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ORIGINAL ARTICLE

In Vitro Biological Studies of Isoflavonoid from *Tridax procumbens* L.

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ABSTRACT
Phytochemical investigation of the aerial part of the methanol extract of T. procumbens leads to isoflavonoid (Genistein glycoside) for the first time from this plant through a silica gel column chromatographic technique. Various spectroscopic methods were used for the structure identification of Genistein glycoside. Further Genistein isoflavonoid evaluated for in vitro biological studies such as antiproliferative, antimicrobial, antioxidant, and antituberculous activities. Genistein isoflavonoid was found significant (P < 0.05) cell viability against MCF-7 cell lines with an IC_{50} value of 8.43 µg/mL and 4.99 µg/mL against MDA-MB-249 cells by MTT assay. Excellent antioxidant activity of Genistein glycoside was found with an IC_{50} value of 1.05 µg /mL (P < 0.05). The MIC of 125 µg/mL was found against S. aureus and P. aeruginosa. Genistein glycoside showed antimycobacterial activity at higher concentration against Mycobacteria tuberculosis (vaccine strain, H37RV strain). This plant serves as a source of Genistein isoflavonoid as an anticancer, and antimicrobial agent and finds applications in many pharmaceutical, food, and cosmetics industries.
Keywords: *Tridax procumbens*; Isoflavonoid; Antiproliferative, Antioxidant activity; Antimicrobial activity; Antituberculosis.

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INTRODUCTION

The *Asteraceae* is one of the largest flowering plant families with 25,000 species in 1600 genera worldwide. Several studies suggested that the species has pharmacological activities such as anticancer, antimicrobials, and antioxidants. *Tridax procumbens* L. herb belongs to the *Asteraceae* family [1, 2]. Nevertheless, various chemical compounds have been examined as potential ant-proliferative or cytotoxic constituents of some *Asteraceae* species [3]. *Tridax procumbens* plant is a source of flavonoids, coumarins, and polysaccharides. Natural products like phytosterols, flavonoids, and saponins are present in dietary food, they do not have harmful side effects such as synthetic anticancer drugs, antimicrobials, and antioxidants [4]. *T. procumbens* is about 15-40 cm high, with a woody stem base, hairy, and 4-30 mm ovate long leaves. The corolla's yellow disc is about 2-5 mm wide [5, 6]. Its native place is Mexico, spread all over the world. In India, this plant has been used since ancient times to cure wound formulation [7]. Natural products used in cancer treatment in recent years due to their low toxicity, and strong effectiveness [8]. Isoflavones and flavonoids are reported as potent antioxidant, anticancer natural product agents [9]. Biological activities of the isoflavones vary with the chemical structure. Isoflavones occur in the plant with glycoside but in the intestine transfer into aglycon [10]. Cell death, mutagenesis, carcinogenesis, and impacts on gene expression have all been linked to isoflavonoids. Isoflavonoids play

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an important role in inhibiting cancer cells via suppressing proliferation, angiogenesis, metastasis, and apoptosis [11].

Thus World Health Organization (WHO) published, that two pathogens *P. aeruginosa*, and *Staphylococcus aureus* nowadays resistant to available antibiotics [12]. Genistein glycoside is a heterocyclic compound known for its antioxidant, anti-inflammatory, anticancer, antibacterial, and anticancer activity [13]. Here we describe the chemical investigation of the Genistein glycoside as well as the biological activities tests such as antimicrobial on *S. aureus* and *P. aeruginosa* strains, antioxidant and antituberculous.

MATERIAL AND METHODS

General experimental Method

The JASCO P-2000 polarimeter was used to record optical rotation. The 1D and 2D experiments (HMBC) were performed using a standard BRUKER (^{1}H NMR 500 MHz and ^{13}C NMR 125 MHz) spectrometer. HRMS spectra were obtained in positive ion mode using a Bruker compass data analysis mass spectrometer. The analytical TLC has been performed on Merck pre-coated TLC plates of silica gel 60F_{254}. Spots were visualized using the *p*-anisaldehyde-H_2SO_4 reagent.

Plant Material

The plant was collected from August to December 2018 in Ambegaon (Bk.), Pune district of Maharashtra, India. It lies between the 18º31'13" North part and 73º51'24" East part of India. The aerial part of the plant was washed thoroughly with tap water. The parts of the plant were shade-dried for about 15 days. The plant was authenticated at the Botanical Survey of India (BSI), Pune. The voucher specimen was (VVI02) also submitted to the herbarium of the BSI, Pune.

Extraction of plant and isolation of active components

In the extraction process, 300.0 g of the aerial parts of the plant material was extracted successively by the polar to non-polar method using the soxhlet extraction method. The yield of methanol extract was obtained at 23.27%. Column (4.5 × 120 cm) was prepared with silica gel 1:40 in pure pet ether. A total of 46.0 g of methanol extract was adsorbed with 1: 1 g of silica gel and dried completely. Further adsorbed extract was loaded onto silica gel column chromatography (60-120 mesh size, ASI to 7661-86-91, Fischer Scientific). Column elution started with petroleum ether followed by successive applications of solvent gradients of pet ether - ethyl acetate 95:5, 90:10, 85:15, 75:25, 65:35, 50:50, 0: 100 % then the solvent gradient of ethyl acetate: methanol 50:50, and 0:100 (solvents AR grade Rankem). The fractions of 50 ml volume were collected in tubes. Preliminary TLC (pre-coated plates of silica gel 60$_{F254}$) spots all fractions were done to check the number of similar pots and their rf values of each fraction. Fractions showing similar spots were combined, concentrated, and dried with a rotary evaporator obtaining 12 major fractions. Out of these, fraction11 (2.0 g) were rechromatographed by CC (2.5 × 60 cm) onto 80.0 g. Isoflavone was obtained as a pure yellowish-orange liquid fraction identified by TLC with the solvent system (30:70 Pet ether: EtOAc). Rf value 0.55 dark orange colour spot seen on TLC and blue fluorescence in 365 nm wavelength. The spot was visualized in anisaldehyde after heating with a hot gun. Selective tests were used for the identification of compounds. The structure of the compound was identified by using 1D, and 2D NMR techniques.

Cytotoxicity assay (MTT Assay)

Cell Culture: MCF-7, MDA-MB-249 (human breast estrogen-dependent adenocarcinoma) cells and Doxorubicin (Standard) were cultured in DMEM, (Dulbecco's modified eagle's medium), MEMB medium composed of ten per cent (FBS) fetal bovine serum. In 96-well plates, cells were placed in a density of 5000 cells per well in 100 µL. They were following an incubation period (37°C) and an atmosphere in a humidified environment with CO_2 (5%). The endpoint MTT assay was then carried out according to the provided protocols [14]. The selected living cells (5×10^3 cells per well) were planted for 24 hours in 96-well plates. MTT (5 mg/mL) each well-received 20 µL was added to each cell before being incubated for 4 hours (37°C). In the MTT assay to dissolve crystals, DMSO control (100 µL) was mixed in each cell. Further plates were incubated overnight (37°C). Each concentration of the tested compound (6.25, 12.5, 25, 50 µg/mL) was added into microplate wells, and incubation was continued (24 hours). Followed by to each well, 10 µL MTT was added, and plates were incubated (4 hours). Florescent and emission excitation were measured at 544 and 570 nm respectively using (1550-800375C Multiskan SkyHigh). By comparing the average absorbance values of the tested compound and medium-containing cells the % inhibition was calculated.

The % inhibition was determined using the formula

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

Where the average absorbance of media **A**, the average absorbance of compound **B** and the average absorbance of the sample compound **C**. Based on the percentage caused by the compound, the IC_{50} value was calculated based on linear regression analysis [14]. The experiment was carried out quarterly.

Antioxidant activity

The antioxidant activity was carried out by using the DPPH radical-scavenging method [15]. In the DPPH radical scavenging activity 0.1 mM DPPH was prepared in ethanol. Different concentrations of compound in a volume of 12.5, 31.5, 62.5, 125, 250, and 500 µg/mL were added to 1.0 mL of 0.1mM DPPH in methanol and finally made up to 3.0 mL with ethanol. A mixture of DPPH and methanol was used as a control. The mixture was kept for 30 min in the dark. The absorbance was determined using a UV spectrophotometer at 517 nm. L-ascorbic acid and gallic acid were used as standard controls. The results were calculated using the formula:

Free radical scavenging rate % (%RSA) = (AC-A)/AC × 100

A is the absorbance of the compound and Ac is the absorbance of control. All samples were tested in triplicate. The average mean of three different experiments was determined as % RSA. The curve was drawn with the concentrations of the compound against % RSA. The IC_{50} value was determined by the linear regression method. [16].

Antibacterial activity

Materials and Chemicals

Incubator at 37° C (Klenzone 2019 model no. country), Pipettes of various sizes, sterile tips, 100 – 1000 µl, Vortex mixer (Make), Petri dishes, Sterile flasks 100 – 1000 ml (Borosil), Sterile normal saline, Sterile nutrient broth (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 model no. country) antibiotic streptomycin.

Bacterial Strains

Medium: 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.

For the evaluation of antibacterial activity, *Staphylococcus aureus* (NClM5021) and *Pseudomonas aeruginosa* (NCIM5029) Gram-positive and Gram-negative bacteria were used. These were obtained from the National Collection of Industrial Microorganisms (NCIM).

2.3 Broth microdilution method

MIC was performed by broth dilution method on isolated compounds by two-fold dilution and some changes of procedure as described by serial dilution using a microplate reader [17,18]. Dimethyl sulphoxide (DMSO) was used for a stock solution to a final concentration of 1.0 mg/L. Six different dilutions were made ranging from 500 µg/mL to 15.5 µg/mL The bacterial suspension was prepared using aseptic techniques an isolated colony of bacteria was transferred into flask *nutrient* broth which was incubated overnight at 37°C. Broth suspension of bacterial cultures of *Staphylococcus* and *Pseudomonas* was ready after 24 hrs of incubation. The absorbance of the culture was recorded at 500 nm. The range obtained was between 0.5 - 1.0 (Cell density 1.5 × 108 CFU/mL) UV- Spectrophotometer (BioEra -2017, Country name). From this suspension, 180 µl suspension was inoculated into each cell was inoculated into each well. A sterility control well and a growth control well were also studied for each strain. The microtiter plates were incubated (37°C), for 24 h for bacteria. For the broth microdilution test, 20 µL of two-fold serially diluted compounds were 20 µL was added to the cells of a 96-well microliter plate already containing µL of each bacterial suspension in a suitable growth medium in a proper growing medium. The final volume of each well was 200 µL. The wells were loaded with 180µL of bacterial suspension, leaving the last column for DMSO only as a 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 (i.e. 500 µg/ml to 15.5 µg/ml – serial dilution). The top and bottom rows of the plate were loaded with sterile PBS (Phosphate buffer saline- 1x, pH 7.4) solution to prevent evaporation of culture media in the incubator. These plates were incubated overnight at 37°C. After the incubation plates were observed and analyzed using a Microplate reader Read well Touch-2019, India. The absorbance was taken at 400 nm [19]. Positive and negative controls were placed in wells with growth medium and pure compounds. The plates were washed with PBS. This second dose of compounds in fresh media having the above-mentioned concentration was added and incubated for 24 hours. All measurements of MIC values were repeated in triplicate [16].

Anti-tubercular activity

Anti-tubercular activity using the 96-well microplate Alamar Blue assay method previously described [20]. This approach is highly agreed with proportional and BACTEC radiometric methods and is non-

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toxic. A thermally stable reagent was used to minimize the medium evaporation in the test wells during incubation using a microplate Alamar Blue assay. The anti-mycobacterial activity of compounds was evaluated against M. tuberculosis by adding 200 µL of sterile deionized water to all outer perimeter wells 96-well plates. To reduce the amount of medium that dried out in the test wells throughout incubation, 200 µL of sterile deionized water was added to all of the sterile 96-well plates outside perimeter wells. The MIC value was determined by a change in colour from pink to blue. The experiment was carried out 5 times.

Statistical analysis

From a minimum of three determinations SD values were obtained. MS Excel was used for data analysis using one-way ANOVA using Microsoft Excel. A significant difference was recorded at levels of *P<0.05*.

RESULT AND DISCUSSION

The compound was obtained as orange oil, soluble in methanol, with concentrated 10% NaOH, and showed a yellow colour indicating flavonoid. The compound also showed a red color with concentrated sulphuric acid indicated as a glycoside. The structure of the Genistein glycoside was established by using various spectroscopic methods UV, FTIR, 1D NMR, 2D NMR, and HRMS data. At λ_{max} 254 nm and 365 nm, the compound showed blue fluorescence indicating that it had a highly conjugated system under UV light.
IR (KBr)-3650-3000 cm^{-1}, 2950 -2800 cm^{-1}, 1700 -1600 cm^{-1}, 1400 -1300 cm^{-1} 1100 -1000 cm^{-1} shown in fig. S1.

Optical rotation $[\alpha]_D^{25}$ = -12.2° (c 0.4352, DMSO) was negative. The molecular formula was established as $C_{45}H_{60}O_{23}$, at m/z 969.0061 $[M + H]^+$ (observed), 968.350 $[M^+]$ (calculated) for in addition, the HRESIMS spectrum of Fig. S2. showed main fragment peaks m/z 648, 446 (evidence 5-methoxy Genistein isoflavonoid, 249 are shown in Fig S2. [21, 22]. m/z 284, 266 strong evidence for the presence of Genistein isoflavonoid [23]. These are characteristic peaks for isoflavone suggesting the bonding of glucose moiety, m/z 249 suggesting a strong evidence peak for the presence of isoflavonoid [24].

The proton NMR spectrum (Fig. S3) displayed seven aryl proton signals at 8.64 (1H, s, H-6), 8.55 (1H, s, H-6′), δ_H 8.40 (1H, s, Hz, H-2), 8.33 (1H, m, H-2′), 7.91 (1H, s, H-8), 7.33 (1H, m, H-5′), 7.11 (1H, m, H-3′), Three anomeric protons 5.18 (1H, d, *J* = 3.7 Hz, H-1'''') indicating α-orientation, δ_H 4.97 (1H, d, *J* = 11.3 Hz), δ_H 4.96 (1H, m, H-1′′) *β*-orientation from coupling constants, the series of overlapping protons of sugar was observed at δ_H 3.80 -2.98. Isoflavonoid showed peaks between 8-9 ppm [24].

On comparison of proton NMR data with the literature, the compound was identified as Genistein 5-methoxy, 7-O- [β-D- rhamnopyranosyl-(2→2)-fructofuranosyl]-4′-O- [β-D- rhamnopyranosyl 1→2]-α-D-rhamnopyranoside.

The ^{13}C NMR spectrum (Table, Fig. S4-S5) represents a total 45 carbons, out of these two carbonyl carbons δc 177 (C-4), 172 (C-7′′′), seven aromatic tetras substituted, 163.1 (C-9), 157.9 (C-4′), 155.4 (C-7) 154.1(C-3), 152.7 (C-5), 117.6 (C-1′), 104 (C-10), methine carbons 143.9 (C-6′), 142.7 (C-2′), 129.9 (C-5′), 128.7 (C-3′), 123.7 (C-5′), Saccharide anomeric carbon signals three trisubstituted 103.5 (C-1′′′′) 97.3 (C-1′′) 92.3 (C-1′′′′′) carbons indicated the presence of three glucopyranose rings and one quaternary anomeric carbon 101.0 (C-2′′′) suggested for fructofuranose ring. All sugar methylene protons were identified at δ_C 81-61.

HMBC correlations (Fig. S6-S7a-e) were used to identify the linkage of the side chain and the attachment to the aglycon. δ_H 4.97 (1H, *d*, J = 11.3 Hz) of anomeric proton and gave cross-correlation with C-7 (155.7) and (δc 4.96) (1H, *d*, *J* = 11.3 Hz) showed correlation with 157.6 (C-4′). The anomeric proton δ_H 5.18 (1H, *d*, *J* = 3.7 Hz), bearing at 92.3 gave relation to δ_C 103.5, suggested linking 1→2-α-D- glucopyranoside.

δ_H 3.29 gave a relation to quaternary carbons at δ_C 101.0 and anomeric carbon 97.3 This suggests that α-(2→2) links to another sugar unit fructofuranose. This data suggests that there are four sugar units in the compound, one rhamnose, two glucose, and one fructose unit attached to isoflavonoid. HMBC spectrum was used to achieve the complete assignment of sugar shown in (Table 1, Fig. S6) [25-27]. δ_H 3.32 correlated to 67 (C-6′′′), δc 172 (C-7′′′), δc 18.02, δ_H 1.29 (C-8′′′) give relation to δc 67 (C-6′′′) confirmed the attached of glycoside.

The structure of the compound was confirmed by comparing the spectroscopic data with the values reported in the literature. The spectroscopic data were used to structure elucidation and compared to the literature. As Genistein, 5-methoxy, 7-O- [β-D- rhamnopyranosyl-(2→2)-fructofuranosyl]-4′-O-[β-D-rhamnopyranosyl 1→2]-α-D- rhamnopyranoside.

Anticancer activity

Anticancer activity was evaluated for Genistein isoflavonoid isolated from *Tridax procumbens* L. Results are presented in Fig. 2-3. Genistein isoflavonoid was screened for cytotoxicity against human breast cancer MCF-7 and MDA-MB-249 cells using an MTT assay using DMSO as a control. The IC_{50} value is 50%

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cell viability, Genistein isoflavonoid was found significant at ($P < 0.05$) 8.43 µg/mL against MCF-7 cell lines and at 4.99 µg/mL ($P > 0.05$) against MDA-MB-249 cells lines. Treated cell viability is reduced in a concentration-dependent manner. An increase in efficacy against tested cell lines suggests that the structure of the phenolic substituent in the third position of isoflavonoids may appear to play a significant role in the class of drugs. Genistein isoflavonoid showed activity against breast cancer cell lines.

Antioxidant activity

Genistein glycoside's antioxidant capacity was evaluated using the DPPH test. It has been found that the compound exhibited good antioxidant capacity with an IC_{50} value of 1.05 µg/mL. Ascorbic acid and gallic acid were used as standard references (Table 2 and Fig. 4). The compound exhibited a strong antioxidant agent that might stop or prevent and slow delay the onset of ageing or various disorders associated with oxidative stress. According to the literature, C-ring glycoside could be unfavourable for antioxidant activity [28].

Antimicrobial activity

The antibacterial activity of Genistein glycoside was evaluated against *P. aeruginosa* and *S. aureus* Fig. 5 and 6 showed that Genistein glycoside proved to have a strong biofilm activity at concentrations ranging from 15.5 to 500 µg/mL. The standard antibacterial agent streptomycin is a positive control. MIC of both selected bacteria was found at 125 µg/mL. The Genistein glycoside exhibited a stronger antimicrobial activity than streptomycin against *P. aeruginosa* at all concentrations. Moderate activity was observed against *S. aureus*. In conclusion, the positive results encourage researchers to search potential of Genistein isoflavone in developing novel drugs.

Anti-tuberculosis activity

The results of antitubercular screening of isolated Genistein glycoside are presented in Fig. S8. The antimycobacterial activity was found at MIC value 50 µg/mL against Mycobacteria tuberculosis (vaccine strain, H37RV strain) ATCC No- 27297, by Alamar Blue assay. This study revealed the antitubercular activity of *Tridax procumbens* L. The anti-tuberculosis activity of *T. procumbens* ethanol and water extracts showed excellent activity with MIC values of 0.8 g/mL and 6.25 g/mL, respectively, compared to standard drugs Pyrazinamide, Ciprofloxacin, and Streptomycin, against Mycobacterium tuberculosis (H37Rv strain). The author suggested that it is due to the presence of tannin, and flavonoids, phenolic compounds [29].

Table 1. 1H NMR, 13C NMR, DEPT (DMSO, 125 MHz), HMBC (DMSO, 500 MHz) **Genistein glycoside**

Position	Δc	DEPT	δH	HMBC
2	140.85	CH	8.40 (s)	C3
3	154.1	C	-	-
4	177.2	C=O	-	-
5	152.7	C		-
6	93.27	CH	8.64 (d, 3.4 Hz)	C7, C-5
7	155.4	C	-	
8	97.9	CH	7.91(s)	C6, C7,C9
9	163.1	C	-	
10	104.5	C	-	
1'	117.6	C	-	
2'	142.5	CH	8.33 (d)	C2'
3'	128.7	CH	7.11	
4'	158.1	C	-	-
5'	129.9	CH	7.33 (d1H)	C6'
6'	143.7	CH	8.55	C5', C1'
5OCH3	59	CH_3	3.77	C5
7-O β–D-Rhamnose				
1''	97.3	CH	4.97	C7, C2'',C3''
2''	71.5	CH	3.29	C1'', C3'',C2'''
3''	72.1	CH	3.04	C2'' C4''
4''	70.2	CH	3.76	C3'',C5''
5''	76.3	CH	3.48	C4'',C6''
6''''	63.4	CH	2.99	C5'''
3''-CH3	18.0	CH	1.28	C3''

Fru (2→2)				
1'''	63.3	CH_2	2.98	
2'''	101.0	-	-	
3'''	77.5	CH	3.59	C2'''
4'''	72.6	CH	3.72	C3'''
5'''	81.3	-	-	-
6'''	63.6	CH2	3.17	C5'''
4′-β-O-D-Rhamnose				
1''''	103.5	CH	4.96	C4', C2''''′ ,C3''''′
2''''	76.7	CH	3.83	C1''''
3''''	71.2	CH	3.42	C2'‴
4''''	72.4	CH	3.32	C3‴,C4‴
5''''	70.7	CH	3.35	C6‴
6''''	62.3	CH	2.99	C5‴
7''''	172.0	CO	-	-
8''''	18.0	CH_3	1.27	C7''''
[1→2]-α-D- Rhamnose				
1'''''′	92.3	CH	5.18	C1'''', C2'''''′
2'''''′	73.2	CH	3.30	C1'‴', C3'‴'
3'''''′	68.0	CH	3.93	C2‴'′, C4'‴'
4'''''′	74.96	CH	3.86	C3'‴', C5'‴'
5'''''′	71.49	CH	3.93	C4‴'', C6'‴'
6'''''′	61.93	CH_2	2.99	C5'‴'
3'''''′-CH_3	18.0	CH_3	1.27	C3'‴'

Table 2. Antioxidant activity Genistein glycoside

Radical scavenging activity (%)						IC_{50} µg/mL
Concentration µg/mL	100	200	300	400	500	
Genistein glycoside	51.27 ± 0.008	53.48 ± 0.011	54.70 ± 0.004	54.98 ± 0.006	59.46 ± 0.009	1.05
Ascorbic Acid RSA	23.85 ± 0.095	47.08 ± 0.057	60.66 ± 0.051	70.04 ± 0.048	88.95 ± 0.055	2.41
Gallic Acid	46.05 ± 0.049	62.64 ± 0.002	70.02 ± 0.001	72.71 ± 0.003	83.02 ± 0.39	0.9233

Ascorbic acid was used as standard, the scavenging capacities were represented as percentage inhibition and values were the means of three replicates (levels significance of $P < 0.01$, Mean ± SD, n = 3).

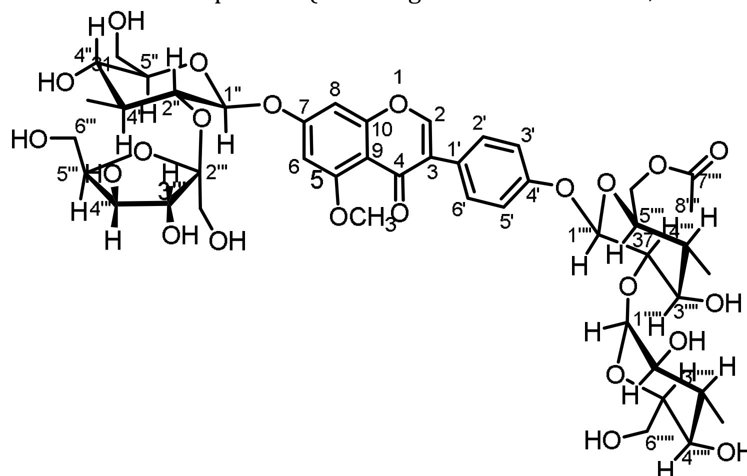

Figure 1. Structure of Genistein, 5-methoxy, 7-O- [β-D- rhamnopyranosyl-(2→2)-fructofuranosyl]-4′-O-[β-D- rhamnopyranosyl 1→2]-α-D- rhamnopyranoside isolated from *T. procumbens* L.

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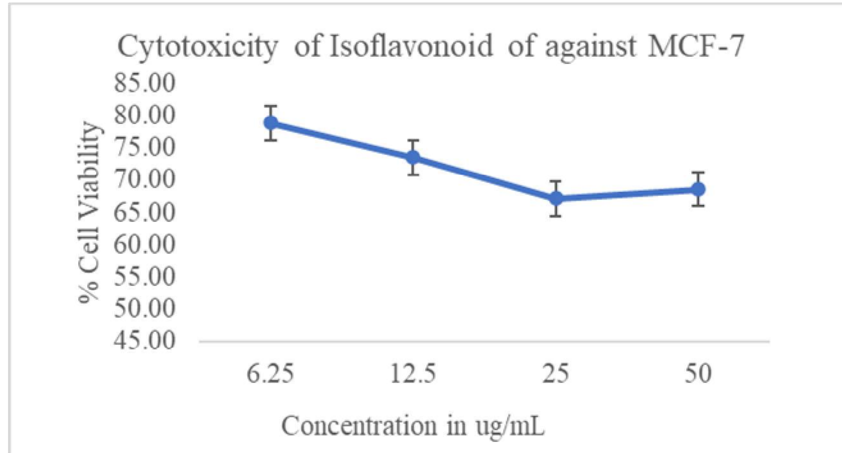

Figure 2. Anti-proliferative activity of Genistein Isoflavonoid against MCF-7

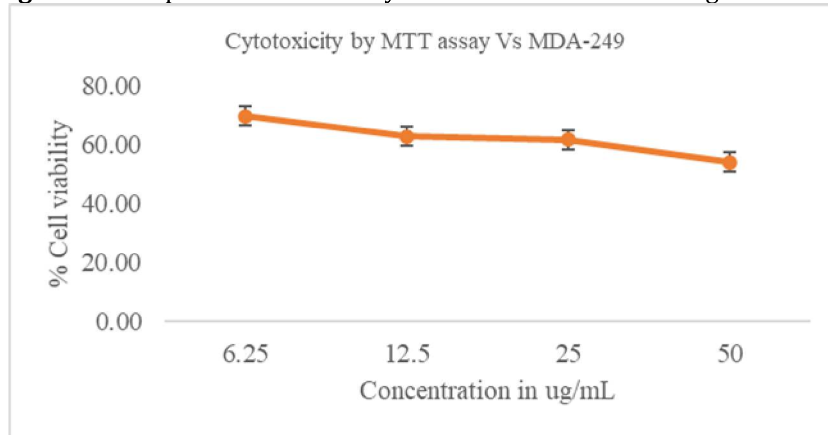

Figure 3. Anti-proliferative activity of Genistein Isoflavonoid against MDA-MB-249

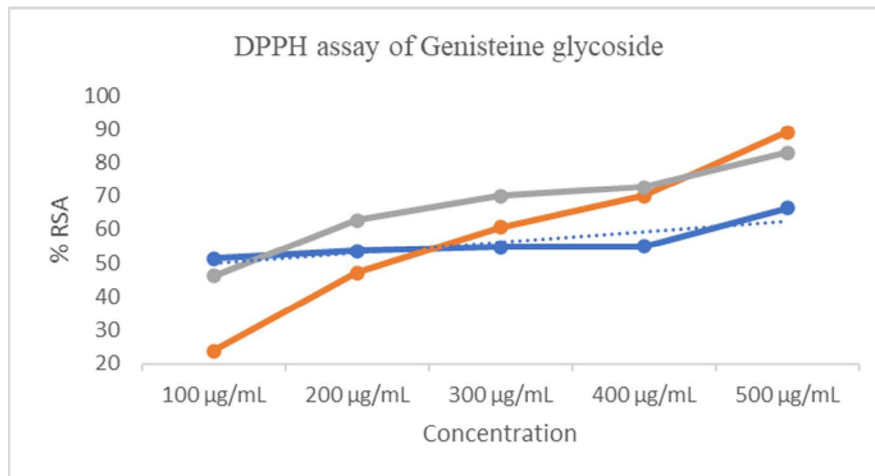

Figure 4. Antioxidant activity of Genistein Glycoside

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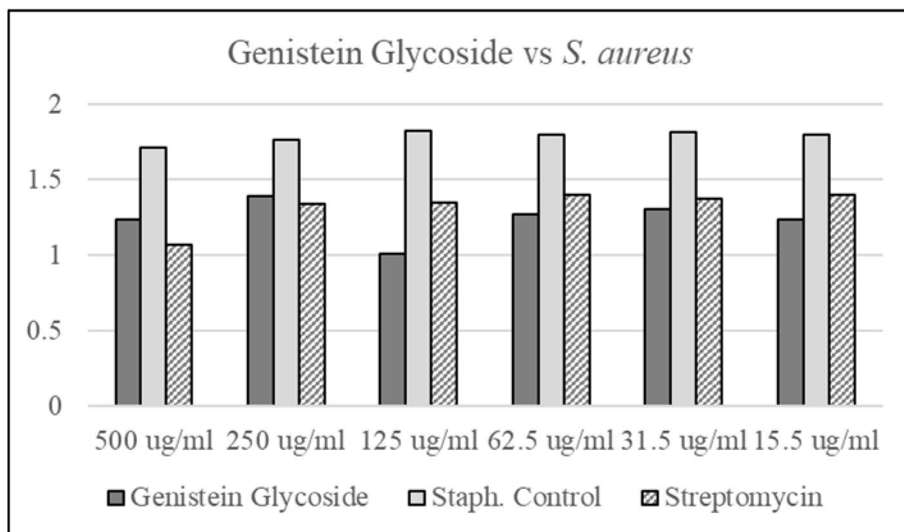

Figure 5. Antibacterial activity of Genistein glycoside against *S. aureus*

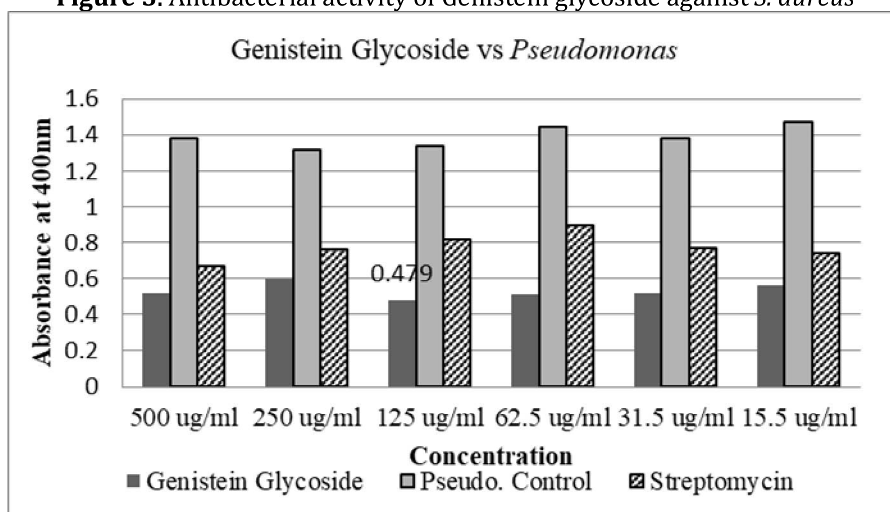

Figure 6. Antibacterial activity of Genistein glycoside against *P. aeruginosa*

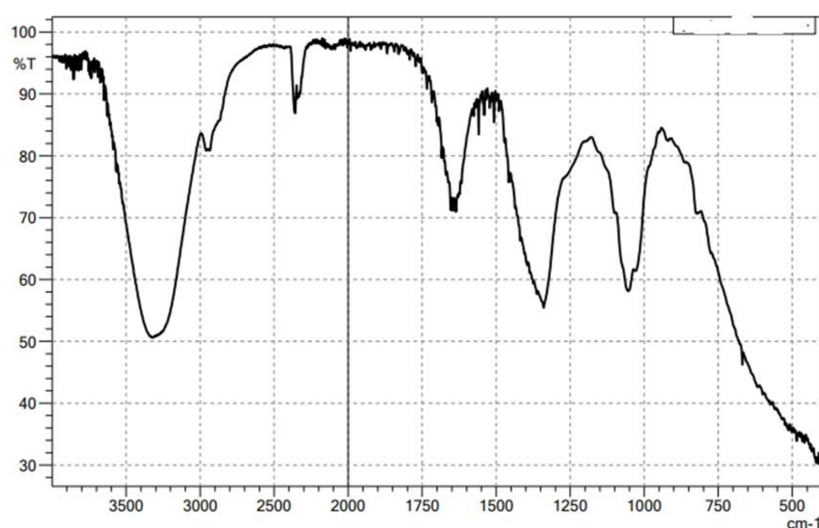

Figure *S*1. FT-IR(KBr) of Genistein glycoside

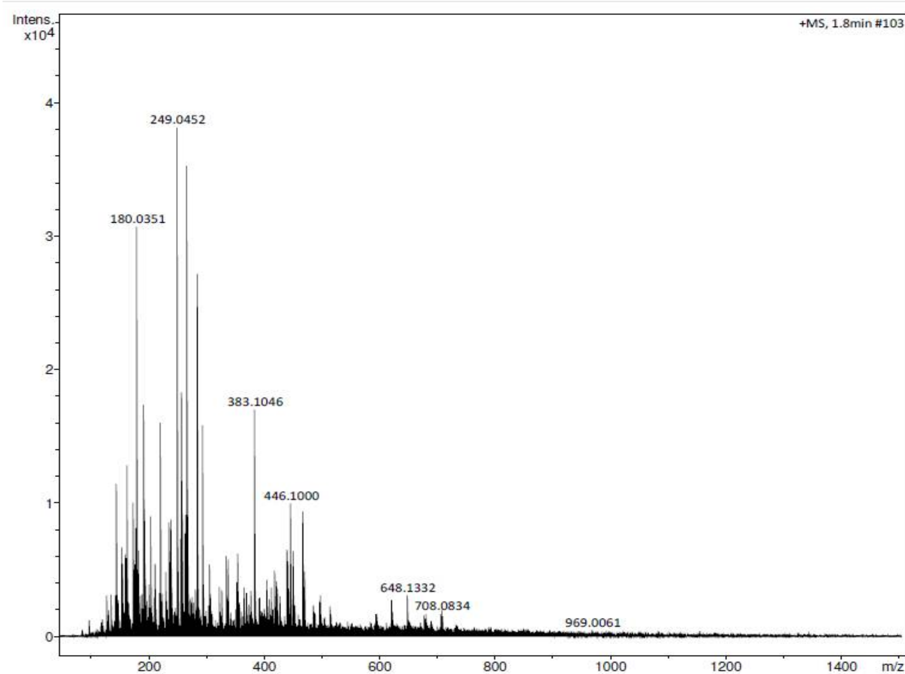

Figure S2. HRESIMS (positive ion mode) of Genistein glycoside

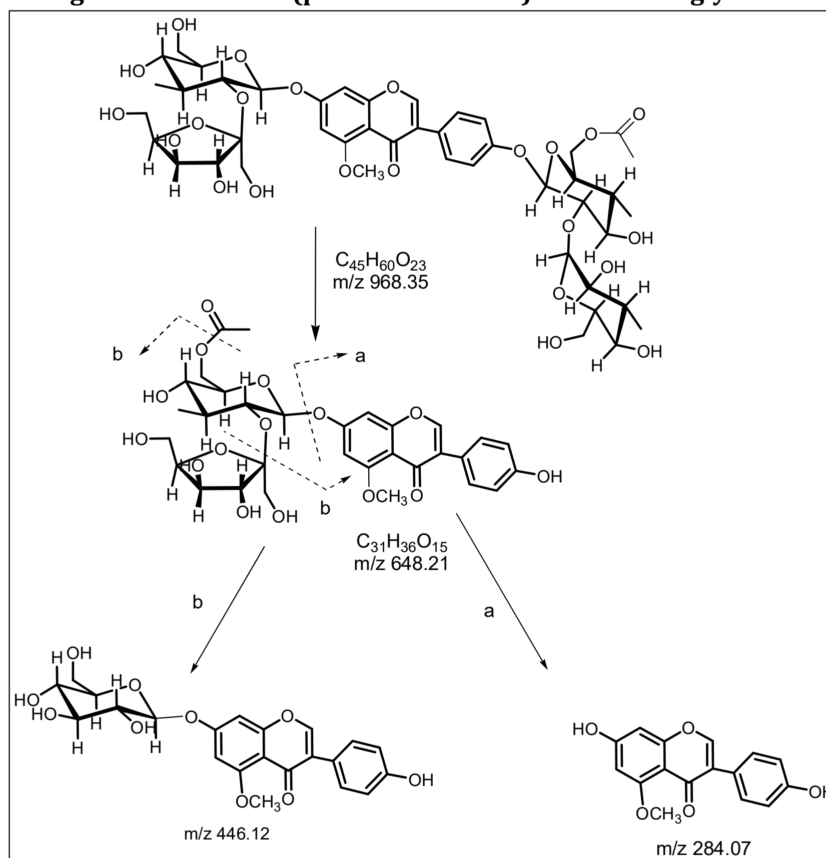

Figure S3. HRESIMS (positive ion mode) *fragments* of Genistein glycoside

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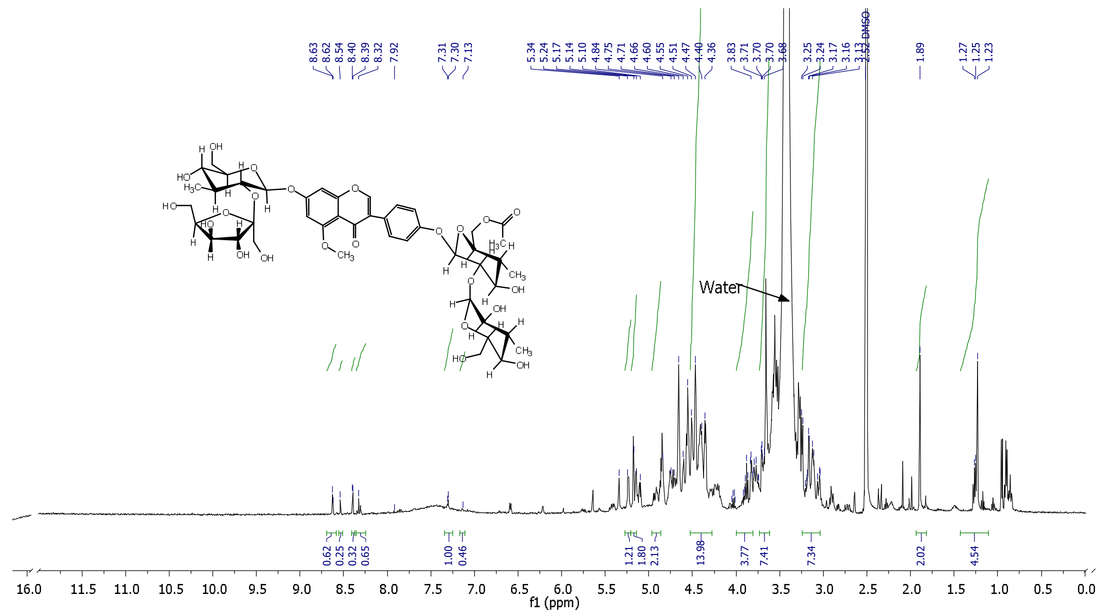

Figure S4. ^{1}HNMR (DMSO 500 MHz) Genistein glycoside

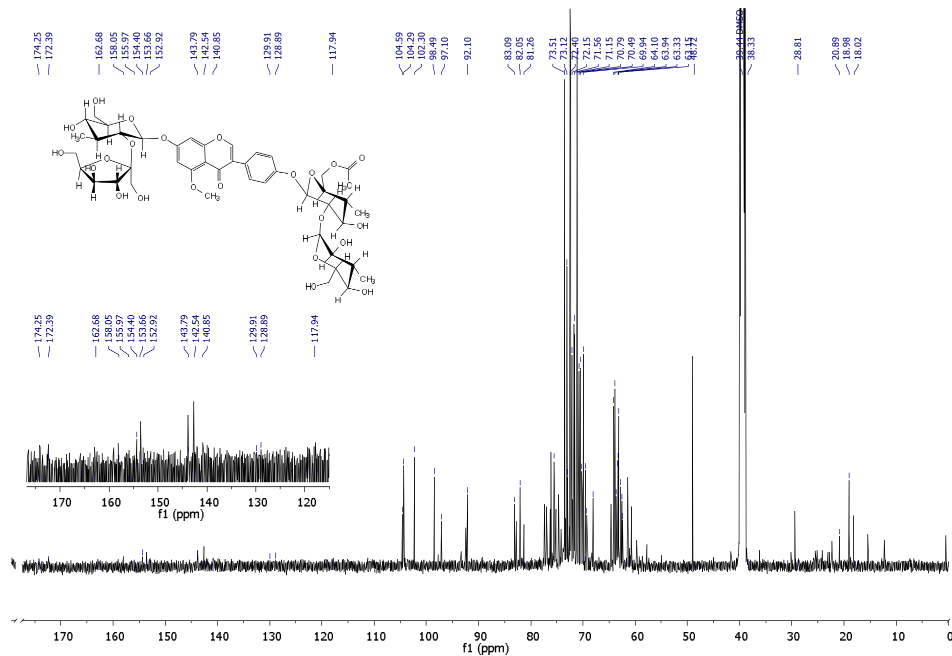

Figure S5. ^{13}C NMR (DMSO 125 MHz) spectrum of Genistein glycoside

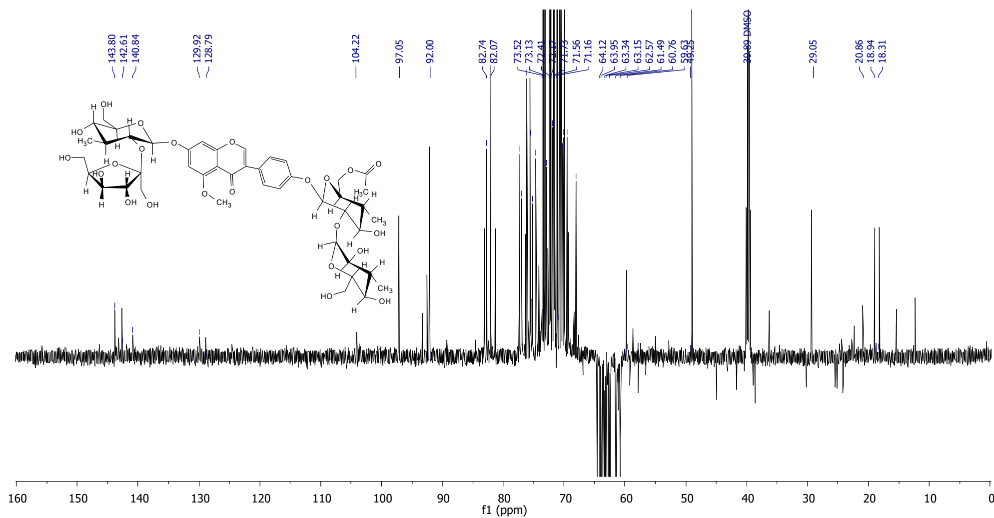

Figure S6. ^{13}C NMR DEPT 135 [DMSO 500 MHz] spectrum of Genistein glycoside

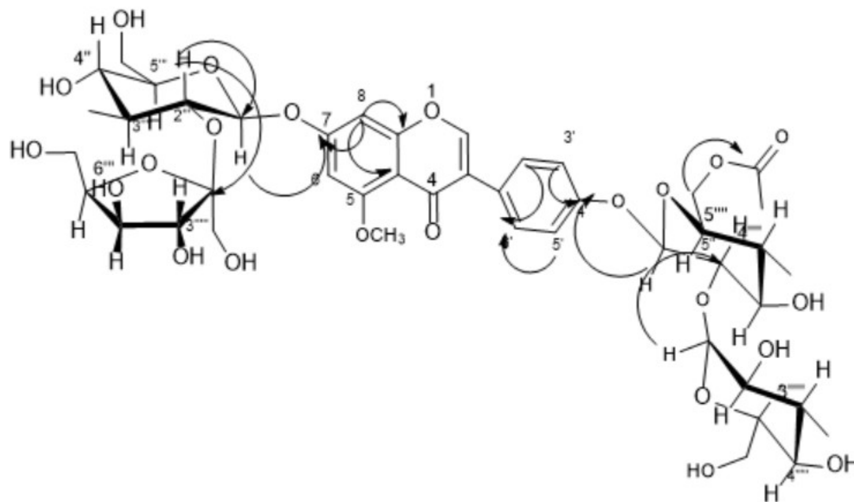

Figure S7. Key HMBC (H→C) correlations are shown by red lines of Genistein glycoside

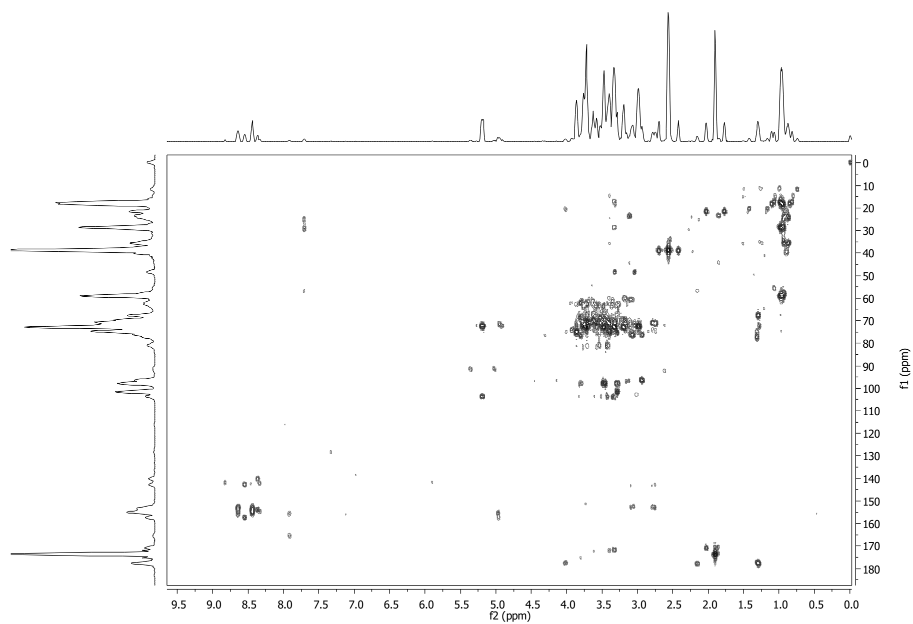

Figure 8a. ^{1}H-^{13}C HMBC spectrum (DMSO 500 MHz) of Genistein glycoside

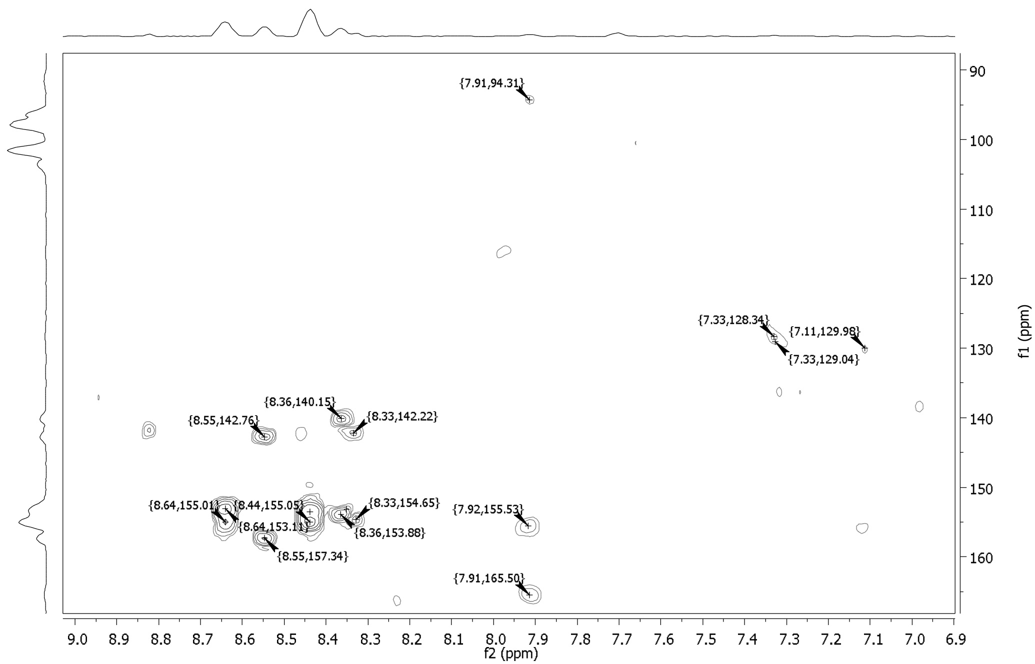

Figure 8b. ^{1}H-^{13}C HMBC spectrum (DMSO 500 MHz) of Genistein glycoside

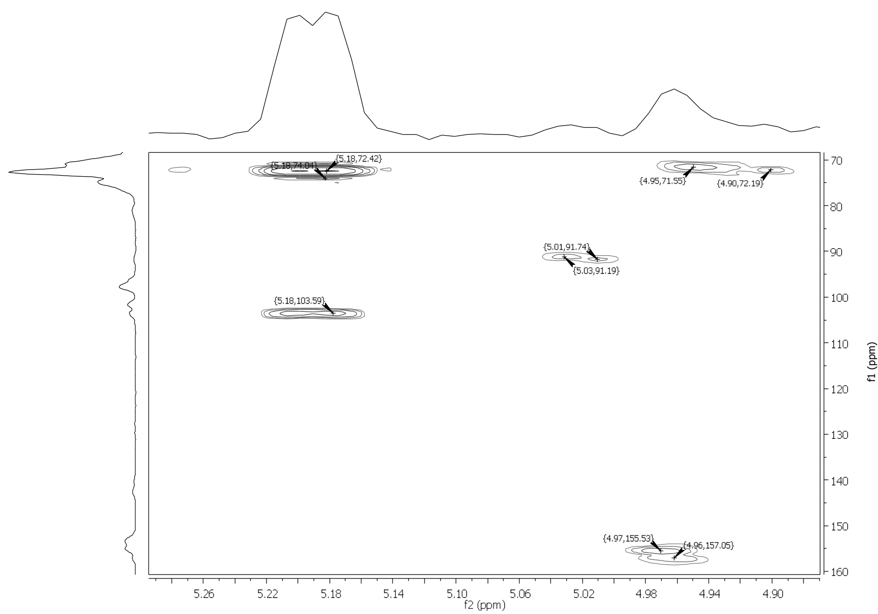

Figure 8c. ^{1}H-^{13}C HMBC spectrum (DMSO 500 MHz) of Genistein glycoside

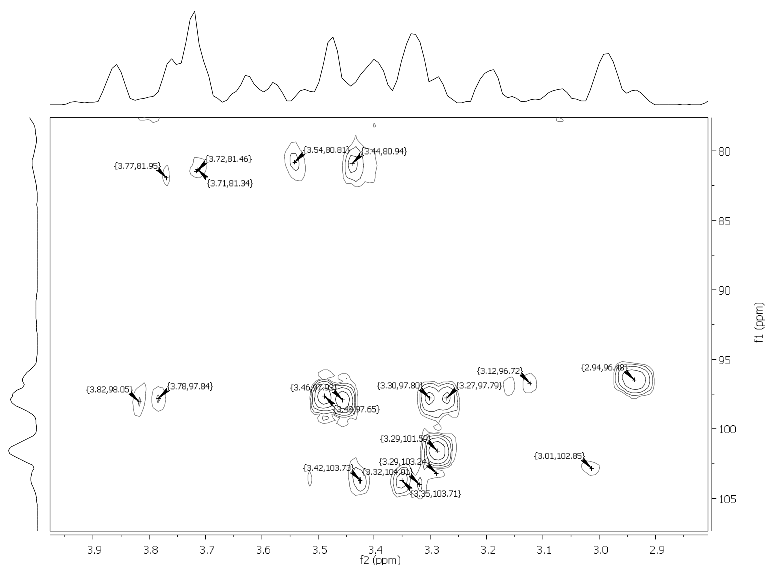

Figure 8d. ¹H-¹³C HMBC spectrum (DMSO 500 MHz) of Genistein glycoside

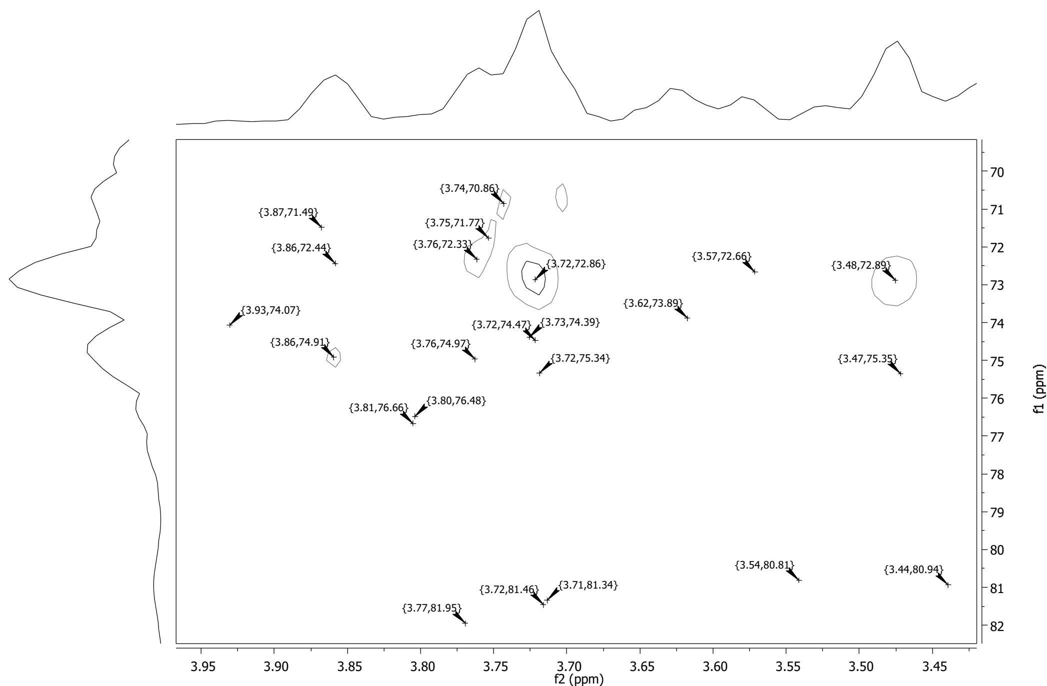

Figure 8e. ¹H-¹³C HMBC spectrum (DMSO 500 MHz) of Genistein glycoside

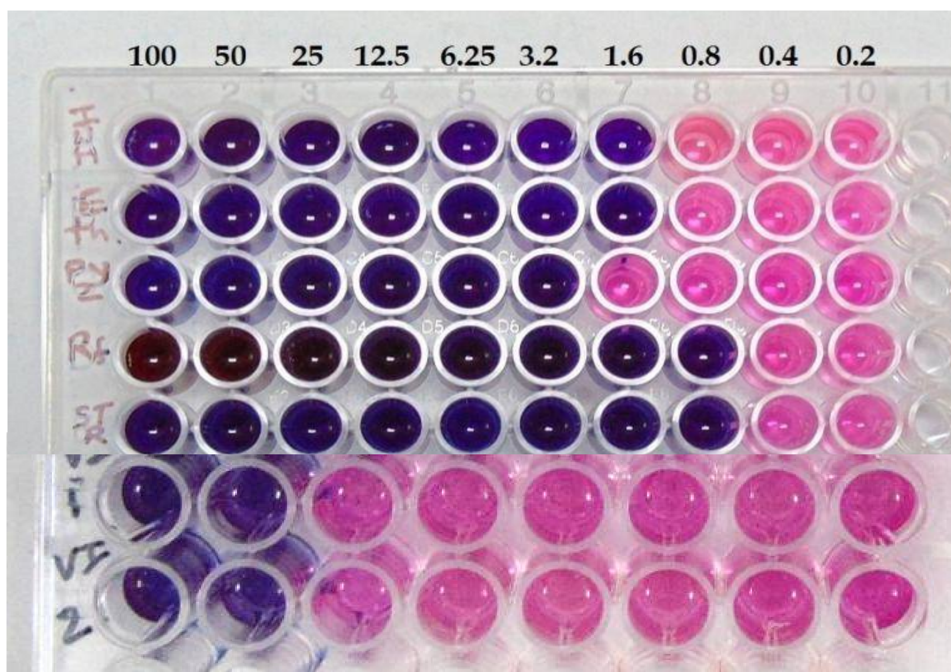

Figure 9. Antitubercular activity of Genistein glycoside (VI 1)

CONCLUSION

This study suggests that the aerial part of *T. procumbens* is a rich source of flavonoids. In this investigation, we presented the isolation, antiproliferative and antituberculosis activity of Genistein glycoside. It confirms the traditional uses of the plant in the treatment of free radical, infectious-related diseases. The strong antioxidant agent could prevent and slow the progress of ageing or various diseases associated with oxidative stress. Significant antibacterial activity was observed for flavonoid, it may have immense potential for the control diseases related to *S. aureus and P. aeruginosa* pathogens at higher concentration activity was greater than streptomycin used standard. The aerial part of *T. procumbens* can be used as a source of water-soluble drugs, nutraceuticals, etc. Therefore, this herbal plant can be used in food, cosmetics, and pharmaceutical applications.

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AUTHORS CONTRIBUTION

S.K. conceptualized the idea. S.K. and V. I. performed the literature search. V. I wrote the first draft of the manuscript. S. K. and P.M. S.B. critically reviewed the revised manuscript. All the authors have read and approved the final version of the manuscript. All data was generated in-house, and no paper was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

REFERENCES

1. Verma RK, and Gupta MM. (1998). Lipid constituents of *Tridax procumbens*. Phytochemistry; 27(2):459-463 (1998).
2. VV Ingole, PC Mhaske, SR Katade. (2022). Phytochemistry and pharmacological aspects of *Tridax procumbens* (L.): A systematic and comprehensive review. Phytomedicine Plus. 2 (1): https://doi.org/ 10.1016/ j.phyplu.2021.100199
3. Rolnik A, & Olas B. (2021). The Plants of the Asteraceae Family as Agents in the Protection of Human Health. International Journal of Molecular Sciences. *22*:6, https://doi.org/10.3390/ijms22063009

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4. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. (2020). Flavonoids as Anticancer Agents. Nutrient. 12:2. https://doi.org/10.3390/nu12020457
5. Powell AM, (1965). Taxonomy of tridax (Compositae). Brittonia. 17:47–96. https://doi.org/10.2307/2805391
6. Rogers S. (1969). Some Observations on the Reproduction of *Tridax procumbens* L II. Development of the embryo sac and embryo. Cytologia. 34 **(**2), 196-201.
7. Udupa SL, Udupa AL, Kulkarni DR. (1991) Influence of *Tridax procumbens* on lysyl oxidase activity and wound healing. Planta Medica. **57**: 325-327.
8. Yuan L, Cai Y, Zhang Liang, Liu Sijia, Li Pan, Li Xiaoli. (2022) Promoting Apoptosis, a Promising Way to Treat Breast Cancer with Natural Products. A Comprehensive Review. Frontiers in Pharmacology. 12.
9. Wei H, Saladi R, Lu Y, Wang Y, Palep SR, Moore J, Phelps R, Shyong E, Lebwohl MG. (2003). Isoflavone genistein: photoprotection and clinical implications in dermatology. J Nutr. 133**.**
10. M. Begoña Ruiz-Larrea, Aarthi R. Mohan, George Paganga, Nicholas J. Miller, G. Paul Bolwell & Catherine A. (1997). Rice-Evans Antioxidant Activity of Phytoestrogenic Isoflavones, Free Radical Research. 26(1): 63-70
11. Razak NA, Abu N, Ho WY, Zamberi NR, Tan SW, Alitheen NB, Long K, Yeap SK. (2019). Cytotoxicity of eupatorin in MCF-7 and MDA-MB-231 human breast cancer cells via cell cycle arrest, anti-angiogenesis, and induction of apoptosis. Sci Rep. 6;9(1):1514. doi 10.1038/s41598-018-37796-w.
12. WHO World Health Organization. Available online: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed [accessed on 9 July 2022].
13. Górniak, I, Bartoszewski R, & Króliczewski J. (2019). A comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev. 18, 241–272.
14. Mossman T. (1983). Rapid colourimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 16(65):55-63. doi: 10.1016/0022-1759(83)90303-4. PMID: 6606682.
15. Williams W, Cuvelier, ME and Berset CL. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology. 28(1): 25-30.
16. Ingole VV, Katade SR. (2024). Chemical composition, antioxidant, antibacterial activity of isolated oil and methanol extract of *Tridax procumbens* L. Int J Pharm Sci & Res. 15(4): 1157-66. doi: 10.13040/IJPSR.0975-8232.15(4).1157-66.
17. Eloff JN. (1998). A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria. Planta Medica. 64: 711-713. Doi: 10.1055/s-2006-957563. PMID: 9933989.
18. Sarker SD, Nahar L, Kumarasamy Y. (2007). 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. 42(4):321-4. Doi: 10.1016/j.ymeth.2007.01.006
19. Adan A, Kiraz Y, Baran Y. (2016). Cell Proliferation and Cytotoxicity Assays. Curr Pharm Biotechnol. 17(14):1213-1221.
20. Linda Feketeová, Christopher K, Barlow, Timothy M, Benton, Simone J. Rochfort, Richard AJ, O'Hair. (2011). The formation and fragmentation of flavonoid radical anions. International Journal of Mass Spectrometry. 301(1–3): https://doi.org/10.1016/B978-0-12-396485-4.00178-X.
21. López-Jácome LE, et al. (2016). Increment Antimicrobial Resistance During the COVID-19 Pandemic: Results from the Invifar Network. Microb Drug Resist. 28(3):338-345. xDoi: 10.1089/mdr.2021.0231.
22. B. A, Prabhushankar HB. (2021). Screening of Novel Source for Genistein by Rapid and Sensitive UPLC-APCI-TOF Mass Spectrometry. Int J Food Sci. 16: 5537917.
23. Ablajan, K. (2011). A study of characteristic fragmentation of isoflavonoids by using negative ion ESI-MS^{n}. J. Mass Spectrom. 46: 77-84.
24. Xiao X, Xu L, Hu H, Yang Y, Zhang X, Peng, Xiao P. (2017). DPPH radical scavenging and postprandial hyperglycemia inhibition activities and flavonoid composition analysis of hawk tea by UPLC-DAD and UPLC-Q/TOF MSE. Molecules. 22(10): 1622.
25. Phansalkar RS, Simmler, Charlotte, Bisson, Jonathan, Chen, Shao-Nong, Lankin, David C, McAlpine, James B, Niemitz, Matthias, Pauli, Guido F. (2017). Evolution of Quantitative Measures in NMR: Quantum Mechanical qHNMR Advances Chemical Standardization of a Red Clover (Trifolium pratense) Extract. Journal of Natural Products. 80:634- 647, https://doi.org/10.1021/acs.jnatprod.6b00923
26. Chen WH, Ma XM, Wu QX, Shi, YP. (2008). Chemical-constituent diversity of *Tridax procumbens*. Can. J. Chem. 86:892–898. https://doi.org/10.1139/V08-097
27. Cui HX, Zhang LS, Yan HG, Yuan K, Jin SH. (2020). Constituents of flavonoids from *Tridax procumbens* L. and antioxidant activity. Pharmacogn. 16:201. https://doi.org/10.4103/pm.pm_229_19
28. Wang R, Li W, Fang, et al. (2023). Extraction and identification of new flavonoid compounds in dandelion *Taraxacum mongolicum* Hand.-Mazz. With the evaluation of antioxidant activities. Sci Rep. 13:2166. https://doi.org/10.1038/s41598-023-28775-x
29. Bhagat VC, Kondawar MS, (2019). The antitubercular potential of dendrophthoe falcate (l.) And *Tridax procumbens* (l.) Plants extract against h37rv stain of mycobacteria tuberculosis. Int. J. Pharmaceut. Sci. Res. 10: 51-259.

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