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RESEARCH ARTICLE

In vitro antioxidant potential and antimicrobial activity of leaves and stem extracts of *Anogeissus pendula* Edgew.

Priyanka Danai, Varsha Pandey & Teena Agrawal*
Department of Bioscience and Biotechnology, Banasthali Vidyapith Rajasthan 304 022, India
*Email: tagrawal024@gmail.com

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ABSTRACT

Anogeissus pendula Edgew. is commonly used in the conventional Indian medicinal system and is reported to contain phenolic compounds which have antioxidant and antimicrobial potential. The goal of our study is to look at the antioxidant function and antibacterial activity of *A. pendula* leaf and stem extracts. The total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) were determined using a spectrophotometric technique (TTC). *In vitro* techniques such as 1, 1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide radical scavenging tests (H_2O_2) and Ferric reducing antioxidant power (FRAP) assay were used in the study. The disc diffusion technique was used to assess antibacterial activity and the minimum inhibitory concentration (MIC) was investigated against four bacterial strains. The TTC of leaf and stem methanol extract was considerably higher which ranged from 15.07 ± 0.506 to 38.77 ± 1.253 mg gallic acid equivalent (GAE) /g in leaves and 19.83 ± 0.084 to 28.56 ± 0.437 mg GAE/g in the stem. The content of flavonoid in the leaf and stem methanol extract varied from 12.53 ± 0.603 to 37.28 ± 0.466 mg rutin equivalent (RE) /g in leaves and 10.01 ± 0.177 to 37.28 ± 0.466 mg RE/g in stems. Hydroalcoholic extract of leaf and stem showed the highest tannin content and ranged from 23.73 ± 0.091 to 34.08 ± 0.261 mg tannic acid equivalent (TAE) /g. In order of efficacy (IC_{50}) of the plant extracts, the effective inhibitor was the methanol extract of leaf and stem in the DPPH and H_2O_2 assay. FRAP value was higher in the hydroalcoholic extract of both leaf and stem. Antimicrobial activity tests revealed that all extracts limit the development of diverse microbial strains such as *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas putida* and *Streptococcus aureus* with a mean zone of inhibition ranging from 0 to 15.67 mm. The MIC of *A. pendula* leaf and stem solvent extracts against bacterial strains ranged from 0.195 to 50 mg/ml. The findings revealed that *A. pendula* has a variety of phytochemicals with substantial antioxidant and antibacterial properties, confirming its usage in traditional medicine.

Introduction

The utilization of conventional medicines in emerging countries leads straightforwardly to the financial situation and prosperity of the countries associated with communities (1, 2). Plants have been the origins of complicated conventional drug systems that have existed for thousands of years and are still giving people new cures (3). Traditional medicine (TM) is the most developed form of health care in the world and is used in the anticipation and healing of physical and psychological sicknesses. Traditionally, various civilizations have developed a range of effective therapeutic approaches to tackle various health and life-threatening disease (4). TM is also known as complementary or ethnic medicine, and it still plays a key role in many countries today (5).

Oxidative stress is a major threat factor for many incurable illnesses in pathogenesis. Any component that slows or prevents oxidative damage to the target molecule can be loosely defined as an antioxidant (6). Herbal plants are also known as strong antioxidants since ancient times. Natural antioxidants are very efficient in mitigating destructive processes related to oxidative stress, whether in the form of crude extracts or chemical components (7). Recently concern in bioactive compounds has grown significantly for use in skincare products, foods and medicinal products, as they have immense scope to correct imbalances (8).

Microorganisms are closely associated with the health and welfare of human beings. Today microbial infection, resistance to antimicrobial drugs have been the biggest challenges that threaten the health of societies and are responsible for millions of deaths

every year worldwide. The problems of multidrug resistant pushed scientists to look for novel antibacterial medicines from unexpected sources, such as medicinal plants (9). Antibiotic indiscriminate usage has resulted in the evolution of resistance strains in bacteria, and a number of antibiotics have lost their efficacy against those pathogens in recent years (10). In this sense, natural medicines derived from medicinal plants may provide an alternative supply of antimicrobial agents that might have a significant influence on infectious illnesses and general human health (11).

From medicinal plants *A. pendula* Edgew. (Combretaceae) known as Button tree in English and Kardhai or Dhonk in Hindi is extensively used by the local communities of Rajasthan, Madhya Pradesh and UP in India for traditional uses. It has long been used in Asia and Africa. Stem, bark, twig, seeds and fruits are the widely used sections of the plant. Traditionally, various illnesses such as gastrointestinal disorders, anemia, diarrhea, wound healing, cough and inflammation are treated with these plant components. *A. pendula* is widely used to treat gastric disorders that could be caused by the presence of the phenols (12). The present research is therefore concerned with the study of phytochemicals and antioxidant activity of different solvent extracts of leaves and stems of *A. pendula*.

Materials and Methods

Plant material

In the month of August, plant material of *A. pendula* Edgew. was collected from the Sariska Tiger Reserve forest of Rajasthan. Compared to the voucher specimen (37392) at the Dehradun herbarium of systematic botany discipline, Forest Research Institute, Dehradun, the plant was identified and its authenticity was confirmed.

Plant extracts preparation

At ambient temperature, the stems and leaves were air dried for 1 week and then crushed using a grinder machine into powder. The fine powder mixture was subjected to a cold maceration process for separation. Approximately 250 g of air dried plant matter was filled in a washed and air dried 3L conical flask and then 2500 ml of various solvents, such as methanol, hydro alcohol, and water was poured. The flask was kept in a rocker shaker for three consecutive days and after then the extract was purified in a round bottom flask using Whatman filter paper No. 1 and the filtrate was procured and dried up with the Rotaevaporator.

Quantitative estimation of phytochemicals

Assessment of Total phenolic contents (TPC)

TPC was measured using the Folin- Ciocalteu (FC) process with some modifications (13). Different aliquots of methanol, hydro alcohol and aqueous extract of *A. pendula* were taken, and 0.5 ml of the FC reagent has been applied and gently mixed. 20% Sodium carbonate (1 ml) and 12.5 ml of purified water was added after 2 min. The contents were carefully combined and made to stand at room temperature for 2 hr. Transmittance was estimated at 720 nm and TPC was represented as equivalent to gallic acid per g of extract.

Assessment of Total flavonoid content (TFC)

TFC was assessed by the aluminum chloride colorimetric process and was calculated using rutin as a standard with some slight modifications (13). Various aliquots (20, 40, 60, 80 and 100 µl) of methanol, aqueous and hydro alcohol extract of *A. pendula* were taken and to this 1.5 ml of $NaNO_3$ reagent (5%) was incorporated and mixed gently. After that 0.15 ml aluminum chloride (10%) followed by 1 ml of NaOH (1N) solution was incorporated. The contents were mixed and left to stand for a while. The absorbances of the samples were taken at 415 nm and TFC was represented as (1mg/1 ml) rutin.

Assessment of Total tannin content (TTC)

TTC was measured by the standard procedure with some slight modification (14). Various aliquots (20, 40, 60, 80 and 100 µl) of methanol, hydroalcohol and aqueous extracts were taken and gently mixed with 0.5 ml Folin Denis reagent. After 5 min of incubation 5 ml of sodium carbonate (15 %) was added and kept in a dark at room temperature for 30 min. The transmittance was assessed at 640 nm and TTC was calculated as equivalent to tannic acid per g of extract.

Antioxidant activity

Assessment of Free radicals scavenging activity (DPPH)

DPPH radical scavenging activity was determined by the standard protocol (15). 2 ml of DPPH methanol solution was then incorporated to the different sample solution (1mg/ml) at different volumes (20, 40, 60, 80 and 100 µl). The samples were vigorously shaken for 10 min in the dark, after which the wavelength was recorded at 517 nm. Greater is the free radical scavenging activity, the lesser the absorbance of the reaction mixture. The standard used was ascorbic acid. Using linear regression analysis, the IC_{50} value was determined. Lower IC_{50} value indicates greater antioxidant activity. The inhibition percentage of the reaction mixture was calculated as.

$$\text{Inhibition \%} = \frac{\text{Ac - As x 100}}{\text{Ac}}$$

Where, Ac = Absorbance of the control

As = Absorbance of the sample

Assessment of Hydrogen peroxide radical scavenging activity (H_2O_2)

The capability of plant extracts to scavenge H_2O_2 was calculated by the standard protocol (16). H_2O_2 solution (40 mM) was made in a phosphate buffer saline (0.05 mM, pH 7.4). The extracts at varying doses were then combined with the H_2O_2 solution (0.6 ml, 40 mM). Hydrogen peroxide absorption was recorded at 230 nm after 10 min of incubation in the dark against the blank solution. The amount of hydrogen peroxide scavenging of plant extracts and standard was measured as follows:

$$\text{\% Scavenged} = \frac{\text{A - B x 100}}{\text{A}}$$

Where, A = Absorbance of the control
B = Absorbance of the sample

Determination of FRAP assay

The FRAP procedure was used to analyse the decreasing ability of the plant extracts with some minor modifications (17). The reagent mixture of FRAP was formed by adding 300 mM Acetate buffer, 10 mM TPTZ in 40 mM of HCl and 20 mM of $FeCl_3.6H_2O$ at 37 ^{o}C in the ratio 10:1:1. Freshly formulated FRAP reagent was then combined with varying amounts of plant extracts. An extreme blue color complex was developed when Fe^{3+} TPTZ complex was reduced to Fe^{2+} and the increase in absorption was examined spectrophotometrically at 593 nm. The antioxidant activity was evaluated using the linear calibration graph and represented as mmol of $FeSO_4$ equivalent per gm of dry weight of the sample

Antimicrobial activity

Microorganisms and culture media used

A total of four bacterial strains *Escherichia coli, Bacillus subtilis, Pseudomonas putida* and *Staphylococcus aureus* were studied which belong to different genera. These organisms were maintained on nutrient agar. Sub culturing was done on fresh medium when required. The master cultures were sub cultured every month and were stored in the refrigerator.

Disc diffusion method

Antimicrobial activity was performed by the standard disc diffusion method (18). Bacterial inoculums were swabbed onto nutrient agar plates. *A. pendula* leaves and stem extracts were impregnated on sterile filter paper discs at 200 mg/ml and 250 mg/ml concentrations. Extract discs were then placed on the agar plates. Each extract was evaluated in triplicates using gentamycin as a bacterial standard. After that, the plates were incubated at 37 ^{0}C for bacteria (24 hrs). The inhibition zone surrounding the discs was measured and represented in mm. The larger the zone of inhibition, the more powerful the test medication (plant extract).

Minimum inhibitory concentration (MIC)

The MIC of the plant extract was determined using the broth micro dilution method, with a few minor changes (19). The experiment was carried out utilising 96-well micro titre plates and nutrient agar broth. To prepare the 96-well plates, 50 µl of NA broth for bacteria was poured into each well. In the first row of the plate, 50 µl of the stock solution of examined extracts (concentration of 200 mg/ml) was added. Then, using a micropipette, two fold serial dilutions were done. The resulting concentration range was 100 - 0.1953 mg/ml and 10 µl of inocula was subsequently applied to each well except a positive control. As a negative control, a plant extract with medium was utilised. The test plates were then incubated for 24 hrs at 37 °C. After 24 hrs, 20 µl of Alamar blue dye (resazurin) was added to the wells and the plates were incubated for an additional 4 hrs. The wells were then examined visually for colour changes. Blue wells showed no bacterial growth, but pink wells indicated bacterial development. After incubation, the MIC value was determined to be the lowest concentration at which no viability was detected in the 96 micro well plates.

Statistical analysis

Anova was used to analyze the results, which was done using the SPSS (20) statistical program. All the tests were done in triplicate and values are given as mean ± SEM. The level of significance was considered at P≤0.05. The antimicrobial activity data were analyzed in triplicates; the average and the relative SD have been calculated using the Excel software.

Results

Evaluation of Total Phenolic content

The TPC of all the three extracts of leaves and stems of *A. pendula* is shown in Table 1. The MeOH extract (38.767 ± 4.638 mg GAE/g) of *A. pendula* leaves showed the higher phenolic compound followed by hydroalcoholic (22.433 ± 3.334 mg GAE/g) and aqueous extract (15.067 ± 2.515 mg GAE/g). In the stem also the methanol extract showed the highest amount of phenolic compound (28.56 ± 3.99 mg GAE/g) followed by the hydroalcohol (21.85 ± 2.66 mg GAE/g) and aqueous extract (19.83 ± 3.47 mg GAE/g). The results were authenticated by the linear association between the absorbance and concentration and an R^2 value of 0.999 was achieved (Fig. 1).

Evaluation of Total Flavonoid content

Table 1. Quantitative investigation of leaf and stem extracts of *A. pendula*

	Methanolic extract		Hydroalcoholic extract		Aqueous extract	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
TPC	38.77±1.253***	28.56±0.437**	22.43±1.003*	21.85±0.307*	15.07±0.506	19.83±0.084
TFC	37.28±0.466*	29.95±0.124*	25.68±0.408*	19.97±0.067	12.53±0.603	10.01±0.177
TTC	23.73±0.091	25.39±0.226	34.08±0.261*	41.71±0.682**	31.68±0.291*	6.66±0.339

The data represent mean± SEM of three determinants
*p< 0.05, significantly different from standard

The TFC of all the three extracts of leaves and stems of *A. pendula* is shown in Table 1. The MeOH extract (37.28 ± 0.466 mg RE/g) of the leaves of *A. pendula* showed the maximum amount of flavonoid content followed by hydroalcoholic (25.68 ± 0.408 mg RE/g) and aqueous extracts (12.53 ± 0.603 mg RE/g). TFC of methanol extract of stem showed the higher flavonoid content (29.95 ± 0.124 mg RE/g) than the hydroalcoholic (19.97 ± 0.067 mg RE/g) and aqueous extract (10.01 ±0.177 mg RE/g). The results were authenticated by the linear association between

absorbance and concentration and an R^2 value of 0.993 was achieved (Fig. 2).

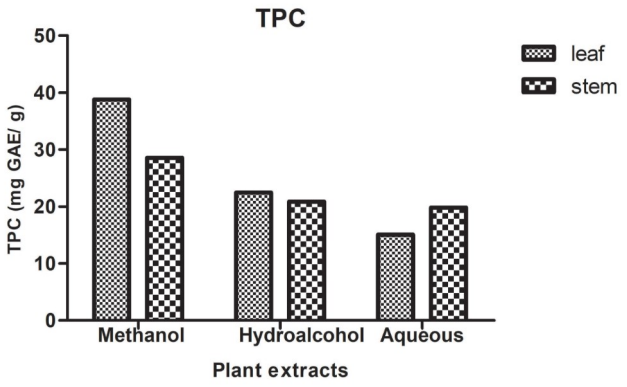


Fig. 1. TPC of leaf and stem extracts of *Anogeissus pendula.*

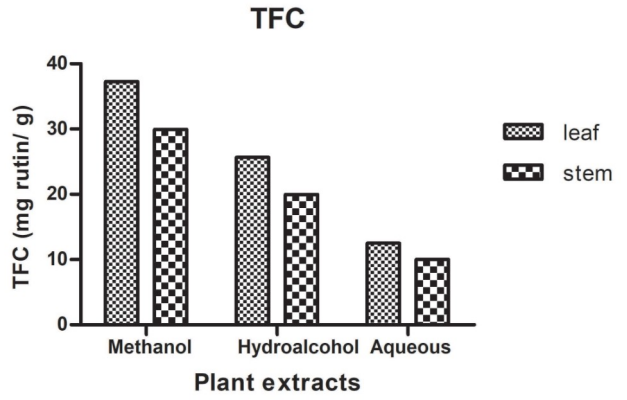


Fig. 2. TFC of leaf and stem extracts of *A. pendula.*

Evaluation of total tannin content

The TTC of leaves and stem extracts of *A. pendula* are listed in Table 1. The hydroalcoholic extract (34.08 ± 0.261 mg TA/g) of the *A. pendula* leaves showed the maximum amount of tannin content followed by aqueous (31.68 ± 0.291 mg TA/g) and methanol extract (23.73 ± 0.091 mg TA/g). The highest amount of tannin content in the stem was found in the hydroalcoholic extract (41.71 ± 0.682 mg TA/g) then the methanol extract (25.39 ± 0.226 mg TA/g) and the least quantity of flavonoid content was found in aqueous extract (6.66 ± 0.339 mg TA/g) of the stem. The results were

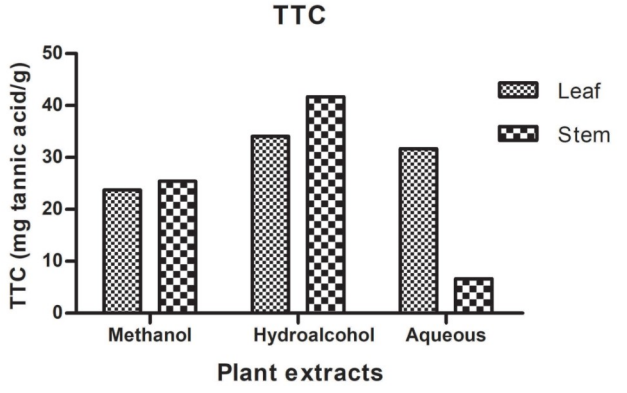


Fig. 3. Total tannin content of various leaf and stem extract of *A. pendula.*

authenticated by the linear relationship between absorbance and concentration and an R^2 value of 0.995 was achieved (Fig. 3).

Antioxidant activity

DPPH Free radical Scavenging assay

Table 2 and Fig. 4 showed the DPPH radical scavenging ability of the leaves and stem extract of *A. pendula.* The solvent extracts of *A. pendula* showed remarkable scavenging activities. Methanol extract of leaves had the maximum scavenging activity with the minimum IC_{50} value (46.22 ± 0.060 µg/ml) followed by hydroalcoholic extract (92.80 ± 0.339 µg/ml). The least radical scavenging activity was found in aqueous extract (114.4 ± 1.202 µg/ml). In stem the methanol extract showed the maximum scavenging activity with minimum IC_{50} value (77.844 ± 0.204 µg/ml) followed by aqueous extract (87.762 ± 0.201 µg/ml) and the least radical scavenging activity was found in hydroalcoholic extract (155.02 ± 0.422 µg/ml) with the highest IC_{50} value. The IC_{50} value of leaf and stem methanol extract was comparable to the IC_{50} value of Ascorbic acid (35.52 ± 0.341 µg/ml).

Hydrogen peroxide free radical scavenging activity

The effect of free radical scavenging activity of hydrogen peroxide is given in Table 2 and Fig. 5. The maximum H_2O_2 scavenging activity was seen in methanol extract of the leaves (79.30 ± 0.107 µg/ml) followed by aqueous extract (128.1 ± 0.448 µg/ml). The least scavenging activity was seen in hydroalcoholic extract with the highest IC_{50}value (186.1 ± 0.536 µg/ml). The methanol extract (110.643 ± 0.216 µg/ml) of the stem showed a higher scavenging activity followed by aqueous extract (153.316 ± 0.507 µg/ml) and the least hydrogen peroxide scavenging activity was in hydroalcoholic extract (170.458 ± 0.146 µg/ml) with the highest IC_{50} value.

Ferric reducing antioxidant potential assay (FRAP)

The FRAP value of leaves extracts of *A. pendula* is shown in Table 2 and Fig. 6A and 6B. Hydroalcoholic extract of the leaves showed the highest FRAP value (529.73± 1.073 mg/g) followed by methanol (454.01±0.171 mg/g) and aqueous extract (420.73± 0.436 mg/g). In stem, the highest FRAP value was found in hydroalcoholic extract (715.89± 1.217 mg/g) followed by methanol extract (519.02±1.439 mg/g). The least FRAP value was recorded in aqueous extract (69.36±0.889 mg/g).

Antimicrobial activity

Methanol, Hydroalcoholic and Aqueous extracts of *A. pendula* leaves and stems were tested for antibacterial activity against two gram- positives and two gram-negative microorganisms. As a positive control, gentamycin was employed. Table 3 and Fig. 7 illustrate the findings of the antimicrobial testing. The MeOH, HA and AQ extracts of *A. pendula* leaves inhibited all of the microorganisms tested in this study. The MeOH extract of stem inhibited *E. coli* and *P. putida* at concentrations of 200 and 250 mg/ml, but the aqueous extract of stem exhibited no zone of inhibition. Zone of inhibition ranged from 1.20 to 15.67 mm. In this study, *E. coli* demonstrated for the greatest sensitivity to HA extract of leaves at 200 and

Table 2. Antioxidant capacity of leaf and stem extracts of *A. pendula*

Extracts	DPPH (IC50 µg/ml)		H_2O_2 (IC50 µg/ml)		FRAP(mg equivalent Ascorbic acid/g of extract)	
	LEAF	STEM	LEAF	STEM	LEAF	STEM
Methanol	46.22±0.060***	77.844±0.204***	79.30±0.107***	110.64±0.216***	454.01±0.171**	519.02±1.439***
Hydroalcohol	92.80±0.339***	155.028±0.422***	186.1±0.536***	170.46±0.146***	529.73±1.073***	715.89±1.217***
Aqueous	114.4±1.202***	87.762±0.201***	128.1±0.448***	153.32±0.507***	420.73±0.436**	69.36±0.889
Ascorbic acid	35.52±0.341		64.37±0.024		-	

The data represent mean± SEM of three determinants
*p< 0.05, significantly different from standard

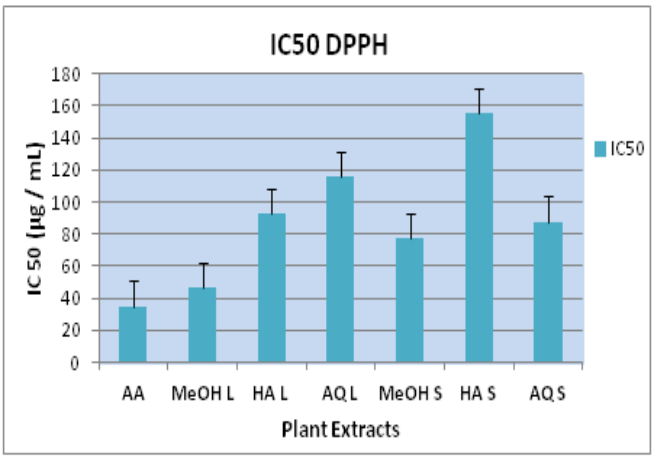


Fig. 4. DPPH antioxidant potential of leaf and stem extract of *A. pendula.*

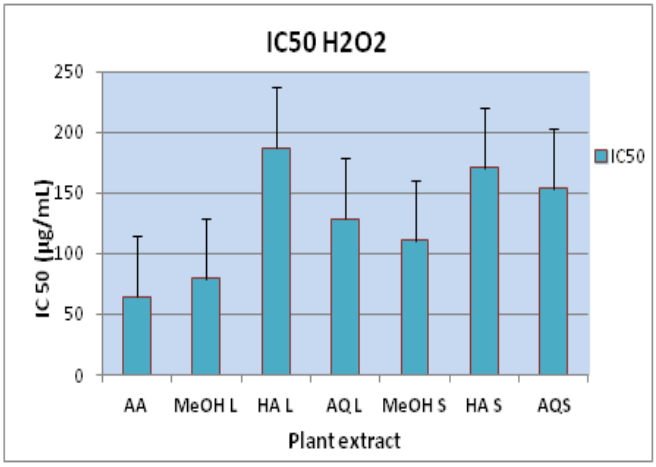


Fig. 5. H_2O_2 scavenging activity of leaf and stem extract of *A. pendula.*

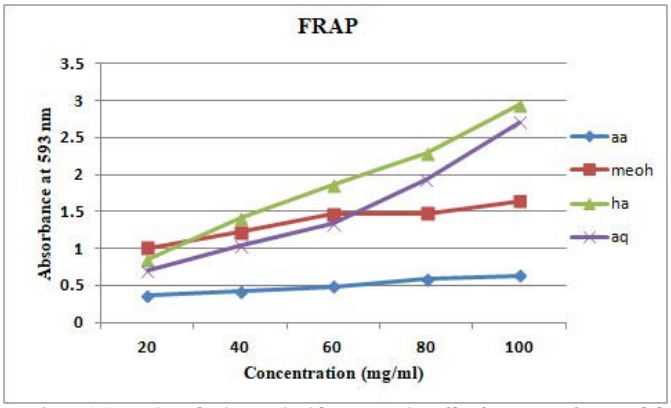
250 mg/ml concentrations, with zones of inhibition of 11.870, 0.264 mm and 15.670, 0.258 mm respectively whereas *B. subtilis* demonstrated for the lowest zone of inhibition of 1.200 and 0.115 mm against MeOH extract of stem at 250 mg/ml concentration. *S. aureus* exhibited no sensitivity to any of the stem extracts examined. The aqueous extract had no inhibitory impact on any of the four microorganisms tested.

Determination of minimum inhibitory concentration (MIC)

The MIC was determined using a bro micro dilution technique as the lowest dosage that stopped bacterial growth. The MIC data shown in Table 4 and Fig. 8 demonstrated that all four bacteria were susceptible to the minimal inhibitory concentration of *A. pendula* leaves and stem extracts. Methanol extract of leaf and stem showed lowest minimum inhibitory concentration values 0.78 and 1.56 µg/ml against *S. aureus*. Hydroalcoholic extract of leaves showed the lowest MIC value 1.56 µg/ml towards *S. aureus*, while aqueous extract of leaf showed 3.12 µg/ml MIC against *P. putida*. Hydroalcoholic extract of stem showed the least MIC of 12.5 µg/ml against *B. subtilis* and *P. putida* while the highest MIC value of 25 µg/ml was observed against *E. coli* and *S. aureus*. Aqueous extract of stem showed least MIC 25 µg/ml against *E. coli* while higher values of MIC 50 µg/ml was observed for *B. subtilis, S. aureus* and *P. putida.*

Discussion

Numerous techniques were used to assess *In vitro* antioxidant activity to enable rapid drug screening because compounds with low *In vitro* antioxidant properties are prone to show little *in vitro* actions (20). In a broad spectrum of pathological forms free radicals are considered to have a definite function. Antioxidants counter free radicals and shield us from a number of illnesses. Whether by scavenging the reactive oxygen molecules or by defending the antioxidant defence mechanism, they exert their action (21).



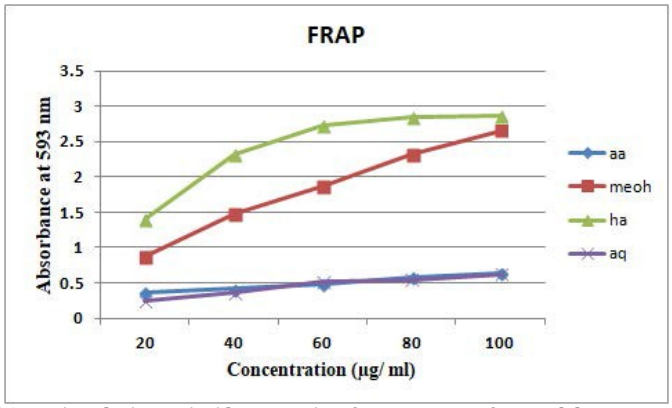


Fig. 6. (A) Ferric reducing antioxidant capacity of leaf extracts of *A. pendula*. **(B)** Ferric reducing antioxidant capacity of stem extracts of *A. pendula*.

Table 3. Comparison of zone of inhibition in mm among different crude extracts of *A. pendula* leaves and stems against gram- positive and gram- negative bacteria.

Plant extracts	Concentrations	Tested bacterial strains			
		E. coli	*B. subtilis*	*P. putida*	*S. aureus*
MeOH leaf	200 mg/mL	5.930 ± 0.058	5.270 ± 0.058	9.130 ± 0.058	3.370 ± 0.058
	250 mg/mL	7.000 ± 0.058	7.330 ± 0.058	11.267 ± 0.115	6.470 ± 0.100
HA leaf	200 mg/mL	11.870 ± 0.264	8.370 ± 0.153	8.430 ± 0.153	2.230 ± 0.058
	250 mg/mL	15.670 ± 0.258	10.970 ± 0.100	9.877 ± 0.058	4.600 ± 0.100
AQ leaf	200 mg/mL	3.100 ± 0.265	2.430 ± 0.058	5.377 ± 0.058	10.470 ± 0.058
	250 mg/mL	4.300 ± 0.231	3.600 ± 0.100	7.377 ± 0.115	13.780 ± 0.058
MeOH stem	200 mg/mL	4.470 ± 0.058	NA	5.133 ± 0.058	NZ
	250 mg/mL	5.100 ± 0.058	1.200 ± 0.115	5.670 ± 0.058	NZ
HA stem	200 mg/mL	8.870 ± 0.058	2.030 ± 0.058	3.630 ± 0.100	NZ
	250 mg/mL	9.350 ± 0.153	4.030 ± 0.058	4.970 ± 0.100	NZ
AQ stem	200 mg/mL	NZ	NZ	NZ	NZ
	250 mg/mL	NZ	NZ	NZ	NZ
Gentamycin	10 µg/mL	22.467 ± 0.115	15.253 ± 0.038	26.180 ± 0.026	21.063 ± 0.031

Data are means of 3 replicates and are represented as mean ± SD
NZ= no zone of inhibition

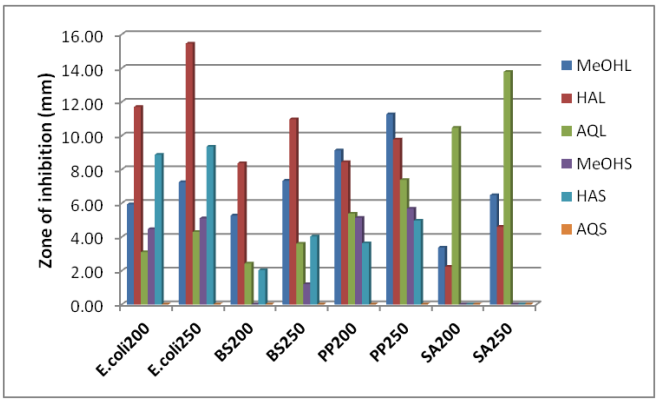


Fig. 7. Comparison of inhibition zone of *A. pendula* leaves and stem crude extracts against four bacterial strains: *E. coli, B. subtilis, S. aureus, P. putida.*

healing process. The antioxidant function of plants has been proposed to be ascribed to the existence of phenolic compounds in them (22). Therefore, in this study, we are evaluating the *A. pendula* leaves and stem extract by different antioxidant parameters in vitro as a new antioxidant agent.

Preliminary phytochemical research done for the leaves and stem extracts of *A. pendula* showed the occurrence of alkaloids, saponins, terpenoids, glycosides, phenols, flavonoids, glycosides, triterpenes, tannins and resins. According to research, the bioactive chemicals contain a wide range of biological and therapeutic activities. Each phytochemical demonstrated potency for a biological function, such as antioxidant potential in flavonoids, antibacterial,

Table 4. Minimum inhibitory concentration (MIC) of the plant extract against microorganisms by broth micro dilution method

Microorganisms	MIC (µg/mL): range (n=3)					
	MeOH L	HA L	AQ L	MeOH S	HA S	AQ S
E. coli	12.5	25	25	25	25	25
B. subtilis	3.12	12.5	25	6.25	12.5	50
S. aureus	0.78	1.56	6.25	1.56	25	50
P. putida	1.56	6.25	3.12	6.25	12.5	50

Data are means of 3 replicates
MeOH= methanol extract, HA= hydroalcoholic extract, AQ= aqueous extract

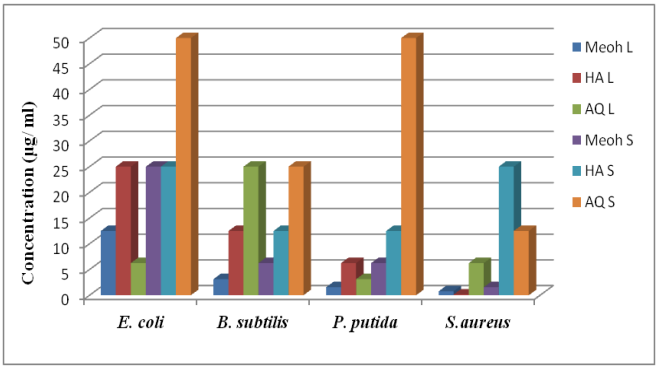


Fig. 8. Minimum inhibitory concentration (MIC) values for methanol, hydroalcoholic and aqueous crude extracts of *A. pendula* leaves and stems against *E. coli, B. subtilis, P. putida* and *S. aureus*.

There are several forms of bioactive compounds with antioxidant properties in the world of therapeutic plants that play a significant role in mitigating tissue injury which stimulates the wound

analgesic and antispasmodic potency in alkaloids and inflammatory potency in steroids (23).

Anti-carcinogenic, anti-inflammatory and anti-atherosclerotic activities have been documented for phenolic compounds such as phenols, tannins and flavonoids (24). The yield of overall phenolic, flavonoid and tannin content of the leaves and stem extract of *A. pendula* is shown in Table 1. In contrast to hydroalcoholic and aqueous extract, the methanol extract of the stem and leaves of *A. pendula* had a higher phenolic content in our sample. This may elucidate the wide folklore use of the plant. The presence of phenolic compounds in this genus has been verified by several phytochemical studies (25). Flavonoids are the secondary metabolites which are also well-known as Vitamin P. They are well-known for their anti-inflammatory, anti-allergic and anti-cancer properties (26). In our study, the methanol extract of leaves and stem of *A. pendula* showed a higher flavonoid content as compared to

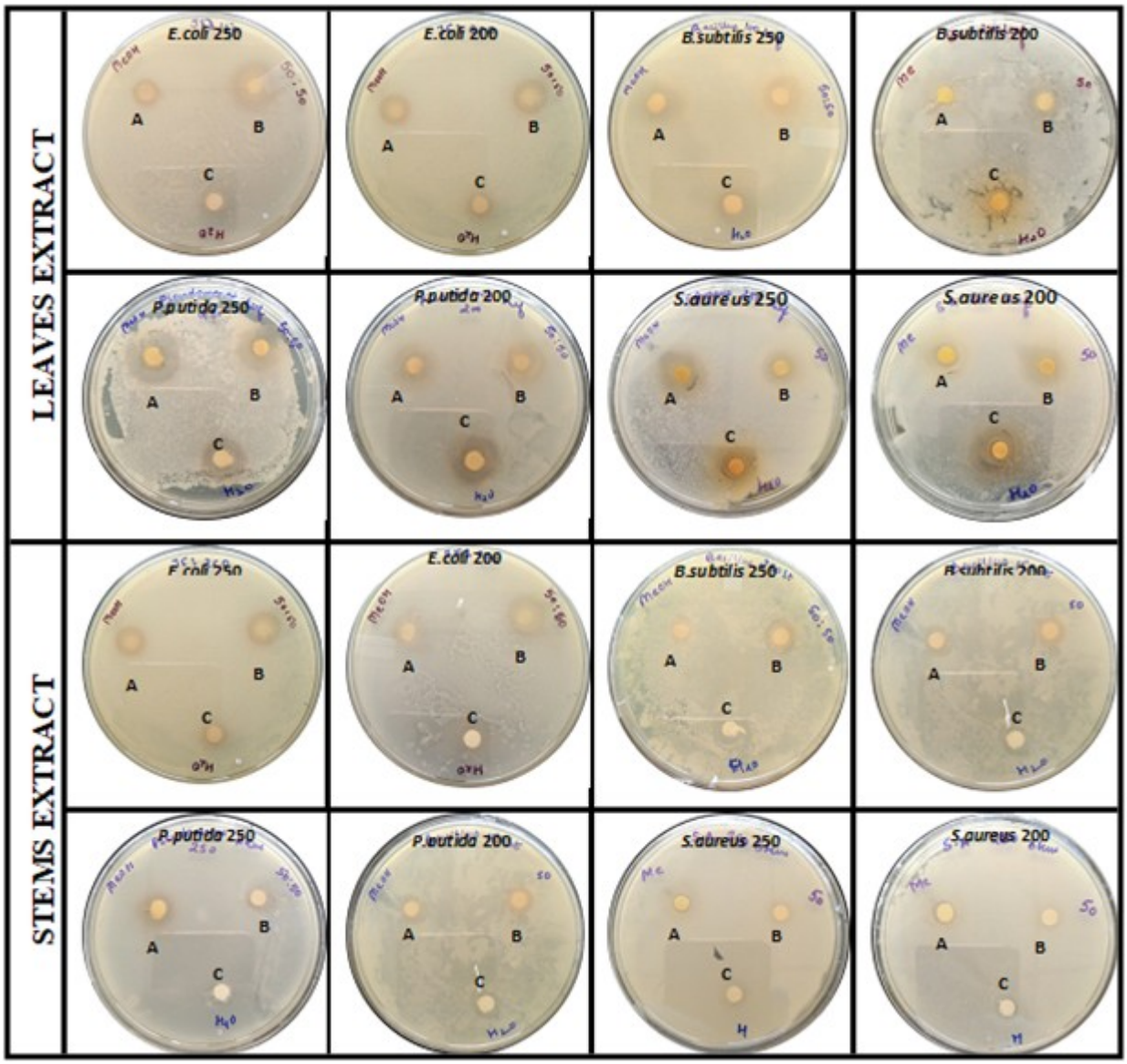


Fig. 9. Zone of inhibition for the different plant extracts against bacterial strains by disc diffusion method
In the figure A, B, C represents different solvent extracts of leaves and stems at two different concentration (200 and 250 mg/ml). **A**-Methanol extract. **B**-Hydroalcoholic extract. **C**-Aqueous extract.

hydroalcoholic and aqueous extract. Tannins possess astringent, antioxidant and antimicrobial activities and are used as tanning agents (27). The bactericidal and fungicidal abilities of the plant extract are due to the inclusion of tannins (28). In our study, the tannin content of the hydroalcoholic extract of leaves and stem extract was higher than the methanol and aqueous extract.

The DPPH technique is usually based on the scavenging of DPPH by incorporating an antioxidant that decolorizes the DPPH solution. A huge decline in the absorption spectrum of the reaction mixture signifies that the compound has considerable free radical scavenging activity (29). The methanol extract of the leaves and stem of *A. pendula* had the strongest free radical scavenging activity in this analysis, followed by the hydroalcoholic and aqueous extract.

Hydrogen peroxide exists generally at little concentration in all the living cells and it must be removed as soon as it is produced in the cell since it is very harmful (30). Despite its low reactivity, H_2O_2 can kill cells by increasing hydroxyl radicals in the cells (31). In our study, the methanol extract of leaves and stem of *A. pendula* revealed the radical scavenging activity which can be due to the existence of phenolic contents that can pass electrons to H_2O_2 and thus neutralize them into water. The FRAP assay evaluates the lowering potential of an antioxidant as it binds with Fe^{3+}- TPTZ complex forming a colored Fe^{2+}-TPTZ (32). The FRAP value increases with a rise in the concentration of the extract and is confirmed by an increase in absorption at 593 nm (33). In our study the higher antioxidant activity was shown by the hydroalcoholic extract of leaves and stem of *A. pendula* with the increase in absorbance which is attributed to the development of Fe^{2+} complex with increased concentration.

The problem of antibiotic resistance persists in many developing and developed countries, posing a significant challenge to the global health sector. The rise and spread of multidrug resistant organisms have significantly jeopardised current antibiotic therapy. This has forced the search for a new source of antimicrobial chemicals, such as plants, which

produce a variety of bioactive molecules with recognised medicinal capabilities (34).

In this study, all four bacteria examined responded similarly to methanol, hydroalcoholic and aqueous extract, with methanol and hydroalcoholic extract having the best results. Considerable activity in all tested bacteria was seen with methanol, hydroalcohol and aqueous extract of leaves (inhibition zone varied from 0 to 15.67 mm; MIC range of 0.195 to 50 µg/ml). In our investigation, methanol and hydroalcoholic extract antibacterial activities were stronger than aqueous leaves extract towards the pathogen examined. This shows that methanol and hydroalcohol are more the sources of polar bioactive chemicals than aqueous extract (35, 36). The action against *E.coli* is consistent with previous findings in *Anogeissus leiocarpus*, indicating that the plant can be used to treat *E. coli* related infected wounds and diarrhoea in ethnomedicines (37). The findings of the study on the isolation and identification of phytosterols and their antibacterial activity are also comparable to ours.

Conclusion

The purpose of this study is to determine the possible antioxidant function of the *A. pendula* Edgew. extract commonly used in indigenous medicinal systems. The results have shown that the methanol and hydroalcoholic extracts of *Anogeissus pendula* leaves and stem has a potent antioxidant function with scavenging capabilities against DPPH, H_2O_2 and FRAP which can be the effective sources of natural antioxidants and can be useful in curing many diseases such as gastric disorders, wound healing, dysentery, burns and diarrhea. The use of herbal items for disease bio control as a unique developing alternative to antimicrobial therapies that leads to non-toxic and more environmentally friendly management is essential. The current investigation validated some of the ethnomedicinal applications of *A. pendula* to treat illnesses caused by microorganisms, confirming the potential utility of developing herbal products as antibacterial agents against resistant and vulnerable bacteria. However, further research is needed before they may be recommended for usage in the healthcare system for the treatment of infectious diseases.

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Authors' contributions

All authors, PD, VP and TA, contributed equally to this work. PD conducted the actual study and wrote the manuscript. VP was involved in developing the idea and designing of the study. TA supervised the work and did the proof reading of the final manuscript. All authors approved the submission of the final version of the manuscript.

Conflict of interests

No conflict of interest has been reported by all the authors.

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