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## Studies on antioxidant and antibacterial activities of crude extracts of *Plantago lanceolata* leaves

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### ABSTRACT

In this study, three different antioxidant activity quantification methods: 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH), reducing power, FRAP and peroxide value determination including total phenolic, TP, total flavonoid, TF assays of crude leaf extracts of *Plantago lanceolata* were carried out. The extracts showed significant activities in all antioxidant assays in a concentration dependent manner. The crude extracts were found to possess higher ferric reducing activity ranging from  $336.58 \pm 0.052$  mgAAE/100g (90% methanol) to  $172.94 \pm 0.032$  mgAAE/100g (petroleum ether) and DPPH activity varied from 90.6% (90% methanol) to 36.04% (petroleum ether). Strong correlation of TPC with AEAC<sub>DPPH</sub> and AEAC<sub>FRAP</sub> ( $R^2 = 0.9033$  and  $R^2 = 0.8538$  respectively) implied that compounds in the extract were proficient to scavenge the DPPH free radical and reducing ferric ions into ferrous ions. Moreover, peroxide values of the extracts were obtained and varied from 19.8 meq/kg (90% methanol) to 379 meq/kg (petroleum ether) and 3.1 meq/kg (90% methanol) to 119 meq/kg (petroleum ether) at 70 °C and room temperature respectively. Therefore, *P. lanceolata* leaf extracts as compared to the control showed effective activity in delaying oxidation of the oil. Antimicrobial capacity evaluation of crude extracts against different gram-positive and gram-negative organisms was also evaluated and a higher degree of antimicrobial activity was observed of crude extracts with MIC and MBC values in the range of 6.25 to 25% were also observed for the crude extracts.

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**Capsule Summary:** Antimicrobial and antioxidant activities of the *Plantago lanceolata* leaves extracts have been examined using different test systems. The extracts showed effective activity in delaying oxidation of the oil as compared to the control. Antimicrobial capacity evaluation of the crude extract against different gram-positive and gram-negative organisms demonstrated significant activity with mean zone of inhibition up to 26 mm.

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### INTRODUCTION

A free radical is any species capable of independent existence that contains one or more unpaired electrons (Halliwell,

2000). Free radicals are well documented for playing a dual role in our body as both deleterious and beneficial species. In low/moderate concentrations free radicals are involved in normal physiological functions but excess production of free

radicals is a harmful process that causes oxidative stress (Golden et al., 2002; Lee et al., 2003). Oxidative stress can damage the cell structures, including lipids, proteins, RNA and DNA which leads to number of diseases (Sen et al., 2010).

The damaging effect of free radical can prevented by a group of substance called antioxidant. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. A great number of natural medicinal herbs and plants have been tested as naturally occurring antioxidant (Hamid et al., 2010).

According to the World Health Organization more than 80% of the world's population of the developing countries relies mainly on the traditional medicines, mostly plant derived. Among the plants that have been used as traditional medicines, the genus *Plantago* is known for their medicinal properties. *Plantago lanceolata* known by a common name ribwort plantain is one of the species of genus plantago. The Leaves of this plant have been used for traditional medicinal activities in various countries (Miser-Salihoglu et al., 2013; Taskova et al., 2002). In most part of the world including Ethiopia, the plant has not been cultivated on piece of land or large farms since it is considered as weed. However, the data generated from leaf extracts of the *P. lanceolata* plant have demonstrated strong antioxidant and antimicrobial properties.

## MATERIAL AND METHODS

### Sample collection and preservation

The leaves of the *P. lanceolata* were washed with tape water to remove dust particles and other waste material from the surface of leaves followed by drying in open air at room temperature. The air-dried leaves of *P. lanceolata* chopped in to small pieces and milled in to a uniform powder.

The bacterial species used to test antimicrobial activities (*Staphylococcus aureus* (ATCC2923), *streptococcus pneumoniae* (ATCC49619), *Escherichia coli* (ATCC2592) and *shigella boydii* (ATCC9289)), were obtained from Biotechnology Laboratory, Biology Department, Gondar University, Ethiopia.

### Extraction and defatting procedure

Five different flasks each containing 20 g non-defatted *P. lanceolata* leaf powder were used for extraction using five different solvents (80% and 90% aqu. methanol, pure methanol, pure chloroform, and pure petroleum ether) at room temperature. After filtration and concentration at 35 oC, crude extracts were labeled as PLS1, PLS2, PLS3, PLS4 and PLS5 for 90% methanol, 100% methanol, chloroform, petroleum ether and 80% methanol solvents respectively. For all analyses we used analytical grade chemicals/reagents and solvents.

In addition, 20 g of powdered leaf sample each was first defatted using soxhlet extractor with petroleum ether and chloroform solvents for 4-5h above 70 OC. After defatting, only 90% methanol was used for further extraction. Finally petroleum ether and chloroform defatted leaf samples which were extracted using 90% methanol were labeled as PDBPE and PDBCM.

### Determination of total polyphenol content, TPC

Total phenolic content of *P. lanceolata* leaf extracts were determined using the Folin ciocalteu method with slight modification (Amin et al., 2006). 5 mL of herb extract was diluted with 45 mL of distilled water followed by addition of 0.25 mL Folin-Ciocalteu reagent and 0.5 mL of 7% sodium carbonate solution. The mixture were diluted to 100 ml and left for 30 min in dark. Finally absorbencies were measured at 750 nm using UV-vis spectrometer.

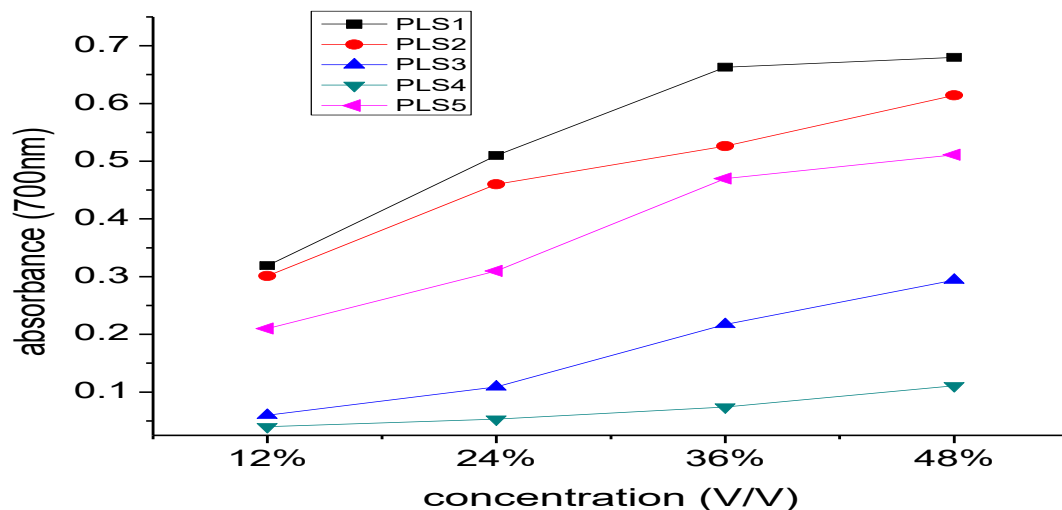
### Determination of total flavonoids

The total flavonoid content of *P. lanceolata* crude leaf extracts were determined using aluminum chloride assay (Chang et al., 2002). 2.00 mL of the extract was mixed with 4 mL distilled water with 10 mL volumetric flask followed by an immediate addition of 0.30 ml of 5% NaNO<sub>2</sub>. 5 min latter, 0.30 mL of 10% AlCl<sub>3</sub> solution was added. After 6 min, 2.00 mL of 1.0 M NaOH solution was added. The absorbance at 510 nm using Uv-vis spectrophotometer was taken after 10 min.

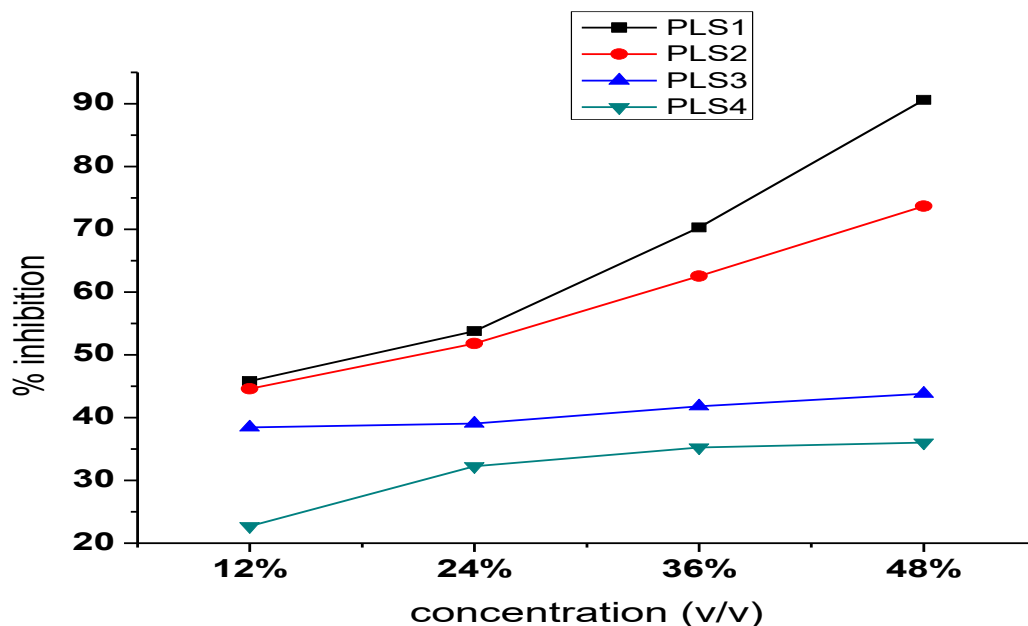
### Antioxidant activity determination

Reducing Power Assay: The reducing power of crude extracts was determined according to the method developed by Oyaizu (Oyaizu, 1986). From each sample (PLS1, PLS2, PLS3, PLS4, PLS5, PDBPE and PDBCM), different concentrations (12%, 24%, 36%, 48%, (v/v)) of *P. lanceolata* leaf extracts were prepared. 2.5 mL extract was taken and mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH= 6.6) and 2.5 mL of potassium ferricyanide solution (1%). The mixture was incubated at 50 oC for 20 min. Finally, 2.5 mL of trichloroacetic acid solution (10% w/v) was added and the resulting mixture was centrifuged at 3000 rpm for 10 min. At last, 2.5 mL of the filtrate (upper layer) was mixed with 2.5 mL distilled water and 0.5 mL ferric chloride solution (0.1% w/v). Finally, absorbance of the mixture was measured at 700 nm.

DPPH radical-scavenging capacity: The antioxidant activities of *P. lanceolata* leaves were assessed according to the method developed by Brand Manzorro using 2,2 - diphenyl-1-picrylhydrazyl (DPPH) (Louli et al., 2004). 90% and 100% methanol, chloroform, and petroleum ether extracts of *P. lanceolata* leaves with different concentration (48%,36%, 24%, 12%(v/v)) were prepared. 5 mL of 0.004% of DPPH solution was mixed with 1 mL of each extract. Solutions were kept in dark for 30 min; absorbencies were measured at 517 nm using Uv-vis spectrophotometer.



**Fig. 1:** DPPH radical scavenging activity of aqueous (Aq) and ethanol/water (hydro eth) extracts of *Hibiscus sabdariffa* Linn and control (ascorbic acid).



**Fig. 2:** DPPH radical scavenging activity of *P. lanceolata* leaf extracts at different concentration

### Peroxide value determination

For peroxide value determination Niger seed oil was used because it is rich in linolic acid which is important component for antioxidant activity research (Gutteridge, 1995). Twelve different samples that contain Niger seed oil and pure Niger seed oil were prepared for the determination of peroxide value. Six of them were placed at room temperature and the other six samples were placed at 70 oC. From each sample, 5 g were taken and transferred to 12 different 250 mL conical flasks. 30 mL of mixture of glacial

acetic acid and chloroform (3:2) were added to each sample. The mixtures were shaken to dissolve and 0.5 mL of saturated KI solution was added to all samples kept both at room temperature and 75 oC. Finally 30 mL of water was added to each sample and titrated with 0.01 N sodium thiosulfate solution. After a yellow color disappears, 5 ml of starch solutions were added to check completion of titration. The titrant was added slowly with continuous shaking until the blue color was disappeared. A blank determination was performed under the same condition.

the turbidity equal to 0.5 MacFarland standard solutions which is equivalent to  $1.5 \times 10^8$  CFU/ml.

### Disk diffusion method

Disk diffusion assay with Muller Hinton agar (MHA) medium was used to analyze antimicrobial activities of different crude extracts of *P. lanceolata* leaves. The MHA was melted and then cooled and finally poured into sterile petri dishes to get a solid plate. Then standardized inoculums (0.5 McFarland) were added and streaked on the agar plate surface. Wells were prepared in the seeded agar plates with sterile cork borer (6 mm diameter). The test compound or crude extract (100  $\mu$ l) were carefully dispensed into the wells. This was done in triplicate parallel with different control antibiotics. Extracts were allowed to diffuse for about 2 h at 37 °C. After overnight incubation, the zone of inhibition was observed and the diameter of inhibition zone was measured.

### Determination of MIC and MBC

MIC and MBC extracts of the plant were determined according to methods described by Shahidi (Cushnie and Lamb, 2005). *P. lanceolata* leaf extracts were diluted to different concentration (1/4, 1/8) of the original solution. To each diluted crude extracts, nutrient broth tubes were seeded both with 100  $\mu$ L of the pathogenic standard and resistant clinical bacteria. Negative control tubes with no bacterial inoculation were simultaneously maintained. Tubes were incubated aerobically at 37 °C for 24h. The lowest concentration of the extract that produced no visible growth was recorded as the MIC. Diluted samples showing no visible growth for the MIC were sub-cultured into a fresh MHA plate and incubated at 37 °C for 24h for the determination of MBC.

### Methods of data analysis

Analytical equation: In this study, the antioxidant activities, total polyphenol and flavonoid contents of *P. lanceolata* leaf extracts were calculated and reported in terms of ascorbic acid (AA), gallic acid (GA), and quercetin (QT) equivalent per gram of extraction. The following analytical equations (1, 2 and 3) were used for calculation.

$$W \left( \frac{mg}{g \text{ of } DW} \right) = \frac{[x(PPM)(Df)(\text{volume of extract}(ml))]}{\text{weight of dry sample in gram}} \quad (1)$$

$$\text{Where: } W = \text{AAE, GE, QE} \quad D_f = \frac{\text{final volume}}{\text{aliquet volume}}, \quad X = \frac{Y-C}{Q},$$

y = absorbance of the sample,

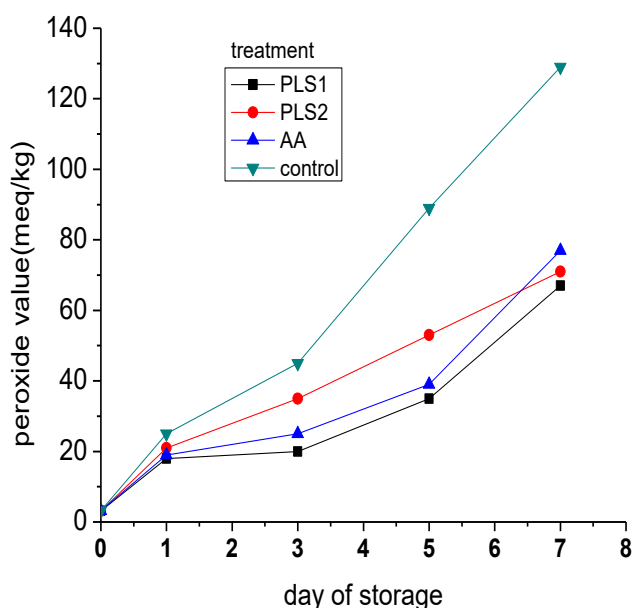
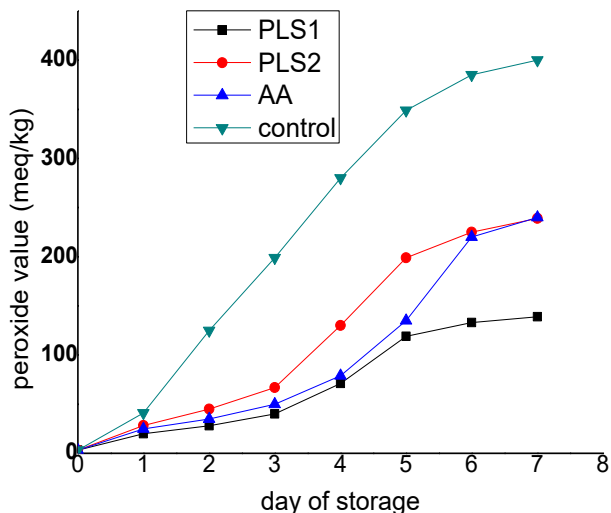
c = y-intercept from calibration curve,

The percentage of DPPH radical scavenging activities of *P. lanceolata* leaf extracts were calculated using:

$$\text{DPPH (\%)} \text{ activity} = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100 \quad (2)$$

Where:  $A_0$  = absorbance of the blank,

$A_1$  = absorbance of the sample.



**Fig. 3:** Change in peroxide value (meq/kg) during storage at 70°C (upper) and room temperature lower) with different treatments

### Antibacterial activity determination

Preparation of inoculum: The microbial stock cultures were maintained at 4 °C on slopes of Muller-Hinton Agar, MHA. Active cultures for experiments were prepared by transferring a loopful of cells from the stock culture to the test tubes containing Muller-Hinton broth and incubated without agitation for 24 h at 37 °C. To 5 ml of Muller-Hinton Broth, 100 $\mu$  of culture was inoculated and kept till it reached

**Table 1:** Result of phytochemical screening of different solvent extracts of *P. lanceolata* leaves

Phytochemicals	Solvents			
	90% MeOH	100% MeOH	Chloroform	Petroleum ether
Phenolic	++	+	+	+
Flavonoids	++	+	-	+
Tannins	++	-	-	-
Saponnins	+	+	+	-
Alkaloids	-	-	-	-
Glycoside	+	+	+	+

++: strong, +: moderate -: absent

**Table 2:** Effect of solvents on TPC, TFC, free radical scavenging capacity and percentage inhibition of *P. lanceolata* leaf extracts

Extraction of plant leaves	TPC mgGAE/100g ext.	TFC MgQE/100g ext.	Ferric reducing power (mgAAE/100g) ext.	DPPH scavenging (%)
90% Methanol	344.70±0.021 <sup>a</sup>	45.23±0.034 <sup>a</sup>	336.58±0.0052 <sup>a</sup>	90.6 ±1.60 <sup>a</sup>
100% Methanol	295.052±0.017 <sup>b</sup>	26.13±0.012 <sup>b</sup>	326.04±0.013 <sup>b</sup>	73.7±1.03 <sup>b</sup>
Chloroform	205±0.032 <sup>c</sup>	10.24±0.037 <sup>c</sup>	184.67±0.054 <sup>c</sup>	43.8±1.42 <sup>c</sup>
Petroleum ether	185.07±0.012 <sup>d</sup>	2.156±0.128 <sup>d</sup>	172.94±0.032 <sup>d</sup>	36.04±1.3 <sup>d</sup>

**Table 3:** Ferric reducing antioxidant power (FRAP) of defatted and non-defatted extracts of *P. lanceolata* leaves.

Sample	AAE (mg/100g ext.)
PDBPE	49.7±0.113
PDBCM	211.3±0.124
PLS1	336.58±0.052

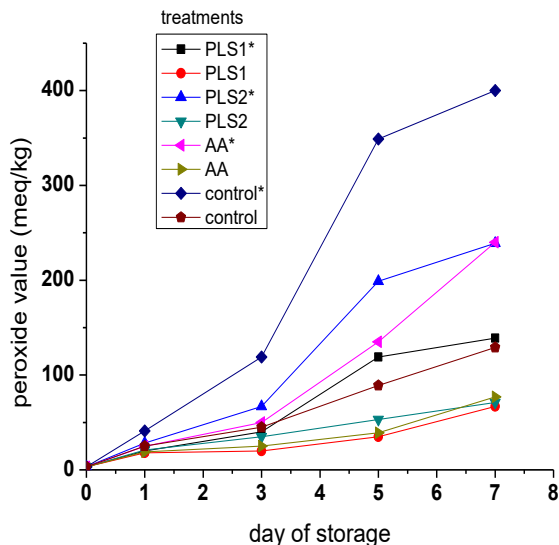
**Table 4:** MIC determination of *P. lanceolata* crude leaf extracts against gram positive and gram-negative bacteria

Ext	Gram negative bacteria								Gram positive bacteria							
	Kp		Sb		EC <sub>1</sub>		EC <sub>2</sub>		Sp <sub>2</sub>		Sp <sub>1</sub>		M		Sa	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Aq	12.	25	6.25	12.5	6.25	12.5	6.25	12.5	12.5	25	6.25	6.25	6.35	12.5	12.5	25
Me	25	25	25	25	25	25	6.25	12.5	12.5	25	6.25	12.5	12.5	12.5	6.25	25
Ac	25	25	6.25	12.5	25	25	12.5	12.5	25	25	6.25	12.5		25	25	25

Kp = *K. pneumoniae*, Sb = *S. boydii* (ATCC9289), EC<sub>1</sub> = *E. coli* (ATCC49619).EC<sub>2</sub> = *E. coli*, sp<sub>2</sub> = *S. pneumoniae*, sp<sub>1</sub> = *S. pneumoniae* (ATCC49619), m = MRSA, Sa = *S. aureus* (ATCC2923).

Aq. = Aqueous, Me = Methanol, Ac = Acetone and Ext = Extracts





**Fig. 4:** Change in peroxide value (meq/Kg) during storage at 70°C and 25 °C. (\*) represents treatment at 70 °C

The peroxide value (Meq/kg oil) *P. lanceolata* leaf extracts against the Niger seed oil was determined using:

$$POV = \frac{(V_0 - V_1)(C)(1000)}{m} \quad (3)$$

Where,  $V_0$  = volume of titrant ( $\text{Na}_2\text{S}_2\text{O}_3$ ) needed for blank  
 $V_1$  = volume of titrant ( $\text{Na}_2\text{S}_2\text{O}_3$ ) needed for sample  
 $C$  = concentration of titrant ( $\text{Na}_2\text{S}_2\text{O}_3$ )

### Statistical analysis

All measurements were carried out in triplicate ( $n = 3$ ), and values expressed are the mean of three repetitions  $\pm$  standard deviation (SD). Results were subject to analysis of variance (ANVOA). The graphs as well as linear regression coefficients ( $R^2$ ) were done using origin 8 software. The difference between mean were determined by the least significant difference test, and significance was defined as a confidence limit of  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Phytochemical screening tests

Three different antioxidant activity quantification i.e., 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH), reducing power, FRAP and peroxide value determination including total phenolic, TP, total flavonoid, TF assays of crude leaf extracts of *P. lanceolata* were performed along phytochemical screening. The results of phytochemical screening test performed on crude leaf extracts of *P. lanceolata* plant are summarized in Table 1. The type of

phytochemicals identified from the leaf extracts of the *P. lanceolata* depends on the types of solvents used for the extraction. For instance, tannins were observed only in 90% methanol extract. High amount of polyphenols, flavonoids and tannins were determined in 90% methanol leaf extract of *P. lanceolata*. Research results from different laboratories showed that secondary plant metabolites such as alkaloids, flavonoids, tannins and saponins exert a wide range of biological activities on physiological systems (Asressu, 2013). Therefore; these phytochemicals found from *P. lanceolata* could also play similar biological activities.

### Determinations of total polyphenol content, TPC

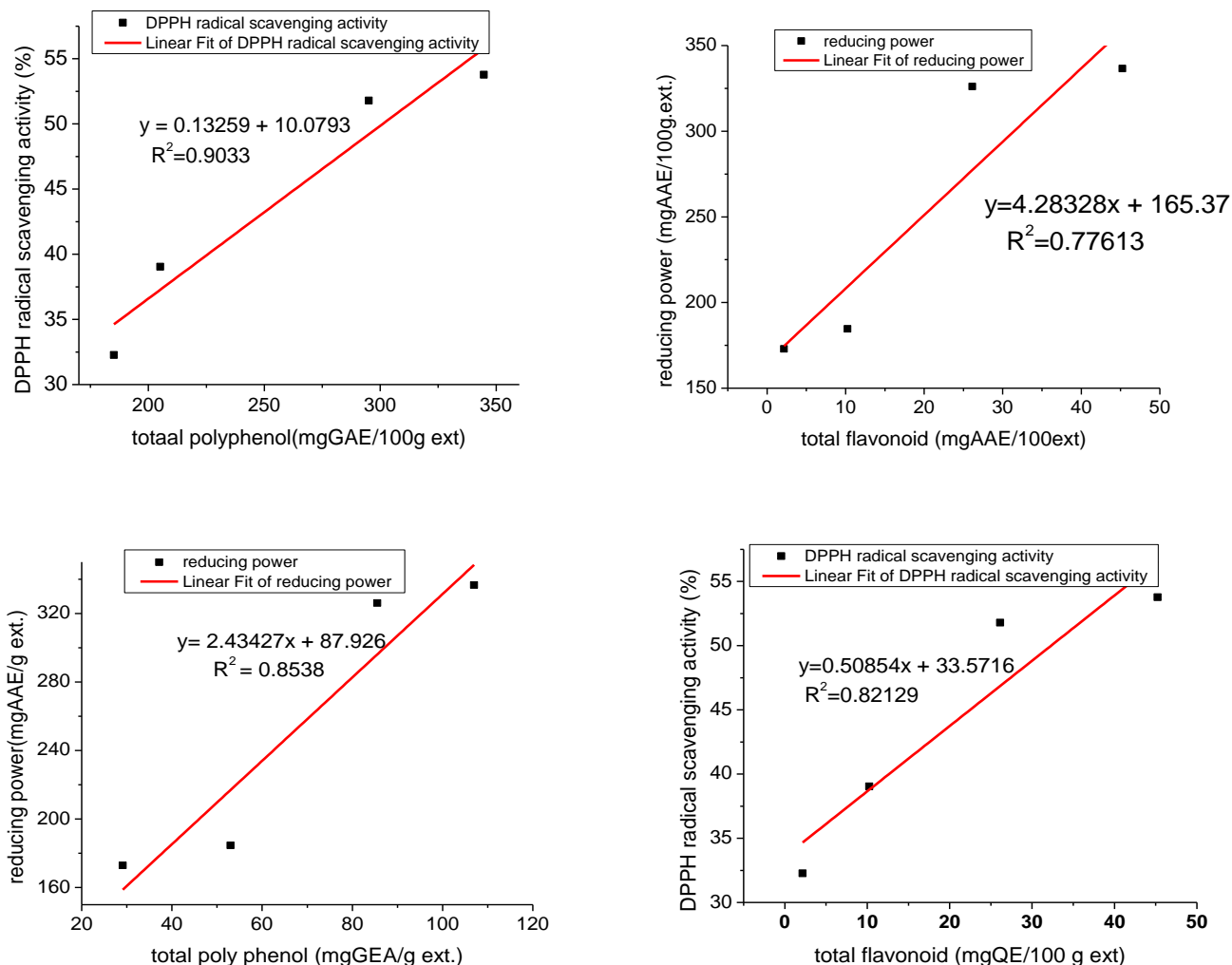
The Folin–Ciocalteu reagent is used to obtain a crude estimate of the amount of phenolic compounds present in the extracts. The Folin–Ciocalteu method was based on the reduction of Mo (VI) by the antioxidant compound and the formation of a blue (Mo (V)) complex with a maximal absorption at 760 nm (Anusuya and Mania, 2013). TP content increases with polarity of the solvent used for extraction and the maximum total phenolic contents were recorded from extracts using highly polar solvents. According to the results obtained, 90% methanol (PLS1) extract contains the highest amount ( $344.70 \pm 0.021$ ) mgGAE/100 g while petroleum ether extracts (PLS4) contains the least amount of phenolic compounds ( $185.07 \pm 0.012$ ) mgGAE/100 g. Furthermore, qualitatively, 90% methanol extract showed very intense blue color as compare to other extracts while petroleum ether extract showed very weak color intensity. This is in agreement with the result reported by Anusuya and coworkers (Ghareeb, 2014). However, the assay summarized in Table 2 might not be specific to just polyphenols but to any other substance that could be oxidized by the Folin–Ciocalteu reagent. Likewise, phenolic compounds, depending on the number and position of hydroxyl groups they contain, respond differently to the Folin–Ciocalteu reagent.

### Determinations of total flavonoid content, TFC

High contents of flavonoid were detected when 90% methanol solvent was used while the least amount was recorded from petroleum ether extracts. The absorbance of the solution was taken from each sample, then, the total flavonoid content of the samples in terms of quercetin equivalent mgQE per 100 g of dry weight of sample was calculated and the result were given in Table 2. The content of flavonoid of different solvent extracts of *P. lanceolata* leaves decrease in the same order as the order of TPC. The concentration of flavonoid compounds in the crude samples increases when polarity of solvents used for extraction was increased. Generally, the result indicates the leaf extracts of *P. lanceolata* is rich in phenolic and flavonoid compounds which makes the plant to possess strong antioxidant activity.

### Antioxidant potential determination

Antioxidant activity of leaf extracts of *P. lanceolata* were evaluated using DPPH radical scavenging, FRAP and peroxide



**Fig. 5:** Correlation between DPPH radical scavenging power with TPC (top left), FRAP with TFC (top right), FRAP and TPC (bottom left), DPPH scavenging activity with TFC (bottom right) of extracts.

value assays. The selection of these assays was due to their ease and reproducibility as well as the availability of required chemicals in the laboratory. Moreover, they are widely used assays to evaluate antioxidant activity of plant metabolites.

Ferric reducing power assay, FRAP: The reducing power of different extracts of *P. lanceolata* leaves was evaluated using potassium ferricyanide method. Presence of antioxidants causes the conversion of the  $Fe^{3+}$ /ferricyanide complex to the ferrous/ $Fe^{2+}$  forms. The reducing power was measured using different concentrations (12%, 24%, 36%, and 48%) (v/v) expressed in terms of ascorbic acid equivalent (AAE) as absorbance per specific amount of extracts. As it is given in Fig. 1, all the extracts exhibited considerable degree of reducing power. From Table 2, the reducing power of extracts increases with increasing concentration and nature of the extracts (polarity of solvents used for extraction). For example, among the different extracts, 90% methanol extract

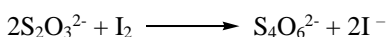
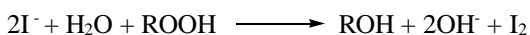
exhibited the maximum reductive capability ( $336.58 \pm 0.052$ ) followed by 100% methanol. This showed that components which can be extracted from the leaves with polar solvents are more responsible for antioxidant activities. Phenols containing several hydroxyl groups would play an important role for antioxidant activity, since they transfer hydrogen easily to scavenge free radicals and convert themselves into stable phenoxide radicals (Miser-Salihoglu et al., 2013). Therefore, these data indicate that *P. lanceolata* plant can be used as a good source of antioxidative compounds that control the level of free radical formation in the body.

DPPH radical scavenging activity: The percentage radical scavenging activity of the *P. lanceolata* leaf extracts is depicted in Fig. 2. 90% methanol extract with a concentration of 48% (v/v) exhibited the highest radical scavenging activity ( $90.6\% \pm 1.60$ ) and petroleum ether extracts showed the least activity ( $36.04\% \pm 1.35$ ) with the same concentration. As

concentration of antioxidative compounds increases, degree of hydroxylation by the phenolic compounds will increase (Muthukrishnan and Subramanian, 2012). That is why 90% methanol extraction of leaf extracts of *P. lanceolata* exhibited the strongest radical scavenging activity in comparison to other extracts. The formation of yellow color solution after the addition of the extract is an indication of conversion of free radicals (DPPH.) to stable DPPH molecule. This was quantified by measuring its absorbance. Degree of inhibition increases as the concentration of the sample increases as it is given in Fig. 2 below but concentration and absorbance of the sample are inversely related in DPPH assay. Thus, the result reveals that *P.lanceolata* plant is rich in antioxidant compounds.

Determination of Peroxide Value, PV: Oxidative stability of Niger seed oil was measured at 70 °C and room temperature using different peroxide value treatments. The graphs of the peroxide values of Niger seed oil with and without antioxidant at 70 oC are shown in Fig. 3. In the first storage day, nothing was observed from Niger seed oil with and without extracts at room temperature. The figure showed an increase in peroxide (PV) of all treatments that contained *P. lanceolata* leaf extracts for the first seven days. Oil containing PLS1 (2000 ppm), PLS2 (2000 ppm) and ascorbic acid (AA) showed slightly higher peroxide value during the first three days compared with the control. In the 4th, 5th, and 6th storage days, rapid increase in peroxide value of oils both with and without antioxidants were observed. For instance, the PV of Niger seed oil was 3.1 meq/kg immediately after it was purchased but it was increased to 129 meq/kg after 7 days of storage at room temperature. According to the data analyzed, the PV of the control was higher than the PV of oil containing PLS1, PLS2 and AA. Oil containing PLS1 showed the lowest PV than all other treatments in all storage days. This indicates that oxidation of Niger seed oil could be highly inhibited by using PLS1 extracts and PLS1 extract exhibited better antioxidant activity than PLS2 and AA until 6th days of storage at 70 oC. Generally, the PV of the treatments containing PLS1, PLS2, AA and control during all storage day at 70oc were higher than same treatments stored at room temperature.

The amount of titrant ( $S_2O_3^{2-}$ ) needed to change the color of the sample to blue increases as temperature and storage days increase. This is because the concentration of  $I_2$  formed from the reaction between peroxide and iodide ion could be increased. This indicates an increase in peroxide concentration and reduction of antioxidant property of the sample (Scheme 1) (Prieto et al., 1999).



**Scheme 1:** The reaction between peroxides and iodide ion in aqueous media.

### Correlation between TPC/TFC with antioxidant activities

Values of different antioxidant assays, DPPH and FRAP, were correlated with values of total phenolic or total flavonoid contents of extracts (Fig. 5). Analysis on the correlations between TPC/TFC and the antioxidant properties measurement is significant. DPPH radical scavenging activity was better correlated with TPC ( $R^2=0.9033$ ) than it correlates with TFC ( $R^2=0.82129$ ). The correlation between FRAP and TPC was also good ( $R^2=0.8538$ ) and moderate with TFC ( $R^2=0.77613$ ). In both cases the correlation between antioxidant activities with TPC was better than they correlates with TFC of *P. lanceolata* leaf extracts. This evaluation informed us that polyphenols may contribute more to antioxidant activities than TFC. Moreover, the correlation coefficient found for DPPH radical scavenging capacity with either TPC or TFC is greater than FRAP. In this study, the strong correlation between the mean values of AEACDPPH and AEACFRAP with TPC indicated that metabolites found in *P. lanceolata* leaves are powerful scavenger of free radicals as well as reducing agents. This is probably due to fast hydrogen transfer from polyphenols than flavonoids to quench DPPH radical and formation of stable DPPH molecule (Thite et al., 2013).

### Comparison of antioxidant activities between defatted and non-defatted extracts

Antioxidant activity comparison by FRAP method: As it is indicated in Fig. 6 and Table 3, the change antioxidant power of 90% methanol extracts of defatted and non-defatted *p. lanceolata* leaf extracts were assessed. *P. lanceolata* leaves were first defatted using petroleum ether or chloroform and then extracted with 90% methanol. This study verifies that non-defatted *P. lanceolata* leaf extracts showed stronger antioxidant activity than the defatted extracts with the same solvent and temperature. This might be due to either the removal of some active components together with solvents used for defatting purpose and/or the heat applied during defatting would decompose some volatile and less stable metabolites. Since, non-defatted extracts showed stronger activities than defatted once; it is possible to conclude that defatting reduces antioxidant activities.

### Determination of antimicrobial activity of *P. lanceolata* leaf extracts

Agar well diffusion method: The result of this study has shown that all the isolated bacteria were susceptible to all extracts because different solvent leaf extracts of *P. lanceolata* showed a significant inhibition zone against each bacterium species (Fig. 7 and 8). The inhibition zone of acetone, methanol and water extracts against *S. pneumonia* (ATCC49619) and *E. coli* (ATCC49619) weaker than that of amoxicillin. There was no great inhibition zone difference observed among different extracts against both gram-positive and gram-negative bacteria. However, the inhibition activity of our extracts was stronger than the control.



For instance, all standard antibiotics had no any activity against *E. coli* and *K. pneumoniae* but sample extracted with water showed stronger inhibition zone against the *E. coli* than acetone and methanol extracts. However, in most cases, methanol extract exhibited better activity against most tested pathogens. In other words, the antimicrobial activity of methanol extracts of *P. lanceolata* leaves was better than acetone and water extracts. Therefore, *P. lanceolata* leaves will provide effective ways to control microbial infection especially caused by those multidrug resistance pathogens.

### Determination of MIC and MBC

Acetone extract of leaf of *P. lanceolata* showed the MIC value at 25% which is the same as MBC value against the multi-drug resistance *S. pneumoniae*. Similarly, the same MIC and MBC of 25% were also analyzed against clinical isolate of *K. pneumoniae* with methanol and acetone extract of *P. lanceolata* leaves as it is indicated in Table 4. The MIC value of acetone extracts against both gram-positive and gram-negative bacteria was 25% which demonstrated weaker antimicrobial activity.

The MIC values of *S. pneumoniae* (ATCC49619) in all extracts were found to be 6.25%. The MIC value of gram-positive bacteria such as *S. pneumoniae* (ATCC49619), MRST and gram-negative bacteria such as *E. coli* (ATCC2592), *E. coli* (multi drug resistance), and *S. boydii* (ATCC9289) were 6.25% (the least value, the highest antimicrobial activity). In all of *P. lanceolata* leaf extracts, MIC values of both gram-positive and gram-negative bacteria were found to be between 12.5% -25%.

MBC of each crude extracts of *P. lanceolata* against *K. pneumoniae*, *S. pneumoniae*, and *S. aureus* (ATCC 2923) was 25% but it was 12.5% against *E. coli* (ATCC49619). The lowest MBC value (6.25%) was exhibited only in water extract of *P. lanceolata* leaves against *S. pneumoniae* (ATCC49619). In each tested bacteria species, the MBC value also varied from 6.25% to 25%.

The aqueous extract of *P. lanceolata* leaves against all gram-negative bacteria species account the minimum MIC values with the highest antimicrobial activity. The methanol extracts of the plant towards each gram-positive bacteria has also exhibit minimum MIC values except against MRSA. Therefore, this implies that the leaf extracts of *P. lanceolata* contain bioactive compounds which can either hinder the growth or reproduction or kill bacteria directly (Dimkić et al., 2016; Guil-Guerrero et al., 2016; Gupta et al., 2016; Netshiluvhi and Eloff, 2016; Rathee et al., 2016; Wang et al., 2016).

### CONCLUSIONS

The antioxidant capacities and total phytochemical contents of both defatted and non-defatted extracts of *P. lanceolata* leaves were examined using different assays. Overall, this medicinal plant has relatively high antioxidant capacities and total phenolic (flavonoid) contents which were observed from significant correlation among the FRAP, DPPH and TPC/TFC

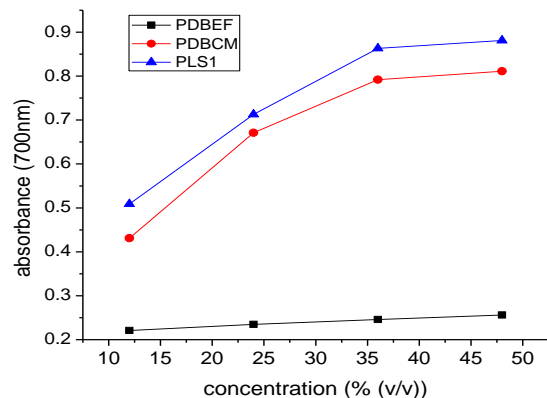


Fig. 6: Reducing power of different solvent extracts of defatted and non-defatted extract of *p. lanceolat* leaves

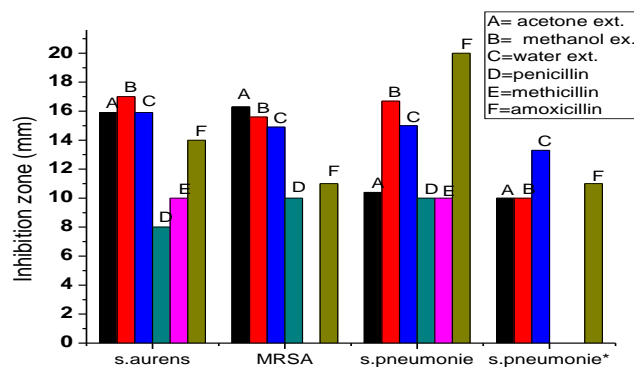


Fig. 7: Comparison of inhibition zone among crude leaf extracts of *P. lanceolata* against standard gram-positive bacteria using different solvents. (\*) for drug resistance bacteria

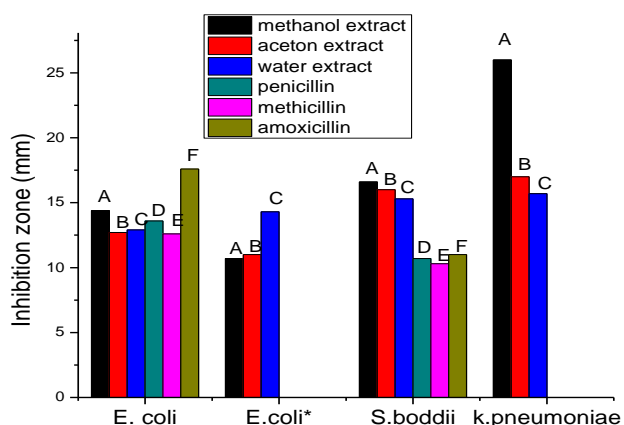


Fig. 8: Comparison of inhibition zone among crude leaf extracts of *P. lanceolata* against standard gram-negative bacteria using different solvents. (\*) for drug resistance bacteria

values. It possesses also strong antimicrobial activities which is believe to be one of the most important component for many pharmacological activities. The findings of this study support the view that *P. lanceolata* plant could be used as a promising and easily accessible source of potential antioxidants or may be used as effective preventive agent against some pathogenesis. These justify the traditional using of this plant's extract as folkloric remedies.

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#### CONFLICT OF INTEREST

We declare that there is no any conflict of interest among the authors as well as between the authors and the funding institution (Bahir Dar University).

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