Studies on Antioxidant and Antimicrobial Activities of Plumbago zeylanica Linn. Traditionally Used for the Treatments of Intestinal Warms and Skin Diseases in Ethiopia

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ABSTRACT
Traditionally, Plumbago zeylanica Linn. is used for the treatments of intestinal warms and skin diseases in Ethiopia. The main objective of this study was to investigate the antioxidant and antimicrobial activities of the crude extract of Plumbago zeylanica root. In this study, two different antioxidant activity quantification methods: 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) and reducing power, FRAP including total phenolic, TPC assays of crude extract of P. zeylanica root were performed. The extracts showed significant activities in all antioxidant assays in a concentration dependent manner. Crude extract was found to possess higher ferric reducing activity (ascorbic acid equivalents 227.01±0.042 mg AA/100 g) and DPPH activity of 156.00±0.048 mg AAE/100 g. Strong correlation of TPC with AEAC_{DPPH} and AEAC_{FRAP} (R = 0.9745 and R = 0.96652, respectively) implied that compounds in the extract were capable of scavenging the DPPH free radical and reducing ferric ions. The free radical scavenging activity and the reducing capacity of the extract in the present study may serve as a significant indicator of its potential antioxidant activity. Antimicrobial activities evaluation of crude extract against S. aureus ATCC, MRSA, S. pneumoniae ATCC, S. pneumoniae (clinical) (gram positive bacteria), K. pneumoniae (clinical), S. boydii ATCC, E. coli ATCC, E. coli (clinical) (gram negative bacteria) and antifungal activity against C. albicans (clinical) (fungus) were also showing a higher antimicrobial activity of the crude extract. The findings of this study support the view that P. zeylanica plant is a promising source of potential antioxidants and may be effective as preventive agent in the pathogenesis of some diseases.

Key words: Antioxidant potential, antimicrobial activity, ferric reducing antioxidant power, DPPH radical, human pathogenic bacteria, Plumbago zeylanica and total phenolic content

INTRODUCTION
Plants have important roles in the lives of humans. History has shown that the leaves, flowers, berries, barks and/or roots of plants were used as antibacterial, antioxidants, antimalarial, analgesics and for several other ailments (Manyakara, 2009).

The World Health Organization (WHO) has reported that approximately 80% of the world's population currently uses herbal medicines as teas, decocts or extracts with easily accessible liquids such as water, milk or alcohol (Khattar and Wal, 2012). Their use continues in the modern world as many conventional drugs are derived from plants (Pan et al., 2014).
Plants have been a source of medicine in pharmacopoeia. Herbs have been utilized to treat acute and chronic disorders for thousands of years. They have recently attracted attention as health beneficial foods (physiologically functional foods) and as a source of materials for drug development (Devi and Thenmozhi, 2011).

Medicinal plants are commonly rich in phenolic compounds such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant and antimicrobial activity (Packer et al., 1999). In vitro experiments on antioxidant compounds in higher plants show how they protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species (Ali et al., 2008). The role of these compounds as potential antioxidants can be inferred by their similarity to synthetic antioxidants of related structures (Mohan et al., 2014).

Plant polyphenols have been studied with the intention to find compounds protecting against a number of diseases related to oxidative stress and free radical-induced damages (Sanda et al., 2012). In this regard, prevention strategies are essential since the risk of oxidative stress increases with age and endogenous antioxidants are gradually consumed over time. Natural antioxidants play key roles in these strategies. Supplementation with antioxidants has received wide spread attention in recent years. There is no doubt that antioxidants are essential in maintaining a healthy body and preventing diseases (Pouillot et al., 2011).

The working principle of antioxidants is either by donating single electron or by donating radical hydrogen. Recent evidence shows that antioxidants can be used to provide photo-protection for the skin. Thus, they achieve, as they prevent and repair damage caused by free radicals (Murray et al., 2008).

Recently, the increased awareness regarding the health protecting properties of non-nutrient bioactive compounds found in fruits and vegetables has directed immense attention to search for antioxidants of natural origin. This is because finding natural antioxidants for use in foods or medicinal materials would enable replacement of synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity. The natural antioxidants may have free-radical scavengers, reducing agents, potential complexes of per-oxidant metals, quenchers of singlet oxygen etc. Natural phytochemicals from plant sources including fruits and vegetables are the main source of antioxidant in the diet, which could decrease the potential stress caused by reactive oxygen species (Salah et al., 1995; Tyagi et al., 2010; Bravo, 1998).

In Ethiopia, there are many plants which are not examined for their antioxidant as well as antimicrobial activity. One of those plants is Plumbago zeylanica. It is only documented as a source of traditional medicine by Getaneh et al. (2014). To the best of our knowledge, there is no report on the anti-oxidant and antimicrobial capacity of the root of Plumbago zeylanica grown in Ethiopia. So, the present study aims to look into the bioactive constituents of the roots of this plant responsible for defending against pathogens and free radicals as well as its in vitro antioxidant and antibacterial activity of root extract of Plumbago zeylanica.

MATERIALS AND METHODS

Chemicals: Gallic acid, Folin-Ciocalteu’s phenol Reagent (FCR), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were all of analytical grade from sigma aldrich products (USA). Hydrochloric acid, potassium iodide and ferric chloride were obtained from BDH, England. Glacial acetic acid (NICE, Lab. reagent), chloroform (WINLAB limited), sodium phosphate monobasic dehydrates and sodium phosphate dibasic were from Merck Chemical Supplies (Damstadt, Germany). Mercuric...
chloride, ascorbic acid and all other chemicals and solvents were procured from Blulux-Laboratories (P) Ltd., India. All the chemicals used were of analytical grade and distilled water was used throughout the experiments.

**Materials:** Double beam Perkin Elmer Lambda 35 UV-visible and single beam UV-visible (NV2003) spectrophotometer were used for absorbance measurements. Chopper, pestle and mortar, grinder, electric shaker, Whatman filter paper No. 1, vacuum pump, rotary evaporator, round bottom flask and volumetric flask were used.

**Plant material and extraction:** Root of *Plumbago zeylanica* plant was collected from around Abay River, Bahir Dar in January 2014 and further identified and authenticated by Dr. Birhanu Andualem, Department of Biology, College of Natural and Computational Sciences, University of Gondar, Ethiopia. For extract preparation, the plant material was first collected from around Abay River. The root part was cut into pieces, washed with tap water and dried at room temperature in shaded place for three weeks. The dried root was made in to small pieces by using pestle and mortar and then ground with a grinder. The 35 g of dry plant powder was soaked in 350 mL of 99.8% methanol for 72 h at 10 RFC using electrical shaker. At the end of extraction, it was passed through Whatman filter paper No. 1. The filtrate was subsequently concentrated under reduced pressure at 37°C in rotator evaporator and dried to a constant weight and the net yield of the crude methanol extract was 7.86%.

**Determination of total phenolic content:** Total phenolic content of the root extract of *P. zeylanica* was estimated using Folin-Ciocalteu reagent based assay (McDonald *et al.* 2001). To 1 mL of different concentrations of the extract 0.125-1.0 mg mL\(^{-1}\) in water, 5 mL of Folin-Ciocalteu reagent (diluted tenfold) and 4 mL (75 g L\(^{-1}\)) of Na\(_2\)CO\(_3\) were added. The mixtures were allowed to stand at room temperature for 30 min and the absorbance of the developed blue colour was recorded at 760 nm using UV-VIS spectrophotometer. One milliliter aliquot of 0.1-0.25 mg mL\(^{-1}\) gallic acid solution was used as standard for calibration curve. All determinations were performed in triplicate.

**Ferric reducing antioxidant power assay:** The reducing antioxidant power of the plant methanolic extract was determined by the method of Oyaizu (Saha *et al.*, 2008). Variable concentrations (0.4-1.0 mg mL\(^{-1}\) extract of the plant (2.5 mL) were mixed with phosphate buffer (3.0 mL, 0.2 M, Ph 6.6) and potassium ferricyanide [K\(_2\)Fe(CN)\(_6\)] \((2.5 \text{ mL, 1%})\). The mixtures were incubated at 50°C for 20 min. Then, 2.5 mL of trichloroacetic acid (10%) was added to the mixtures, which was then centrifuged for 20 min at 112 RCF. The upper layer of solution (5 mL) was mixed with distilled water (5 mL) and FeCl\(_3\) \((0.5 \text{ mL, 0.1%})\). The absorbance of developed blue color was measured against a blank using UV-Vis spectrophotometer at 700 nm. Increment in absorbance of the reaction mixture indicates increase in reducing power. Ascorbic acid was used as standard and water with all reagents except the analyte was used as a blank. The percentage reduction power (%RP) of the sample was calculated.

**Scavenging activity against DPPH:** The crude methanol extract of the plant was screened for DPPH radical scavenging activity. An aliquot of 2 mL of 0.1 mM DPPH solution in methanol and 2 mL of plant extract in methanol at various concentrations 0.6-1.0 mg mL\(^{-1}\) were mixed and
incubated at 25°C for 30 min and absorbance of the test mixture was measured using a spectrophotometer at 515 nm against a DPPH control containing only 2 mL of methanol in place of the extract. The fresh DPPH solution in methanol was prepared before the absorbance measurements. The DPPH is a purple colored stable free radical. When, reduced it becomes the yellow colored diphenyl picrylhydrazine. Ascorbic acid was used as a standard and percent inhibition of the sample was calculated.

**Antimicrobial activity determination**

**Media preparation and its sterilization:** For agar well diffusion method antimicrobial susceptibility was tested on solid (Agar-agar) media in petri plates. For bacterial assay Nutrient Agar (NA) and for fungus PDA was used for developing surface colony growth. The Minimum Inhibitory Concentration (MIC), the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) values were determined by serial micro dilution assay.

**Preparation of inoculum**

**Antibacterial activity:** The antibacterial assay was carried out by micro-dilution method in order to determine the antibacterial activity of bioactive compounds tested against standard and clinical pathogenic bacteria. The pathogenic bacteria were inoculated and spread over on Muller-Hinton agar and inoculated for 24 h. The 2-3 colonies were peaked up by wire loop aseptically in to sterile saline solution and the turbidity was adjusted to the 0.5 McFarland’s standard solution to a concentration of 1.5×10⁸ CFU mL⁻¹. Each MH agar plate was uniformly seeded by means of sterile swab dipped in to the suspension and streaked on the agar plate surface and the plates were left on the bench for excess fluid being absorbed. Using a sterile cork borer (6 mm diameter, 4 mm deep and 2 cm apart) wells were made the agar medium. Using a micropipette, 100 µL of sample with the concentration of 50% w/v was added in to the wells in the plate. The plates were incubated at 37°C for 24 h. The mean diameters of inhibition zones were measured in mm and the results were recorded. The same procedure was performed for antifungal susceptibility test. All experiments were performed in triplicates.

**Determination of minimum inhibitory concentration:** The Minimum Inhibitory Concentrations (MIC), MBC and MFCs were performed by a serial dilution technique using 96-well microtitre plates. Different plant extracts (methanol, acetone and aqueous) were diluted to concentrations ranged from 6.25-25% w/v. To each dilution of extracts, 100 µL of clinical and standard bacterial inoculum were seeded in nutrient broth tubes. Negative control tubes with no bacterial inoculation were simultaneously maintained. Tubes were incubated aerobically at 37°C for 24 h. The MIC was defined as the lowest concentration of extracts those completely inhibits the growth of the organism.

**Determination of minimum bacterial concentration:** Dilutions showing no visible growth for MIC was sub-cultured on to a fresh MH agar plate and inoculated at 37°C for 24 h. The lowest concentration of the extracts yielding no growth on MH plate was recorded as MBC. The same procedure was performed for MFC.

**Statistical analysis:** All the analyses of total antioxidant activity, total phenolic content and antimicrobial activity analyses were performed in triplicates. The data was recorded as
Means±Standard Deviation and analyzed using Microsoft Excel windows 7. Origin 6.1 was used for drawing of calibration curve. One-way analysis of variance (ANOVA) was performed for both antioxidant and antimicrobial activity analysis. Significant differences between means were analyzed by Tukey’s multiple regression tests. p-values≤ were regarded as significant.

RESULTS AND DISCUSSION
Total Phenolic Content (TPC) determination of the root extract: The Folin-Ciocalteu phenol reagent is used to obtain a crude estimate of the amount of phenolic compounds present in the extract. When the reagent was added to the extract and then neutralized by sodium carbonate solution, the original yellow color of the reagent was changed to blue that indicates the presence of phenolic compounds. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the reagent. The phosphomolybdenum 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. The increasing series of absorbance with concentration of root extract (Table 1) showed us the existence of phenolic compounds in the sample. However, the assay has been shown not specific to just polyphenols but to any other substance that could be oxidized by the Folin. In addition, phenolic compounds, depending on the number of phenolic groups they have, respond differently to the Folin-Ciocalteu reagent (Gulcin, 2012).

The result of TPC (317.04 mg GAE/100 g sample) indicates the root extract of Plumbago zeylanica is rich in phenolic compounds which make it a potential antioxidative species. These results are in agreement with reported data. The phenolic content of ethyl acetate/diethyl ether root extract of P. zeylanica has been reported ranging from 0.27±0.04 to 0.93±0.28 mg g⁻¹ Tannic Acid Equivalent (TAE) (Raimi and Oyedapo, 2009). It is also reported that the total phenolic content of Plumbago rosea root extract is 235 µg g⁻¹ in comparison to 204 µg g⁻¹ in Plumbago zeylanica (Nahak and Sahu, 2011). This variation may be due to drying condition, types genes, extraction technique, solvent type or climatic condition of the species to be grown as well as sensitivity of the instrument.

Phenolics are the most widespread secondary metabolites in the plant kingdom and have received much attention as potential natural antioxidants in terms of their abilities to act as both efficient radical scavengers and metal chelators. In this study, Plumbago zeylanica plant was investigated for its total phenolic content, antioxidant and antimicrobial properties.

Antioxidant activity determination: There is increasing evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and in the protection of biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants (Nahak and Sahu, 2011). Antioxidant activity of methanolic extract of root of P. zeylanica was measured to exhibit scavenging of DPPH radical and reducing of ferric reducing potential. These activities of the extract were evaluated using DPPH scavenging and FRAP assays. The selection

<table>
<thead>
<tr>
<th>Concentration of extract (mg mL⁻¹)</th>
<th>Absorbance</th>
<th>TPC (mg GAE/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.125</td>
<td>0.29±0.05c</td>
<td>317.04±0.00</td>
</tr>
<tr>
<td>0.25</td>
<td>0.31±0.03d</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.39±0.04e</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.48±0.01f</td>
<td></td>
</tr>
</tbody>
</table>

Differences between values at the same column followed by different super scripts c, d, e and f are highly significant at p<0.01, TPC: Total phenolic contents.
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 (Litescu et al., 2010).

**Ferric reducing power assay:** Ferric reducing power of the methanolic extract was analyzed using potassium ferric cyanide method. The reducing power of the extract, which was determined using potassium ferricyanide method may serve as a significant indicator of its potential antioxidant activity.

*Plumbago zeylanica* is a potential antioxidative species and the calculated antioxidant activity in milligram equivalent of ascorbic acid using the regression equation \( y = 0.07492 + 0.7783x \) as well as the reducing potential of the extract are shown in Table 2.

As it is indicated in the result, the extract has a potential to reduce ferric into ferrous ion. This result showed that the methanolic root extract of *P. zeylanica* has a potent ferric reducing power indicating that it is rich in reductants (antioxidative compounds). The redox reaction was confirmed when the yellow color of potassium ferric cyanide was changed into Prussian blue. The reducing capacity increases with the concentration of the extract. Generally, the reducing power of this plant could be due to the presence of flavonoids and phenolic acids that is capable of reducing ferric ion (Fe\(^{3+}\)) to ferrous ion (Fe\(^{2+}\)).

**DPPH scavenging capacity assay of the sample:** The methanolic root extract was tested for the DPPH free radical scavenging ability. The original purple color of DPPH was changed in to yellow when the reagent was added to the extract, which confirmed the reaction of the extract with the DPPH radical. This can be quantified by measuring its absorbance. The result reveals that *P. zeylanica* is rich in antioxidant compounds. This method is somewhat difference in determination of antioxidant capacity of the sample. The concentration and absorbance of the sample are inversely related. The absorbance was decreased as the concentration of the sample increased i.e., slope of the graph is negative. Antioxidant power of the extract could be calculated using the regression equation \( y = 0.3771x + 61.3885 \) and described as mg AAE/100 g of sample which was resulted 156.048 mg AAE/100 g (Table 3).

**Correlation between antioxidant capacities and total phenolic content:** Analysis on the correlations between TPC and the antioxidant properties measured is significant. The strong correlation between the mean values of AEAC\(_{DPPH}\) with TPC and AEAC\(_{FRAP}\) with TPC of the plant

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Table 2: Reducing power of sample and its antioxidant activity (mg of ascorbic acid equivalent per 100 g of sample at 700 nm)

<table>
<thead>
<tr>
<th>Concentrations of extract (mg mL(^{-1}))</th>
<th>Absorbance of extract</th>
<th>Reducing of extract (%)</th>
<th>Power FRAP (mg AAE/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>0.224±0.007(^a)</td>
<td>66.465±0.378(^a)</td>
<td>227.014±0.0442</td>
</tr>
<tr>
<td>0.6</td>
<td>0.356±0.005(^b)</td>
<td>78.952±0.035(^b)</td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0.441±0.003(^c)</td>
<td>82.994±0.101(^c)</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.693±0.001(^d)</td>
<td>89.183±0.018(^d)</td>
<td></td>
</tr>
</tbody>
</table>

All data was performed in triplicate and values in the same column followed by different super scripts a, b, c, d are significantly different \( p < 0.05 \).

Table 3: DPPH scavenging activity of sample extract at different concentration

<table>
<thead>
<tr>
<th>Extract concentrations (mg mL(^{-1}))</th>
<th>Absorbance of extract</th>
<th>Inhibition of extract (%)</th>
<th>DPPH (mg AAE/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>0.150±0.001(^a)</td>
<td>83.748±0.125(^a)</td>
<td>156.048±0.002</td>
</tr>
<tr>
<td>0.8</td>
<td>0.136±0.001(^b)</td>
<td>85.333±0.125(^b)</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.119±0.000(^c)</td>
<td>87.135±0.000(^c)</td>
<td></td>
</tr>
</tbody>
</table>

Values were triplicate determinations. Those values in the same column followed by super scripts a, b, c are significantly different at \( p < 0.05 \).
samples deserves detailed attention. This could be explained from the basic concept that antioxidants are reducing agents and capable of donating a single electron or hydrogen atom for reduction. However, not all reducing agents are antioxidants (Wong et al., 2006). In this study, the strong correlation between the mean values of AEAC_{DPPH} and AEAC_{FRAP} with TPC indicated that phenolic compounds present in the methanol extracts capable of reducing DPPH radicals were also able to reduce ferric ions. In other words, metabolites found in P. zeylanica root are powerful scavenger of free radicals as well as reducing agents. Similar trend was reported in earlier studies (Wangcharoen and Morasuk, 2007).

Linear regression analysis was used to analyze the correlation between the antioxidant capacity and the total phenolic content of P. zeylanica plant. For the correlation between FRAP values and total phenolic contents, the regression equation \( Y = 7.46x - 0.0937 \) was statistically significant \((p = 0.02545)\) and the coefficient of determination \( R \) was 0.9745. In the same case, DPPH and TPC were also correlated with regression equation of \( Y = 32.07856x + 74.81228 \) with R-value of 0.96653 but not significant at \( p<0.5 \) \((p = 0.16517)\). So, the correlations of TPC against the antioxidant activity based on the DPPH and FRAP assays involving P. zeylanica plant was excellent.

In general, in this study ferric reducing power is better to evaluate methanolic extract of P. zeylanica than DPPH radical scavenging assay. Besides, ferric reducing power assay is a simple, accessible and repeatable parameter to estimate the antioxidant activity of the extract. The probable reason for the lower AEAC_{DPPH} values of the plants compared with AEAC_{FRAP} could be due to the presence of compounds not reactive towards DPPH. Antioxidant compounds such as polyphenols may be more efficient reducing agents for ferric ion but some may not scavenge DPPH free radicals as efficiently due to stearic hindrance (Wong et al., 2006).

**Antimicrobial activity of Plumbago zeylanica:** Results in the present study revealed that the tested medicinal plant, P. zeylanica root extracts posses a potential antibacterial activities against S. aureus ATCC, MRSA, S. pneumoniae ATCC, S. pneumoniae (clinical) (gram positive bacteria), K. pneumoniae (clinical), S. boydii ATCC, E. coli ATCC, E. coli (clinical) (gram negative bacteria) and antifungal activity against C. albicans (clinical) (fungus).

Different extracts of P. zeylanica were tested for their antibacterial and antifungal activities against different strains. As shown in Fig. 1-3, these different extracts of P. zeylanica have resulted with good antimicrobial and antifungal activities with relative to their antibiotic standards. For their antibacterial activity, acetone extract showed a highest result compared to the other extracts to inhibit all gram positive bacterial strains (S. aureus ATCC with inhibition zone of 41.00±1.00 mm, MRSA; 36.67±1.53 mm, S. pneumoniae ATCC; 44.00±1.00 mm, S. pneumoniae (clinical); 25.00±1.00 mm followed by methanol extract with inhibition zones of S. aureus ATC; 40.33±0.58 mm, MRSA; 36.00±1.00 mm, S. pneumoniae ATCC; 21.00±1.00, S. pneumoniae (clinical); 21.67±0.58 mm and aqueous extract (S. aureus ATC; 14.67±0.58 mm, S. pneumoniae ATCC; 17.00±1.00 mm and S. pneumoniae (clinical); 16.67±0.58 mm). It also showed that acetone extract has highest values of inhibition zones against gram negative bacterial strains (E. coli (clinical); 14.67±0.58 mm, E. coli ATCC; 18.33±0.58 mm and K. pneumoniae (clinical); 14.00±1.15 mm and methanol extract has the highest value of inhibition zone against S. boydii ATCC; 35.00±1.00 mm). For their antifungal activity, methanol extract has the highest value of inhibition zone against the tested fungal strain; C. albicans (clinical) 30.67±0.58 mm followed by acetone, 29.33±1.15 mm and aqueous extracts, 13.33±1.15 mm.
Fig. 1: Inhibition zones of extracts and standards against gram positive bacterial strains (susceptibility of organisms towards *Plumbago zeylanica* root extracts), MeOH: Methanol, Act: Acetone, Aq: Aqueous

Fig. 2: Inhibition zones of extracts and standards against gram negative bacterial strains (susceptibility of organisms towards *Plumbago zeylanica* root extracts), MeOH: Methanol, Act: Acetone, Aq: Aqueous extracts

Fig. 3: Inhibition zones of extracts and standards against fungal strain, *Candida albicans* (clinical), MeOH: Methanol, Act: Acetone, Aq: Aqueous extracts
As their MIC and MBC indicated in Fig. 4 and 5, the methanolic and acetone extract of *Plumbago zeylanica* root were shown more effective for all tested organisms than aqueous extract. *Klebsiella pneumonia* (clinical) was not sensitive for aqueous root extract. The MFC result also showed (Fig. 6) that the methanol and acetone extracts are more effective against the tested fungal strain than the aqueous extract. It is reported that the MIC of ethanolic root extract of *P. zeylanica* against *S. aureus* and *M. luteus* is 1250 and 2500 µg mL⁻¹, whereas the MIC of ethanolic callus extract against these microorganisms is 5000 µg mL⁻¹ (Mittal and Sharma, 2010).

But in the present study, all methanolic, acetone and aqueous extracts of root extracts showed a very good effectiveness with a minimum concentration of these extracts with respective values...
Fig. 6: MFC values of *Plumbago zeylanica* root extracts against fungal strain, *Candida albicans* (clinical), MeOH: Methanol, Act: Acetone, Aq: Aqueous

of 62.5, 62.5 and 250 µg mL⁻¹. The high susceptibility of these tested organisms against the whole root extracts of *P. zeylanica* might be due to the bioactive constituents in the extracts.

**CONCLUSION**

The antioxidant capacities and total phenolic contents of *P. zeylanica* plant were evaluated using the FRAP and DPPH assays as well as the Folin-Ciocalteu method, respectively. Overall, this medicinal plant has relatively high antioxidant capacities and total phenolic contents which were observed from a significant correlation among the FRAP, DPPH and TPC values. It possesses also strong antimicrobial activities which is believe to be one of the most important components for many pharmacological activity.

The preliminary qualitative screening of major secondary metabolites of the extract of *P. zeylanica* showed that the presence of alkaloids, flavonoids, tannins, saponins and anthraquinones as per the phytochemical tests. The above mentioned metabolites have been reported to possess antioxidant and antimicrobial activity. Research results from different laboratories showed that secondary plant metabolites such as alkaloids, flavonoids, tannins and terpenoids exert a wide range of biological activities on physiological systems. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on human body. Therefore, these phytochemicals found in *P. zeylanica* may play similar biological activities and pharmacological properties of the plant.

The findings of this study support the view that *P. zeylanica* plant is a promising source of potential antioxidants and may be effective as preventive agents in the pathogenesis of some diseases. These justify the traditional using of this plant’s extract as folkloric remedies. However, in the future, the specific compounds with high antioxidant and antimicrobial capacities should be isolated and identified from the plant to further develop natural antioxidants and investigate antibacterial drugs either in crude or pure form.

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