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In vitro Antioxidant and Antibacterial Activity of Root Extracts of *Pycnostchyus abyssinica* Fresen

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ABSTRACT:

The aim of this study was to assess phytochemicals, in vitro antibacterial and antioxidant activity from the root of *pycnostchyus abyssinica.* Antioxidant activity was investigated byusing 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and phosphomolybdenun assays, and the total flavonoid content was determined by aluminum chloride method. In vitro antibacterial activities of crude extracts were evaluated via disc diffusion method. Minimum inhibitory concentration (MIC) was determined by Broth dilution method. Phytochemical screening of root extracts revealed the presence alkaloids, phenols, flavonoids, triterpenes, tannins and steroids. The acetone extract was showed greater radical scavenging activity (IC_{50}=85.40±14.26), and total flavonoid contents (10.09 ±59 mg CE/g of dried extract). The acetone, chloroform/methanol(1:1), and methanolic root extracts showed that growth suppression at a concentration above 25mg/mL, and the minimum inhibitory concentration (MIC) value of the extracts against *B. cereus and S. aureus S. typhi, and E. coli* was found to be 25 mg/mL in both acetone and methanolic extracts, 50mg/mL in chloroform/ methanol (1:1) extracts.ANOVA test of the antibacterial activity in all solvent system extracts at concentration 100 mg/mL revealed had not significant effect ($p < 0.05$) on the level of inhibition respect to reference antibiotic (Chloramphenicol). The results proved that polar extracts of the root of *pycnostchyus abyssinica*possess antibacterial activity.

Keyword: *Phytochemicals, Antibacterial, Antioxidant, pycnostchyus abyssinica*

INTRODUCTION

Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. According to the World Health Organization plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world drugs are of natural product origin [1]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated the search for new antimicrobial substance from other sources. Enhancing of the knowledge of screening of medicinal plants for antimicrobial and antioxidant activities as well as the determination of the structure of the active chemical constituents may help in sustaining the use of natural products. It is important for finding potential new compounds for therapeutic uses [2]. Moreover, medicinal plants play a role in the treatment of various human and their livestock ailments in rural communities traditionally through the world including the country Ethiopia.

The genus *Pycnostachys* (Lamiaceae) is native to tropical Africa and South Africa. There are about 40 species of woody stemmed perennials; they can grow about 3m high with evergreen narrow leaves. Among the species in *Pycnostachys, P. abyssinica* Fresen is recognized as endemic species to the country Ethiopia [3]. The vernacular name of *P. abyssinica* Fresen, in the study area, is Olomuwa, (Wolaita People, the language of Ethiopia). An ethnobotanical study showed that leaves of *P. abyssinica* are boiled in water and the vapor is inhaled for the treatment of "Mitch disease", an infectious and inflammatory disorder. After warming, the leaves are pressed and the juice is used in the treatment of skin and eye infections. Leaves are used as a tea for the treatment of dysentery, and the aerial parts of this plant are also applied as termite repellant [4]. *P. abyssinica* is known to produce essential oil capable of repelling insects, thus underscoring the plants' use as live fences [5]. However, phytochemicals, antioxidant activity and antibacterial efficacy against microbes in *P. abyssinica* have not been investigated. Thus, the present study aimed at investigating invitro antioxidant and anti bacterial activities of root extracts of *P. abyssinica.*

1. MATERIALS AND METHODS

2.1. Chemicals

DPPH, Sodium phosphate, ammonium molybdate, BHT, L-ascorbic acid, catechin, aluminium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals and solvents used in this experiment were of analytical grade reagents.

2.1. Plant material collection

Roots of *P. abyssinica* were collected from around Wolaita Sodo town, Wolaita zone, Ethiopia. Wolaita Sodo town is located at 330 km from Addis Ababa, the capital of Ethiopia. The collected plant specimens were identified by comparison with the specimens of Herbarium in the Department of Biology, Addis Abba University, Ethiopia.

2.2. Preparation of extracts

At room temperature, air dried root collections of *P. abyssinica* were powdered by using a mechanical grinder. About 300g of plant powder was socked into cold percolation with 1.5 L different solvent system (chloroform/methanol (1:1), acetone, and methanol) three times for 48h while shaking at room temperature. The solution was filtrated by using suction filtration and the filtrate was concentrated by using a rotary evaporator at a temperature 40^{o}c to yield brown crude extracts. The percentage yield of the crude extract was calculated as (equation 1), and then collected in labeled sterile evaporating dish and put in deep freezer until the next experiment.

$$Extract\ yield = \frac{Dry\ weight\ of\ extract}{Intial\ powder\ weight} X100 \ldots\ldots\ldots\ldots \text{Equation (1)}$$

2.3. Phytochemical screening

Phytochemical screening tests were done to determine the class of secondary metabolites present in the crude extract following the standard protocols [**6, 7**].The results were reported as (+Ve) for the presence and (-Ve) for absence (Table 1).

2.5.3. *Determination of total flavonoid content (TFC)*

Total flavonoid contents of extracts weredetermined by a colorimetric assay according to Chun OK, et al [**8**]. The plant extracts (1 mL, 1 mg/mL) were diluted with 1.25 mL distilled water and 75 µL 5% $NaNO_2$ was added to the mixture. After 6 min, 150 µL 10% $AlCl_3$ was added and after another 5 min, 1 mL1M NaOH was added to the mixture. Immediately, the absorbance of the mixture, pink in color, was determined at 510 nm versus prepared water as a blank. A standard curve was prepared using 5 – 120 µg/mL of catechin. Results were expressed as milligram of catechin equivalents per gram of dry plant extract,by reference to standard curve ($Y = 0.022x + 0.041$, $R^2 = 0.99$, $P < 0.001$).

2.5 Determination of Antioxidant Activity

2.5.1 DPPH assay

The DPPH free radical scavenging activity of the extracts of root of *P. abyssinica* was assayed according to the method of Szollosi*et al.,* [**9**], with slight modification. Different concentrations (50 to 1000µg/mL) of the extracts were taken in different test tubes. Freshly prepared DPPH solution (2 mL, 0.006%, w/v) in methanol was added in each of the test tubes containing 1 mL of the extract. The reaction mixture and the reference standards such as ascorbic acid and butylatedhydroxytoluene(BHT) were vortexed and left to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was then taken at 520 nm. Methanol was used as a blank. The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenged\ (\%) = \frac{(Ac - As)}{Ac} x100$$

Where *A*c is the absorbance of the control and *A*s is the absorbance in presence of the sample of the extracts.

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/mL) of extracts that scavenges the DPPH radical by 50%. The high IC_{50} value indicates less antioxidant capacity.

2.5.2 Total antioxidant using phosphomolybdenum assay

The total antioxidant activities of the crude extracts were evaluated by the phosphomolybdenum method reported by Prieto*et al*., [**10**], with slight modification. 0.3 mL plant extract (0.5 and 1 mg/mL) was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The samples were incubated at 95 ^{0}C for 90 min, was cooled to room temperature and absorbance was measured at 695 nm. 3 mL of methanol was used as a blank. The total antioxidant activity was expressed as milligram ascorbic acid equivalent/gram of dried extract (mg AAE/g) ($y = 0.321x + 0.019$; $R^2 = 0.996$, $P < 0.001$) based on the calibration curve.

2.5.3 *Microorganism strain*

Four human pathogenic bacterial strains: two Gram-positive *i.e. Staphylococcus aureus and Bacillus cereus* and two Gram-negative i.e. *Escherichia coli* and *Salmonella typhi* was used to test the antibacterial activity of *P. abyssinica.* These microorganism strains were fresh cultured and isolated in food science laboratory, Hawassa University, Ethiopia, following standard laboratory procedures.

2.3.1. *Evaluation of antibacterial activity*

Antibacterial activities of the extracts were evaluated by the disc diffusion method in accordance with the guidelines of National Committee for Clinical Laboratory Standards [**11**] with minor modification. Chloramphenicol was used as a standard antibacterial agent. With a loop, touched the top of 24 hours old culture, individual bacteria were selected and transferred to a tube of saline (0.85%) where the turbidity was adjusted to the standard inoculums of MacFarland scale 0.5 [~10^6 colony forming units (CFU) per milliliter]. To avoid further growth before inoculation, the media were sterilized in a flask and cooled to 40-45°C. Petri dishes containing 20 mL of Mueller-Hinton agar were used to inoculate bacterial suspensions, and then the medium was distributed in petri dishes homogeneously. Filter paper discs (Whatman no. 1, 6 mm diameter) was impregnated with the extract solution. The solution of extracts prepared in DMSO (100, 50, 25 and 12.5 mg/mL) were placed on the inoculated plates, and petri dishes were incubated for 24 h at 37°C. The filter paper disc was also impregnated with chloramphenicol used as positive control and DMSO as a negative control. The inhibition zone diameters were measured in millimeters. The experiments were carried out in triplicate, and the results of antibacterial activity of extracts were analyzed in terms of inhibition zones with respect to a standard antibiotic drug.

2.5.4 MIC determination procedure

Culture: Overnight dextrose broth cultures of *S. aureus, E. coli,Bacillus cereus and salmonella typhi* at 37^{0}C were prepared. A drop plate method was used to analyze MIC and a culture media of mannitol salt agar for *s. aurous*, salmonella shigella agar for *S. typhi*, ECD agar for *E. coli* and bacillus cereus agar for *B. cereus* were used respectively. After the bacteria inoculated in to the appropriate media for the four microorganisms at 37^{o}c were incubated for 24hour and all microorganism also inoculated into dextrose broth in order to confirm the MIC after incubator for 24h the MIC was measured and recorded.

2.6 Statistical Analysis

The quantitative data of antioxidant and antibacterial activity tests were presented as mean ± SD of triplicate measurements. The comparisons for the tests between the control group (chloramphenicol) and the test groups (plant extracts) were performed by one-way analysis of variance (ANOVA). Statistical significance was considered when the p-values were less than or equal to 0.05 at 95% confidence level.

3 RESULTS AND DISCUSSION

3.1. Phytochemical screening and percentage yield

The acetone, methanol, and chloroform/methanol (1:1) root extracts *P. abyssinica* were yielded 9.3%, 7.2%, and 6.3% (w/w) with brown color crude extracts, respectively. The extracts were subjected to various qualitative tests for phytochemicals such as alkaloids, Phenols, steroids, flavonoids, tannins, and terpenoids. Thus, as indicated in table 1, phytochemical screening of root extracts of *P. abyssinica* revealed the presence of alkaloids, Phenols, steroids, terpenoids, tannins, and flavonoids in root extract of *P. abyssinica*.

Table 1: Preliminary phytochemical screening of root extracts from *P. abyssinica*

Chemical constituents	Reagents	Results of extracts		
		Solvent System		
		Chloroform/methanol(1:1)	Acetone	Methanol
Alkaloids	Mayer reagent	++	++	++
Phenol	$FeCl_3$	+	++	++
Steroids	CCl_3 and conc. $H2SO_4$	++	+	+

Terpenoids	CCl_3 and conc. H_2SO4	++	+	+
Tannins	$FeCl_3$	+	++	+
Flavonoids	Dilute NH_3 solution	+	++	++

3.2. Antioxidant activity

3.2.1. DPPH Assay

The antioxidant activity of the root extract of *P. abyssinica* in different solvent systems was assessed by the DPPH assay, the extracts to act as a donor of hydrogen atoms or electrons in the transformation of DPPH· into its reduced form DPPH-H was investigated. The result revealed that the examined root extract of *P. abyssinica* was able to reduce the stable, purple colored radical DPPH into yellow colored DPPH-H. As indicated table 2, the acetone extract wasshowed greater radical scavenging activity (85.40±14.26), than those extracted in methanol and chloroform/methanol (1:1) solvent system exhibiting IC_{50} values 190.22±14.60 and 455.52±45.80, respectively. The smaller decrease in absorbance of extract indicates a lower rate of oxidation of DPPH and higher antioxidant activity in the presence of crude extract. The lower the IC_{50} value, the higher is the scavenging potential. Thus, in the present study, stronger scavenging activity (lower IC_{50} values) was recorded for acetone extract. This showed that root Extract of *P. abyssinica* in acetone extract was exhibited better antioxidant activity than extracts in methanol and chloroform/methanol(1:1) solvent system (In Fig. 1). The IC_{50} values of L-ascorbic acid and BHT tested as references were found to be significantly lower (stronger DPPH scavenger) ($p < 0.05$) than that of methanol, chloroform/methanol (1:1) and acetone extracts. The phytochemical screening test showed that the presence bioactive phenolic compounds such as alkaloids, phenols, steroids, flavonoids and tannins in the root extract of *P. abyssinica*. Thus, the antioxidant activity of *P. abyssinica* extracts might be attributed to the presence of these phenolic compounds.

Table 2: IC50 values of DPPH scavenging activities in various solvent extracts from *P. abyssinica*.

Plant extracts in different solvent system	**IC_{50} (µg/mL)**
Root extract of*P. abyssinica* in acetone	85.40 ± 14.26b
Root extract of *P. abyssinica* in Methanol	190.22 ± 14.60c
Root extract of *P. abyssinica* in chloroform/methanol(1:1)	455.52 ± 45.80d
BHT	34.27 ± 2.24a
AA	23.94 ± 1.33a

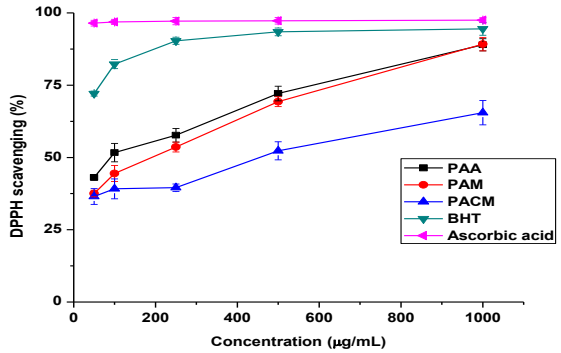

Figure1: DPPH radical scavenging activity (%) of acetone (PAA), methanol (PAM), and chloroform/methanol (1:1) (PACM) root extracts of P. abyssinica and controls (L-ascorbic acid and BHT). Values are the average of triplicate measurements (mean ± SD).

3.2.2. Total antioxidant capacity by phosphomolybdenum method

Total antioxidant capacity was reported as milligram ascorbic acid equivalents (mg AAE/g of dried extract). The method is utilized for the spectrophotometric quantification of total antioxidant capacity, based on the reduction of Mo(VI) to Mo(V) in presence of antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acidic pH and at higher temperature [12].As indicated in Figure 2, results revealed that acetone extract of P. abyssinicahad also the highest total antioxidant activity followed by methanol and the lowest total antioxidant activity was found in the Chloroform: methanol (1:1) extract. No significant difference ($p > 0.05$) was found in the total antioxidant activity of methanol and Chloroform: methanol (1:1) extract ($p > 0.05$).

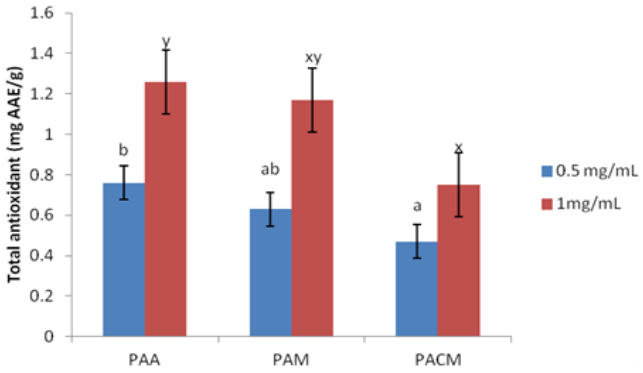

Figure 2: Total antioxidant activity (mg AAE/g of dw) of acetone, methanol, and methanol: chloroform extracts from PA, PAM, and PACMat concentrations of 0.5 and 1.0 mg/mL.Values are the average of triplicate measurements (mean ± SD). Values within the same concentration with different letters in the histogram bar are significantly different at $p < 0.05$.

3.2.3. Total flavonoid content (TFC)

Total flavonoid contents values were ranging from 2.56±0.50 to 10.09±59 mg CE/g of root extract P. abyssinica(Table 3). The best flavonoid contents were achieved by acetone extract with 10.09±59 mg expressed as the Catechin equivalentgram of dry plant extract, followed the order: acetone > methanol > chloroform/ methanol (1:1).ThePresence of high amount of flavonoid content indicates the presence of phytochemicals in P. abyssinicawith high antioxidant potential .Flavonoids comprise the most widespread and diverse group of polyphenolic plant secondary metabolites. These compounds play an important role in biological activities includes antibacterial, antiviral, and anti-inflammatory, antiallergic, antithrombotic, vasodilatory actions and also exhibit free radical scavenging properties by either through scavenging or chelating process[13-15].

Table 3: Total flavonoids content of *P. abyssinica*

Extracts in different solvent system	**Total flavonoid content (** mg CE/g)
Root extract of *P. abyssinica* in acetone	$10.09±59^{c}$
Root extract of *P. abyssinica* in methanol	$4.90 ±0.14^{b}$
Root extract of *P. abyssinica* inchloroform/methanol(1:1)	$2.56±0.50^{a}$

3.3. In vitro Antibacterial Activity

3.3.1. Disc Diffusion Method

Antibacterial activities were determined by measuring the 'inhibition zone' for root extract of P. abyssinica in the different solvent system against two gram positive i.e. B. cereus, S. aureus, and two gram negative i.e. S. typhi and E. coli bacteria strains. The activity of root extracts has also been compared with the broad spectrum commercially available antibiotic (chloramphenicol). Chloramphenicol showed the growth suppression for B. cereus , S. aureus, S. typhi, and E. coli, (23.3±2 mm) at the concentration 50 µg/mL; while acetone root extract of P. abyssinica recorded for B. cereus (17.8 ±0 mm), S. aureus (18.7±2 mm), S. typhi (20.0±1 mm) and E. coli (18.0±5 mm), at 100mg/mL concentration. And methanol root extract showed the growth suppression forB. cereus (16.0 ±4 mm), S. aureus (13.0±3 mm), S. typhi (10.7±1 mm) and E. coli (19.3±2 mm), at 100mg/mL concentration. Furthermore, chloroform/methanol (1:1) extract showed thatthe growth suppression of B. cereus (17.0 ±3 mm), S. aureus (13.7±2 mm), S. typhi (11.3±2 mm) and E. coli (18.3±1 mm), at 100 mg/mL concentration. However, in the half fold diluted concentration, (50 - 25 mg/mL) the values were decreased in all extracts (Table 4).ANOVA test of the antibacterial activity in all solvent system of root extracts P. abyssinicaat concentration 100 mg/mL revealed had not significant effect ($p < 0.05$) on the level of inhibition respect to reference antibiotic (Chloramphenicol).

The acetone, chloroform/methanol (1:1), and methanol extracts were examined MIC. The results revealed that acetone, chloroform/methanol(1:1), and methanolic root extract of P. abyssinica showed growth suppression at a concentration above 25mg/mL, andMIC of the extracts against B. cereus and S. aureus S. typhi, and E. coli was found to be 25 mg/mL in both acetone and methanolic extracts, 50mg/mL of chloroform/ methanol (1:1) extracts. The results proved that polar extracts of the root of P. abyssinica possesshigh antibacterial activity. The phytochemical screening tests were confirmed that the dominant secondary metabolities in the root extracts in those polar solvents are Alkaloids, phenols, flavonoids, and tannins. Thus, the highest antibacterial activity in acetone and methanolic root extract of P. abyssinica might be attributed to the presence of these phenolic secondary metabolites

Table 4: Antibacterial activities of root extracts of *P. abyssinica*

		Zone of inhibition (mm)		
Strains	Concentration mg/mL	Acetone extract	Chloroform/methanol (1:1) extract	Methanol extract
B. cereus	100	17.8 ±0a	17.0 ±3a	16.0 ±4 b
	50	8.7±2c	8.3±2 b	9.3±2 c
	25	2.3±1 d	ND	2.3±1 d
S. aureus	100	18.7±2a	13.7±2 b	13.0±3 b
	50	6.7±1 b	7.0±1 c	7.7±2 c
	25	1.3±1 d	ND	1.3±1 d
S. typhi	100	20.0±1a	11.3±2 b	10.7±1 b
	50	14.0±1 b	5.0±1 d	7.7.0±4c
	25	3.3±1 c	ND	1.7±1 c
E. coli	100	18.0±5a	18.3±1a	19.3±2a
	50	11.7±6 b	11.3±1 b	10.3±1 b
	25	3.0±3 d	ND	4.2±0 d
Chloramphenicol	50 µg/mL	23.3±2 a		

The data represent the mean ± SD, n=3. No significant differences were observed with respect to the reference antibiotic compound at 0.05 level of Dunnett test, ($p > 0.05$), ND=not detected.

4. CONCLUSION

The finding of present study revealed that the extracts of P. abyssinica have shown the significant antioxidant activity depending upon the type of solvent used for extraction. Acetone was found to be good solvent followed by methanol, and chloroform/methanol (1:1) respectively. The flavonoid content and the antioxidant activity were highest in acetone extract when compared with other solvent system. Thus, the work indicated that the root of P. abyssinica is good source of phytochemicals that can be extracted by using proper solvent system. It was concluded that the polar extracts of the root of P. abyssinica possesshigh antibacterial activity.

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