In Vitro Anticancer Screening of Methanolic Extract of Stachytarpheta Mutabilis

Ramesh Londonkar(✉) and Maithilee Kesralikar

Department of Biotechnology, Gulbarga University, Gulbarga, Karnataka, India
londonkarramesh53@gmail.com

Abstract. In therapeutic plants, phytochemicals are found in abundance. A family of verbena known as vervain (verbenaceae) consists of over 100 genera and nearly 2600 species. Among the Verbenaceae family is *Stachytarpheta,* a green perennial herb. The species is being examined in this study for its phytochemical, antioxidant, and anti-inflammatory properties. *Stachytarpheta mutabili*s methanol extracts were studied for their phytochemical screening, antimicrobial, anti-inflammatory, and anticancerous effects in the current study. Phytochemical study revealed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, carbohydrates, and saponins, among other phytoconstituents. One fungus, *Aspergillus niger, Staphylococcus aureus*, and *Bacillus subtilis*, as well as four-gram (+) pathogens, including *Staphylococcus aureus* and *Bacillus subtilis*, and two gram (-) pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Klebsiella pneumoniae*, were disc diffusion tested for antibacterial activity. The nitric oxide scavenging assay was used to test for anti-inflammatory efficacy in vitro. DPPH, ABTS, and total antioxidant tests were used to evaluate antioxidant activity. Analyses such as TLC, GC-MS, and were performed as per common procedure. A nitric oxide scavenging experiment was utilised to determine the anti-inflammatory properties of the drug in vitro. Tests for antioxidant activity were conducted using DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and total antioxidant tests. MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) analysis has been used to do anticancer screening. Extracts from *Stachytarpheta mutabilis* were found to include flavonoids, tannins and phenolic compounds as well as terpenoids and steroids in their phytochemical analysis. Squalene, octadecanal, and butan-1-one were found in the GC-MS study as were thirty other bioactive chemicals such as 4-isopropylcinnamic acid and octadecanoic acid. The methanolic fractions of plant extracts were shown to be effective against all bacteria. Anti-inflammatory and antioxidant experiments performed in the lab revealed the compounds' potent inhibition activity. Analysis of the GC-MS data revealed the existence of thirty distinct components. MTT assay on the extract revealed anticancer activity. The data shows that the methanolic extract has a significant properties. *Stachytarpheta mutabilis* has therapeutic value and other pharmaceutical qualities may be assessed as a result of these results, which support the traditional usage of folk medicine as well.

Keywords: Medicinal plants · phytochemical analysis · antimicrobial · anti-inflammatory · anticancer

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1 Introduction

The fauna and flora of India are very diverse. For centuries, people have relied on plants and weeds for therapeutic purposes. Compounds that plants produce for various purposes, including defence against insects, fungus, illnesses as well as herbivorous animals, are known as phytochemicals. Weeds are generally undesirable plants, but there are several that have therapeutic value and a wide array of secondary metabolites, making them worth the effort to remove from the landscape [1]. Weeds that have anticancer, antibacterial, and antifungal properties are many. Phytochemicals are found in abundance in medicinal plants. There are roughly 2600 species in the verbena (also known as vervain) family, which includes 100 genera. *Stachytarpheta mutabilis*, a perennial plant, is also a member of this family. Changeable velvet berry, coral porter weed, pink snakeweed, and red snakeweed are just a few of the numerous names it goes by. To the west of Maharashtra and south of Karnataka in India, as well as in the Caribbean and South America. As an invasive species, it may be found in a wide variety of locations. Perennial plant or subshrub, normally 10 to 20 cm in height, but may reach half a metre in height at times. The leaves on the hairy stalks are placed in opposing directions. A lance-shaped or oblong form to the blades of the leaves. They may grow up to a length of 12 cm. Inflorescences may grow up to 60 cm in length and are composed of a single slender spike. The blossoms are red and pink in colour. Abortifacients and menstrual pain relief are common uses of this species in traditional medicine [2]. The phytochemical screening of methanol extracts of *Stachytarpheta mutabilis* leaves and assessment of their in vitro anti-inflammatory, antioxidant, and antibacterial activities was the subject of a recent research. When the methanolic extract was subjected to GC-MS analysis, different phytoconstituents were discovered in the methanolic extract. Analysis shows that methanolic extract has considerable effects, which supports its usage as a folk medicine for its medicinal characteristics and may be tested for more pharmacological properties.

2 Material and Methods

2.1 Phytochemical Analysis

Stachytarpheta mutabilis leaves collected at More Nursery Pune Maharashtra's January 2021 and identified in Department of Botany, Gulbarga University, Kalaburagi. The leaves were washed with water, dried at room temperature, then coarsely pulverised using a grinder to eliminate any residues. Methanol was used in a 1:25 soxhlet extraction of crushed powder [3]. It was discovered in the phytochemical study that secondary metabolic products such as phenolics and flavonoids as well as steroid and saponin chemicals and alkaloid compounds were present. The research methodology used antibacterial, thin-layer chromatography (TLC), in vitro anti-inflammatory, and Gas Chromatography (GC-MS) to further characterize the crude extract. Using phytochemical research, [4] identified roughly 7–8 distinct phytochemicals. Quantitative estimation of secondary metabolites such as total phenol, total tannins, total flavonoids, and total terpenoids were expressed in terms of gallic acid, tannic acid, quercetin, and linalool equivalent respectively.

2.2 Antimicrobial Studies

The antibacterial activity was determined by well diffusion methods [5]. About 25 mL of molten Mueller Hinton agar was poured into a sterile Petri plate (Himedia, Mumbai, India). The plates were allowed to solidify, after which 18 h grown (OD adjusted to 0.6) 100 μl of above said pathogenic bacteria were transferred onto plate and made culture lawn by using sterile L-rod spreader. After five min setting of the pathogenic microbes, a sterile cork borer was used to make 5 mm well on the agar. The test samples were dissolved in sterile saline and loaded in to wells with various concentrations such as 50 μg/well, 100 μg/well, 150 μg/well and 200 μg/well. The solvent sterile water loaded well served as negative control and azithromycin (30 μg/ml) well served as positive control for bacteria and clotrimazole (30μg/ml) well served as positive control for fungi. The plates were incubated at 37 °C in a bacteriological incubator for 24 h. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale (Himedia, Mumbai, India).

2.2.1 Antifungal Activity

The antifungal activity was determined by well diffusion methods [6]. About 25 mL of potato dextrose agar was poured into a sterile Petri plate (Himedia, Mumbai, India). The plates were allowed to solidify, after which three days grown fungal suspension were made 10^5 cfu/ml, of which 100 μl of suspension was swabbed using sterile swab (Himedia, Mumbai, India). The test samples loaded in to wells various concentrations such as 50 μg/well, 100 μg/well, 150 μg/well and 200 μg/well. The standard antifungal clotrimazole was added at 30 μg/well. All the samples loaded plates were kept for 48–72 h. The antifungal activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale (Himedia, Mumbai, India).

2.2.2 Minimum Bactericidal and Fungicidal Activity

Fresh nutrient agar and potato dextrose agar plates were inoculated with 10 μl of culture from each to assess the MBC and MFC [7]. Plates used in the MBC test were incubated for 24 h, whereas plates used for the MFC test were incubated for five days. MBC and MFC values for this extract were defined as the lowest concentration of the extract that did not cause any bacterial or fungal growth on the solid medium throughout the incubation periods.

2.2.3 Test Microbial Strain

Antibacterial testing in vitro was performed on Bacillus cereus (MTCC 430), *Staphylococcus aureus* (MTCC 96), *Salmonella typhi* (MTCC 98), *Staphylococcus aureus* (MTCC 96), *Klebsiella pneumoniae* (ATCC 109), and *Salmonella typhi* (IMTECH, Chandigarh, India).

2.3 In Vitro Anti-inflammatory Activity

The following investigation was used to verify the anti-inflammatory properties in vitro.

2.3.1 Nitric Oxide Scavenging Activity

At physiological pH, the Griess reaction was used to measure the nitric oxide generated by sodium nitroprusside [8]. 150 min of incubation at 25 °C resulted in the colourless reaction mixture (3 ml) with the test extracts (10, 25, 50, and 100 μg/ml) and the reaction mixture (3 ml) containing the Griess reagent (1% orthophosphoric acid, 2% sulphanilamide, and 0.1% napthylethyline diamine hydrochloride). At 546 nm, the chromophore's absorbance was measured. The formula was used to determine the percentage inhibition of nitric oxide scavenging [9].

$$\text{Percentage Inhibition} = \frac{(\text{A of Control} - \text{A of Sample})}{\text{A of Control}} \times 100$$

A- absorbance.

2.4 In Vitro Antioxidant Activity

Antioxidant activity was tested using the following techniques:

2.4.1) DPPH-Assay 2.4.2) ABTS Assay 2.4.3) Total antioxidant Assay.

2.4.1 DPPH Assay

Samples were tested for their effect on the radical DPPH [10]. Two ml of DPPH solution were added to a 50 μl methanolic solution to test the sample. Absorbance measurements using a spectrophotometer at room temperature, measured the reduction in absorbance at 515 nm for 16 min. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 m in duration. All of the measurements were obtained during three distinct experiments. DPPH was performed according to [11] procedure.

2.4.2 ABTS Assay

The free radical scavenging capacity of samples was tested using ABTS radical cation decolorization assay [12]. ABTS dissolved in water to get 7 mM concentration. ABTS radical cation ($ABTS^{*+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark room temperature for 12–16 h before use. The free radical was stable for more than two days, when stored in the dark room temperature. For the study of the test samples, the $ABTS^{*+}$ solution was diluted with absolute ethanol to an absorbance of 0.700 (±0.02) at 734 nm and equilibrated at 30 °C. Reagent blank reading was taken (A_0). After addition of 2.0 mL of diluted $ABTS^{*+}$ solution ($A734\ nm = 0.700$ (±0.02) to 50 μL of test sample (20 mg/mL), the absorbance reading was taken at 30 °C exactly 6 min after initial mixing (A_t). Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times. The percentage inhibition of absorbance at 734 nm was calculated using the above formula and decrease of the absorbance between A_0 and A_t.

PI = $[(AC(0) - AA(t))/AC(0)] \times 100$ where $AC(0)$ is the absorbance of the control at t = 0 min; and $AA(t)$ is the absorbance of the antioxidant at t = 6 min.

2.4.3 Total Antioxidant Assay

Plant extract in different concentration ranging from 100 μg to 500 μg were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution according to the procedure [13]. These tubes were kept incubated at 95 °C for 90 min. After incubation, these tubes were normalized to room temperature for 20–30 min and the absorbance of the reaction mixture was measured at 695 nm. Percent inhibition of nitric oxide scavenging was calculated using the formula.

$$\text{Percentage Inhibition} = \frac{(\text{A of Control} - \text{A of Sample})}{\text{A of Control}} \times 100$$

A- absorbance.

2.5 Thin Layer Chromatography

A silica gel TLC plate grade F254 was used to assess the number of compounds found in a sample (E-Merck, Darmstadt, Germany). Using capillary tubes, 5 μL of material was observed on silica gel plates at a distance of one cm from the bottom. Metabolites were profiled using a variety of solvents in varying mixtures and concentrations. A filter paper liner was wetted to provide a saturated atmosphere in the closed tanks used for the chromatogram development [14]. To see the chromatogram, UV light was used (254 nm). The following formula was used to get the compounds' (Retardation factor) values.

$$R_f = \frac{\text{distance travelled by compound}}{\text{distance travelled by solvent front}}$$

Fig. 1. Thin Layer Chromatography of Methanolic Extract (Chloroform: Methanol (8:2))

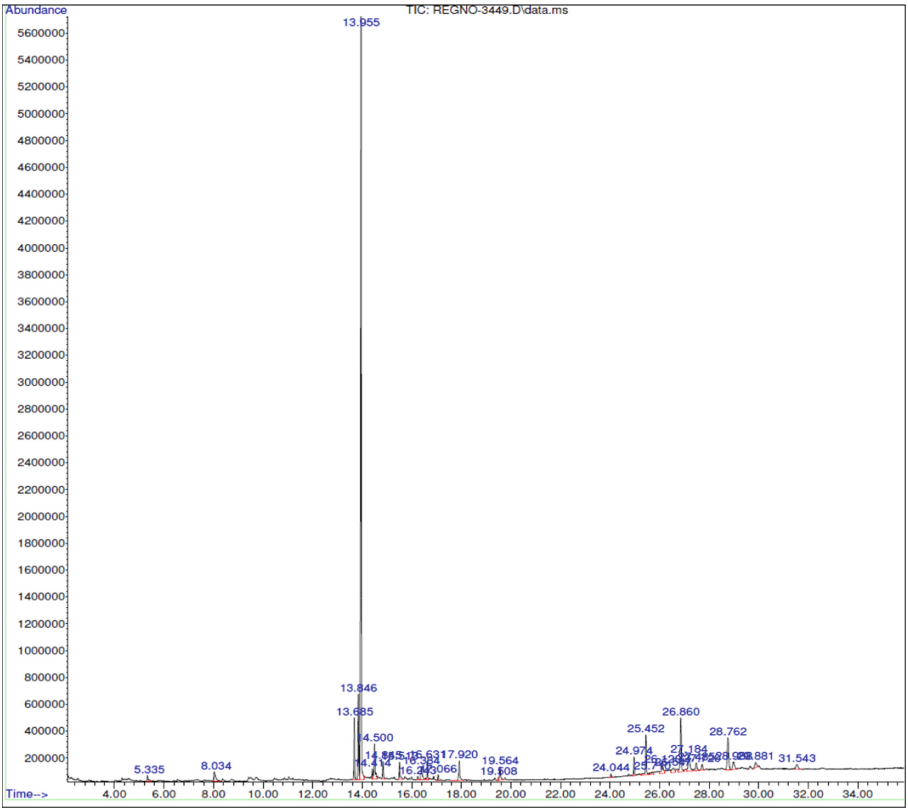


Fig. 2. GC chromatogram of *S. mutabilis*

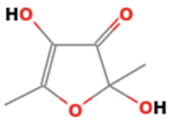
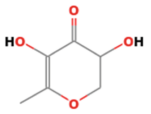
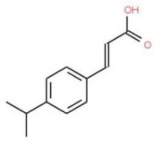
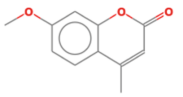
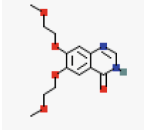
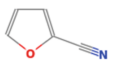
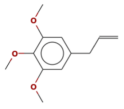
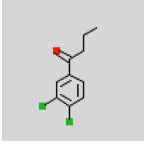
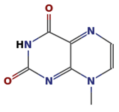
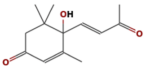
2.6 GC-MS Analysis

To separate the constituents of a sample combination, the analytical method of gas - chromatography-mass spectrometry, or GC-MS, is used. We performed GC-MS using the Agilent Technologies, USA-based, 7890B-GC with the MSD 5977A (Agilent Technologies). The presence of roughly 29 components was found by GC-MS analysis of methanolic plant extract. *Stachytarpheta mutabilis* GC chromatogram is guided by Fig. 1. Table 1 lists the GC-MS-identified chemicals (Fig. 2).

2.7 MTT Assay

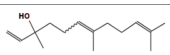
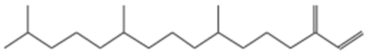
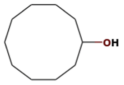
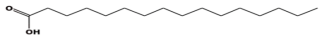
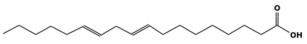
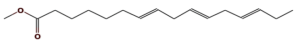
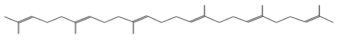
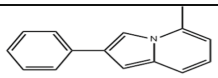
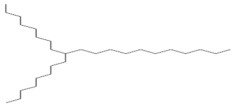
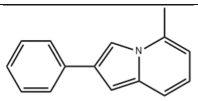
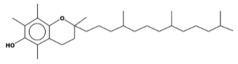
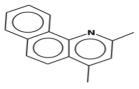
A standard approach for performing the MTT test. It has an OD of 570 nm range. A healthy and 80% confluent HeLa cells from T25-flask were detached by using 0.25% trypsin EDTA (with phenol red) prepared in PBS. Cell viability and total cell count were checked. Generally, cells are plated in triplicates to minimize the variability of the results. 100 µl of cell suspension (1000 cells/well) is used. Approximately 72,000 live cells with 97% viability were seeded into 96-well culture plate (100 µl each well), leaving the first and last row empty (i.e., 72-wells total). The plate was observed under microscope and

Table 1. The list of compounds identified through the GC-MS analysis is as follows

Sr. No.	Phytoconstituent	Structure and Activity
1	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one $C_6H_8O_4$	
2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl $C_6H_8O_4$	
3	4-Isopropylcinnamic acid $C_{12}H_{14}O_2$	
4	4-Methyloxy-7-methylcoumarin $C_{11}H_{10}O_3$	
5	6,7-Methylenedioxy-4(3H)-quinazolone $C_{14}H_{18}N_2O_5$	
6	3-Furancarbonitrile C_5H_3NO	
7	Benzene, 1,2,3-trimethoxy-5-(2-pro penyl)- $C_{12}H_{16}O_3$	
8	1-(3,4-Dimethoxyphenyl)butan-1-one $C_{10}H_{10}Cl_2O$	
9	2,4(1H,3H)-Pteridinedione 1,3 dimethyl- $C_8H_8N_4O_2$	
10	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)- $C_{13}H_{18}O_3$	

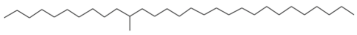
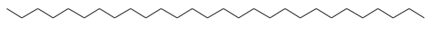
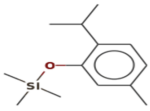
(*continued*)

Table 1. (*continued*)

11	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl $C_{15}H_{26}O$	
12	Neophytadiene $C_{20}H_{38}$	
13	Cyclodecanol $C_{10}H_{20}O$	
14	n-Hexadecanoic acid $C_{16}H_{32}O_2$	
15	Linoelaidic acid $C_{18}H_{32}O_2$	
16	7,10,13-Hexadecatrienoic acid, methyl ester $C_{17}H_{28}O_2$	
17	Eicosane $C_{20}H_{42}$	
18	Squalene $C_{30}H_{50}$	
19	Nonacosane $C_{29}H_{60}$	
20	5-Methyl-2-phenylindolizine $C_{15}H_{13}N$	
21	Eicosane 9-octyl-	
22	5-Methyl-2-phenylindolizine $C_{15}H_{13}N$	
23	Hentriacontane $C_{31}H_{64}$	
24	dl-.alpha.-Tocopherol $C_{28}H_{48}O_2$	
25	Benzo[h]quinoline, 2,4-dimethyl $C_{15}H_{13}N$	

(*continued*)

Table 1. (*continued*)

26	11-Methylnonacosane $C_{30}H_{62}$	
27	Octacosane $C_{28}H_{58}$	
29	Thymol, TMS derivative $C_{10}H_{14}O$	

incubated for 24 h. The test compound was stable at room temperature and stored at 27–30 °C until their use. The test compound was added to 96-well plate at 805.2 μg, 402.65 μg, 201.32 μg, 100.66 μg, 50.33 μg, 25.16 μg/100 ul (w/v) concentrations. Complete media (DMEM/F12 + 10% FBS) was used as Media control, 3% Methanol (v/v) was used as vehicle control, whereas, 10 μg/100 μl, 08 μg/100 μl, 06 μg/100 μl, 04 μg/100 μl, 02 μg/100 μl, 00 μg/100 μl Cisplatin was used as positive control. HeLa cells were exposed to test compounds by incubating for 24 h to determine the viability of cells against each test compound in an incubator with 5% CO_2 at 37 °C and 90% Rh. After 24 h incubation time, add 1:10 (10 μl MTT + 90 μl Complete medium) ratio of MTT solution (5 mg/mL). Shake the plate for 5 min on a plate shaker by slowly increasing the shaking speed to a maximum of 900 shakes/min. Then incubate the plate for another 4 h at 37 °C in a CO_2 incubator. The OD is measured at 570 nm. The measured data are copied into an excel sheet and with the use of the formula, the percentage of living and dead cells was determined: The HeLa cell survival (Viability) is calculated by: (OD of treated well/mean OD of control well × 100). For more reliable results the experiment was carried in triplicate. After adding DMSO, dissolving the formazan crystals thoroughly the plate has to rest for 10 min before measuring.

3 Results and Discussion

3.1 Plant Extraction Yield

The primary and secondary metabolites of *S.mutabilis* may be determined by conducting a phytochemical analysis. Fats and oils were detected in the methanolic extract using primary metabolite assays. There are many multiple kinds of compounds found in the methanolic extract, such as phenolic and flavonoid compounds as well as steroid compounds as well as saponins and alkaloids as well. Using methanol to extract 90 g of dried plant material generated plant extract residues ranging from 10 to 11 g. A 12% yield was found for methanolic plant extract. Table 2 shows the methanolic extract's phytochemical analysis.

Table 2. Phytochemical Analysis of methanolic plant extract

Sr. No.	Phytochemical Test	Methanolic Extract
1.	**Test for phenols** 1) Phenol Test 2) Ellagic Test	+ +
2	**Test for Flavanoids** 1) Flavanoids test 2) Shinoda test	+ +
3	**Test for Steroids** 1) Salkowski test 2) Leibermann Burchards test	+ +
4	**Test for saponins** **1)** Foam test	+
5	**Test for alkaloids** 1) Mayers test 2) Wagners test 3) Hagers test	**+** **+** **+**
6	**Test for Tannins** 1) Ferric Chloride test 2) Gelatin Test	**+** **+**
7	**Test for glycosides** 1)Killers Killani test 2) Conc.sulphuric Acid test	**+** **+**
8	**Test for Terpenoids**	+

Table 3. Quantitative analysis of secondary metabolites

Sr. No.	Analysis of Secondary Metabolite	μg/mg equivalent
1.	Total Phenol	300 (Gallic acid equivalent)
2	Total Tannins	100 (Tannic acid equivalent)
3	Total Flavanoid	25 (Quercetin equivalent)
4	Total Terpenoids	120 (Linalool equivalent)

3.2 Quantitative Analysis of Secondary Metabolites

Quantitative estimation of secondary metabolites such as total phenol, total tannins, total flavonoids, and total terpenoids were expressed in terms of gallic acid, tannic acid, quercetin, and linalool equivalent respectively.

3.3 Antibacterial and Antifungal Activity of Methanolic Plant Extract

Using the disc diffusion method, investigated the antimicrobial activity of *Stachytarpheta mutabilis* Methanolic Extract against two Gram (+) bacteria, *Staphylococcus aureus* and *Bacillus cereus*, and three Gram (−) bacteria, *Escherichia coli, Salmonella typhi*, and *Klebsiella pneumoniae*. As outlined in Table 2 and shown in Fig. 1, the method for evaluating antibacterial and antifungal activity was thoroughly described. Only *Escherichia coli* was shown to be resistant to the methanolic extract's antibacterial properties. In other words, the extract is very effective against bacteria. Antimicrobial effects of tannins, flavonoids, and terpenoids may be found in a wide range of foods. *Aspergillus niger* was shown to be resistant to the extract's antifungal effects. As positive controls for the bacterial and fungal assays, we utilised 30 μg/ml Azithromycin and 30 μg/ml Clotrimazole. The extract was then submitted to the MBC test, which showed its antibacterial activity (Fig. 3).

At a dose of 200 μg/ml, the strongest growth inhibition zones were discovered. It signifies that the inhibitory zone has the highest density among all bacteria and fungi. Table 3 shows the growth inhibition zones. The inhibitory zone expands as the concentration rises, [15]. The total antibacterial activity is shown in the following graph. *Bacillus*

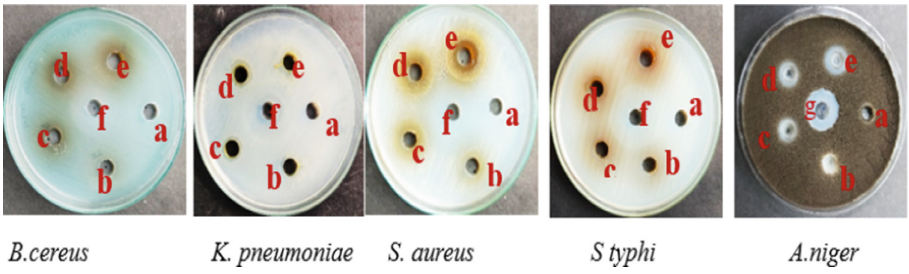


Fig. 3. Growth inhibition zones of Bacteria, a: 0 μg/ml; b: 50 μg/ml; c: 100 μg/ml, d: 150 μg/ml, e: 200 μg/ml, f: 30 μg/ml (Azithromycin); g: 30 μg/ml (Clotrimazol)

Table 4. The tabular representation of growth inhibition zone

Microorganisms	Zone of Inhibition (mm)				
Concentration	50 μg/ml	100 μg/ml	150 μg/ml	200 μg/ml	30 μg/ml (Standard drug)
Bacillus cereus	11	13	14	16	21*
Kleibsella pneumoniae	10	11	12	14	15*
Salmonella typhi	07	08	10	13	25*
Staphylococcus aureus	07	08	09	11	25*
Aspergillus niger	–	07	08	10	18#

*Azithromycin, #Clotrimazol

Table 5. MBC and MFC results showing the activity

Sr. No.	Organisms	Parameter	Results
1	*Bacillus subtilis*	MBC	75 µl/ml (7.5 mg/ml)
2	*Kleibsella pneumoniae*		100 µl/ml (10 mg/ml)
3	*Salmonella typhi*		100 µl/ml (10 mg/ml)
4	*Staphylococcus aureus*		100 µl/ml (10 mg/ml)
5	*Aspergillus niger*	MFC	100 µl/ml (10 mg/ml)

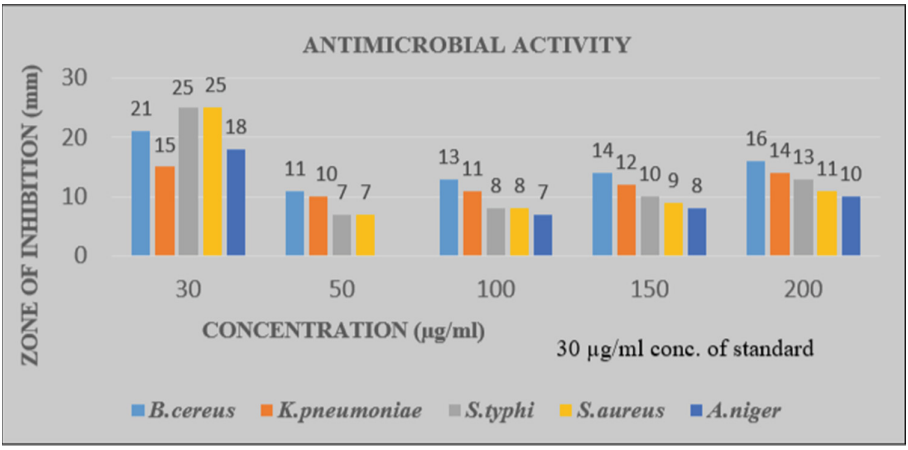


Fig. 4. Graphical presentation of comparative analysis of zone of inhibition

subtilis had a minimum bactericidal concentration of (7.5 mg/ml), which was the same for all other organisms. On the other side, the bactericidal and fungal concentrations are shown in Table 4. Some strains of bacteria have been shown to be effective at concentrations of 10 mg/ml *K. pneumoniaeae*; *Staphylococcus aureus*; and *A. niger*). The ethanol and aqueous extracts of *S.indica* [16] shown antibacterial and antifungal activity. Phytochemicals a have the strong potential to defend against drug resistant biologically important microorganisms [17] (Table 5).

Comparison of inhibition zones shows that *S.aureus* has an 11-mm zone of inhibition and a 7-mm zone of inhibition. *B.cereus, K.pneumoniae*, and *A. niger* showed maximal inhibitory zones of 16 mm, 14 mm, and 10 mm, respectively, while the least was 11 mm, 10 mm, and 7 mm. It has been shown that the greatest inhibition zone for *Salmonella typhi* is 13 mm, whereas the lowest is 7 mm. [18] proved some antimicrobial properties of plant extracts from euphorbiaceae, lamiaceae and sapindaceae against some pathogenic organisms.

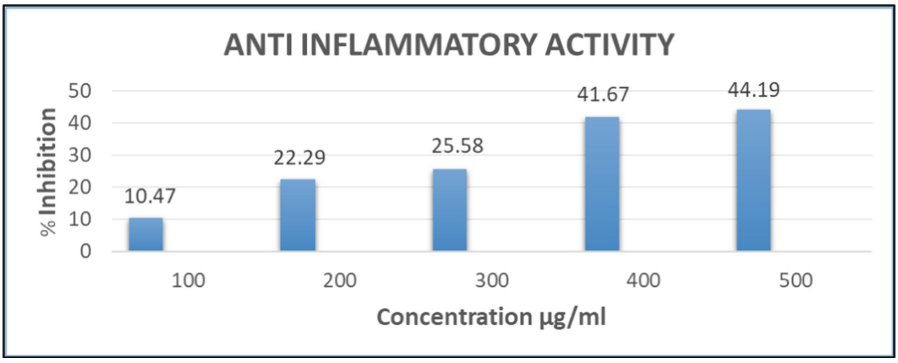


Fig. 5. Graphical presentation of anti-inflammatory activity.

3.4 In Vitro Radical Scavenging and Anti-inflammatory Studies

A nitric oxide scavenging test was used in an in vitro anti-inflammatory study. Irritation or infection may cause inflammation that results in redness, swollen areas that are painful and impeded movement, and decreased function. As a defensive mechanism, inflammation removes foreign substances and prevents additional harm to the host [19]. The anti-inflammatory activity was 44.19% at a concentration of 500 µg/ml. Anti-inflammatory efficacy was shown to be closely linked to the ethanolic extract concentration. Antioxidant tests were performed using the DPPH, ABTS and the total antioxidant test. According to the findings of this study, *Stachytarpheta mutabilis* methanolic extract has a significant amount of antioxidant activity. It became more effective as the methanolic extract concentration grew. This drug has anti-inflammatory properties, as seen in Fig. 4.

The anti-inflammatory activity was determined to be 10.47% at 100 µg/ml.

Antioxidant compounds found in so many therapeutic plants, such as phenolic compounds and polyphenols, serve a critical function in removing free radicals from the body [20]. An antioxidant test based on electron transfer is performed using the DPPH (2, 2-diphenyl-1-picrylhydrazyl-hydrate) free radical technique [21]. Antioxidant molecules reduce this free radical to a colourless ethanol solution. In spite of all three radical scavenging tests exhibiting substantial activity, the ABTS assay revealed the most significant percentage of inhibition and it was about 71.66%. A maximum activity of 55.89% and a low activity of 12.37% were found in the overall antioxidant test. According to the DPPH test, the percentage inhibition was found to be 58.06% when used at 500 µg/ml and 18.97% when used at 100 µg/ml. [22] proved that the extracts from different plant sources have significant free radical scavenging and anti-inflammatory activity (Figs. 5 and 6).

3.5 TLC and GC-MS Analysis of Plant Extract

The TLC sheet revealed five spots during thin layer chromatography of the crude methanolic extract. The Rf value was computed and then subjected to GC-MS analysis. The chemical formula and structure of the plant extract were investigated further

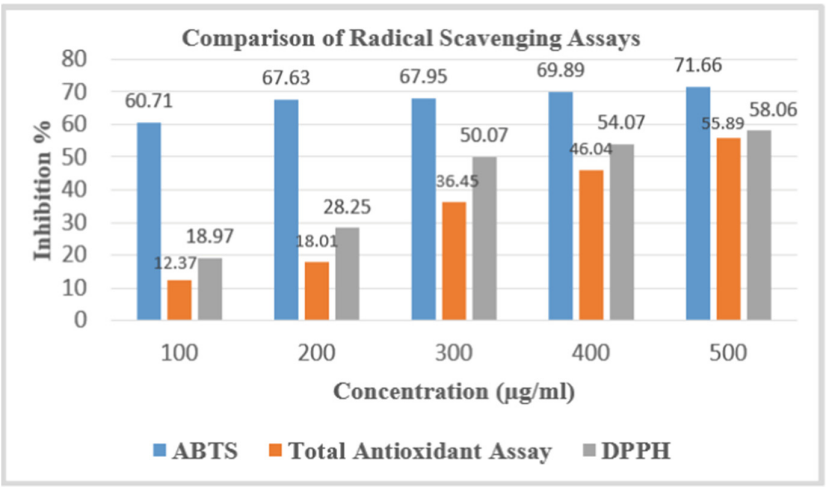


Fig. 6. Graphical presentation of comparative analysis of radical scavenging assays

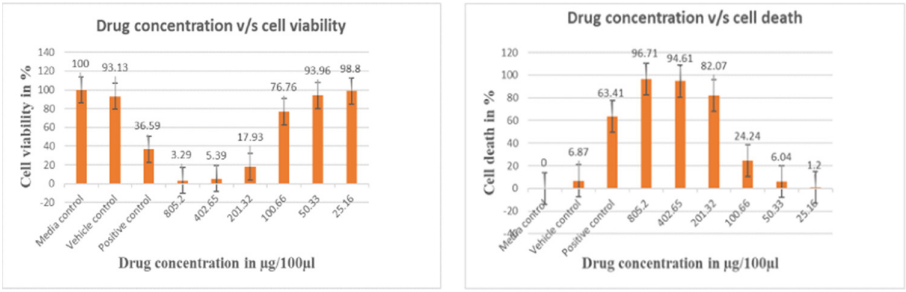


Fig. 7. Effect of drug concentration against cell viability and cell death

using GC-MS analysis. The methanolic extract of *Stachytarpheta mutabilis* was found to have 29 distinct phytochemical components. Components in crude methanolic extract include antioxidant, anti-inflammatory, antibacterial, antifungal, and antiseptic properties. GC-MS analysis confirmed the existence of a number of different components, suggesting that the methanolic extract may be further studied for its pharmacological effects. The structure and names of different compounds given in Table 1. The test drug induced cell death within 24 h in a dose dependent manner [23]. Whereas, media control, positive control (10 µg) and vehicle control 3% (v/v) concentrations did not induce any cell death even after 24 h. The cell viability of each test category was determined after 24 h exposure by the OD values using ELISA plate reader (Biobase) at 570 nm. The highest cell death was found at drug concentration 805.2 µg. Least cell death was found at 25.16 µg. No cell death was observed in media control. Overall, the test drug shows very good anticancer activity against HeLa cells in a dose dependent manner [24] as shown in Fig. 7.

4 Conclusion

Finally, the results of this investigation show that *Stachytarpheta mutabilis* methanolic extract contains a wide range of phytochemicals, including phenols, flavonoids, saponins, tannins, and terpenoids, among others. Chemical diversity in natural products from medicinal plants [21] is unmatched. This means that new drug leads may be generated from either pure compounds or standardized extracts. There are several biological activities that result from the presence of various types of phytoconstituents. There are various bioactive compounds in the *S. methanol* leaf extract that can be exploited to build effective drugs to treat a wide range of ailments, including cancer and disorders.

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Author's Contribution. M.K. has written the manuscript and Dr. R.L. has revised and edited the research paper.

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