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Research Article

ANTIBACTERIAL ACTIVITY OF DIFFERENT PHYTOCHEMICAL EXTRACTS FROM THE LEAVES OF *T. PROCUMBENS* LINN.: IDENTIFICATION AND MODE OF ACTION OF THE TERPENOID COMPOUND AS ANTIBACTERIAL

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ABSTRACT

The aim of the present study was to identify the bioactive compounds and to investigate the antibacterial activity from the leaves of *Tridax procumbens*. The antibacterial activity of *T. procumbens* phytochemical extracts was determined by agar disc diffusion and minimum inhibitory concentration (MIC) against *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, and *Vibrio cholerae.* The phytochemicals of the plant extract were analysed by thin layer chromatography (TLC) and antibacterial compounds were determined by TLC-bioautography. Furthermore, the structural elucidation of the antibacterial compound was confirmed by IR, Mass spectrum and NMR. Significant inhibitory activity was observed with terpenoid extract of the plant against the test bacteria while less antibacterial activity was observed in alkaloid, flavonoid and glycoside extracts. Terpenoid compound treated microbes resulted in the leakage of reducing sugars and proteins through the membrane. It also induced the activity of respiratory chain dehydrogenase. Therefore it was justified that terpenoid compound was able to destroy the permeability of bacterial membrane. We also concluded that the plants rich in terpenoids can be used as an alternative for bactericidal drugs.

Keywords: *T. procumbens*; Antibacterial activity; Terpenoid compound; TLC-bioautography

INTRODUCTION

In general, bacteria have the genetic ability to transmit and acquire resistance against the drugs used as therapeutic agents. One way to prevent antibiotic resistance is by using new compounds which are not based on the existing synthetic antimicrobial agents[1]. According to Zahid Zaheer et al.[2], antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Researchers are increasingly turning their attention to the medicinal plants and it is estimated that, plant materials are present in, or have provided the models for 25-50% Western drugs[3]. Many commercially proven drugs used in modern medicine was initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable in the treatment of various illness[4]. Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action may likely to differ[5]. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity [6, 7].

T. procumbens is a semi-prostrate annual or short-lived perennial herb. Leaves are membranous, scaberulous above, glabrate beneath, auricled at base, irregularly toothed. Flower heads have long stalk, Yellow hard, rounded, 2.4-3.9 cm across, often 2-5 clustered together in the axils of leaves or terminal. Involucral bracts are many, ovate-lanceolate, long pointed, purple, rigid and hairless. Receptacle bristles are very long. Petals are about 2 cm long, tubular, yellow in color. Anther tails are fimbriate. Achenes are curved, compressed *ca*. 8mm long, tip narrowed, with one rib on each face. The hard achene is covered with stiff hairs and having a feathery, plume like white pappus at one end. In the Indian systems of medicine (*Ayurveda, Siddha,* and *Unani*) *T. procumbens* is used either as a single drug or in combination with other drugs. Traditionally, it is used for the treatment of bronchial catarrh, dysentery, malaria, stomachache, diarrhoea, high blood pressure. It is also used to check haemorrhage from cuts, bruises and wounds and it prevents falling of hair. The leaf extract has been extensively used in Indian traditional medicine as anticoagulant, anticancer, antifungal and insect repellent[8]. Dexamethasone luteolin, glucoluteolin, β-sitosterol quercetin, β-sitosterol-3-O- β -D-xylopyranoside and flavonoid procumbenetin have been isolated from leaves and flower of *T. procumbens*[9]. The different concentrations of triterpenoid compound from the leaves of Compositae member (*Elephantopus scaber*) were investigated for the inhibitory effect on platelet aggregation invitro[10]. The main aim of this report was to evaluate the phytochemicals from the leaves of *T. procumbens*, to identify the antibacterial compound and its mode of action against the pathogenic bacteria. To the best of our knowledge, this is the first report on antibacterial activities of different phytochemical extracts and mode of action of the bacterial susceptibility compound from the leaves of *T. procumbens*.

MATERIALS AND METHODS

Plant material

Tridax procumbens leaves were collected during Feb-March 2009, from Sri Sairam Siddha Medical College and Research Centre, Tamilnadu, India. The plant was identified by the taxonomist. Collected plant material was air-dried under shade at room temperature, ground with an electric grinder into fine powder and stored in airtight containers.

Bacterial strains

Microorganisms used for the determination of antibacterial activities of isolated compounds were Gram positive; *Staphylococcus aureus* MTCC 29213, Gram negative; *Escherichia coli* MTCC 25922, *Proteus mirabilis* MTCC 13315, *Vibrio cholerae* MTCC 12657. All bacterial strains were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Sector 39-A, Chandigarh – 160036, India. Different bacterial strains were maintained on nutrient agar and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring two to three colonies into a tube containing 20 ml nutrient broth and grown overnight at 37 °C.

Phytochemical analysis of the plant extract

The extract was subjected to phytochemical tests for plant secondary metabolites such as, tannins, saponins, flavonoids, alkaloids, terpenoids and glycosides in accordance with Trease and Evans[11] and Harborne[12] with little modification.

Extraction of phytochemicals from the leaves of *T. procumbens*

Extraction of Alkaloid

Ground leaf material was extracted with cold distilled methanol (CH_3OH) with occasional swirling. After filtration, the solvent was removed under reduced pressure at 40 °C, to minimise any thermal degradation of the alkaloids. The crude alkaloid mixture was then separated from neutral and acidic materials, and from water solubles, by initial extraction with aqueous acetic acid (CH_3CO_2H) followed by dichloromethane. Then basification was done on the aqueous solution and further the organic layer of dichloromethane contained crude alkaloid extract[13].

Extraction of Terpenoids

Ground leaves were extracted with hot (60 °C) 95% EtOH. After filtration, the dark green solvent was evaporated to dryness under reduced pressure at 40 °C. The residue was partitioned between H_2O and CHCI. The organic layer was separated and condensed to yield dark green syrup. The CHCI, extract was then partitioned between hexane and 10% aq. MeOH. The aq. MeOH extract was then used for antibacterial activity as terpenoid extract[14].

Extraction of Flavonoids

The dried leaf powder of *T. procumbens* was defatted with petroleum ether (40-60 °C). The extract was then percolated with methanol until exhaustion at 40 °C by rotary evaporator. The condensed material was partition using ethyl acetate. This ethyl acetate extraction contained crude flavonoids[15].

Extraction of Glycosides

Dried leaf powder of the plant was extracted three times with methanol at 25 °C for 24 hours and then concentrated in vacuo. The extract was washed with n-hexane and then the methanol layer was further concentrated to a gummy mass. The later was suspended with water and extracted with equal volume of ethyl acetate to give glycosides extract of the plant[16].

Analysis of Isolated Compound

^1H NMR and ^{13}C NMR spectra were recorded at 399.952 and 100.577 MHz, respectively, on a Varian UNITY-400 spectrometer and with $CDCl_3$ and $(CD_3)_2CO$ as solvent. The resonances of residual $CHCl_3$ and C_3H_6O at δH 7.25 and 2.04 and signals of $CDCl_3$ and $(CD_3)_2CO$ δc 77.0 and 206.0, respectively were used as internal reference for ^1H and ^{13}C spectra. Mass spectra were obtained using a VG 1250 or a Kratos MS80 RFA instrument at 70 eV. The IR spectra were recorded on a BioRad FTS-7.

Antibacterial activity of four different phytochemical extracts of *Tridax procumbens* tested against pathogenic bacteria

Agar disc diffusion assay

The antibacterial activity was studied using the disc-diffusion method[17]. Bacteria were grown overnight on Muller Hinton agar plates. Five young colonies were suspended with 5ml of sterile saline (0.9%) and the density of the suspension adjusted to approximately 3×10^8 colony forming units (CFU). The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90 ° to ensure an even distribution of the inoculums. The medium was allowed to dry for about 3 min before adding a sterile paper disc of 5 mm diameter. Each disc was tapped gently down onto the agar to provide uniform contact. Phytochemical extracts (50µg) were weighed and dissolved in 1ml of 7% Methanol. 5, 10, 15 and 20 microlitres of the compounds were introduced on each disc (five replicates) and 7% methanol alone served as a negative control. The plates were incubated at 37 °C for 24 h; inhibition zones were measured and calculated.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the isolated compounds was determined by dilution method[18]. The strains were grown in Mueller Hinton broth to exponential phase with an *A*560 of 0.8, representing 3×10^8 CFU/ml. Different dilutions of the *T. procumbens* phytochemical extracts were prepared to give concentrations at 5, 10, 15 and 20 µg/ml respectively. 0.5 ml of each concentration was added into separate test tubes containing 4ml of MH broth inoculated with 0.5 ml bacterial suspension at a final concentration of 10^8 CFU/ml. Each MIC was determined from five independent experiments performed in duplicate. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% methanol were used as bacterial controls, 4.5 ml of uninoculated Mueller Hinton broth and 0.5 ml PBS served as a blank. The tubes were incubated at 37 °C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at *A*560 nm.

Bioautography on TLC plates

The bioautography of four different phytochemical leaf extracts of *Tridax procumbens* were studied using *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Vibrio cholerae* as test microorganisms. Overnight bacterial cultures were centrifuged at 3000 rpm for 10 min, the pellets redissolved in 10 ml of fresh Mueller–Hinton agar (Hi-Media). Developed TLC plates were sprayed with these cultures and incubated overnight at 37 °C in 100% relative humility[19]. After incubation, the plates were sprayed with a 2 mg/ml iodonitrotetrazolium chloride (INT) and incubated at 37 °C for 6 h. Active compounds were localized by comparing the zones of bacterial growth inhibition (white spots against a violet TLC background) with a duplicate TLC developed under identical conditions as described by Reid et al.[20].

Effect of *T. procumbens* compound on the leakage of membrane of in pathogenic bacteria

A volume of 10 ml culture containing 10^8 CFU/ml of the four pathogenic bacteria were inoculated into 5,10, 15 and 20 µg/ml *T. procumbens* compound containing MH medium and were incubated at 37± 2 ºC, shaken at 150 rpm for 18 hours. The samples were centrifuged at 12,000 rpm and the supernatant were collected and frozen at -30 ºC. The concentrations of reducing sugar and proteins were determined using Miller[21]; and Braford[22] methods.

Effect of *T. procumbens* compound on the respiratory chain dehydrogenase enzyme activity in pathogenic bacteria

The enzyme activity was determined by iodonitrotetrazolium chloride method with slight modifications[23, 24]. In different concentrations (5, 10, 15 and 20 µg/ml) of *T. procumbens* compound containing MH medium, 10 ml culture containing 10^8 CFU/ml of the four pathogenic bacteria were inoculated and incubated at 37±2 ºC, shaken at 150 rpm for 18 hours. Pathogenic cells boiled for 20 minutes were used as negative control while non pathogenic cells were observed as positive control. A volume of 1 ml of each culture was collected and centrifuged at 12,000 rpm. Then the precipitate was collected and washed by phosphate buffered saline (PBS) twice and suspended with 0.9 ml PBS. A volume of 0.1 ml of 5% INT (Iodonitrotetrazolium) solution was added to the suspension and incubated at 37± 2 ºC in dark for 2 h and then 50 µg/ml formaldehyde was added to terminate the reaction. The culture was again centrifuged and the bacteria were collected by discarding the supernatant. 250 µl acetone and ethanol in 1: 1 ratio were added to distill INF (Iodonitrotetrazolium formazan) twice. The supernatant was measured spectrophotometrically at 480 nm.

RESULTS AND DISCUSSION

The phytochemical screening of the *T. procumbens* studied showed the presence of alkaloids, flavonoids, glycosides and terpenoids (Table-1). The phytochemical screening revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), in *T. procumbens*[25]. The presence of terpenoid was reaveled by Ali and jahangir[26] in their study on a bis-bithiophene from *T. procumbens*.

Antibacterial activities of different phytochemical extract from the leaves of *T. procumbens* against selected pathogenic bacteria

The susceptibility of bacteria towards the plant phytochemical extracts was assessed. All the four phytochemical extracts were tested on selected Gram positive and negative bacteria. The screening showed a broad spectrum of antibacterial activity towards all the bacterial strains. But the terpenoid extract alone exhibited more inhibition activity when compared with the other phytochemicals. The terpenoid extract was more promising against *S. aureus, E. coli* and *V. cholerae,* when compared with *P. mirabilis* (Table 2). Antibacterial natural products can be classified according to a general biogenetic source, such as terpenoids, alkaloids, flavonoids and simple phenols. One of the most active compounds is the triterpenoids, which comprises different types of compounds which can be further divided into more important chemical structure groups. The main groups of triterpenoids are represented by tetracyclic and pentacyclic derivatives. Pentacyclic triterpenoids are all based on a 30-carbon skeleton comprising five six-membered rings (ursanes and lanostanes) or four six-membered rings and one five-membered ring (lupanes and hopanes)[27]. This result was comparable to the preliminary study on the pentacyclic terpenoids like α-amyrin, betulinic acid and betulinaldehyde against clinical isolates of methicillin resistant strains of *Staphylococcus aureus* that showed inhibition at concentrations in the range of 8 to 32 μg/ml[28].

Table 1: Phytochemical screening of leaf extract of *T. procumbens*

S/No.	Phytochemical Constituents		Observation	Methanol extract of *T. procumbens*
1	Alkaloids	Dragendorff's test	Orange / red precipitate	+
		Mayers test	Yellowish pricipitation	
2.	Flavonoids Alkali Reagent		Intense yellow colour	+
3.	Glycosides Bornbager's test		Pink colour (Ammonia layers)	+
4.	Tannin $FeCl_3$ test		Violet colour	--
5.	Saponins Frothing test		Foam	--
6.	Terpenoids Nollers test		Purple colour to Red	+
7.	Anthraquinones Benzene Ammonia Test		Pink colour	--

-- = Negative (absent); + = Positive (present)

Table 2: Antibacterial activity of different phytochemical extracts against bacterial species tested by disc diffusion assay

Phyto chemical extracts of *T. procumbens*	*Escherichia coli*				*Vibrio cholerae*			
	5μg/ml	10μg/ml	15μg/ml	20μg/ml	5μg/ml	10μg/ml	15μg/ml	20μg/ml
Alkaloid extract	11.5±0.5	13.2±0.25	15.5±0.5	17.2±0.25	9.1±0.32	12.5±0.50	14.3±0.57	16.1± 0.45
Glycoside extract	7±0.5	8.9±0.51	10±0.28	12.5±0.5	8.2±0.76	11.6±0.60	12.1±0.40	13.9 ± 0.45
Flavonoid extract	9.1±0.56	10.4±0.61	12.1±1.00	13.9±0.54	7.8±0.79	9.2±0.47	11.53±0.50	13.10± 0.75
Terpenoid extract	13.7±0.62	16.3±0.72	20.9±0.83	23.7±0.32	12.5±0.45	15.1±0.37	19.4±0.89	23.9± 0.7

Phyto chemical extracts of *T. procumbens*	*Staphylococcus aureus*				*Proteus mirabilis*			
	5μg/ml	10μg/ml	15μg/ml	20μg/ml	5μg/ml	10μg/ml	15μg/ml	20μg/ml
Alkaloid extract	10.1±2.8	12.83±0.76	14±0.5	16.1±0.28	9.6±0.57	11.5±0.86	13.8±0.76	16.5±0.5
Glycoside extract	8.1±0.5	10.2±0.76	12.4±0.6	13.9±0.5	8.3±0.81	9.8±1.17	10.9±0.28	12.1±0.36
Flavonoid extract	9.3±0.40	11.6±0.55	13.2±0.45	14.3±0.5	10.03±0.37	12.05±0.45	13.9±0.5	15.4±0.36
Terpenoid extract	14.03±0.55	16.9±0.36	21.3±0.35	24.6±0.36	11.7± 0.70	13.9±0.72	17.6±0.77	21.8±0.72

*The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SD of three replicates.

Phytochemical extracts thus obtained were further subjected for determination of minimal inhibitory concentration by two-fold micro broth dilution method against the bacteria studied. Table-3 indicates that the terpenoid extract was found to be most significant inhibitor than the other extracts. MIC of this extract showed gradient value against the concentration used to inhibit the bacteria. Furthermore, gram positive bacterial species was found most sensitive as compared to gram negatives.

Table 3: Minimal Inhibitory Concentration (MIC) of different phytochemical extracts against bacteria

Phyto chemical extracts	*Escherichia coli*				*Vibrio cholera*			
	5μg/ml	10μg/ml	15μg/ml	20μg/ml	5μg/ml	10μg/ml	15μg/ml	20μg/ml
Alkaloid extract	0.610±0.01	0.393±0.02	0.312±0.01	0.185±0.01	0.694±0.01	0.560±0.02	0.333±0.05	0.209±0.03
Glycoside extract	0.579±0.05	0.462±0.07	0.296±0.01	0.233±0.04	0.660±0.04	0.569±0.09	0.469±0.04	0.409±0.06
Flavonoid extract	0.544±0.02	0.425±0.01	0.315±0.01	0.213±0.01	0.580±0.01	0.459±0.03	0.364±0.04	0.241±0.04
Terpenoid extract	0.396±0.05	0.245±0.01	0.142±0.01	0.106±0.03	0.367±0.02	0.247±0.03	0.132±0.01	0.122±0.01

Phyto chemical extracts	***Staphylococcus aureus***				***Proteus mirabilis***			
	5µg/ml	**10µg/ml**	**15µg/ml**	**20µg/ml**	**5µg/ml**	**10µg/ml**	**15µg/ml**	**20µg/ml**
Alkaloid extract	0.629±0.04	0.542±0.04	0.283±0.04	0.121±0.05	0.667±0.04	0.506±0.03	0.384±0.05	0.230±0.02
Glycoside extract	0.719±0.02	0.617±0.05	0.498±0.03	0.438±0.03	0.712±0.04	0.587±0.04	0.455±0.06	0.382±0.01
Flavonoid extract	0.752±0.01	0.641±0.01	0.516±0.02	0.456±0.02	0.647±0.03	0.557±0.02	0.424±0.01	0.332±0.01
Terpenoid extract	0.356±0.03	0.263±0.01	0.160±0.01	0.112±0.01	0.376±0.02	0.270±0.02	0.162±0.02	0.135±0.02

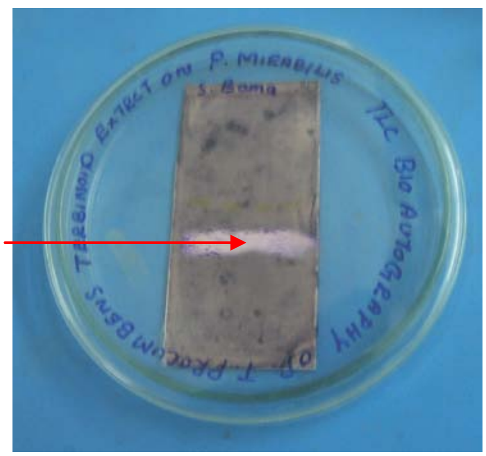
*The Minimal Inhibitory Concentration was determined by measuring the turbidity of the bacterial culture that is the mean of triplicates ± SD of three replicates.

Identification of the antibacterial terpenoid compound, analysed by thin layer bioautography

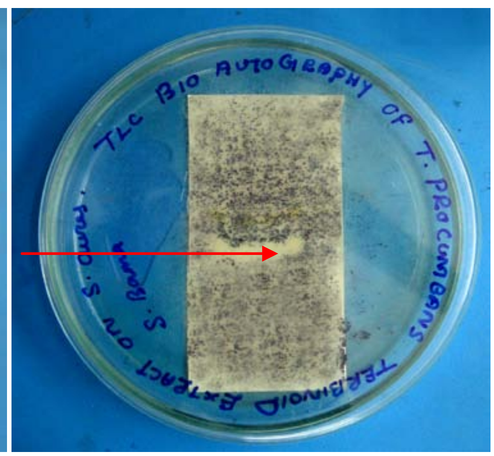
Bioautography of the TLC Plate showed a large area containing substances that inhibited the growth of pathogenic bacteria over the region containing the active components. The active compound was present in the terpenoid extracts of *T. procumbens* (Rf 0.66) (Fig. 1)

Antibacterial compound characterization from the leaves of *T. procumbens*

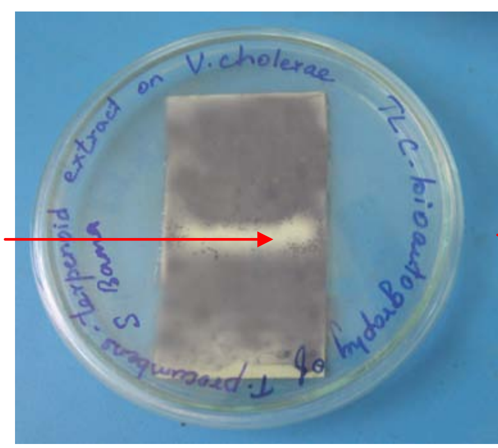
The *T. procumbens* compound with Rf value 0.66 under IR spectrum of intensely broad band at 331, 3320 and 3320 cm^{-1}, showed presence of OH stretching and so they were assumed to be a triterpenoid. The ^1H NMR spectrum showed the presence of multiplets tertiary methyl's at δ 0.77, 0.79, 0.84, 0.97, 0.98 1.04 and 1.69, appeared as singlets except the signal appeared at δ 1.69 which showed allylic coupling (*J*=1.3Hz). These chemical shifts and biogenetic consideration lead to the conclusion of β- orientation of the hydroxyl function at C-3. Proton doublet of triplet at δ 2.39 (J= 10.6, 10.6, 5.3 Hz) was assigned to 19 β-H on comparison with literature values[25]. The structural assignment of *T. procumbens* compound was further substantiated by its ^{13}C NMR spectrum which showed seven methyl groups at [*δ*: 28.0 (C-23), 19.3 (C-30), 18.0 (C-28), 16.1 (C-25), 15.9 (C-26), 15.4. (C-24), 14.5 (C-27)], an exomethylene group at [*δ*: 150.8 (C-20), 109.3 (C-29)] and a secondary hydroxyl bearing carbon at [*δc*: 78.9 (C-3)], in addition to ten methylene, five methine and five quaternary carbons. The molecular formula was established by HR-EIMS at *M/Z* 426.3855 ($C_{30}H_{50}O$) (Fig. 2), which was diagnostic for pentacyclic triterpenes with an isopropenyl moiety [29].



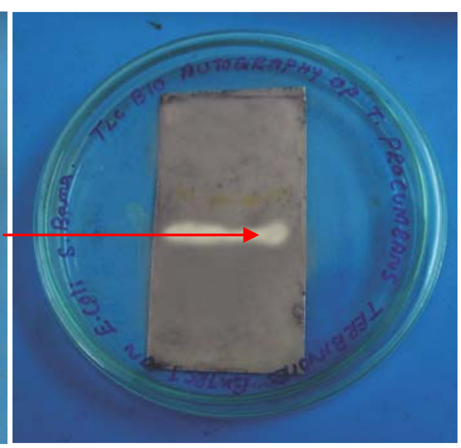
Proteus mirabilis



Staphylococcus aureus



Vibrio cholerae



Escherichia coli

Fig. 1: TLC – bioautography of *T. procumbens* Terpenoid Extract tested against Pathogenic Bacteria

*The Red arrow indicates Rf(0.66) value – suppressed Pathogenic Bacteria growth

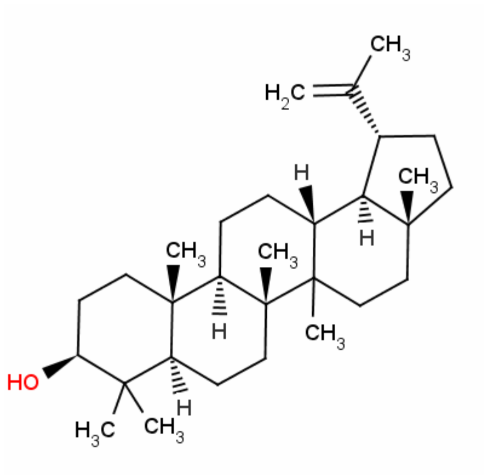


Fig. 2: Structure of Terpenoid compound of *T. procumbens*

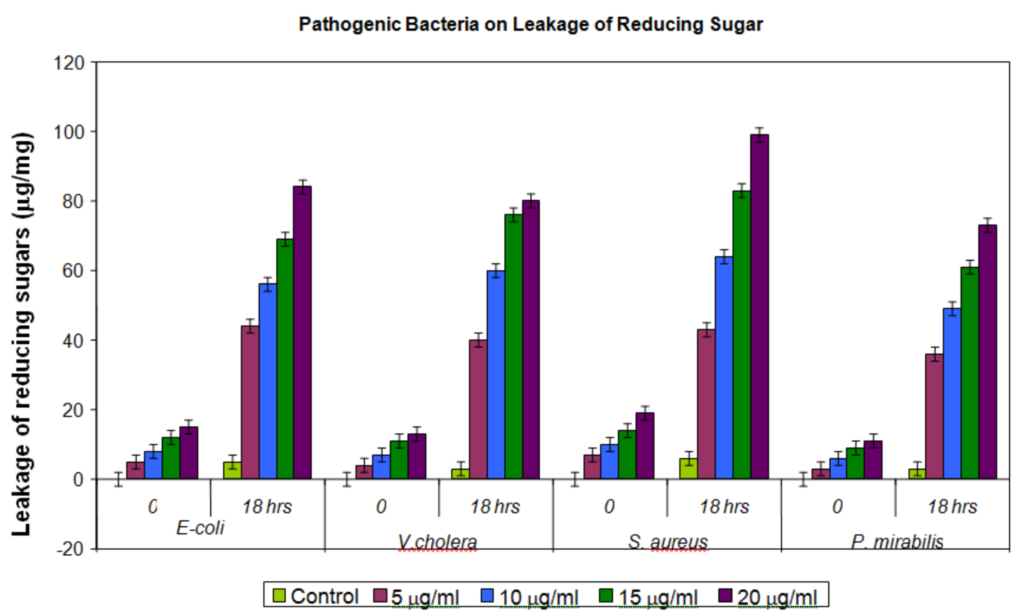
Effect of *T. procumbens* compound on the leakage of membrane of the pathogenic bacteria

Assay on the presence of reducing sugar in the control at starting time showed absence of reducing sugar indicating no leakage of the cell membrane. In the *T. procumbens* compound treated bacterial cell cultures, the presence of reducing sugar was detected, which revealed the leakage of cell membrane. Initially the leakage of sugars through the membrane was detected in all the four concentrations which ranged from 5 to 15 µg/mg of bacterial dry weight in *E. coli*; 4-13 µg/ml in *V. cholarae*; 7-19 µg/mg in *S. aureus* and 3 to 11 µg/mg in *P. mirabilis.* After 18 hours of treatment (Fig.3.), the amounts of reducing sugars were estimated to be higher than the initial value. This study revealed that the amount of the reducing sugars increases with increase in the concentration of the compound and duration of the time. This confirmed that the terpenoid compound has the ability to cause leakage in the membrane. Among the four pathogens studied *S. aureus* was found to be more susceptible to release high amount of reducing sugar (99 µg/mg).

In the antibacterial compound treated bacterial cell cultures, presence of protein were also detected, which in turn revealed the leakage of cell membrane. In the initial stage (Fig 4) the estimation of protein was found to be high when compared to the control which implies that antibacterial compound was potent against the pathogens. After 18 hours of the treatment the amount of the protein estimated was much higher than control and initial value. This study compromises that the higher the concentration and longer the duration, the higher will be the leakage of the membrane. The amount of protein was high (60.12 µg/mg) in the terpenoid compound treated culture of *S. aureus.* This again confirmed that *S. aureus* was most susceptible than the other bacteria studied. Similar result was observed by Wen-Ru Li et al.[30] on the antibacterial activity and mechanism of Silver nanoparticles on *E. coli*.

Effect of *T. procumbens* compound on respiratory chain dehydrogenase of pathogenic bacteria

The effect of terpenoid compound of *T. procumbens* on respiratory chain dehydrogenases of the four bacteria where shown in Figures 5-8. The activity of enzyme in the all four bacteria increased in positive control with increasing time, but there was no change in the negative control. Initially the enzymatic activity of the cell treated with 5 µg/ml of terpenoid compound was even higher than the positive control, but the activity fell down with increasing incubation time. The activity of the enzyme decreased with increasing concentration of terpenoid compound. After being treated for 30 minutes the enzymatic activity was almost feeble. The result revealed that the activity of respiratory chain dehydrogenases of four bacterial pathogen would be inhibited by the terpenoid compound of *T. procumbens*. It also explained that the higher the concentration of terpenoid compound, the lower be the activity of enzyme. Similar accordance results were described on the antibacterial activity and mechanism of silver nanoparticle of *E. coli*[30]. Similarly Holt and Bard[31] found that Ag^+ inhibited respiration of *E. coli* by determining change oxygen dissolving culture resolution.



Bar chart represent the average data of the duplicate experiments. Error bars are the representation of S.D. of duplicate incubation.

Fig. 3: Effect of Different concentration of Terpenoid Compound of *T. procumbens*

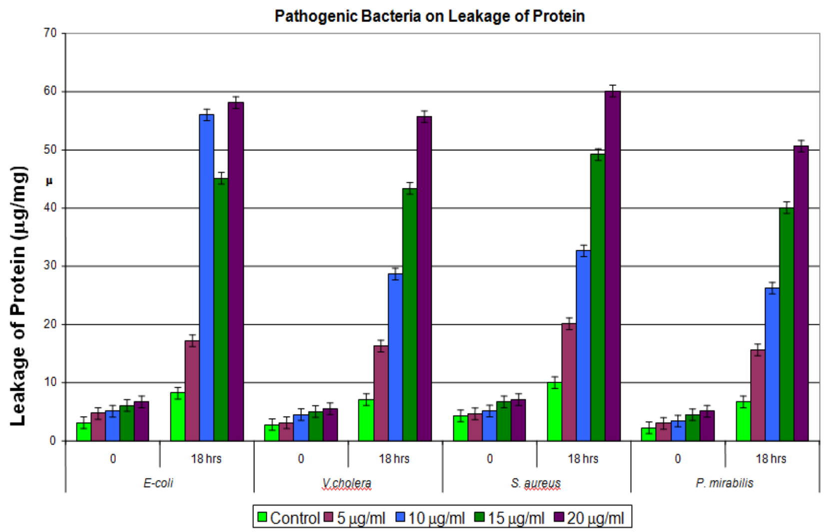


Fig. 4: Effect of Different concentration of Terpenoid Compound of *T. procumbens*

Bar chart represent the average data of the duplicate experiments. Error bars are the representation of S.D. of duplicate incubation.

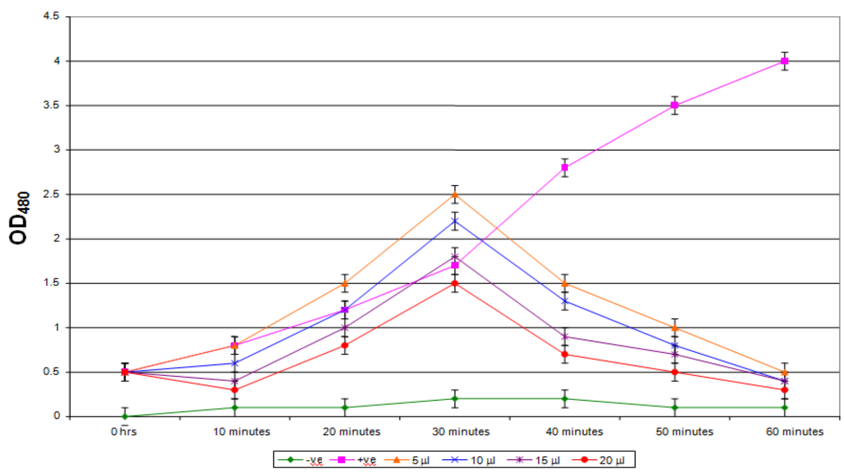


Fig. 5: Effect of Terpenoid Compound on respiratory chain dehydrogenase of *S. aureus*

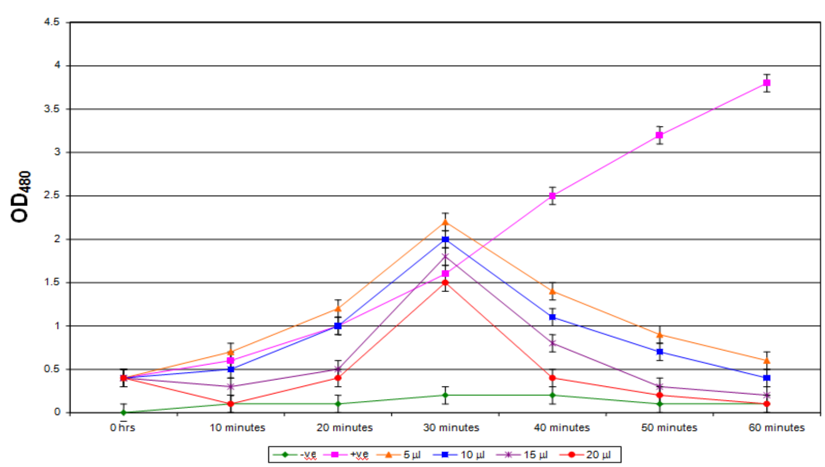


Fig. 6: Effect of Terpenoid Compound on respiratory chain dehydrogenase of *E. coli*

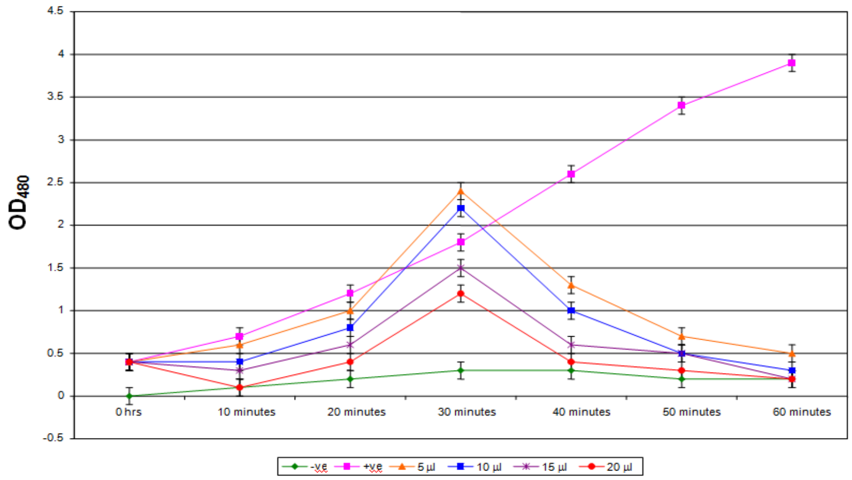
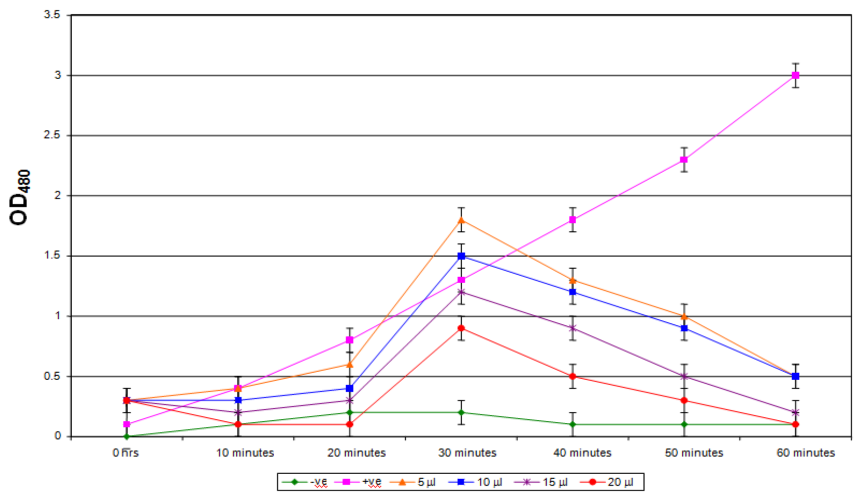


Fig. 7: Effect of Terpenoid Compound on respiratory chain dehydrogenase of *V. cholera*



Error bars are the representation of S.D. of duplicate incubation.

Fig. 8: Effect of Terpenoid Compound on respiratory chain dehydrogenase of *P. mirabilis*

CONCLUSION

The present study clearly indicates that the terpenoid compound of *T. procumbens* posses antibacterial activity on both gram positive and gram negative bacteria. The result is validated as it interferes with the permeability of the membrane of the pathogenic bacteria. The futuristic perspective demands animal toxicity studies, so that the chemopreventive nature can be ascertained on both gram positive and negative bacteria.

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