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Research Article

In-vitro Antioxidant, Antimicrobial and Anticancer Potential of Polysaccharide from *Tridax procumbens* L.

Varsharani V Ingole[1], Pravin C Mhaske[2], Sushma R Katade[1*]

[1]*Department of Chemistry, PES's Modern College of Arts, Science and Commerce, Ganeshkhind, Pune, Maharashtra, India.*
[2]*Post-Graduate Department of Chemistry, S. P. Mandali's, Sir Parashurambhau College, Pune, Maharashtra, India.*

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ABSTRACT

Cancer ranks among the primary causes of death on a global level. Natural products are crucial for both cancer therapy and treatment. Traditionally, *Tridax procumbens* has been used to cure wound infections. The present study investigates the isolation, identification, *in-vitro* antioxidant, antimicrobial, and anticancer activities of isolated polysaccharides from *T. procumbens* L. The polysaccharide (4-deoxy-5-α-D-Rhamnonic acid (1→2) β-D-fructofuranosyl (2→1)-β-D-fructofuranosyl (2→1)-2-D-fructofuranoside) was identified using modern NMR spectroscopic techniques. *In-vitro* anticancer activity was assessed against MDA-MB-249 and MCF-7 using an MTT assay. The IC_{50} value for polysaccharide was found to be 5.06 µg/mL against MCF-7 and that of MDA MB 249 cell lines 15.68 µg/mL. The statistical analysis indicated significance at $p < 0.05$. The polysaccharide showed effective antibacterial activity at MIC 15.5 µg/mL against bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa,* respectively. The DPPH antioxidant potential of the polysaccharide was found to be outstanding, with an IC_{50} value of 1.01 µg/mL. It showed statistical significance at $p < 0.05$. Therefore, the findings reveal that polysaccharides could be used as therapeutic drugs to develop anticancer and antibacterial agents from *T. procumbens* L.

Introduction

Cancer is a leading contributor to the global mortality rate, and it is becoming more prevalent in developing nations. Cancer currently accounts for one in every six deaths worldwide, and this figure is expected to more than double by 2030.[1] The WHO predicts that by the year 2040, overall, 29.5 million new cancer cases will be diagnosed worldwide, resulting in 16.5 million deaths.[2] Cancer growth is frequently caused by cell cycle disruptions that allow cancer cells to proliferate indefinitely and provide apoptosis resistance.[3] Researchers have shown a growing interest in estrogen receptor breast cancer cells.[4]

Moreover, since 2006, no antifungal drug has been derived from natural products; instead, all newly developed drugs are structurally similar to old azole chemistry.[5] It is crucial to explore novel compounds that possess antimicrobial capabilities.[6,7] According to WHO Antimicrobial Resistance (AMR), new viral and fungal diseases are spreading worldwide; hence, plants have the potential to significantly contribute to the development of safe and new antibiotics in modern medicine.[8, 9] Phytoconstituents such as steroids, flavonoids, and polyphenols play a crucial role in cancer treatment and antimicrobial infections. Polysaccharides extracted from plants have been proven to have outstanding antitumor activity. Fructose-containing polysaccharide effects are becoming a current focus of study in cancer treatment, most likely due to distinct action methods.[10] Over 50 carbohydrate-based medications were approved as diagnostic tools between 2000-2021, including antibacterial, antiparasitic, anticancer, antidiabetic and cardiovascular [11], due to their hydraulic nature, high water solubility, excellent pharmacokinetics, and low toxicity.[12] The most active area of research and development

***Corresponding Author:** Dr. Sushma R Katade

Address: Department of Chemistry, PES's Modern College of Arts, Science and Commerce, Ganeshkhind, Pune, Maharashtra, India.

Email ✉**:** sushmarkatade@gmail.com

Tel.: +91-7028865295

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for polysaccharides or carbohydrate drugs right now is cancer, where several promising therapies are in development.[13] Polysaccharides could potentially decrease rectal and colon carcinogenesis.[14] Several saccharides are also available as antibiotic drugs, such as vancosamine, gentamycin, streptomycin, and dihydrostreptomycin.[15] Polysaccharides are carbohydrates and play essential biological roles in food, pharmaceuticals, etc.[7,16] *T. procumbens* L. herb belongs to the family Asteraceae. For its therapeutic potential, this herb has been identified for its healing properties in treating gastrointestinal disorders, fever, bronchial disorders, wounds, and hair growth.[17] Previous research findings have indicated that *T. procumbens* is a source of powerful antioxidant, anticancer, and antibacterial secondary metabolites.[18–20] Various extracts have been found to have several biological properties. Moreover, it has been revealed that polysaccharide's role in medicine is important because of their ability to biodegrade, dissolve in water, lack toxicity, and be non-immunogenic.[21] A few isolated compounds from *T. procumbens* L. were explored for anticancer potential. Unfortunately, the presence of fructose-rich saccharide and its bioactivities was reported for the first time in *T. procumbens* L. Therefore, *in-vitro* antiproliferative, antibacterial, and antioxidant activities were evaluated. The outcomes of this study include not only the isolation of polysaccharides but also the potential to aid in the creation of innovative functional foods or herbal products to manage breast cancer and antibiotics in humans. Therefore, this study aims to isolate a potential anticancer agent from *T. procumbens* L. polysaccharides reduced effective cell viability against MCF-7 and MDA-MB-249 cell lines using an MTT assay and assessing its antioxidant and antibacterial properties. This novel finding adds to the library of polysaccharides and reveals promising medicinal uses for the development of the pharmaceutical sector.

Materials And Methods

Collection and Identification of Plant Material

The plant material was collected from Ambegaon (BK.), Pune, India, from month August to December 2018. The plant was identified at the Botanical Survey of India (BSI) Pune. The voucher specimen VVI02 was also deposited to that institute.

Extraction, Isolation, and Analysis of Methanol Extract

The dried powder of the aerial part (300.0 g) of the plant was extracted with methanol (3.5 L) by the soxhlet extraction method. This process was carried out in triplicate. A rotary vacuum evaporator was used to complete the evaporation of the solvent until the dryness of extract. A rotary vacuum evaporator was used to evaporate the solvent until the extract was completely dry. The dried extract (61.311 g) was stored in the bottle. A crude methanol extract of 46.0 g was loaded onto silica gel at a ratio of 1:5. The slurry of silica gel (60-120 mesh size, ASI to 7661-86-91, Fischer Scientific) in packed 100% petroleum was put onto a column CC (4.5 × 120 cm) (1:40). The initial column was eluted with Then, with an increasing percentage of solvents (petroleum ether-EtOAc and methanol), a total of 12 major fractions (A-K) were obtained. A (0.1492), B (3.0092), C (0.2086), D (2.8165), E (2.1836), F (0.1578), G (6.1578), H (1.1653), I (3.1072), J (3.202), J' (2.012), and K (17.326). Fraction Kc (8.500 g) was further rechromatographed on CC (4.5× 120 cm) onto 240.0 g of silica gel. The column was started with the solvent pure ethyl acetate, followed by different proportions of ethyl acetate: methanol, continuing with a gradual rise in methanol percentage. TLC identified kc9 (1.115) with the solvent system (60:40, MeOH: EtOAc). Rf value of 0.35, a dark orange color spot was seen on TLC, and a black spot was observed in the presence of anisaldehyde.

Antibacterial Activity

Materials and chemicals

Incubator at 37°C (Klenzone 2019 model no. country), sterile tips, various sizes of pipettes, 100 to 1000 µL, Vortex mixer, Petri dishes, sterile flasks, 100 to 1000 mL (Borosil), sterile nutrient agar, sterile normal saline, sterile nutrient bath (Hi Media, Mumbai), sterile nutrient agar, DMSO, microplate reader (Readwell Touch-2019), sterile 96-well plates (polystyrene), sterile Eppendorf tubes (polystyrene), UV spectrophotometer (BioEra 2017 India), antibiotic streptomycin.

Medium

A nutrient medium was used in this assay. Although the recommended Muller Hinton media is required for the antibacterial susceptibility assay, nutrient broth media had comparable results for the bacteria used in this experiment.

Bacterial Strains

The organisms *Staphylococcus aureus* (NClM5021) and *Pseudomonas aeruginosa* (NCIM5029) were purchased from the National Collection of Industrial Microorganisms (NICM) for antibacterial activity.

Broth Microdilution Method

The Minimal Inhibition Concentration (MIC) of polysaccharide was determined by two-fold broth microdilution with some changes in procedure as described by serial dilution.[22,23] Stock solutions of compounds were prepared in a sterile micro-centrifuge. In Eppendorf tubes, the compound was dissolved in 1-mg/L of DMSO at the final concentration. Using Muller-Hinton broth (Hi Media Mumbai), six serial dilutions were prepared from stock solutions ranging from 15.5 to 500 µg/mL in

96-well microplates. An isolated colony of bacteria was transferred into 100 mL of nutrient broth, and the bacterial suspension was prepared using aseptic techniques. It is incubated overnight at 37°C. Broth suspensions of bacterial cultures of *Staphylococcus* and *Pseudomonas* were ready after 24 hours of incubation. At 500 nm the absorbance of the culture broth was recorded. The range obtained was between 0.5 to 1.0 (cell density: 1.5 × 108 CFU/mL) using a UV spectrophotometer (Bio Era 2017, India). About 180 µL of these suspensions were injected into each well. A control well for growth and sterility was examined for every strain. The microplates were incubated at 37°C for a whole day.

During the broth microdilution test, 180 µL of each bacterial culture in the right growth medium was already present in the sterile 96-well microliter plate when 20 µL of the twofold serially diluted compounds were introduced. Each well's ultimate capacity was 200 µL. About 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 (500–15.5 µg/mL, serial dilution). To prevent the culture medium from evaporating in the incubator, the top and bottom rows were filled with sterile PBS (1X, pH 7.4) solutions at 37°C, the plates were incubated. After the incubation, plates were plates were analyzed using a microplate reader (Readwell Touch-2019, India). The absorbance was taken at 400 nm.

Positive control wells had bacterial suspensions in suitable growth media and bacterial suspensions in DMSO at concentrations equivalent to the highest level in the broth dilution experiment. Pure compound and growth medium-filled wells served as negative controls. After the addition of the second dose of compounds in fresh media at the above-mentioned concentration, the mixture was incubated for 24 hours. A triplicate test was performed on each MIC measurement.[24]

In-vitro Antioxidant Activity

DPPH assay

The DPPH assay was performed to determine the antioxidant activity of polysaccharides.[25] The detailed procedure, with some changes, is described as in previous work. Methanol was used to determine 0.1 mM DPPH 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. After 30 minutes in the dark and mixtures, absorbance at 517 nm was determined using a UV spectrophotometer. As our reference, we used 2 mL of pure ether and 1-mL of DPPH. By plotting the DPPH scavenging, the calibration curve was established using gallic acid and ascorbic acid as standards.

DPPH free radical scavenging rate (%) = (AC-A)/AC×100

The experiment was carried out in triplicate. The IC_{50} was determined by the curve of absorbance against the concentration of compounds.[24]

MTT assay

- *Cell culture*

Human breast estrogen-dependent adenocarcinoma cells, MCF-7, MDA-MB-249, and doxorubicin (Standard) were in Dulbecco's eagle's medium Dulbecco's modified eagle's medium (DMEM) cultured and MEMB medium composed of 10% fetal bovine serum (FBS). At a density of 5000 cells were placed in 96-well plates, followed by an incubation period at 37°C and in an environment of 5% CO_2.

- *Cell viability (MTT assay)*

The endpoint MTT assay was executed according to the provided protocols.[26] Doxorubicin, an anticancer drug, was used as a reference for MCF-7 and MDA cells. The living cells (5 × 10^3 cells per well) were planted for 24 hours in 96-well plates. MTT (5 mg/mL) in each well-received 20 µL was introduced to each cell before incubation for 4 hours at 37°C. To dissolve MTT assay crystals, 100 µL of DMSO control was mixed in each well. At 37°C, the plates were incubated overnight. Each concentration of the tested compound was added into microplate wells, and a further 24 hours of incubation were continued. With the addition of 10 µL of MTT was added to each cell and the plates were incubated for 4 hours. Florescent and emission excitation were measured at 544 and 570 nm, respectively, using 1550-800375C Multiskan SkyHigh. To determine the percentage inhibition average absorbance values of the tested compound and blank media and medium-containing cells. It was determined by using the following formula.

%Cell viability = (B-A) – (C-A)/(B-A)*100

Where A = average absorbance of media, B = average absorbance of the media with cells, and C = average absorbance of the compound. Based on the percentage caused by the compound, the IC_{50} value was calculated based on linear regression analysis.

Statistical Analysis

The standard deviation values were assessed from a minimum of three determinations, and the concentration-response curve was drawn in Microsoft Office. Using a one-way ANOVA in Microsoft Excel, the data was analyzed statistically significant difference was considered the level below $p < 0.05$ [24].

Results

Identification of Polysaccharide

Polysaccharides, orange syrup, - 6.8 (c, 0.26 DMSO), IR (KBr) (Fig. S1), λ_{max} cm^{-1}, displayed bands at 3600 to 2500 (carboxylic acid and glycoside OH groups), 2950 to

2800 (asymmetric and symmetric stretching skeletons of CH), 1720 to 1750 (carboxylic acid, stretching vibrations of C=O), 1500 to 1250 (C-OH), and 1100 to 1000 (C-O). The molecular formula $C_{25}H_{42}O_{21}$ and molecular weight were determined by HR-ESI-MS (positive ion mode), Fig. S2. Observed mass 679.0593 [M^++H] and calculated mass 678.2293 [M^+]. The HR-ESI-MS illustrated the high-intensity m/z of 621, 487, 441, 335, 249, and 179, which confirmed a rhamnose unit, β-D-fructofuranose (2→1) linkage. These fragments strongly support the confirmation that polysaccharide affords four sugar units. The ^{1}H-NMR spectrum (Table 1 and Fig. S3) indicates the sugar protons in the 3.20 to 4.44 ppm range. The signal observed at δ 5.14 ppm indicated the H-1 of the α-D-rhamnopyranosyl residue. Acid OH proton attributed at δ12.0 and methyl group of rhamnose at δ1.23. These data suggest that the α-D-rhamnopyranosyl residue attaches to carboxylic acid. The presence of sugar protons is in the range of δ4.20 to 4.65 ppm for fructofuranose units. Spectral data was compared with previously published data.[27,28]

Table 1: ^{1}H-NMR, ^{13}C NMR DEPT-135, HSQC, HMBC spectral data (DMSO, 500 MHz,125 MHz) of polysaccharide

Carbon No.	δ_C	*DEPT*	δ_H	*HSQC*
Rha				
1	96.6	CH	5.14	5.14
2	72.3	CH	-	3.38
3	71.9	CH	3.40	3.40
4	57.2	CH	-	2.55
5	92.4	CH	-	4.93
6	173.6	C = O	-	
-OH	-		12.0	-
4-CH_3	21.7	CH_3	1.23 (d, 1H)	1.23
FF				
1''	61.1	CH_2	-	3.37
2''	102.1	-	-	-
3''	76.4	CH	-	3.05
4''	74.9	CH	-	3.80
5''	81.7	CH	-	3.78
6''	63.7	CH_2	-	3.34
FF'				
1'''	61.2	CH_2	-	3.50
2'''	198.4	-	-	-
3'''	76.2	CH	-	3.68
4'''	75.2	CH	-	3.78
5'''	81.5	CH	-	3.49
6'''	63.5	CH_2	-	3.22
FF''				
1''''	63.0	CH_2	-	3.34
2''''	104.0	-	-	-
3''''	75.9	CH	-	3.78
4''''	73.1	CH	-	3.40
5''''	81.1	CH	-	3.69
6''''	63.1	CH_2	-	3.34

δ_H 2.55, 3.55, ^{13}C = 49.18 for internal methanol and water in DMSO peaks.

In the ^{13}C-NMR (Table 1 and Figs S4-S5) δ 173.8 was identified for the acid carbonyl carbon. The δ96.6 C-1 and δ 92.2 C-5 identified for α-D-rhamnopyranosyl. From DEPT and ^{13}C-NMR spectral data, three quaternary carbon signals at δ 104.3, 102.1, and 98.4 are identified for three fructose units of C-2 of β-(2→1)-D-fructose. δ92.3 for C-5 is assigned to the carbon-bearing carboxylic group and δ81-83/C-5 is composed of three fructofuranose units. According to spectral data, the compound has four monosaccharides, a rhamnose, and three fructofuranose sugar units in the HSQC spectrum Table 1. Fig. S6 sugar unit protons were confirmed by correlation of ^{1}H →^{13}C, Protons at δ 2.55 (H-4), 3.40 (H-3), 3.38 (H-4), 3.22 (H-5), and 0.96 (H-6), showed correlation with carbon signals for δ96.6 (C-1), 57.2 (C-4), 71.9 (C-3), 70.4 (C-4), 64.0 (C-5), and 18.1(C-6), respectively. The position and sequence of sugar unit linkage were confirmed from the ^{13}C NMR downfield shift of carbon values. From these findings, the above compound was determined as 4-deoxy-5-*α*-D-Rhamnonic acid-(1→2)-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-2-D-fructofuranoside (Fig. 1). Its spectroscopic data is compared with previously published data.[27–29] The isolation of polysaccharides was reported first time from *T. procumbens* L.

Antibacterial Activity

The present study used polysaccharides for the antibacterial activity by a broth dilution assay against *S. aureus* and *P. aeruginosa* organisms. The results are shown in Tables 2 and 3 and Figs 2 and 3, respectively. The isolated compound demonstrated good bactericidal activity at a MIC of 15.5 µg/mL for selected bacteria.

Antioxidant Activity

The antioxidant potential of polysaccharides isolated from *T. procumbens* was assessed using the DPPH technique (Table 4 and Fig. 4). Gallic acid and ascorbic acid were used as standards. The percentage radical scavenging inhibition varies linearly with concentration from 100 to 500 µg/mL. The IC_{50} value of the polysaccharide was found to be 1.01 µg/mL. It showed statistical significance at $p < 0.05$. It was found to be higher than the standard reference gallic acid.

Anticancer Activity

To date, this study has evaluated for the first time the *in-vitro* antiproliferation activity of polysaccharide from *T. procumbens* L. The cell viability of polysaccharides was investigated against MDA-MB-249 and MCF-7. The treated cell exhibited dose-dependent cell viability, as indicated by

Table 2: The absorbance of compounds (dilution assay from higher to lower concentration) against *S. aureus* organism (Mean ± SD, n =3)

Concentration µg/mL	*500*	*250*	*125*	*62.5*	*31.2*	*15.62*
Polysaccharide	1.733 ± 0.15	1.684 ± 0.16	1.526 ± 0.03	1.539 ± 0.11	1.47 ± 0.03	1.376 ± 0.18
Control	1.714 ± 0.06	1.764 ± 0.06	1.823 ± 0.02	1.8002 ± 0.07	1.821 ± 0.03	1.802 ± 0.07
Streptomycin	1.067 ± 0.1	1.343 ± 0.1	1.352 ± 0.1	1.402 ± 0.06	1.375 ± 0.05	1.397 ± 0.08

Table 3: Broth dilution assay absorbance of compounds (dilution assay from higher to lower concentration) against *P. aeruginosa* (Mean ± SD, n = 3), significance value ($p < 0.05$) calculated by One-way ANOVA

Concentration µg/mL	*500*	*250*	*125*	*62.5*	*31.25*	*15.62*
Polysaccharide	0.708 ± 0.20 0.1	0.85 ± 0.32	0.699 ± 0.21	0.534 ± 0.07	0.849 ± 0.49	0.51 ± 0.11
Control	1.381 ± 0.43	1.315 ± 0.36	1.337 ± 0.41	1.448 ± 0.29	1.380 ± 0.44	1.47 ± 0.32
Streptomycin	0.671 ± 0.05	0.763 ± 0.14	0.816 ± 0.0	0.894 ± 0.21	0.767 ± 0.10	0.74 ± 0.05

Table 4: The percent DPPH radical scavenging of isolated compounds from *T. procumbens* L. Mean ± SD, n =3), significance value ($p < 0.05$) calculated by One-way ANOVA

	Compound Concentration (µg/mL) The Percent DPPH radical scavenging					
Compound	*100*	*200*	*300*	*400*	*500*	*IC_{50} (µg/mL)*
Polysaccharide	45.82 ± 0.01	59.42 ± 0.01	77.01 ± 0.01	77.31 ± 0.01	84.10 ± 0.05	1.01
Ascorbic acid	23.85 ± 0.09	47.08 ± 0.05	60.66 ± 0.05	70.04 ± 0.04	88.95 ± 0.05	1.45
Gallic acid	46.0 ± 0.04	62.64 ± 0.002	70.02 ± 0.001	72.71 ± 0.03	83.02 ± 0.3	1.01

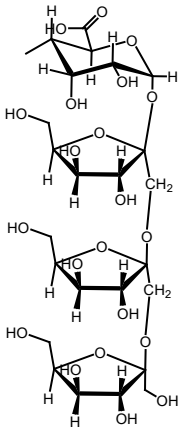

Fig. 1: Structure of polysaccharide

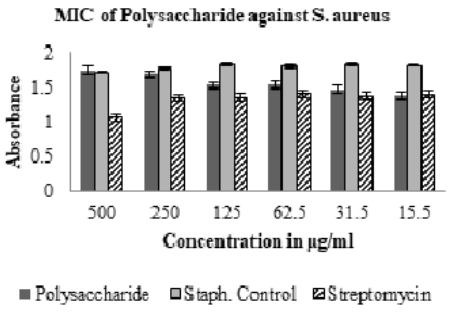

Fig. 2: The graph represents the MIC of polysaccharides against *S. aureus*

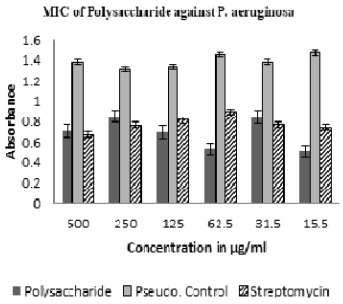

Fig. 3: The graph represents the MIC of polysaccharide against *P. aeruginosa*

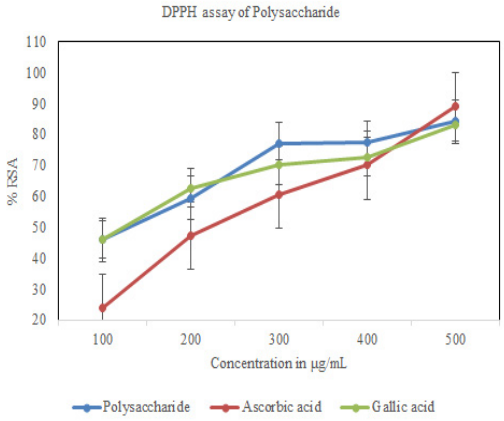

Fig. 4: Antioxidant activity as measured through DPPH assay of polysaccharide significance at $p < 0.05$.

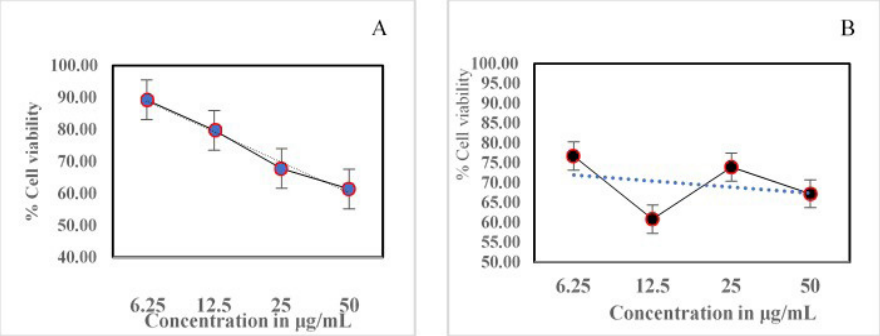

Fig. 5: MTT assay, the dose-response plot of human breast cancer cell lines A) MCF-7 B) MDA-MB-249 Cell with different concentrations for 48 hours. The bars are means ± SEM (n= 4) significance at $p < 0.05$

Table 5: Antiproliferation activity of isolated compounds against MCF-7 and MDA-MB-249 Mean ± SD, n =4), significance value ($p < 0.05$) calculated by One-way ANOVA

Drugs/ Compound	*Concentration in µg/mL*	*MCF-7*			*MDA-MB-249*		
		Average	*%Viability against control*	*IC_{50} (µg/mL)*	*Average*	*%Viability against control*	*IC_{50} (µg/mL)*
Polysaccharide	6.25	1.225 ± 0.07	89.25	5.06	1.348 ± 0.12	76.71	15.68
	12.5	1.095 ± 0.03	79.78		1.080 ± 0.04	60.84	
	25	0.931 ± 0.05	67.80		1.300 ± 0.10	73.89	
	50	0.842 ± 0.07	61.37		1.188 ± 0.07	67.22	
Standard (Doxorubicin)				3.5			8.7

the decreased viability with an increase in concentrations from 6.25, 12.5, and 25.0 to 50 µg/mL. The IC_{50} value is the 50% cell viability of the compound. The percentage cell viability is plotted against the concentration of the compound shown in Figs 5A and 5B. Potent cell viability of polysaccharide demonstrated strong cell viability against MCF-7 cell lines with an IC_{50} 5.06 µg/mL and that of 15.68 µg/mL against MDA-MB-249 cells (Table 5). As compared to positive reference, good results were obtained. The results clearly state that polysaccharides showed remarkable anticancer properties to words MCF-7 cell lines. The study concluded that tested polysaccharide units composed of rhamnose and fructofuranose monosaccharides are responsible for antiproliferation activity.

Discussion

Herbal medicine has gained interest for its various treatments due to its significant biological activities and the presence of phytoconstituents. The benefits of phytochemicals include their extensive pharmacological knowledge, minimal toxicity, and strong potential for natural healing. Modern analytical methods have proliferated to support the innovative discovery of phytochemicals in pharmacological research. *T. procumbens* possesses antioxidant, anticancer, antibacterial, and antifungal properties. It has strong antibacterial efficiency against various bacteria. [30] According to the published reports, antimicrobial activity research has been conducted on extracts and essential oils on a large scale compared to isolated compounds of *T. procumbens*. Traditionally, this plant is used to cure wound infections, kidney stones, diabetes, and hair-related problems. In the present study, the polysaccharide compound was able to reduce remarkably the growth of biofilms of *S. aureus* and *P. aeruginosa* at all doses. Previous studies reported that fructose-rich polysaccharides exhibit a different structure and reactivity compared to those produced by glucose. It was strongly recommended to prevent illness through protein glycation inhibition mediated by fructose.[31] From this perspective, it was possible to conclude that fructose-rich polysaccharides have the potential to be produced as a reliable protective barrier for bacterial use in industries.[32] *S. aureus* bacteria frequently cause bacterial infections in diabetic patients. The study suggested that polysaccharides can cure diabetic patients' infections caused by *S. aureus* and *P. aeruginosa*. Moderate antioxidant activity of the fructooligosaccharide was reported in streptozotocin-induced diabetic animals.[33] The polysaccharides isolated from the edible mushroom *P. eryngii* showed significant antioxidant potential with

an IC_{50} value of 0.52 ± 0.02.[34] The excellent antioxidant activity of the fructose-rich fraction of *Ganoderma lucidum* was also reported.[29] Previous studies of *T. procumbens* L. reported that ethanol extract showed a strong reductive potential due to the presence of a high percentage of phenolic compounds.[35] Ethanol and aqueous extracts were found to be potential antioxidants demonstrated at 61.52 ± 0.32% and 82.5 ± 1.1%.[36] High antioxidant activity was reported in crude extracts and essential oils of *T. procumbens* L. This study investigated the antioxidant activity of polysaccharides was carried out, and it showed strong reduction potential by the DPPH assay.[24] The findings revealed that the polysaccharide has a high antioxidant capacity to its antioxidant characteristics, which may prevent and slow down the progression of aging in various diseases associated with oxidative stress.` There are a few reports on the anticancer activity of extracts as well as isolated compounds. [24] Delphi *et al*. reported that pectin acid anticancer activity in cancer cells. The study investigated the impact of this compound on MDA-MB-249 without having any discernible impact on HUVEC non-cancerous cells. These findings revealed 20 to 80% cell viability after 24 hours in 5 mg/mL of polysaccharides. It has been concluded that tested polysaccharides showed the potential to reduce the cell viability of breast cancer cells.[37,38]

Conclusion

In conclusion, the identification and biological activities of the polysaccharide are thoroughly explored and reported from the aerial part of *T. procumbens* L. It showed outstanding antimicrobial, antioxidant, and an *in-vitro* anticancer effect on MCF-7 cell lines. This is the first report of the polysaccharide in the methanol extract from *Tridax procumbens* L. This could contribute to significant natural bioactive medicine for wound cures and anti-radical agents and is anticipated to be non-toxic. Therefore, this study offers a potential pharmacological justification for the therapeutic potential of *T. procumbens* polysaccharide in the formulation of drugs. As a result, the future focus of this study will be on the broad investigation of biomedical applications and the formulation of polysaccharides.

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References

1. Rahim A, Mostofa MG, Sadik MG, Rahman MA, Khalil MI, Tsukahara T, et al. The anticancer activity of two glycosides from the leaves of Leea aequata L. Nat Prod Res. 2021; 35(24):5867–71. Available from: https://doi.org/10.1080/14786419.2020.1798661
2. Shah SC, Kayamba V, Peek RM, Heimburger D. Cancer control in low- and middle-income countries: Is it time to consider screening? J Glob Oncol. 2019; (5):1–8. Available from: https://doi.org/10.1200/JGO.18.00200
3. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: A changing paradigm. Nat Rev Cancer. 2009; 9(3):153–66. Available from: https://doi.org/10.1038/nrc2602
4. Song H, Zhang S, Mou J, Gong G, Huang Y, Ma R, et al. Cytotoxic activities against MCF-7 and MDA-MB-231, antioxidant and α-α-glucosidase inhibitory activities of Trachelospermum jasminoides extracts in vitro. Biotechnology & Biotechnological Equipment. 2019; 33(1):1671–9. Available from: https://doi.org/10.1080/13102818.2019.1694436
5. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 2020; 83(3):770–803. Available from: https://doi.org/10.1021/acs.jnatprod.9b01285
6. Wang J, Zhang Y, Lu Q, Xing D, Zhang R. Exploring carbohydrates for therapeutics: A review on future directions. Front Pharmacol. 2021;16:12. Available from: https://doi.org/10.3389/fphar.2021.756724
7. Sandhya T, Lathika KM, Pandey BN, Mishra KP. The potential of traditional Ayurvedic formulation, Triphala, as a novel anticancer drug. Cancer Lett. 2006; 1(2):206–14. Available from: https://doi.org/10.1016/j.canlet.2005.01.035
8. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015; 109(7):309-18. doi: 10.1179/2047773215Y.0000000030.
9. Dhingra S, Rahman NA, Peile E, Rahaman M, Sartelli M, Hassali MA, et al. Microbial resistance movements: An overview of global public health threats posed by antimicrobial resistance, and how best to counter. Front Public Health. 2020; 4:8. Available from: https://doi.org/10.3389/fpubh.2020.535668
10. Pang G, Wang F, Zang LW. Dose matters direct killing or immunoregulatory effects of natural polysaccharides in cancer treatment. Carbohydr Polym. 2018; 195:243-56. Available from: https://doi.org/10.1016/j.carbpol.2018.04.100
11. Cao X, Du X, Jiao H, An Q, Chen R, Fang P, et al. Carbohydrates-based drugs launched during 2000-2021. Acta Pharm Sin B. 2022; 12(10):3783-821. Available from: https://doi.org/10.1016/j.apsb.2022.05.020
12. Zhang H, Shi LE, Zhou J. Recent developments of polysaccharide-based double-network hydrogels. Journal of polymer science. 2023; 61(1):7-43. Available from: https://doi.org/10.1002/pol.20220510
13. Zhang Y, Wang F. Carbohydrate drugs: current status and development prospect. Drug Discov Ther. 2015; 9(2):79-87. Available from: https://doi.org/10.5582/ddt.2015.01028
14. Ghosh D, Karmakar P. Insight into antioxidative carbohydrates polymers from medicinal plants: structure-activity relationships, mechanism of actions and interactions with bovine serum albumin. Int J Biol Macromol. 2021; 1:1022-34. Available from: https://doi.org/10.1016/j.ijbiomac.2020.10.258
15. Kim J-H, Baek J, Sa S, Park J, Kim M, Kim W. Kestose-enriched fructooligosaccharide alleviates atopic dermatitis by modulating the gut microbiome and immune response. J Funct Foods. 2021; 85:104650. Available from: Available from: https://doi.org/10.1016/j.jff.2021.104650
16. Li Y, Guo X, Zhong R, Ye C, Chen J. Structure and characterization and biological activities evaluation of two hetero-polysaccharides from Lepista nuda: Cell antioxidants, anticancer and immune-modulatory activities. Int J Biol Macromol. 2023; 31:244:125204. Available from: https://doi.org/10.1016/j.ijbiomac.2023.125204
17. Udupa S, Udupa A, Kulkarni D. Influence of *Tridax procumbens* on lysyl oxidase activity and wound healing. Plant Med. 1991; 57(04):325-7. Available from: https://doi.org/10.1055/s-2006-960108
18. Ingole VV, Mhaske PC, Katade SR. Phytochemistry and pharmacological aspects of *Tridax procumbens* (L): A systematic and comprehensive review. Phytomedicine plus. 2022; 2(1):100199. Available from: https://doi.org/10.1016/j.phyplu.2021.100199
19. Ali M, Ravinder E, Ramachandram R. A new flavonoid from the

aerial parts of *Tridax procumbens.* Fitoterapia. 2001; 72(3):313-5. Available from: https://doi.org/10.1016/S0367-326X(00)00296-3
20. Cui HX, Zhang LS, Yan HG, Yuan K, Jin SH. Constituents of flavonoids from *Tridax procumbens* L. and antioxidant activity. Pharmacogn Mag. 2020; 16(67):201-5. Available from: Available from: https://doi.org/10.4103/pm.pm_229_19
21. Dawood DH, Elmongy MS, Negm A, Taher MA. Extraction and chemical characterization of water-soluble polysaccharides from two palm species and their antioxidants and antitumor activities. Egyptian Journal of Basic and Applied Sciences. 2020; 7(1):141-58. Available from: https://doi.org/10.1080/2314808X.2020.1773126
22. Eloff J. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998; 4:711-3. Available from: Available from: https://doi.org/10.1055/s-2006-957563
23. Sarkar SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods. 2007; 42(4):321-4. https://doi.org/10.1016/j.ymeth.2007.01.006
24. Ingole VV, Katade SR. Chemical composition, antioxidant, antibacterial activity of isolated oil and methanol extract of *Tridax procumbens* L. Int J Pharm Sci Res. 2024; 15(4):1157-66. Available from: https://doi.org/10.13040/IJPSR.0975-8232
25. Brand- Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology. 1995; 28(1):25-30. Available from: https://doi.org/10.1016/S0023-6438(95)80008-5
26. Xu M, McCanna DJ, Sivak JG. Use of the viability regent presto blue in comparison with Alamar blue and MTT to assess the viability of human corneal epithelial cells. J Pharmacol Toxicol Methods. 2015; 71:1-7. Available from https://doi.org/10.1016/j.vascn.2014.11.003
27. Hong T, Yin JY, Nie SP, Xie MY. Applications of infrared spectroscopy in polysaccharide structural analysis: Progress, challenge and perspective. Food Chem X. 2021; 100168. Available from: https://doi.org/10.1016/j.fochx.2021.100168
28. De Bruyan A, Van LJ. The identification by 1H-and 13C-n.m.r. spectroscopy of sucrose,1-ketose, and neokestose in mixtures present in plant extracts. Carbohydr Res. 1991; 2: 131-6. Available from: https://doi.org/10.1016/0008-6215(91)84151-4
29. Le TH, Le LS, Nguyen DC, Tran TT, Vu Ho XA, Tran TM, et al. Rich d-fructose-containing polysaccharide isolated from Myxopyrum smilacifolium roots toward a superior antioxidant biomaterial. ACS Omega. 2022; 27:47923–32. Available from: https://doi.org/10.1021/acsomega.2c05779
30. Jeevitha J, Ramanan R. The efficiency of phytobiotics of Indian medicinal plant *Tridax procumbens* L. against wound infecting bacteria. MOJ Curr. Res. Rev. 2018; 1:278-80.
31. Huong PT, Trang DT, Thu VK, Mai NT, Nhiem NX, Yen PH, et al. Four new triterpene glycosides from the aerial parts of chenopodium album and their cytotoxic activity. Phytochem Lett. 2021; 44:7–13. Available from: https://doi.org/10.1016/j.phytol.2021.05.004
32. Choucry MA, Shalabi AA, El Halawany AM, El-Sakhawy FS, Zaiter A, Morita H, et al. New pregnane glycosides isolated from Caralluma hexagonal lavranos as inhibitors of α-Glucosidase, pancreatic lipase, and advanced glycation end-product formation. ACS Omega. 2021; 27:18881–9. Available from: https://doi.org/10.1021/acsomega.1c02056
33. Jeevitha J, Ramanan R. The efficiency of phytobiotics of Indian medicinal plant Tridax procumbens L. against wound infecting bacteria. MOJ Curr. Res. Rev. 2018; 1:278-80.
34. Syed A, Benit N, Alyousef AA, Alqasim A, Arshad M. In-vitro antibacterial, antioxidant potentials and cytotoxic activity of the leaves of Tridax procumbens. Saudi journal of biological sciences. 2020; 1:757-61. https://doi.org/10.1016/j.sjbs.2019.12.031
35. Fischer D, Geyer A, Loos E. Occurrence of glucosyl sucrose [α-D-glucopyranosyl-(1→ 2)-α-D-glucopyranosyl-(1→ 2)-β-D-fructofuranoside] and glucosylated homologues in cyanobacteria: structural properties, cellular contents and possible function as thermoprotectants. FEBS J. 2006; 13:137–49. Available from: https://doi.org/10.1111/j.1742-4658.2005.05050.x
36. Petraglia T, Latronico T, Fanigliulo A, Crescenzi A, Liuzzi GM, Rossano R. Antioxidant activity of polysaccharides from the edible mushroom Pleurotus eryngii. Molecules. 2023; 26:2176. Available from: https://doi.org/10.3390/molecules28052176
37. Bush PL. pectin: chemical properties, uses and health benefits. Nova Science Publishers, Incorporated. 2014, 1-288.
38. Delphi L, Sepehri H, Khorramizadeh MR, Mansoori F. Pectic-oligosaccharides from apples induce apoptosis and cell cycle arrest in MDA-MB-231 cells, a model of human breast cancer. Asian Pacific Journal of Cancer Prevention. 2015; 3:5265-71. Available from: https://doi.org/10.7314/APJCP.2015.16.13.5265

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Supplementary Data

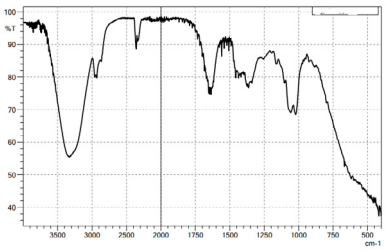


Figure S1: FTIR of Polysaccharide(KBr)

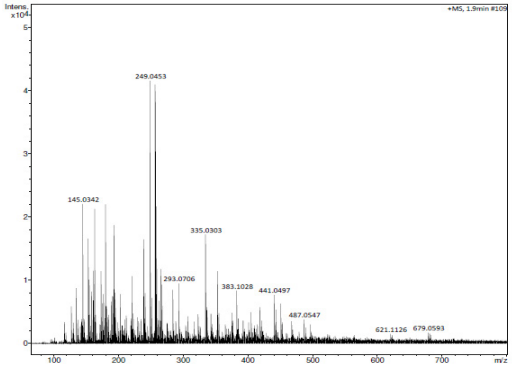


Figure S2: HRESIMS (Positive ion mode) fragmentation of Polysaccharide

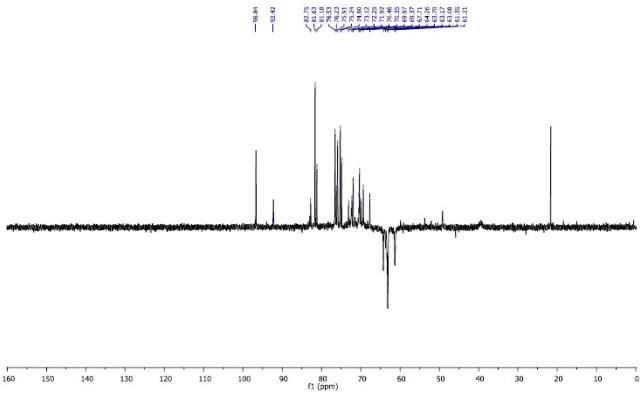
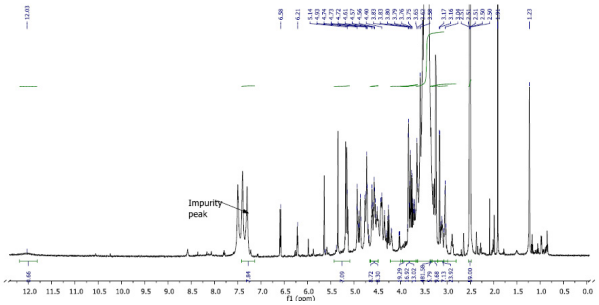


Figure S5: DEPT 135 [DMSO, 125 MHz] of Polysaccharide



^{1}H= 2.55, 3.55, methanol and water in DMSO peaks.

Figure S3: 1H-NMR [DMSO, 500 MHz] of Polysaccharide

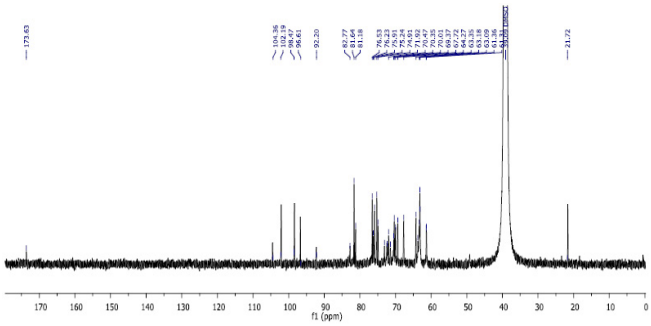


Figure S4: ^{13}C -NMR [DMSO, 125 MHz] of Polysaccharide

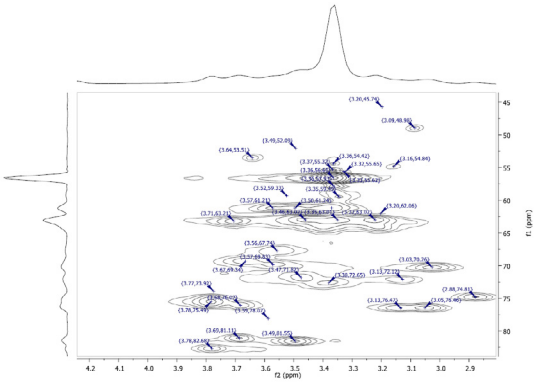


Figure S6: 2D ^{1}H→^{13}C Heteronuclear single-quantum correlation spectroscopy (HSQC) [DMSO 500, 125 MHz] spectrum of Polysaccharide