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Bio-efficacy and Radical scavenging activity of *Terminalia paniculata*

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Abstract

The aim of this study is to qualitatively screen the phytochemicals, to assess antimicrobial and radical scavenging activity and to analyse the fingerprints of bioactive compounds by GCMS (Gas Chromatography and Mass Spectroscopy) in methanolic extract of *Terminalia paniculata*. Secondary metabolites including phenols, tannins, saponins and glycosides were present. The antimicrobial potential of methanolic extract of *Terminalia paniculata* by agar well diffusion assay showed maximum activity. The MIC (Minimum inhibitory concentration) of *Terminalia paniculata* by 96 well plate method ranged from 1.255×10^{3}µg/mL to 5×10^{3}µg/mL. The extract was screened for anti-oxidant activities by free radical scavenging activity DPPH assay (IC_{50}value=3.499µg/mL), ABTS assay *T. paniculata* (IC_{50}value=3.406µg/mL). In Ferric reducing antioxidant power (FRAP) assay *Terminalia paniculata* exhibited higher reducing ability. The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid as a standard. Total phenolic contents in the extracts were determined spectrophotometrically according to Folin-Ciocalteu method. Gallic acid was used to set up the standard curve. Extract *Terminalia paniculata* demonstrated the highest phenolic content of 88.043 mg GAE/g and the GC-MS analysis was performed to evaluate the chemical constituents in the plant methanolic extract of *Terminalia paniculata*. With regard to the results the methanolic extract of *Terminalia paniculata* leaves could be an important source for the discovery of new plant-based drugs. Further research is needed for the identification and purification of chemical compounds and proving the efficacy of medicinal plants used by local people.

Keywords: *Terminalia paniculata*; Gas Chromatography and Mass Spectroscopy (GC-MS); Anti-oxidant activity; Free radical and Anti-microbial activity

1. Introduction

Medicinal plants have been used for centuries as a remedy for various human diseases. These plants owe their therapeutic power to substances, which they contain. For the evaluation of the biological activity of these plants, it is imperative to use appropriate biological tests and chemical screening methods [1]. The knowledge and the progress of the medical benefits of herbs have grown in both, developing and developed countries. Medicinal herbs have constituted the basis of alternative medicine and lead to be the main pathway for conceptualizing new drugs [2].

Natural products have been traditionally accepted as remedies for many diseases. The beneficial medicinal effects of plant products typically result from the combinations of secondary metabolites present in the plants. Plant extracts have been known since antiquity to possess notable biological activities, including antibacterial, antioxidant, and anticancer properties [3]. Therefore, there is a lot of on-going research on such substances for their potential usefulness as dietary supplements and as adjuvants for use in the therapeutic management of free radicals related disorders. And the importance of searching for and exploiting natural antioxidants, especially of plant origin, has increased greatly in

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recent years [4]. During the search of plants as a source of natural antioxidants having anti-mutagenic and anticancer potentials, some medicinal plants and fruits have been extensively investigated [5].

The present study aims to determine the bio efficacy and radical scavenging activity of *Terminalia paniculata*, an ethno-medicinal plant. It involves collecting taxonomic characters, extracting crude compounds, screening phytochemicals, assessing antimicrobial and radical scavenging activity, and analyzing bioactive compounds fingerprints using GCMS. The extracts from medicinal plants exhibit antimicrobial, anti-inflammatory, and antioxidant activities, potentially inhibiting bacteria, fungi, viruses, and protozoa growth and potentially treating resistant microbial strains. The study also aims to analyze the fingerprints of bioactive compounds in the plants extract [6].

2. Materials and Methods

2.1. Collection and identification of plant samples

Healthy plant samples were collected aseptically, photographed, documented, and placed in paper bags. Taxonomic features were identified using standard floras and manuals, and documented in the laboratory [7].

2.2. Preparation of plant materials

Plant samples were thoroughly washed, dried, and pulverized in a mechanical grinder to obtain a fine powder. The pulverized materials were stored in zip lock polythene covers until used for extraction [8].

2.3. Extraction of Phyto-compounds from plants

The crude compound from plants was extracted using cold maceration. The pulverized plant material was immersed in methanol and agitated for 48-72 hours. The solvent-containing phyto-compounds were separated and filtered. The solvent was evaporated, and the crude extract was dried to obtain a persistent weight. The physical properties of the extract were examined, and the percentage yield was calculated. The dried extract was stored in a refrigerator for further use [8].

$$\text{Yield of crude extract } (\%) = \frac{\text{Weight of the plant crude extract}}{\text{Weight of the pulverized plant material taken}} \times 100$$

2.4. Qualitative screening of secondary metabolites in plants extract

The groups of secondary metabolites in plants extract were determined by implementing the standard protocol of qualitative tests of Phyto-chemicals [9].

2.4.1. Test for Phenols (Ferric Chloride Test)

The Ferric Chloride Test indicates the presence of phenols when 2 mL of plant extract is treated with 5% $FeCl_3$ solution, resulting in a deep blue or black color.

2.4.2. Test for Tannins (Gelatin Test)

A mixture of plant extract, gelatin, and 10% NaCl was mixed, resulting in a white precipitation indicating the presence of tannins.

2.4.3. Test for Alkaloids (Mayer's Teat)

To the 2 mL of extract, 2 mL of Mayer's reagent was added. An organic dull white or cream color precipitate indicates the presence of alkaloids.

2.4.4. Test for Flavonoids (Alkaline Reagent Test)

About 2 mL of plant extract was treated with 20% NaOH solution. A formation of intense yellow colouration confirms the presence of flavonoids.

2.4.5. Test for Terpenoids (Salkowski's Test)

The presence of terpenoids was confirmed by adding 2 mL of extract, 2 mL of chloroform, and 2 mL of concentrated H_2SO_4 to the mixture, resulting in a reddish-brown monolayer colouration.

2.4.6. Test for Steroids (Liebermann-Burchard Test)

The presence of steroids was detected by adding 2 mL of acetic anhydride to 0.5gm of plant extract and 2 mL of concentrated H_2SO_4, resulting in a color change from violet to blue or green.

2.4.7. Test for Saponins (Foam Test)

The plant extract was agitated with 20 mL of distilled in a graduated cylinder for 15minutes. The formation of foam layer about 1cm indicates the presence of saponins.

2.4.8. Test for Glycosides (Keller-Killiani Test)

The presence of glycosides was detected when 2 mL of plant extract was treated with glacial acetic acid, $FeCl_3$ solution, and concentrated H_2SO_4, resulting in a dark brown color ring.

2.4.9. Test for Protein and Amino acids (Ninhydrin Test)

To 2 mL of plant extract, 3-5 drops of freshly prepared 2% ninhydrin reagent was added and heated on water bath. The reaction mixture turns to blue color confirms the presence of proteins and amino acids.

2.4.10. Test for Carbohydrates (Fehling's Test)

To 2 mL of extract, equal volume Fehling's solution A and Fehling's solution of B was added and then heated on boiling water bath. The formation of brick red precipitate indicates the presence of carbohydrates.

2.5. Antimicrobial activity of methanol extract of plants

2.5.1. Agar well diffusion assay

The study investigated the effectiveness of a methanolic extract of plants in inhibiting pathogenic microorganisms using an Agar well diffusion assay. The extract was inoculated onto Mueller Hinton Agar and Sabouraud's Dextrose Agar plates and a concentration of different plants extracts was added to the plates. The zone of inhibition around the wells was measured, indicating the compound's antimicrobial activity. The larger the zone, the more effective the extract was in inhibiting the pathogens [10].

2.5.2. Minimum Inhibitory Concentration (MIC) of plants extract

The study tested the effectiveness of plants extracts in inhibiting pathogenic microorganisms using a modified resazurin 96-well micro titre plate broth dilution assay. The extract was diluted in a concentration range of 5000 to 9.76≈10µg/mL for bacterial pathogens and 10,000 to 19.53≈20µg/mL for fungal pathogens. The MIC value of the pigment extract was determined by incubating the extract with resazurin dye for 1-2 hours. The lowest concentration without a color change was considered the MIC value [10].

2.6. Antioxidant activity of plants extract

2.6.1. $DPPH^+$ assay

The 1,1-Diphenyl-2-picrylhydracyl ($DPPH^+$) radical scavenging activity of plants extract was performed as described by Baliyan et al. [11]. The assay involved dissolved plant extract and ascorbic acid in methanol, with DPPH radical solution added to each tube. The reaction mixture was incubated at 37°C for 30 minutes, with methanol used as a control. The effectiveness of plant extract to scavenge the $DPPH^+$ radicals was determined by using the bellow equation.

$$DPPH^+ \text{ radical scavenging activity } (\%) = \frac{(Ac - As)}{Ac} \times 100$$

Where, Ac is absorbance of the control and As is the absorbance of the tested sample.

2.6.2. ABTS•+ assay

The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS•+) radical scavenging activity of the plants extract was performed [12].

The assay involved mixing 0.2 mL of methanol-prepared plant extract with 1.8 mL of ABTS•+ radical solution, using ascorbic acid as a reference standard, and measuring optical density at 734nm. The percentage of ABTS•+ free radicals scavenged was calculated by using fallowing formula

$$\text{ABTS}^{\bullet+} \text{ radical scavenging activity (\%)} = \frac{(A0-A1)}{A0} \times 100$$

Where, A0 is absorbance of the control and A1 is the absorbance of the tested sample.

2.6.3. FRAP assay

Ferric reducing power Assay (FRAP) of plants extract was determined [13], A mixture of plant extract, phosphate buffer, and potassium ferricyanide was mixed and incubated at 50°C for 20 minutes. After cooling, trichloroacetic acid and ferric chloride were added, and absorbance was measured at 700 nm against a blank, with ascorbic acid as the standard. An increase in the absorbance with increase in concentration of plant extract/standard indicates the increasing capacity of ferric ion reducing power of plant extract.

2.6.4. Total antioxidant activity

The total antioxidant activity of plants extract was determined by phospho-molybdenum assay as described by Chaves et al. [12]. Plant extract was mixed with phospho-molybdenum reagent, incubated at 95°C for 90 minutes, and absorbance measured against methanol, using ascorbic acid as a standard.

2.6.5. Total phenolic content in the plants extract

The total phenolic content of the plants extract was determined by using Folin-Ciocalteu (FC) method. The study involved mixing plant extract with FC reagent, adding sodium carbonate solution, and incubating tubes for 30 minutes. Gallic acid concentration was used as a standard. The total phenolic content of the plants extract will be express as gallic acid equivalent in mg/g (GAE mg/g extract) [14].

2.7. GC-MS (Gas Chromatography and Mass Spectroscopy) analysis of plants extract

The array of Phyto-compounds present in the plants extract was determined by GC-MS analytical technique. GC-MS analysis on plants extract was performed using a Shizuzu QP 2010 Plus instrument. The RtxR-5 column was used and the sample was filtered using a Whatman filter paper No 1. The ion sources were maintained at 250°C and the mass spectrum of compounds was obtained by ionization of electrons at 70eV. The procedure took 22.5 minutes, including a three-minute solvent delay, and involved 30 and 500 Da atomic units [15].

3. Results

3.1. Area of sampling

The plant samples were collected from Kuvempu University at Shankaragatta (13°35'48.3"N 75°49'21.3"E) of Shivamogga district (Figure 1).

Figure 1 Location and geographical area of sampling plots

3.2. Identification of plant samples by morphological characters

Terminalia paniculata Roth. (TP), which belongs to Combretaceae family, is a tropical tree with a large natural distribution in Western Ghats, India [16] and taxonomic classification was summarized in Table 1. The tree is commonly known as Kindal Golden coloured flowers and deep red colored fruits of *T. paniculata* give splendid colouration to the entire forest area due to the formation of a large number of flowers and fruits in a single individual and population dominance (Figure 2) [17].

Figure 2 *Terminalia paniculata*

3.3. Physical properties and yield of the extract

The crude methanolic extract of *Terminalia paniculata* was yellowish-brown in color, yield and percentage of the obtained crude extract was 1.7749g and 7.0996% for the dried plant powder.

3.4. Qualitative screening of secondary metabolites in plant extract

This work investigates the phytochemical characteristics of medicinal plants, encompassing bioactive components such as proteins, amino acids, alkaloids, flavonoids, terpenoids, phenols, and tannins. Qualitative phytochemical screening was used to confirm the presence of phenols, tannins, saponins and glycosides in the methanolic extract of *T. paniculata* (Table 1).

Table 1 Phytochemicals in *Terminalia paniculata* extract

Sl. No.	Phytochemicals	Plants Sample
01.	Phenols	Present
02.	Tannins	Present
03.	Alkaloids	Absent
04.	Flavonoids	Absent
05.	Terpenoids	Absent
06.	Steroids	Absent
07.	Saponins	Present
08.	Glycosides	Present
09.	Proteins/Amino acids	Absent
10.	Carbohydrates	Absent

3.5. Antimicrobial activity of methanol extract of plant

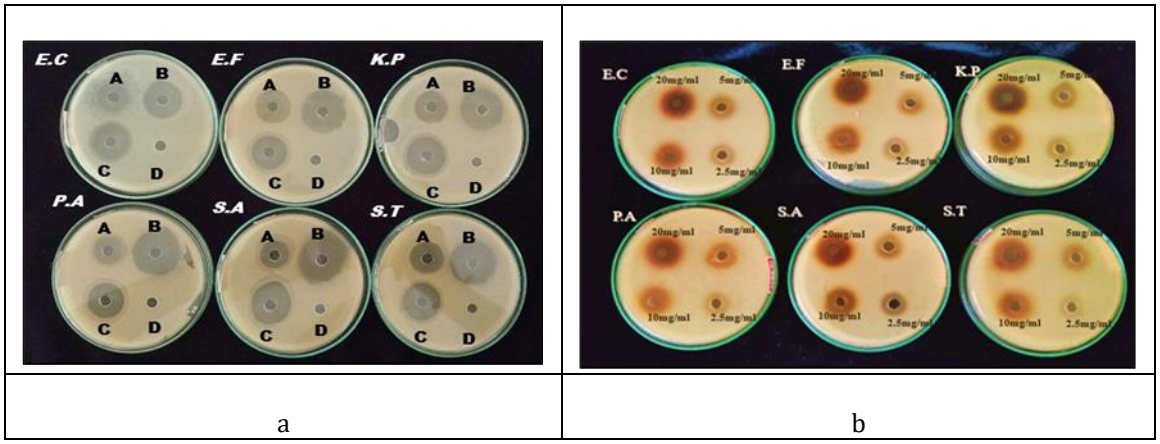
The study evaluates the antimicrobial potential of *Terminalia paniculata* leaves' methanolic extract against pathogenic bacterial and fungal strains was tabulated in table 2 and table 3. Results show *Terminalia paniculata*'s leaves exhibit antibacterial activity against both gram-positive and gram-negative bacteria (Figure 3 & 4).

Table 2 Antimicrobial activity of standard antibiotics and antifungal agents

Test Pathogenic Bacteria	**Antibacterial Activity of Standard Antibiotics(1mg/mL)**			**Negative control**
	Streptomycin	**Chloramphenicol**	**Ciprofloxacin**	**DMSO (10%)**
	Zone of inhibition in diameter (mm)			
E. coli	21	24	25	00
E. faecalis	23	22	28	00
K. pneumoniae	22	25	28	00
P. aeruginosa	19	23	30	00
S. aureus	19	17	29	00
S. typhi	22	24	30	00
Test Pathogenic Fungi	**Antifungal Activity of Standard Antifungal Agents(10mg/mL)**			**Negative control**
	Fluconazole		**Clotrimazole**	**DMSO (10%)**
	Zone of inhibition in diameter (mm)			
A. brasiliensis	20		17	00
C. albicans	13		11	00

Table 3 Antimicrobial activity of methanolic extract of *Terminalia paniculata*

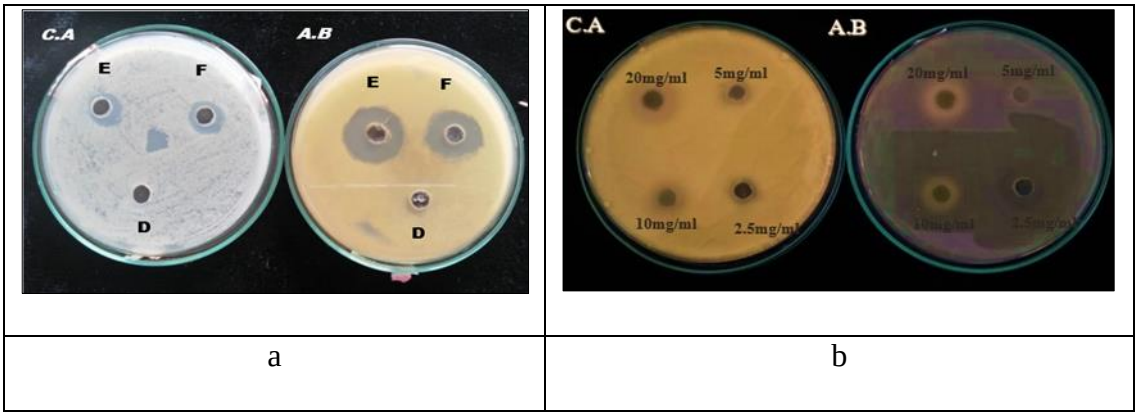
Test Pathogenic Bacteria Plant Extract (mg/mL)	**Antibacterial Activity of Plant Extract**			
	Terminalia paniculata			
	20	**10**	**05**	**2.5**
	Zone of inhibition in diameter (mm)			
E. coli	20	18	17	16
E. faecalis	19	19	16	15
K. pneumoniae	23	18	16	15
P. aeruginosa	24	19	18	16
S. aureus	23	19	16	16
S. typhi	24	20	19	16
Test Pathogenic Fungi Plant Extract (mg/mL)	**Antifungal Activity of Plant Extract**			
	Terminalia paniculata			
	20	**10**	**05**	**2.5**
	Zone of inhibition in diameter (mm)			
A. brasiliensis	23	21	19	16
C. albicans	19	17	12	10



a b

Figure 3 Antibacterial activity of (a) standard antibiotics (b) *Terminalia paniculata*

Note: A: Streptomycin; B: Chloramphenicol; C: Ciprofloxacin; D: DMSO (10%); E.C: *E. coli*; E.F: E. faecalis; K.P: *K. pneumonia*; P.S: *P. syringae*; S.A: S. *aureus*; S.T: *S. thypi*;



a b

Figure 4 Antifungal activity of (a) standard antibiotics (b) *Terminalia paniculata*

Note: D: DMSO (10%); E: Clotrimazole; F: Fluconazole. C.A: *C. albicans*; A.B: *A. brasiliensis*

3.5.1. Minimum inhibitory concentration (MIC) of plant extract

The MIC of *Terminalia paniculata*, a methanolic extract, was determined to be 1.255×103µg/mL, which effectively prevents visible growth of the test strain under controlled conditions.

3.6. Antioxidant activity of plants extract

3.6.1. DPPH+ assay

The study tested the free radical scavenging and hydrogen atom donating properties of plant extracts using DPPH radical scavenging assay. *T. paniculata* showed the highest antioxidant potential, while standard ascorbic acid had the lowest yield. *T. paniculata* exhibited the highest antioxidant potential (IC_{50}value=3.499µg/mL) and the antioxidant potential of standard ascorbic acid was found to be (IC_{50}value = 1.353 µg/mL) shown in Figure 5a.

3.6.2. ABTS•+ assay

The ABTS assay was used to determine the free radical scavenging activity of plant samples, revealing its sensitivity to antioxidant activity due to its faster reaction kinetics. In the present study *T. paniculata* exhibited the anti-oxidant potential of (IC_{50} value=3.406µg/mL) and standard (ascorbic acid) was found to be (IC_{50}value = 1.353 µg/mL) (Figure 5b).

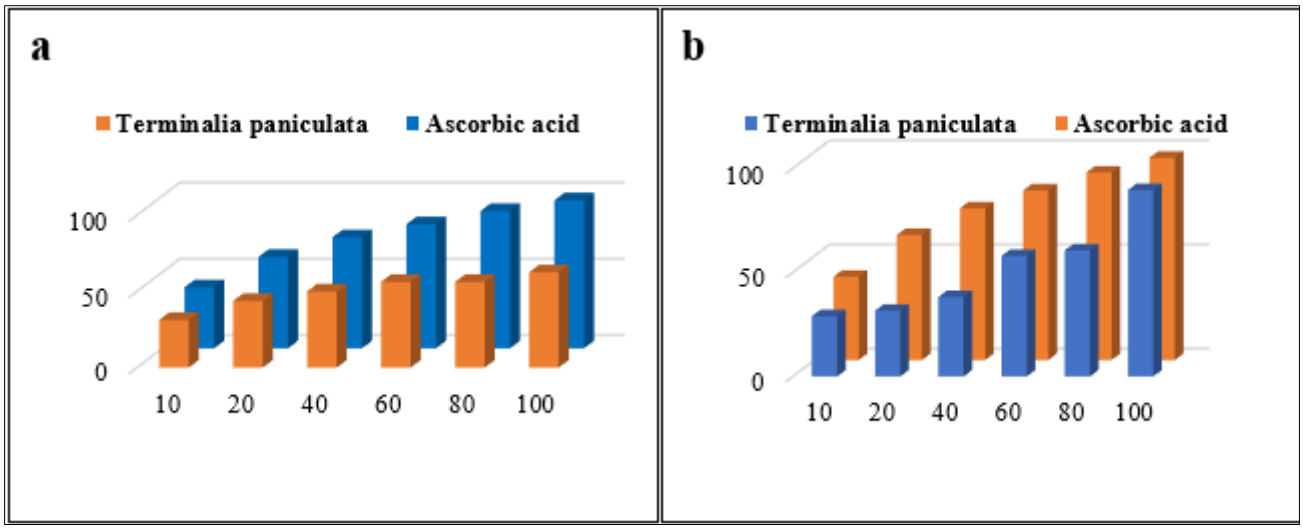


Figure 5 (a) DPPH⁺ radical scavenging activity of extracts and reference standard and (b) ABTS radical scavenging activity of extracts and reference standard

3.6.3. FRAP assay

This method is based on the principle of reduction of ferric tripyridyl-s-triazine complex (colour less complex) to ferrous coloured (blue coloured complex). Increased absorbance of the reaction mixture indicates increased reducing capacity. *Terminalia paniculata* exhibited higher reducing ability (Figure 6).

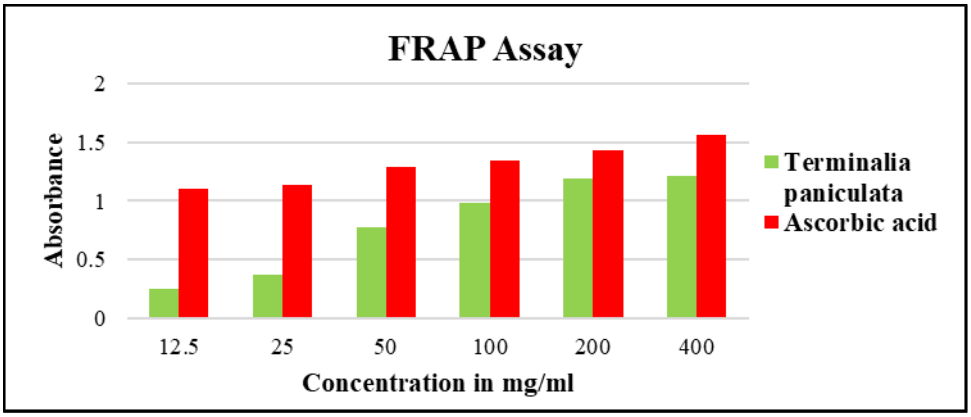


Figure 6 FRAP assay values of *Terminalia paniculata* with standard

3.6.4. Total antioxidant activity

The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid as a standard in this review the total antioxidant activity of methanolic extract of *Terminalia paniculata* were studied which exhibited minimum activity when compared to standard (ascorbic acid) (Figure 7). Similar results were recorded by Elghiet et al. [18] which demonstrated a bad correlation with flavonoids and phenolics content due to its ability to detect other compounds as ascorbic acid, carotenoids and α-tocopherol.

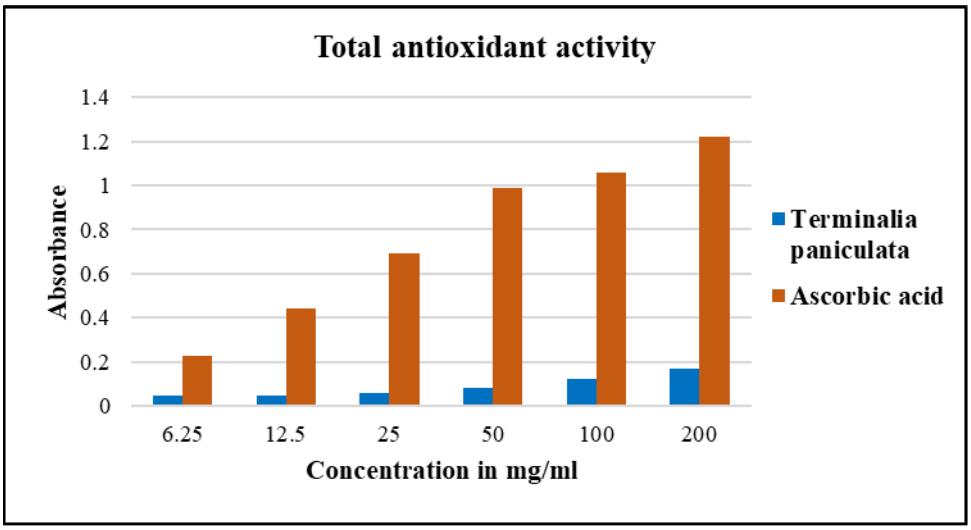


Figure 7 Comparison of Total antioxidant activity between *Terminalia paniculata* and standard

3.6.5. Total phenolic content in the plant extract

Total phenolic contents in the extracts were determined spectrophotometrically according to Folin-Ciocalteu method. Gallic acid was used to set up the standard curve. The content of phenolic compounds of the samples was expressed as gallic acid equivalents (GAE) in mg per gram dry weight and the absorbance was measured at 765 nm using a spectrophotometer. Extract *Terminalia paniculata* demonstrated the highest phenolic content of 88.043 mg GAE/g summarized in Figure 8a & 8b.

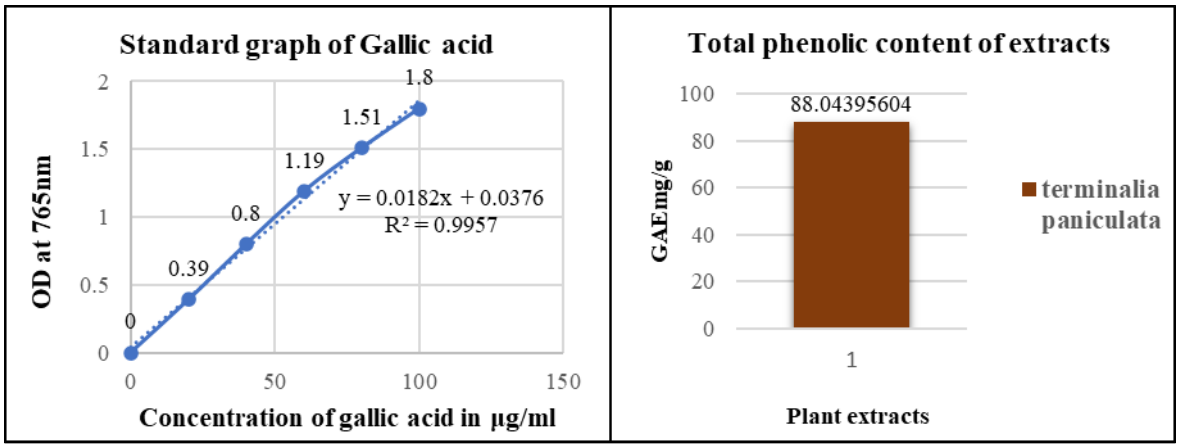


Figure 8 (a) Standard graph of Gallic acid (b) Total phenolic content of extract

3.7. GC-MS analysis of plants extract

The GC-MS Analysis of *Terminalia paniculata* was carried out for the identification of bioactive phytochemicals. The GC-MS chromatogram spectra obtained for the extract revealed that *T. paniculata* is plenteously rich in bioactive compounds in leaf (Figure 9). A total of 45 effective compounds were identified from the chromatogram. The bioactive compounds were predicted by their retention time (RT), peak area percentage (%) and molecular weight with the help of NIST Library summarized in Table 6.

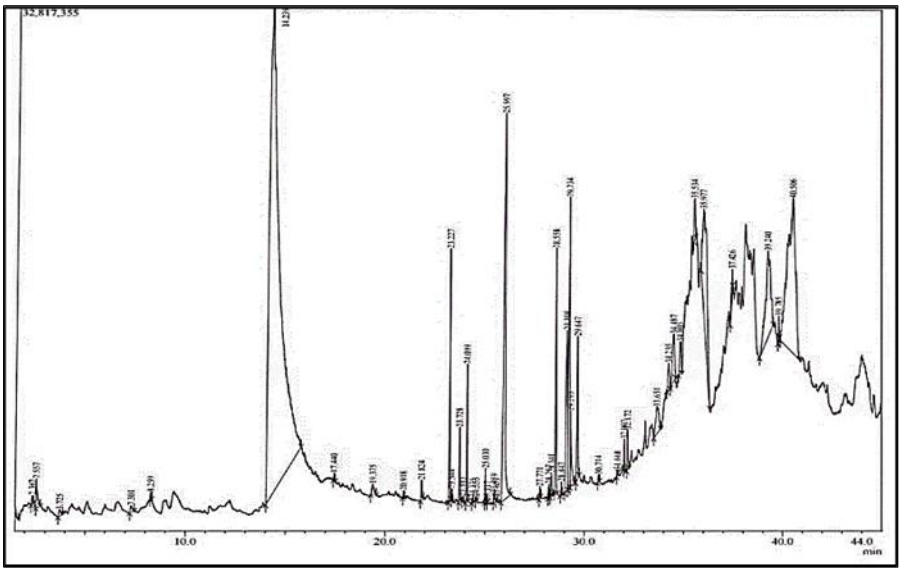
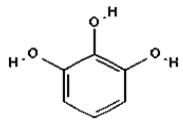
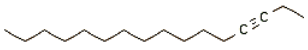
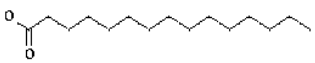
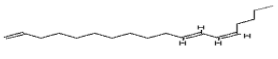
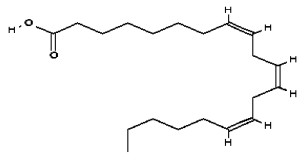
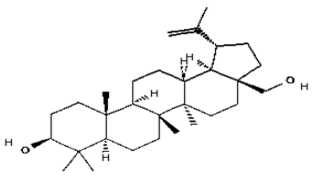


Figure 9 GC MS chromatogram of methanol extract of *Terminalia paniculata*

Table 5 Some of highest percentage of bioactive compounds present in *Terminalia paniculata* extract.

Sl. no	Compound name	Structure	Significance
	1,2,3-Benzenetriol		Trihydroxybenzene or more commonly known as benzenetriol are compounds that have three hydroxyl groups attached to a benzene ring.
	3-Hexadecyne		Hexadecane is a straight-chain alkane with 16 carbon atoms.. It has a role as a plant metabolite, a volatile oil component and a non-polar solvent
	Pentadecanoic acid		Pentadecanoic acid is a straight chain saturated fatty acid containing 15 carbon atoms. It has a role as a plant metabolite, a human blood serum metabolite and a food component. It is a conjugate of a pentadecanoate.
	Phytol		Phytol is an aromatic ingredient used in many fragrance compounds and it may be found in cosmetic and non-cosmetic products. n medicinal fields, phytol has shown antinociceptive and antioxidant activities as well as anti-inflammatory and antiallergic effects
	1,E-11,Z-13-Octadecatriene		Anti-inflamatory, antioxidant, anti tumor, and antimicrobial activity.

	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-		8,11,14-Eicosatrienoic acid is a 20-carbon-chain omega-6 fatty acid, unsaturated at positions 8, 11, and 14. is also known as Dihomo-gamma-linolenic acid (DGLA)
	Betulin		These compounds display anticancer, antiviral, antibacterial, antifungal, and anti-inflammatory properties, which have been described in the literature multiple times.

4. Discussion

Medicinal plants have been the most productive source of leads for the development of drugs from ancient times. Current research in drug discovery involves a multifaceted approach combining botanical, biological, and molecular techniques. Medicinal plant-based drug discovery continues to provide novel and important leads against several diseases. Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The chemical substances present in plants, which have defensive effects, are called phytochemicals. The phytoconstituents found in the plants, which are responsible for antioxidant potential, are mainly flavonoids, phenols, anthocyanin, iso-flavones, flavones, lignin's, catechins, iso-catechins, and coumarins. These phytochemical constituents are mainly determined by measuring total phenolic contents (TPC) and total flavonoid contents (TFC), and the antioxidant effects are determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP), and 2,2-azinobis-ethylbenzothiozoline-6-sulphonic acid (ABTS) assays.

The present study highlights the bio-efficacy and radical scavenging activity of the ethno-medicinal plant *Terminalia paniculata*. *Terminalia paniculata* Roth is a tropical tree with a large natural distribution in Western Ghats, India. Traditionally, flower juice and bark of *Terminalia paniculata* have been used as a remedy for cholera, for the treatment of inflamed parotid glands and in menstrual disorders. Terminalia's are especially known for cyclic triterpenes, flavonoids and tannins and *T. paniculata* is the best source of tannins. The tree is commonly known as Kindal.

The color of the crude methanolic extract of *T. paniculata* is yellowish brown in color and the yield of the obtained methanolic extract is 7.0996%. Phytochemical screening revealed the presence of phenols, tannins, saponins, glycosides but alkaloids, terpenoids, amino acids and steroids were absent.

The antimicrobial activity of the plant extract against some microbial pathogens were performed. The inhibitory activity of *Terminalia paniculata* was nearer to that of standard antibiotic values. Kumar et al. [7] stated that the ethanolic and methanolic extracts of the leaf showed good zone of inhibition at 50mg/mL and 75mg/mL in case of ethanolic extract and 25mg/mL and 50mg/mL in case of methanolic extract. In both the solvents even at 0mg/mL zone of inhibition was observed at 12, 24 and 48 hours with a marginal difference of 0.1 or 0.2 mm. This can be attributed to the inbuilt and natural antimicrobial and microbicidal nature of the ethanol and methanol solvent.

The minimum inhibitory concentration (MIC) in µg/mL of the plant extract was carried out by Broth dilution method. The minimum inhibitory concentration (MIC) as well as minimum bactericidal concentration (MBC) in case of the investigated plant that is *Terminalia paniculata* ranged to 1.25mg/mL.

The antioxidant potential of *T. paniculata* is (IC_{50}value=3.499µg/mL). The antioxidant potential of standard ascorbic acid was found to be (IC_{50}value= 1.353 µg/mL). The antioxidant potential was exhibited by *T. paniculata* was found to be (IC_{50}value=3.406µg/mL). The antioxidant potential of standard ascorbic acid was found to be (IC_{50}value = 1.353 µg/mL). The ABTS radical scavenging potential of different plant samples is presented. Polu et al. [19], stated that The IC_{50} values of extracts were found within the range of 4.17 ± 0.29-132.14 ± 1.14 µg/mL. n-butanol fraction shown a marked ABTS free radical scavenging activity with an IC_{50} values of 29.48 ± 2.23 µg/mL. Agrawal et al., (2011), stated in

the ABTS test, TP and ML had effective scavenging activity. The effects of various concentrations of ML, TP and standard (ASC) on the ABTS were found to be 98.41, 60.94 and 47.71%, respectively, at 25 µg/mL concentration There was a significant decrease ($p<0.01$) in the concentration of ABTS due to the scavenging capacity of TP and ML with ASC standard at 25 µg/mL concentration. The IC50 value of TP and ML were found to be 64.32 and 53.82 µg/mL.

The reducing power of Fe2+ by selected plants was evaluated in that *Terminalia paniculata* exhibited higher reducing ability followed by *Myristica fragrans* and *Tinospora cordifolia*. Agrawal et al. [4] stated that in reducing power assay, EETP showed 50 to 60% activity in comparison to ascorbic acid. The presence or absence of particular component(s) plays a major role in deciding the antioxidant property of medicinal plant.

The ability of the plant extracts to reduce ferric ions was determined by FRAP assay. *T. cordifolia* showed high activity compared to other samples. Premanath and Lakshmidevi [20] stated that the FRAP values for the extracts were lower than that of BHT (63±0.35 µm/g fw). Among the extracts tested, ethanol extract had a total anti-oxidant activity of 41.4±0.45 µm/g fw followed by methanol 33.9±0.49 µm/g fw. Aqueous extract had the least reducing ability of 4.8±0.30 µm/g fw.

The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid as a standard. In the presence of extracts, Mo (VI) is reduced to Mo (V) and forms a green colored phosphor molybdenum V complex. The absorbance was measured at 760 nm using a spectrophotometer.in this review the total antioxidant activity of methanolic extract of three plants were studied which exhibited minimum activity when compared to standard (ascorbic acid). Elghiet et al. [18] reported in their work specified that the assay demonstrated a bad correlation with flavonoids and phenolics content due to its ability to detect other compounds as ascorbic acid, carotenoids and α-tocopherol.

The total phenolic content of extracts ranges from 33.098 mg GAE/g to 88.043 mg GAE/g of Extract. *Terminalia paniculata* demonstrated the phenolic content of 88.043 mg GAE/g, these results co-related with the earlier studies of Rajashekar and Raju [21] according to their observation high content of phenols were present in methanol extracts of stem bark (21.7 mg/g), whereas low content was found in petroleum ether extracts of leaf (0.8 mg/g). Total flavonoid content was high in methanol extracts of stem bark (30.8 mg/g), while low content was found in petroleum ether of fruit (1.35 mg/g) extract. Total antioxidant content was high in methanol extract of stem bark (25.43 mg/g), low content was found in petroleum ether extracts of fruit (1.8 mg/g). These findings were reported earlier by Upadhyay et al. [22] on Tinospora cordifolia stated that, the ethanol stem extract having highest phenol content (66.28 ± 0.82 mg/g) also had the better scavenging or antioxidant activity when compared to methanolic stem extract.

The GC-MS Analysis of *Terminalia paniculata* was carried out for the identification of bioactive phytochemicals. The GC-MS chromatogram spectra obtained for the extract revealed that *T. paniculata* is plenteously rich in bioactive compounds in leaf. A total of 45 effective compounds were identified from the chromatogram. The bioactive compounds were predicted by their retention time (RT), peak area percentage (%) and molecular weight with the help of NIST Library. The methanolic fraction by GC-MS analysis was reported by Uthirapathy and Ahamad [23] according to their study 16 compounds were identified in chromatogram and the compounds were further analysed by Mass spectrometer and identified as 2- Furancarboxaldehyde, 5-(hydroxymethyl) (19.31 %). Mass spectrum of this compound is matched with mass spectral data from then the name & molecular formula of the compound has been obtained.

5. Conclusion

Therapeutic potential of medicinal plants as a source of noble natural anti-oxidants and anti-microbial agents has been well recognized all across the globe. In this study, phenolic compounds, in vitro anti-oxidant activity and anti-microbial properties have been investigated in methanolic extract of *Terminalia paniculata*. The study demonstrated that the selected medicinal plant possessed good antibacterial and antioxidant activity. The results of the present study showed that, the presence of various groups of phytochemicals in *T. paniculata* extracts are responsible for showing considerable antibacterial, antioxidant, and anticancer activities. The best results were obtained from *Terminalia paniculata* extract shows high phenolic concentration, very high FRAP, Antimicrobial activity. With regard to the results. The methanolic extract of *Terminalia paniculata* leaves could be an important source for the discovery of new plant-based drugs. Further research is needed for the identification and purification of chemical compounds and proving the efficacy of medicinal plants used by local people.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests.

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