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CHEMICAL COMPOSITION, ANTIOXIDANT, ANTIBACTERIAL ACTIVITY OF ISOLATED OIL AND METHANOL EXTRACT OF *TRIDAX PROCUMBENS* L.

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Keywords:

T. procumbens L, 2-pyrrolidinone, 1-methyl, 2 (4H)-benzofuranozone, Antioxidant activity, Antimicrobial activity

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ABSTRACT: *Tridax procumbens* L. is an annual herb that has been used in Ayurveda, Unani, and traditional medicine in India to cure various microbial infections, skin diseases, bronchial disorders, and wound healing. The current study aims to investigate the chemical composition, antioxidant, and antibacterial activities of isolated oil (denoted as TPMG oil) from methanol extract using column chromatography. GC-MS analysis identified a total of six compounds among which 2-pyrrolidinone, 1-methyl (48.68 %), 2, (4H)- benzofuranozone, 5, 6,7, 7a-tetrahydro-4, 4-dimethyl (31.94 %), 2, 2-Dimethoxybutane (8.46%). The antioxidant capacity of TPMG oil with the IC_{50} value 18.34 µg/mL and significance at $P < 0.05$ was investigated. The oil was found to be potent bactericidal activity at MIC of 125 µg/mL and 250 µg/mL for *S. aureus* and *P. aeruginosa* respectively. Subsequently, the MIC of methanol extract was varied between 125-62.5 µg/mL against selected microorganisms. The ZOI 16.2 ± 0.8366 mm and 15.8 ± 0.8360 mm against *P. mirabilis* and *A.niger* were observed. It could be used as an alternative source of antimicrobial and antioxidant agents with the potential drug in the preparation of herbal, and pharmaceutical industrial applications.

INTRODUCTION: Gram-positive bacteria *Staphylococcus aureus* and Gram-negative pathogen *Pseudomonas aeruginosa* are two pathogens that have come to prominence as still severe causes of infections in humans [1]. The majority of *S. aureus* and *P. aeruginosa* strains are resistant to most antibiotics now in use [2].

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In particular, burn wounds, complicated urinary tract infections (cUTIs), AIDS, pneumonia, cystic fibrosis (CF), bloodstream infections (BSIs), and immunodeficiency are frequently caused by *P. aeruginosa*. The clinical data that has been reported on both new and current medicines for the treatment of *P. aeruginosa* is limited.

During the COVID-19 pandemic, there was an increase in antibiotic resistance, and this increase was attributed to a rise in the antibiotic resistance for *S. aureus* and *P. aeruginosa* [4]. Thus World Health Organization (WHO) published, carbapenem-resistant *P. aeruginosa*, and *S. aureus*, methicillin- resistant, vancomycin-intermediate and

resistant as one of the most priority pathogens for research and development of new antibiotics (WHO, 2022). Moreover, since 2006, there has been no natural product-related antifungal drug, all the new drugs are functionally based on old azole chemistry [5]. Nowadays, new viral, fungal, and pathogenic diseases spread worldwide. More than 80% population in the world uses traditional medicines as their primary healthcare, due to less expensive and low lethal side effects. Indeed, India is rich in flora and fauna, native, indigenous, and endemic plants that are frequently used in traditional systems such as Ayurveda, Unani, and folk medicine from ancient times [6]. Besides, plants could play an important role in the development of safe and new antibiotics in modern medicine [7]. Plant synthesis major group of secondary metabolites which attracts scientific researchers due to their therapeutic potential [8]. The antimicrobial activity of various extracts of medicinal plants is an advanced search for antibiotics. Further, it has been found that various essential oils have an impact on the mechanism used by bacteria to match gene expression with microbial population density [9]. Therefore, it is important to search for new compounds with higher antimicrobial potential. Consequently, there is an increasing interest regarding the investigation of the antibacterial, and antioxidant activity of the isolated oil fraction from *T. procumbens* against Gram- positive bacteria *S. aureus* and the Gram-negative pathogen *P. aeruginosa*.

Tridax procumbens L. is an active genus of 30 species found primarily in the tropics [10]. Different parts of the plant have been used in Ayurveda, Unani, and traditional medicine in India to cure different microbial infections, skin diseases, and bronchial disorders, and promote wound healing. This plant's fresh juice leaves have been used since ancient times [11]. The purpose of this article is to search for new antioxidant and antimicrobial agents from *T. procumbens* L.

MATERIALS AND METHODS:

Chemicals and Equipment: All solvents used are of AR grade (Rankem). The extract was concentrated using a rotary evaporator under pressure (Rotavap). For column chromatography Fischer Scientific Silica gel 60-120 mesh size, ASI to 7661-86-91. Silica gel precoated Alumina TLC plates of 60F254 (Merk Germany) were used for TLC analysis. Chemical structures have been drawn using Chem Draw ultra 8.0.

Plant Material: The plant material of *T. procumbens L.* was collected from Ambegaon (Bk.), Pune district, Maharashtra, India, from December 2018 to February 2019. It lies between the 18º31′13″ North and 73º51′24″ East of India. The entire material was washed disinfected and shade- dried for 20 days. The plant was authenticated at the Botanical Survey of India (BSI), Pune. The voucher specimen was designated as (VVI02) and submitted to the institute.

Preparation of Methanol Extract: The dried aerial part of the plant was ground into a fine powder using a mechanical grinder. Three different solvents, namely methanol, ethyl acetate, and pet ether were used for the sequential using Soxhlet extractor. The powdered plant materials were filled in cotton bags packed in a Soxhlet apparatus, and fitted with a round bottle flask containing polar to non-polar solvents at about 12 hours at 20 ºC. Extracts were concentrated using a rotary vacuum under reduced pressure. The extraction process was carried out in triplicates with each solvent. Crude extracts were dried completely and collected in the bottles. The yields of methanol extracts were found to be nearly 5 to 20%, ethyl acetate extract of 0.2 to 0.4%, and pet ether extract of 0.05 to 0.1% to be found.

$$\% \text{ Extract} = \text{Weight of the extract} / \text{Weight of plant raw material}$$

Isolation of Oil from Methanol Extract: In the process of extraction, 300.0 g of the plant's aerial part was extracted in methanol as the solvent using the Soxhlet extraction method. The percentage yield of extract was obtained as 21.0. A total of 46.0 g of extract mixed with 1: 5 of silica gel and subjected to column chromatography over silica (60-120 mesh size, ASI to 7661-86-91, Fischer Scientific) CC (4.5 × 120 cm) (1:40) was prepared and poured into the column in 100% petroleum ether. 100 % petroleum was used to begin the column elution process, and then subsequent applications of solvent gradients of petroleum ether: ethyl acetate 95:5, 90:10, 85:15, 75:25, 65:35, 50:50, 0: 100 % then the solvent gradient of ethyl acetate: methanol 50:50, and 0:100 (solvents

of AR grade Rankem). The fractions of 50 ml volume were collected in tubes. Preliminary TLC was performed on pre-coated plates of silica gel 60F254) spots in all fractions were done to check the number of similar spots and their rf values of each fraction. After concentration and drying following 12 major fractions were obtained. Fractions A - K were collected by using 100 % hexane 95:05, 90:10, 90:15, 80:20, 75:25, 50:50, 100% ethyl acetate, 50:50 EtOAc: MeOH, and 100% MeOH respectively. Upon complete evaporation of the solvent fractions G (6.1578) (Pet Ether: Ethyl acetate 1:1), a colorless oil was obtained from a green sticky mass. The colorless oil visualized three violet-blue color spots on TLC when a solution of anisaldehyde containing a trace of glacial acetic acid in ethanol and H_2SO_4 was heated at 103ºC in the hot air gun.

Chemical Composition of Fraction TPMG Oil: The chemical composition of colorless oil (TPMG) was investigated using the fragmentation pattern by Gas chromatography-Mass spectroscopy (GC-MS) analysis. The analysis was conducted using the spectral database and the NIST Library. Based on similarity index major compounds were identified. The investigation was carried out by comparing the mass spectra of unknown chemicals to those of recognized compounds kept in the library. The parameters such as Retention time, Area %, Height %, Molecular Formula, Name of compounds, and structures of six major compounds, from TPMG oil are presented in **Fig. 1** and **Table 1.**

Analysis was carried out on a GCMS (Shimadzu TQ 8030). Separation was carried out on an RTX-5MS capillary column (30 m x 0.25 mm), (Agilent Technologies Schweiz AG). The temperature of chromatic separation was programmed following the method detailed [12]. With a starting temperature 50°C for 1min then increased 250°C at a rate of 10°C/min, then from 250°C to 300°C, at a rate of 4°C/min and held at 300°C for 65 minutes. The carrier gas was helium with a constant flow of 1 mL/min. The sample was injected in split mode with a solvent delay of 4 min by the autosampler. The injector temperature was maintained at 250°C. For MS Detection, ions were formed by electron impact at 230 using a mass-selective detector. Masses were scanned in the quadrupole at 150 from m/z 50 to 800 u. The obtained mass spectra were further evaluated employing the NIST database (MS search; NIST) [14].

Antimicrobial Activity of Methanol Extract Testing Organisms: The antimicrobial activity of methanol extract was investigated using three types of organisms two gram-positive bacterial, *Staphylococcus albus* (NICM2178), Escherichia coli (ATCC 35218), and two gram-negative bacterial, *Bacillus subtilis* (NICM2063), *P. mirabilis* (NCIM2388) and two fungal organisms such as *Candida albicanus* (NCIM 3100) and *Aspergillus niger* (ATCC504). All strains were obtained (NCIM 3100) and *Aspergillus niger* (ATCC504). All of the strains were from the NICM, NCL, Pune, India.

Preparation of Medium: A total of 500 mL of nutrient agar was prepared by mixing 11.5 g of nutrient agar in 500 mL of water, and 500 mL of potato dextrose agar was prepared by dissolving in a minimum amount of water 19.5 g of potato dextrose agar then diluted up to 500 mL of water (Hi media Mumbai). Then the medium was autoclaved at 121ºC, 15 psi for 30 min.

Minimum Inhibitory Concentration (MIC) of Methanol Extract: The MIC of the TPM extract was determined by the standard tube dilution method. This was used to test the lowest concentration of the TPM extract and antibacterial effectiveness by observing visible growth in the agar broth. The MIC was calculated using serial two-fold dilutions of TPM extract ranging from concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, and 12.5 µg/mL. The microorganism concentration (108 CFU/mL) was adjusted in broth. The control was incubated at 37°C for 24 hours with solely inoculation broth. The TPM extract concentration at which there is no discernible growth in the tubes is known as the MIC endpoint. To evaluate the MIC value the visible turbidity of the tubes was measured both before and after the experiment using a spectrophotometer (UV800SCMATAZU) at 530 nm [14].

Antimicrobial Activity of Methanol Extract (Agar Well-diffusion Method): The agar well-diffusion method was used to test the antimicrobial activity of the methanol extract against the selected

test pathogens [15]. Mueller Hinton Agar plates were prepared by pouring 20.0 mL into each sterile plate for the bacterial assay and then being allowed to solidify. In this experiment, bacteria grown on a diet of nutrient-rich culture medium were harvested using a UV spectrophotometer (UV800SCMATAZU) at 530 nm and diluted to attain a viable cell count with an absorbance of 0.1. To ensure equal growth, 500 microliters of freshly prepared, and 48-72 hours old bacterial solution were placed on the agar plates. 5 mm diameter wells were drilled on the agar plate using a cork borer. Weighing and dissolving the test samples in DMSO to a concentration of 2 mg/mL DMSO. 80-90 µL of the sample was added to each well.

On one agar plate, DMSO was applied as a negative control. As a positive control for bacteria streptomycin and fungus fluconazole, were used. The antibiotic used had a concentration of 100 µg/mL. For bacteria, the plates were incubated for 24-48 hours at 37°C, and for fungus, for 48-72 hours at 20°C. The plates were checked for a clear zone of inhibition after the incubation period. Using a measuring scale determines the zones of inhibition in mm after the incubation period, and the mean was computed. Five different experiments were run in parallel.

Antimicrobial Activity of TPMG Oil: The broth dilution method was used to determine the minimum inhibition concentration. The MIC of TPMG oil was performed by two-fold dilution with some changes in procedure as described by serial dilution method [16, 17]. Organisms *S. aureus* (NClM5021) and *P. aeruginosa* (NCIM5029) were utilized for the evaluation of antibacterial activity.

The National Collection of Industrial Microorganisms provided these Sterile 96 well ultimate capacity of 200 µl. 180 µl of bacterial suspension was loaded into wells, leaving the last column for DMSO only as the negative control. One loaded with only bacterial suspension and another with bacterial suspension and streptomycin drug. The streptomycin was also diluted as per the scheme (500 µg/mL to 15.5 µg/mL) serial dilution. After the incubation, plates were analyzed using a microplate reader (Read well Touch-2019, India). The absorbance was taken at 400 nm. TPMG oils and growth medium-filled wells served as negative controls. A triplicate test was performed on each MIC measurement.

Antioxidant Activity of TPMG Oil: The method of DPPH assay from Brand-Williams *et al.* 1995 was used to determine the antioxidant capacity of the TPMG oil. In ethanol, 0.1 mM DPPH was prepared. Each fraction of the volumetric solution is given 1 mL of 0.1 mM DPPH was prepared in methanol and mixed with solutions of each compound in a volume of 12.5, 31.5, 62.5, 125, 250, and 500 µg/mL before being built up to 3 mL with ethanol. The mixture was allowed to stand in the dark for 30 minutes and absorbance at 517 nm was measured by using a UV spectrophotometer. As our reference, pure ethanol (2 mL) and DPPH (1 mL). By plotting the DPPH scavenging, the calibration curve was established using ascorbic acid and Gallic acid as standards.

$$\text{DPPH free radical scavenging rate (\%)} = (AC-A) / AC \times 100$$

Where AC is the absorbance of the control and A is the absorbance of the sample.

All samples were tested in triplicate. Meanwhile, the standard curve was drawn with the concentration of compounds against free radicals.

Statistical Analysis: Concentration-response curves were plotted on Microsoft Excel, and, the data from a minimum of five measurements-reported as mean ± SD values, were collected. The mean and standard deviation are the only data expressions. Utilizing Microsoft Excel, data were analyzed using one-way ANOVA for antibacterial activity and one-way ANOVA for antioxidant activity. Antimicrobial and antioxidant activities were analyzed at $P < 0.05$.

RESULTS AND DISCUSSION:

Yield of Plant Extract and TPMG Oil: The maximum yield of methanol extract (almost 5 to 20%), ethyl acetate extract (0.2 to 0.4%), and per ether extract (0.05 to 0.1%) from each portion of the plant was achieved. Each plant part's output of pet ether extract was minuscule, hence, they were disregarded for the antibacterial study. The yield is 0.05%. of TPMG oil was obtained.

Chemical Composition of the Fraction TPMG Oil: The composition of isolated essential oil was

determined quantitatively and qualitatively based on retention time, peak area, molecular formula, molecular weight, and molecular structure by the GC-MS method shown in **Fig. 1** and **Table 1.**

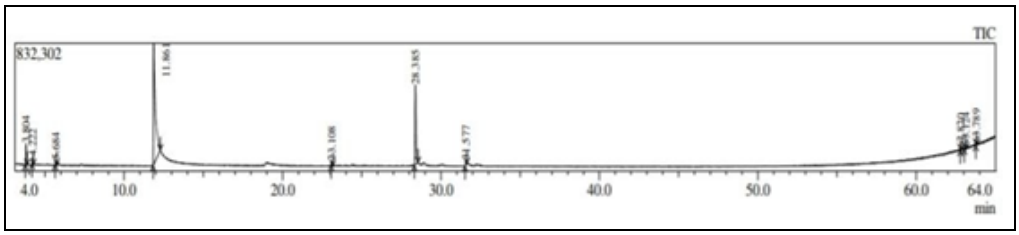
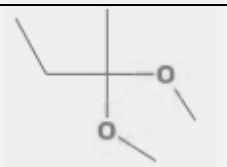
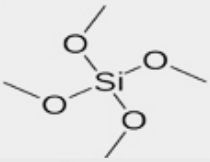
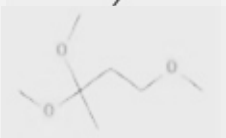
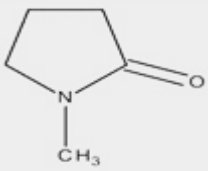
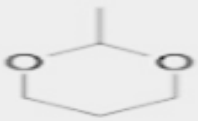
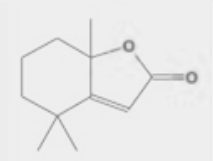


FIG. 1: GC-MS ANALYSIS CHROMATOGRAM OF TPMG OIL OF *T. PROCUMBENS*

TABLE 1: GC-MS ANALYSIS INDICATING CHEMICAL COMPOSITION OF TPMG OILS OF *T. PROCUMBENS* L.

Peak	Retention Time	Area %	Height %	Molecular Formula	Name	Structure
1	3.804	3.89	8.46	$C_6H_{14}O_2$	2, 2-Dimethoxybutane	
2	4.222	0.96	2.90	$C_4H_{12}O_4Si$	Tetra methyl silicate	
3	5.684	0.60	1.66	$C_7H_{14}O_3$	1, 3, 3-Trimethoxybutan	
4	11.861	68.43	48.63	C_5H_9NO	2-Pyrrolidinone, 1-methyl	
5	23.108	1.03	1.93	$C_5H_{10}O_2$	1,3-Dioxane, 2-methyl	
6	28.385	22.87	31.94	$C_{11}H_{16}O_2$	2(4H)-Benzofuranone, 5, 6, 7, 7a-tetrahydro-4,4	

Compounds are listed in order of their retention time. Data comparison with NIST databases.

The TPMG oils were colorless initially, while slightly yellowish later on, and have an intense and pleasant scent. Briefly, six different compounds were identified in the TPMG oils of *T. procumbens L*. TPMG oil was composed mostly of 2-pyrrolidinone, 1-methyl (48.68 %), 2 (4H)-benzofuranozone, 5, 6, 7, 7a-tetrahydro-4, 4 dimethyl (31.94 %) 2, 2-Dimethoxybutane (8.46 %) 14. Other compounds **Table 1** are found less than 1%. The bicyclic lactone benzofuranozone, 5, 6, 7, 7a-tetrahydro-4, 4 dimethyl is a volatile naturally occurring terpene that was previously found among the volatile mixtures from some species, such as *Calamintha grandiflora* (L.) 19. 2, (4H)-benzofuranozone, 5, 6, 7, 7a-tetrahydro-4, 4 dimethyl (31.94 %), identified in different parts in Scorzonera undulate [20, 21, 22]. 2, 2- Dimethoxy-butane (8.46 %) is present in many plants with antibacterial and antifungal properties [15]. *C. balaji* Ayyanar, reported *T. procumbens L.* showed good

antibacterial activity [23]. The GC-MS study revealed that the compounds 2-pyrrolidinone and 1-methyl were reported first time from this plant. In a previous study 2-pyrrolidinone, 1-methyl was evaluated for degradation of gram-positive and gram-negative bacteria in activated sludge [24]. It is also identified in the methanol root extract of *Solanum khasianum* and reported as an anticancer, antioxidant, antibacterial, antifungal, anticonvulsant, and surfactant [25]. The Pyrrolidine moiety is found in many natural compounds, particularly in plant-derived alkaloids [26]. Minmin Tang, *et al.,* 2021 identified 2, (4H)-benzofuranozone 5, 6, 7, 7a- tetrahydro-4, 4 dimethyl from flower buds of Musa nana [27, 28]. Reported from ethyl acetate extract of Xero phyta speaking (Baker) Grewia nemesis.

Antimicrobial Activity:

Minimum Inhibitory Concentration of Methanol Extract: The TPM extract was screened for Minimum Inhibitory concentration (MIC) shown in **Fig. 2** with concentrations ranging from 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, and 12.5 µg/mL. After 24 hours of incubation time at 37°C turbidity was not seen in 62.5, 125, 250, 500, or 1000 µg/mL containing TPM extract indicating bactericidal concentrations against *S. albus* whereas 31.25, 12.5 µg/mL were seen turbidity, exhibiting no bactericidal effect. The MIC of the TPM extract was found 62.5 µg/mL with *S. albus*, *P. mirabilis*, and *A. niger*. Moderate MIC was observed against gram-positive *B. subtilis* and the fungus *C. albicans* at 125 µg/mL and lowest MIC at 250 µg/mL. Overall, the obtained data showed that *T. procumbens* L. entire extract has a lesser inhibitory activity for Gram-positive, Gram-negative, and fungal strains than previously reported in scientific publications [23, 29].

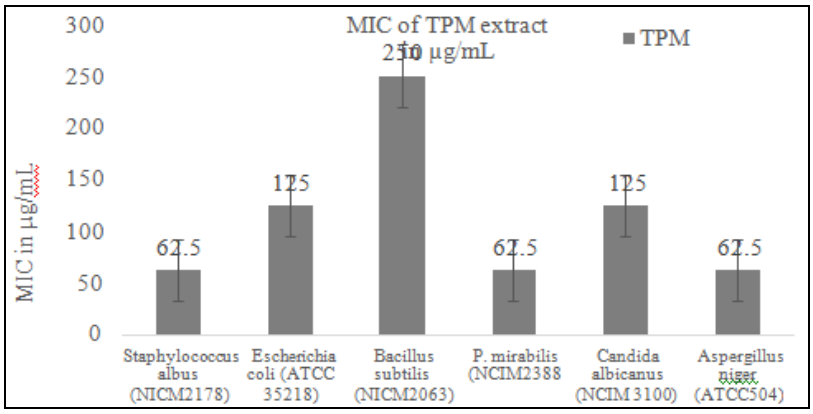


FIG. 2: MINIMUM INHIBITORY ACTIVITY OF METHANOL EXTRACT AGAINST DIFFERENT GRAM-POSITIVE, GRAM-NEGATIVE, AND FUNGAL STRAINS (N = 5)

Antimicrobial Activity of Methanol Extract: In terms of the antimicrobial activity concerned extract demonstrated effectiveness against selected strains shown in **Table 2** and directed in **Fig. 3** and **4**. The positive controls, used were streptomycin for bacteria and fluconazole for fungi. A negative control of 100 µg/mL DMSO was also employed. Standards were prepared in 100 µg/mL concentrations whereas extract was prepared in 2 mg/mL concentration and wells were filled up with 80 µg/well. After incubation zone of inhibition was measured in millimeters and shown in **Fig. 3**. The experiments were carried out 5 times. The average mean and SD value were calculated, and have been presented in **Table 2**. The highest inhibition zone was observed against the showed Gram-negative bacteria *P. mirabilis* (16.2 ± 0.8366) followed by the zone of inhibition against bacteria *S. albus* (15.4 ± 1.6335). TPM extract exhibited good and moderate activity against *B. subtilis* and *E. coli*. The TPM extract showed good activity against both tested fungus organisms *A. niger* (15.8 ± 0.8366) and *C. albicanus* (14.2 ± 0.8336). Effective antimicrobial activity is due to the presence of different bioactive components present in methanol extract. Previous studies reported the antimicrobial activities of various parts of extracts of *T. procumbens* L.[23 29].

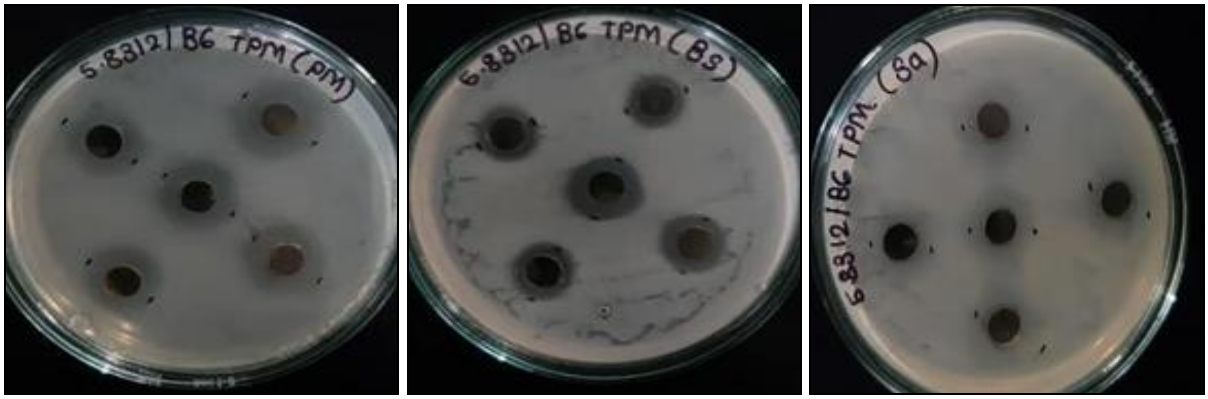


FIG. 3: THE ANTIMICROBIAL ACTIVITY OF TPM EXTRACT OF *T. PROCUMBENS* AGAINST MICROORGANISMS

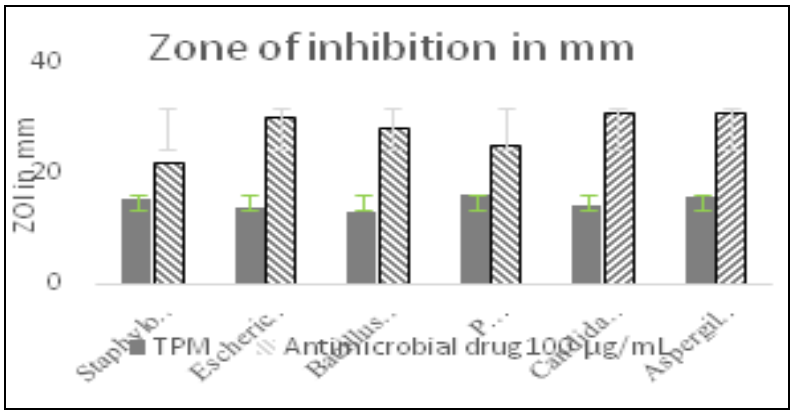


FIG. 4: ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACT BY AGAR WELL METHOD MEASURED ZONE OF INHIBITION IN DIAMETER. (VALUES ± SD; n = 5)

TABLE 2: ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACT (TPM)

Organisms	Inhibition zone in diameter (mm)		
	TPM	Streptomycin	Fluconazole
Proteus mirabilis (NCIM2388)	16.2 ± 0.8366	25	NA
Escherichia coli (ATCC 35218)	13.8 ± 1.0954	30	NA
Bacillus subtilis (NICM2063)	13.0 ± 1.2247	28	NA
Staphylococcus albus (NICM2178)	15.4 ± 1.6335	22	NA
Candida albicanus (NCIM 3100)	14.2 ± 0.8336	NA	31
Aspergillus niger (ATCC504)	15.8 ± 0.8360	NA	31

The concentration of Extract and Reference =80 µg/well; NA: Not Applicable. (Values ± SD; n = 5)

Antibacterial Activity of TPMG Oil: The results of the MIC using the broth dilution method of TPMG oil are presented in **Fig. 5** and **6**.

The fraction TPMG demonstrated bactericidal activity at the MIC of 125 µg/mL and 250 µg/mL against *S. aureus* and *P. aeruginosa* respectively.

The growth of biofilm of *S. aureus* and *P. aeruginosa* was markedly reduced by TPMG oil. At MIC values, the TPMG oil demonstrated considerable antibacterial activity. At all used doses, TPMG oil exhibited efficiency against both selected bacteria.

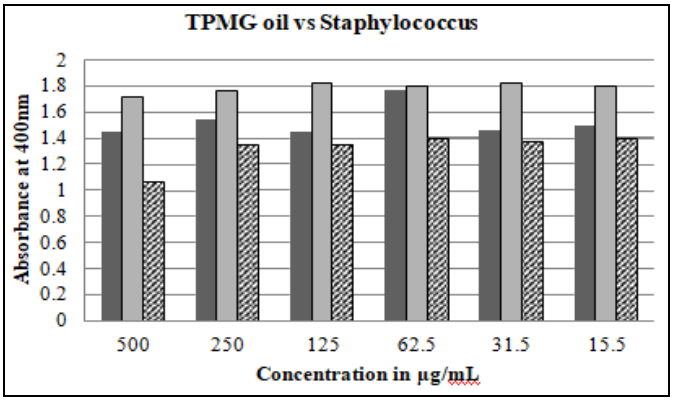


FIG. 5: ANTIBACTERIAL ACTIVITY FOR DETERMINATION OF MIC OF TPMG OILS AGAINST *S. AUREUS*

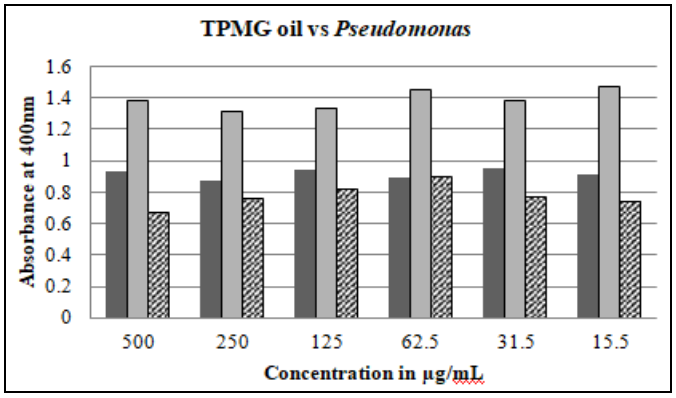


FIG. 6: ANTIBACTERIAL ACTIVITY FOR DETERMINATION OF MIC OF TPMG OIL AGAINST *P. AERUGINOSA*

Antioxidant Activity of Fraction TPMG Oil: Plant antioxidants have been shown to effectively protect plant cells from free radicals. The antioxidant properties of plant species should be evaluated using a variety of techniques due to the complex nature of phytochemicals [30]. In the present study, the DPPH method was used to evaluate the antioxidant capacity of TPMG oil from *T. procumbens*. **Fig. 7**. shows plots of the percentage DPPH antioxidant activity of TPMG oil against its concentration. Gallic acid and ascorbic acid were used as standard references. The result shows that DPPH radical scavenging percentage inhibition varies linearly with concentration from 100 - 500 µg/mL. The IC_{50} value for the essential oil was 18.34 µg/mL. Significant antioxidant activity at $P < 0.05$. TPMG oil showed an antioxidant activity that could prevent and slow the progression of aging in various diseases associated with oxidative stress.

The presented findings suggest that the TPMG oil has a high antioxidant capacity towards the characteristics of methanol extract antioxidants. In the majority of investigations of *T. procumbens*, extracts in methanol ethanol, and ethyl acetate reported high antioxidant capacity, demonstrating a clear correlation between the contents of secondary metabolites such as oils and fatty compounds in the plant [6, 29].

TABLE 3: DPPH ANTIOXIDANT ASSAY OF TPMG OIL (VALUES ± SD; N = 3; SIGNIFICANCE AT P < 0.05 COMPARED WITH POSITIVE CONTROL ASCORBIC ACID AND GALLIC ACID)

	Mean Absorbance			
Concentrationµg/mL	Control	TPMG oil	Ascorbic acid	Gallic Acid
31.5 µg/mL	0.499 ± 0.023	0.415 ± 0.001	0.441 ± 0.006	0.288 ± 0.01
62.5 µg/mL	0.499 ± 0.023	0.425 ± 0.002	0.389 ± 0.004	0.149 ± 0.008
125 µg/mL	0.499 ± 0.023	0.402 ± 0.004	0.378 ± 0.008	0.131 ± 0.003
250 µg/mL	0.499 ± 0.023	0.371 ± 0.003	0.357 ± 0.003	0.101 ± 0.002
500 µg/mL	0.499 ± 0.023	0.368 ± 0.007	0.34 ± 0.004	0.061 ± 0.008

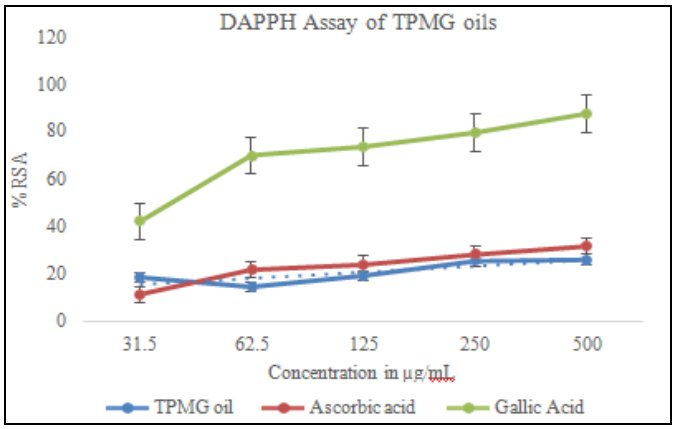


FIG. 7: RADICAL SCAVENGING ACTIVITY OF TPMG OIL

CONCLUSION: In this study, the chemical composition, antibacterial, and antioxidant activities of essential oils, and antibacterial activity of methanol extract *T. procumbens* L were investigated. However, isolated oil was found effective against Gram-positive *S. aureus* and Gram-negative *P. aeruginosa* bacteria. Moreover, oil showed significant antioxidant activity. Methanol extract demonstrates significant MIC at very low concentrations. The TPMG oil or extract can be used as a natural source of antimicrobial agents and fungi causing infections like wound infections endocarditis, sinus infections, and other inflammatory infections. The extracted oil has a pleasant scent. It showed good antioxidant and antimicrobial component that supports the traditional use of *T. procumbens* L. against wound healing and other infections. This plant will boost global acceptance as herbal medicine and can be used in cosmetics, perfumes, and many pharmaceutical applications.

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