Nepeta cataria*

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-	$C_8H_9NO_2$	2.849	151.16 g/mol	3.685
2	Eucalyptol	$C_{10}H_{18}O$	1.513	154.249 g/mol	5.004
3	alpha. -Methyl-. alpha. - [4-me	$C_6H_{11}NO_2$	1.026	129.16 g/mol	5.292
4	(+)-2-Bornanone	$C_{10}H_{16}O$	6.365	152.2334 g/mol	5.984
5	endo-Borneol	$C_{10}H_{18}O$	3.083	154.25 g/mol	6.191
6	(E)-2,6-Dimethylocta-3,7-die	$C_{10}H_{18}O_2$	2.572	170.25 g/mol	6.217
7	1,7-Octadiene-3,6-diol, 2,6-	$C_{10}H_{18}O_2$	0.819	170.25 g/mol	7.049
8	Cyclohexene,1-propyl-	C_9H_{16}	1.093	124.22 g/mol	7.507
9	Hotrienol	$C_{10}H_{16}O$	2.947	152.23 g/mol	7.692
10	Cyclopentanecarboxylic acid,	$C_6H_{10}O_2$	2.496	114.14 g/mol	8.040
11	1-Isopropylcyclohex-1-ene	C_9H_{16}	29.552	124.22 g/mol	8.206
12	Caryophyllene oxide	$C_{15}H_{24}O$	6.868	220.35 g/mol	11.452
13	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	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

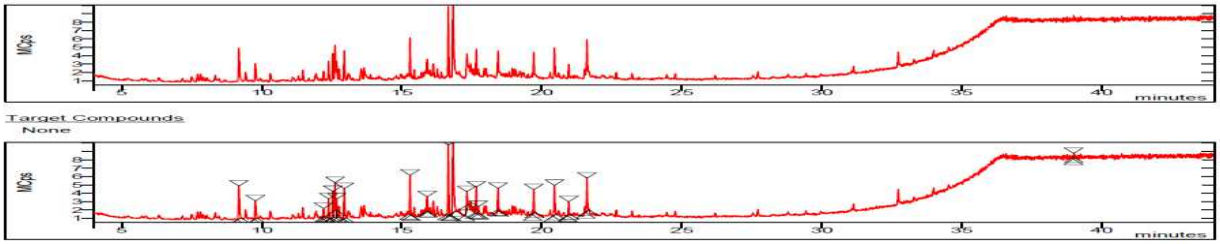

Figure 11 GCMS Spectral Chromatogram of Hexane Extract of *Nepeta cataria*

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	$C_{11}H_{14}O_3$	2.535	194.23 g/mol	15.905
4	Methyl 6,9,12,15,18-heneicos	-	11.008	-	16.663
5	Diethyl Phthalate	$C_{12}H_{14}O_4$	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	$C_{17}H_{24}O_3$	1.956	276.4 g/mol	20.957
8	Dibutyl phthalate	$C_{16}H_{22}O_4$	5.877	278.34 g/mol	21.611
9	Tetracontane, 3,5,24-trimeth	$C_{43}H_{88}$	2.939	605.2 g/mol	8.975
10	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	8.551	166.14 g/mol	10.181
11	7,9-Di-tert-butyl-1-oxaspiro	$C_{17}H_{24}O_3$	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

Figures

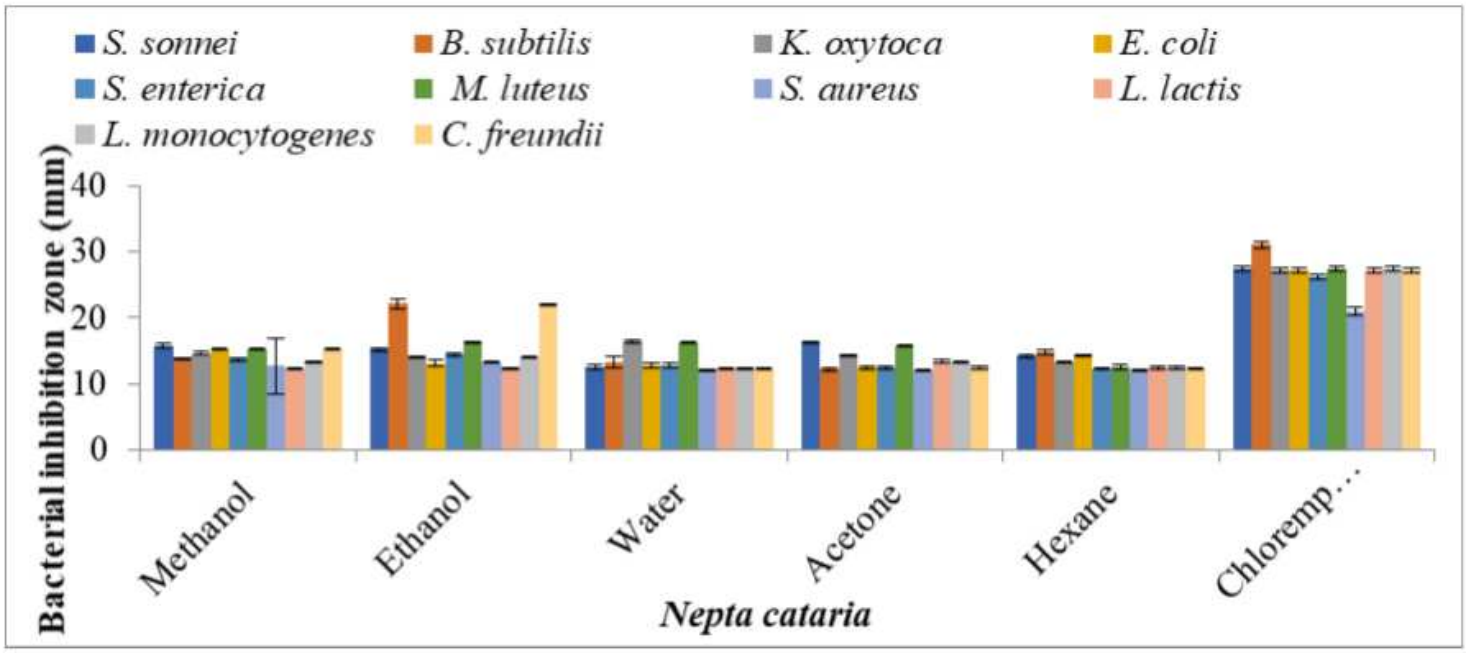

Figure 1

Antimicrobial efficacy of Nepeta cataria based plant extracts in different solvents against bacterial isolates.

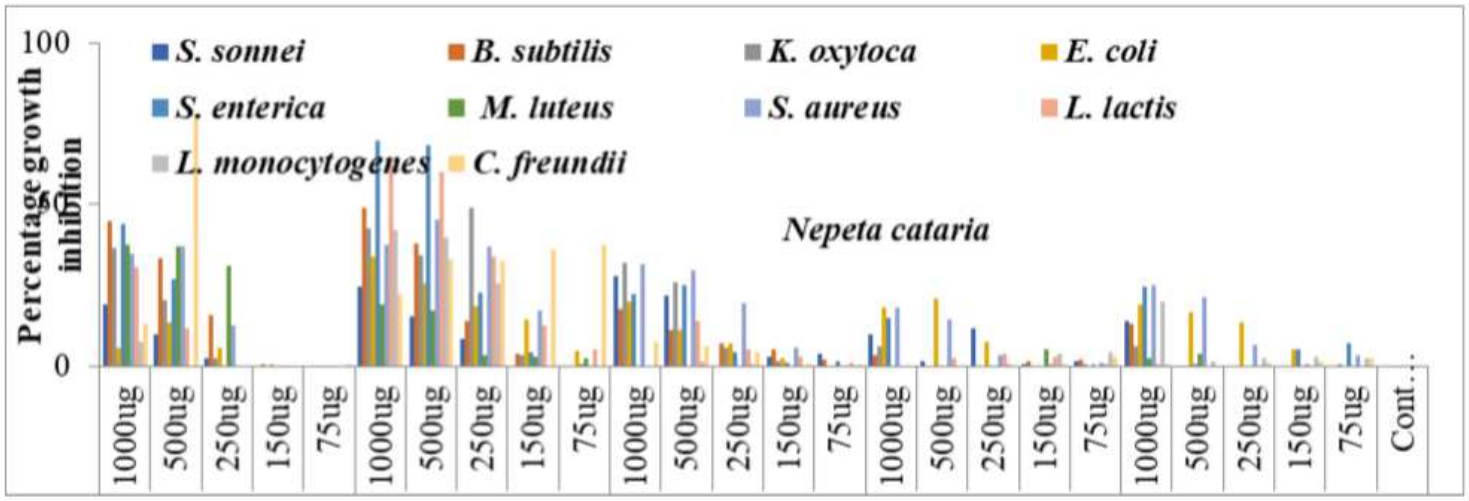

Figure 2

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

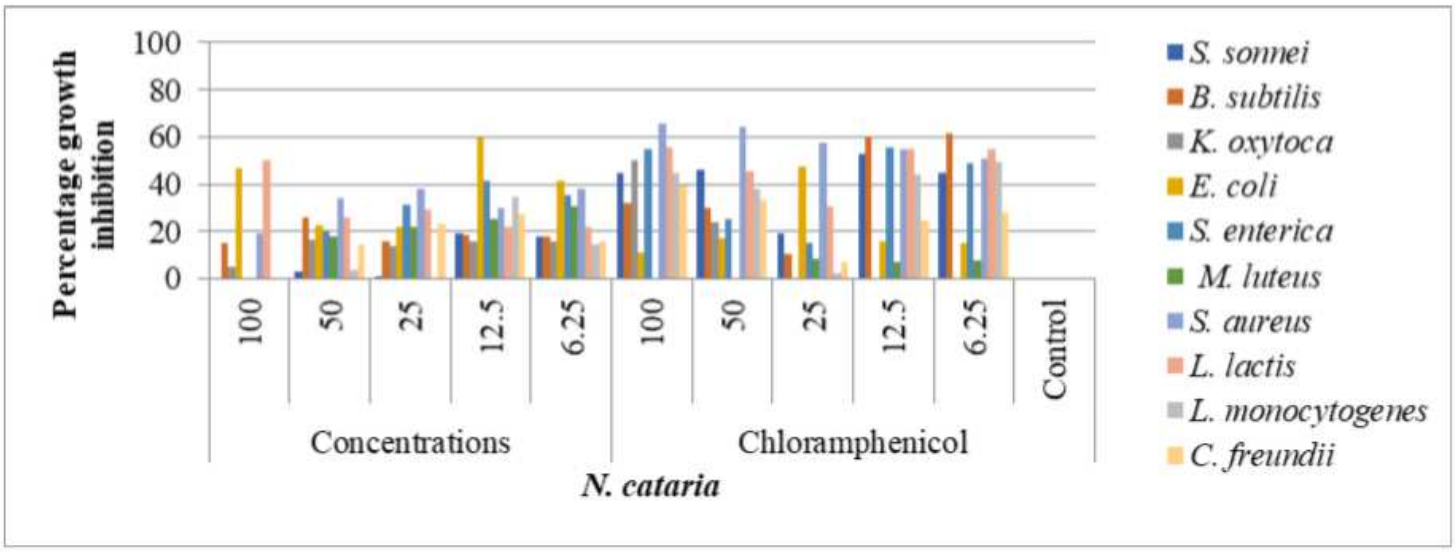

Figure 3

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

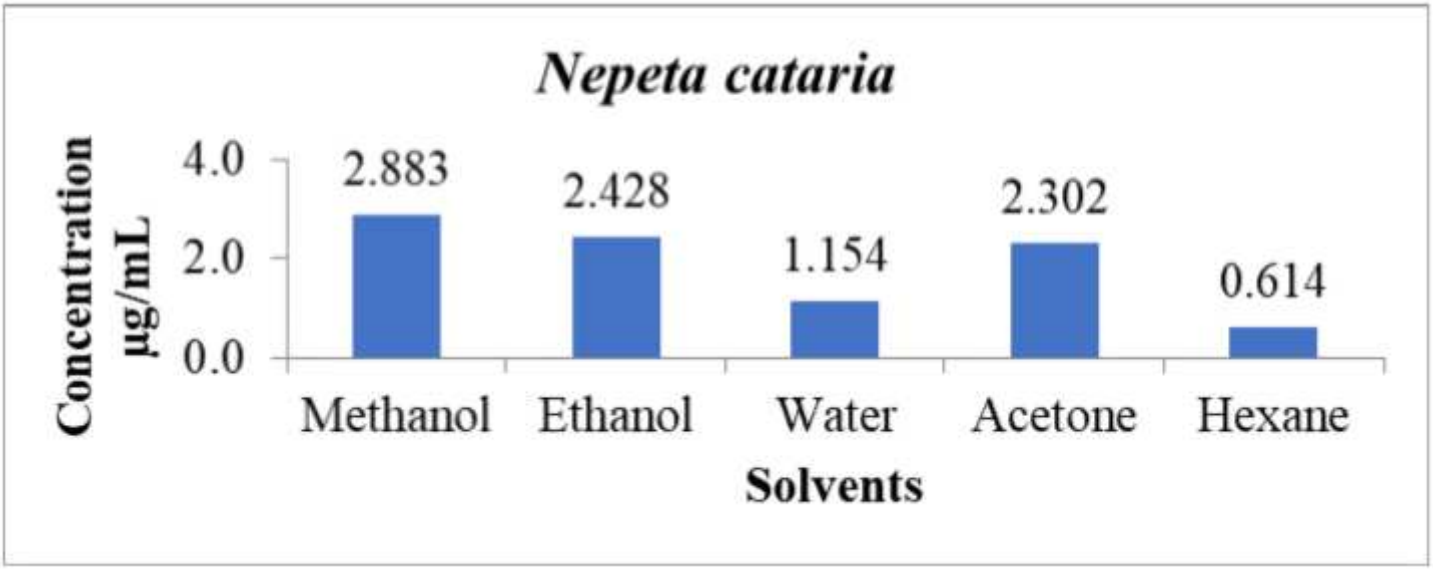

Figure 4

Concentration of phenols in Nepeta cataria extracts in different solvents.

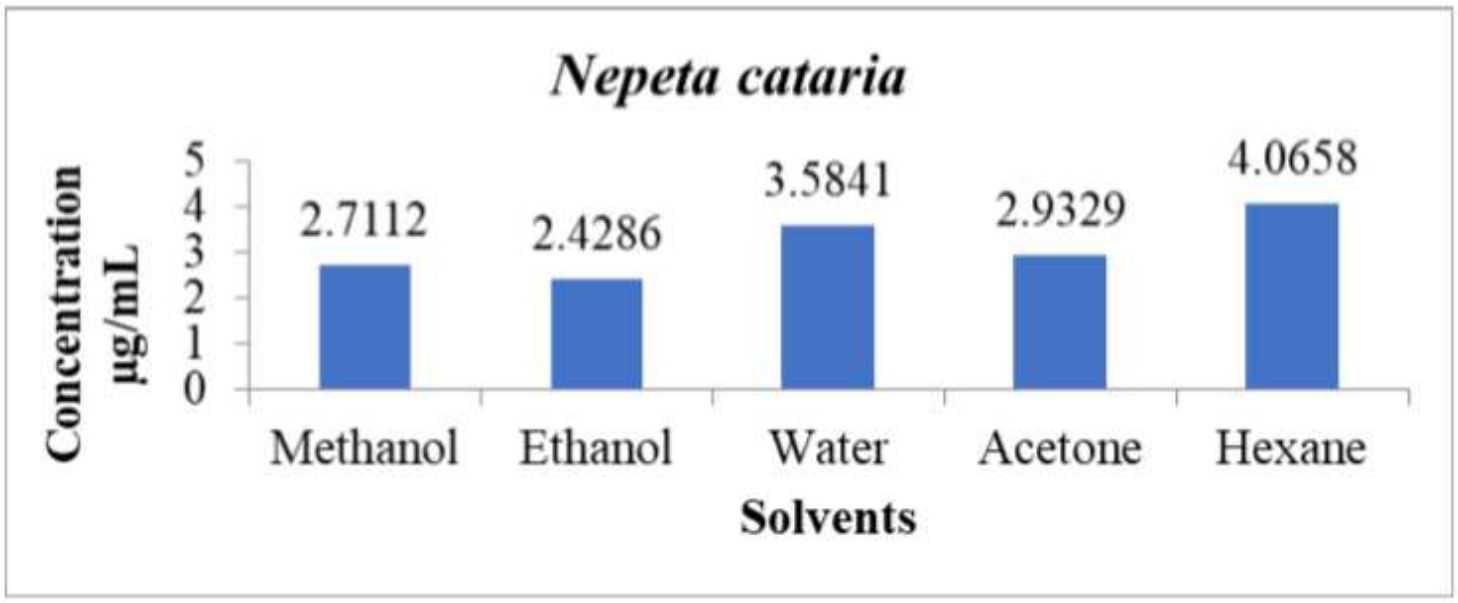

Figure 5

The concentration of Flavonoids in Nepeta cataria extracts in different solvents.

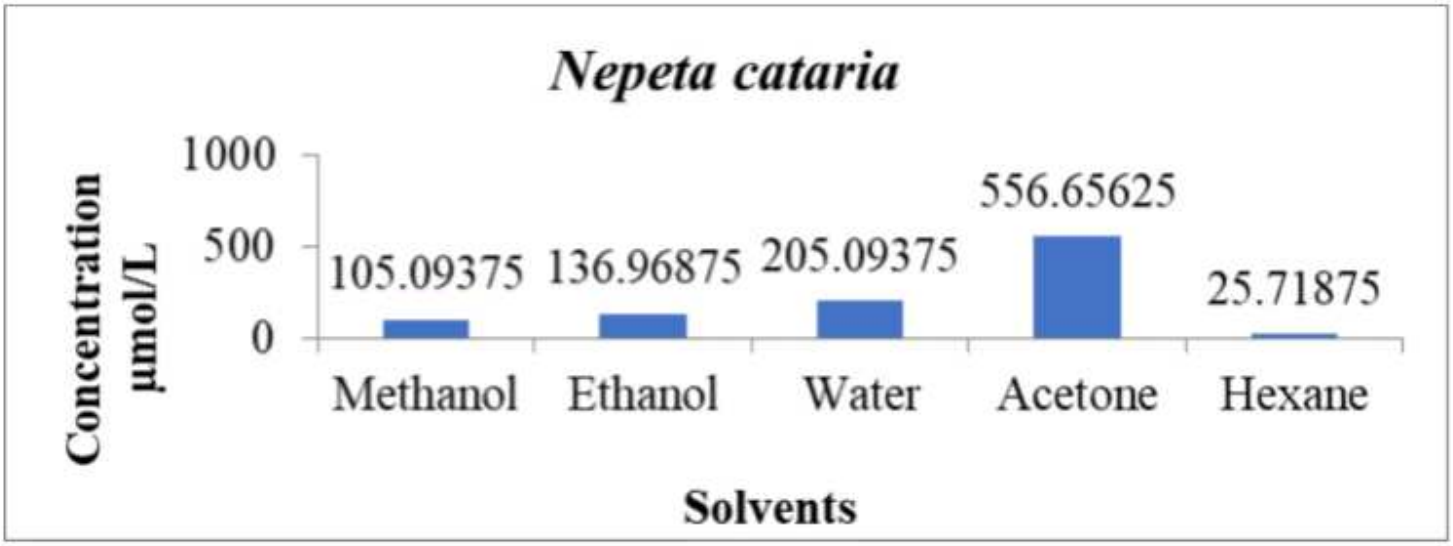

Figure 6

The concentration of DPPH in Nepeta cataria plant extracts prepared in different solvents.

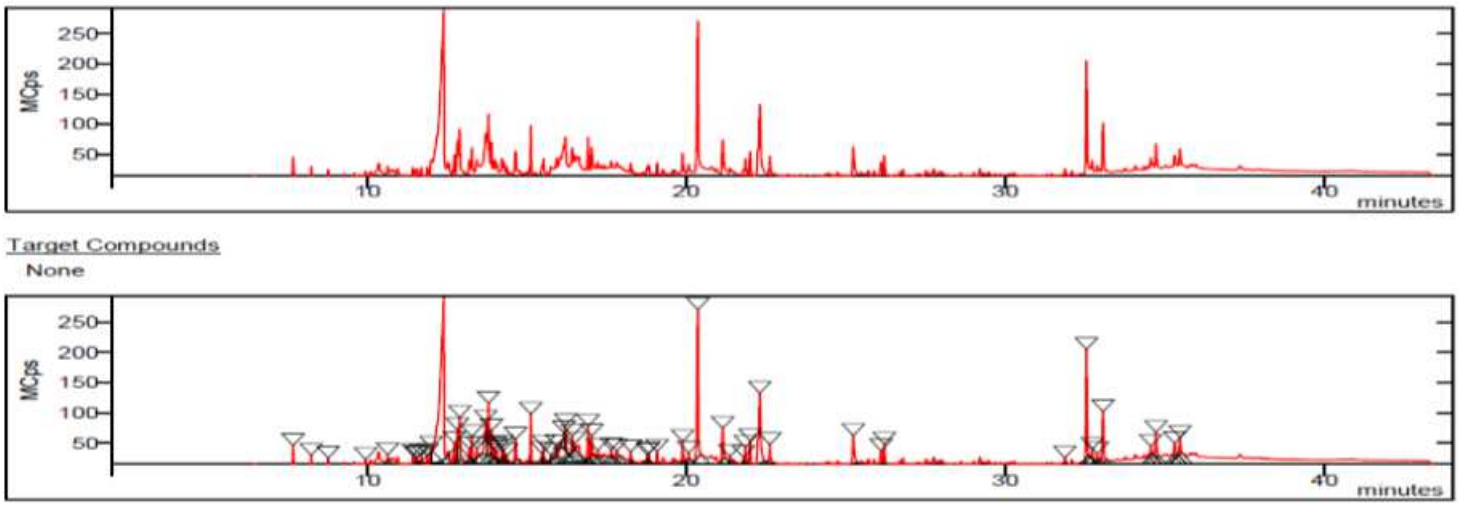

Figure 7

GCMS Spectral Chromatogram of Methanolic Extract of Nepeta cataria

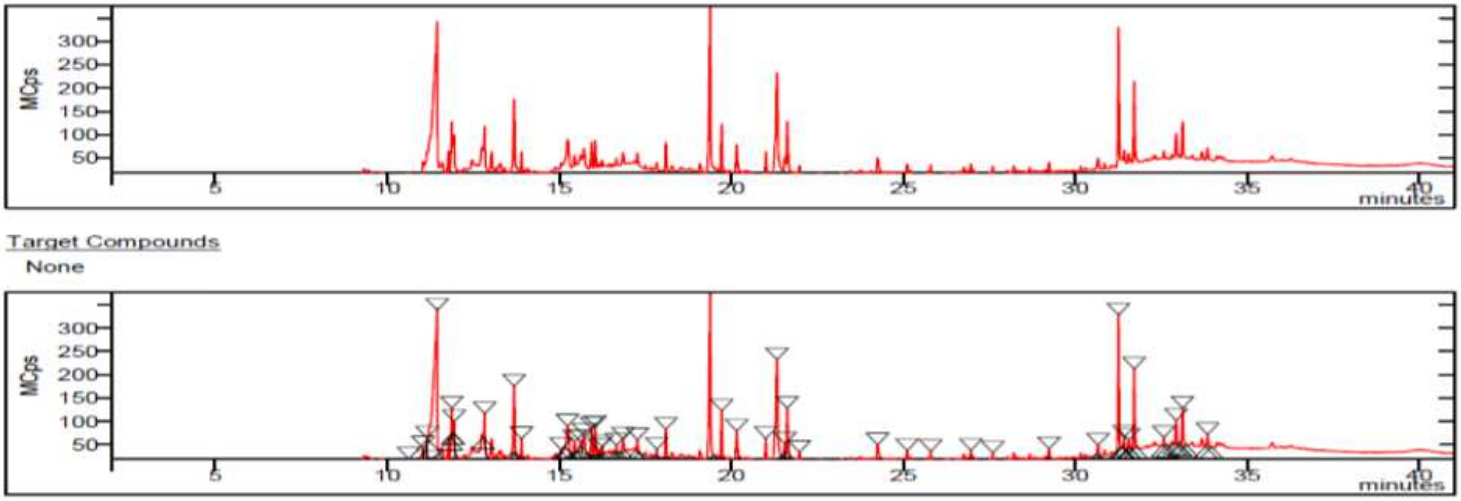

Figure 8

GCMS Spectral Chromatogram of Ethanolic Extract of Nepeta cataria.

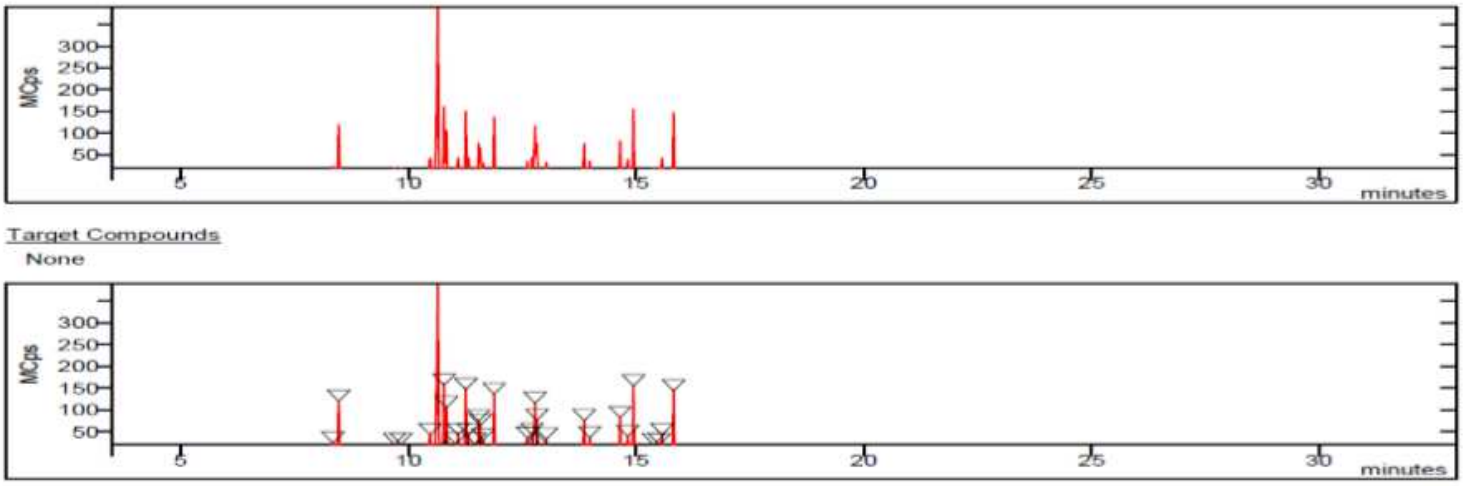

Figure 9

GCMS Spectral Chromatogram of Water Extract of Nepeta cataria.

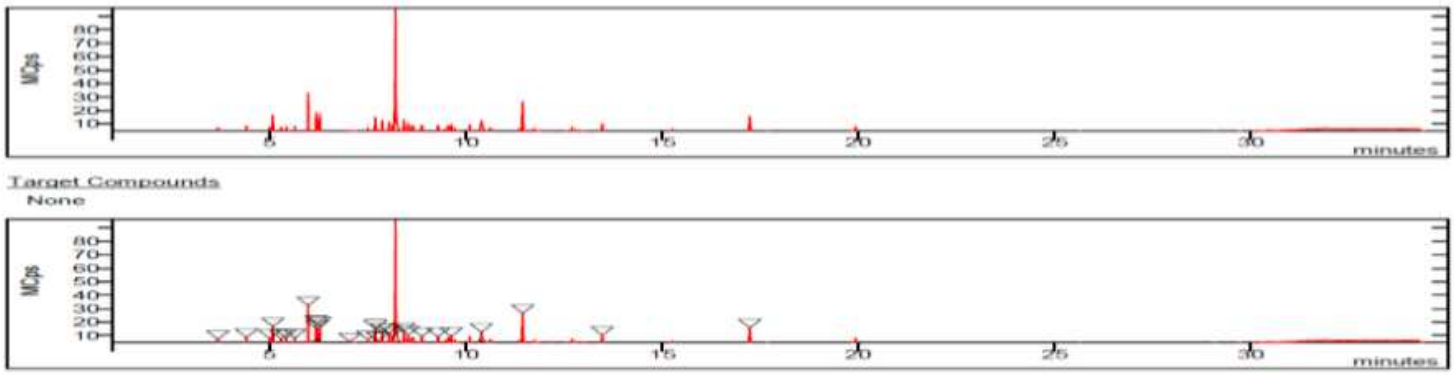

Figure 10

GCMS Spectral Chromatogram of Acetone Extract of Nepeta cataria

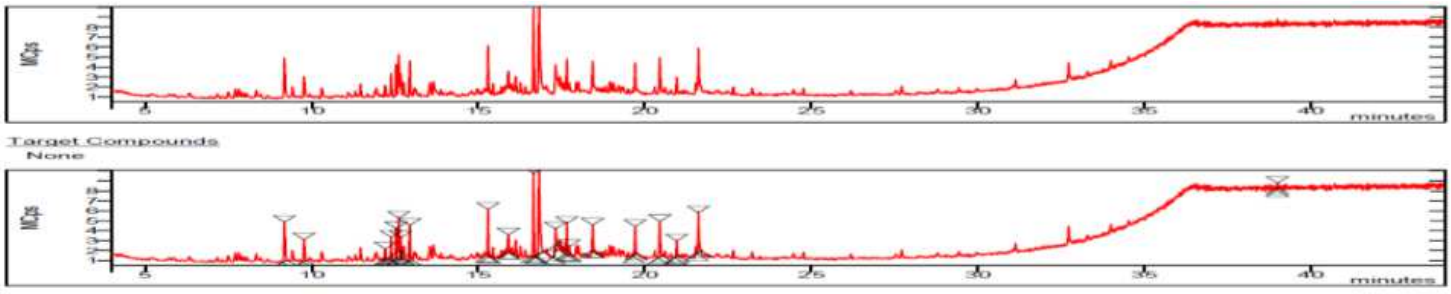

Figure 11

GCMS Spectral Chromatogram of Hexane Extract of Nepeta cataria