Phytochemical Screening, Total Phenolic Content, Total Flavonoid Concentration and Antioxidant and Antimicrobial Activities of Two Myanmar Medicinal Plants

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Abstrct

The aim of this study was to evaluate the phytochemical screening, total phenolic concentration, total flavonoid content and antimicrobial and antioxidant activities of two medicinal plants collected from Sagaing region, Myanmar. Preliminary phytochemical screening was performed with standard procedures. The antimicrobial activities were determined by agar dilution-streak method. Antioxidant activity was analyzed by using 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay. From phytochemical screening results, *Garciniaxanthochymus* showed the presence of all chemical constituents except alkaloids whereas *Naucleasessilifolia*contained all phytochemical constituents except flavonoids. *Garciniaxanthochymus* extract showed no antimicrobial activity on five tested microorganisms while *Naucleasessilifolia* extract revealed low antimicrobial sensitivity on *Bacillus pumilus* and *Escherichia coli*. Among these two plants, the methanolic extract of *Garciniaxanthochymus* showed the highest phenolic content by the FolinCiocalteu's method and the greatest flavonoid content by an aluminium chloride colorimetric method. The methanolic extract of *Garciniaxanthochymus* showed high antioxidant activity (EC_{50} 22.45 μg/mL) whereas *Naucleasessilifolia* exhibited the medium antioxidant activity (EC_{50} 40.21μg/mL). This study demonstrates that these two plants could be a potential source of natural antioxidants.

Keywords: Phytochemical, antimicrobial, antioxidant, potential, scavenging

Introduction

Medicinal plants are used in treatment of various diseases such as diabetics, inflammation and cancer etc. They were reported to have antimicrobial and antioxidant activities. Several studies have shown that plant derived antioxidants scavenge free radicals. Free radicals which are delivered as a consequence of typical biochemical responses in the body are involved in cancer, heart disease, inflammation, diabetes, aging, atherosclerosis, immune suppression, and neurodegenerative disorders (Chanda *et al*., 2011). Recently, researchers have focused on increasing human infections caused by bacteria and fungi. Medicinal plants contain a rich source of antimicrobial agents. Because microorganisms have developed resistance to many antibiotics (Muslim *et al*., 2012), the use of plant extracts and their isolated compounds as a resistant against microorganisms has been increased. The present study is an attempt to performphytochemical screening, total phenolic content, total flavonoid concentration, antioxidant and antimicrobial activities of two medicinal plants (*Garciniaxanthochymus*and *Naucleasessilifolia*collected from Sagaing Region.

Materials and Methods

Plant Material

The medicinal plants of *Garciniaxanthochymus* Hook. f.and *Naucleasessilifolia* (Roxb.) Merr.were collected from MaharMyaing forest, Kalay-wa Township, Sagaing Region (Myanmar) and voucher specimens were deposited in the Department of Chemistry, Kalay University, Myanmar. The collected samples were

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allowed to dry for one week in well ventilated shade. Then, the air dried samples were grounded into powder by grinding machine.

Phytochemical Analysis of the Samples

The preliminary phytochemical analysis of alkaloids, flavonoids, phenolics, glycosides, tannins, saponins, steroids and terpenoids in the extracts were carried out using standard methods (Tiwari *et al*., 2011).

Antimicrobial Assay by Agar Dilution-streak method

The agar-streak method was used to assess the antimicrobial activities of the methanol extracts of two medicinal plants against *Bacilliussubtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*and *Escherichia coli*. Cultures of bacteria and fungus were grown on nutrient broth (Basal Media) at 37°C for 24 h and were maintained on respective agar slants at 4°C. The test organisms maintained on agar slants were recovered for testing by inoculating into nutrient broth and incubated at 37°C in a shaker at 180 rpm. Antibacterial and antifungal activity was carried out by agar dilution-streak method (Mitscher*et al*., 1972). Plant extract was incorporated into the media and poured into the different petri plates and allowed to solidify. Bacteria or fungi inocula were then streaked at different areas on the respective agar plates. Plates were incubated at 37°C and observed after 24 h. Blank plates each containing only NA were prepared. Growth of bacteria and fungus were observed after one day. The results were compared with neomycin as a standard.

Determination of Total Phenolic Contents in Plant Extracts

Total phenolic content of methanol extract of two medicinal plants were determined with Folin-Ciocalteu's method (Singleton *et al*., 1999). The Folin–Ciocalteu's (F–C) reagent is sensitive to reducing compounds, polyphenols and thus produces a blue colour complex. The F-C assay relies on the transfer of reducing equivalents (electrons), in the alkaline medium, from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes, manifested in the formation of blue colour complexes [possibly (PMoW11O40)4−] that are determined on a UV-visible spectrophotometer by monitoring the absorbance at 765 nm (Singleton *et al*., 1999). Ethanolic solution of the extract in the concentration of 1 mg/mL was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of ethanolic solution of extract, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 mL of ethanol, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 mins. The absorbance was determined using spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. To obtain a calibration curve, various concentrations of gallic acid solutions (100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL) were prepared. Based on the measured absorbance, the concentration of phenolics was read (mg/mL) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Total Phenolic Content = $c\frac{V}{m}$ where c = concentration from calibration curve, m = mass of the extract used, v = volume of the extract

Determination of Total Flavonoid Contents in Plant Extracts

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method (Quettier*et al*., 2000). The sample contained 1 mL of ethanol solution of the extract in the concentration of 1 mg/mL and 1 mL of 2% $AlCl_3$ solution dissolved in ethanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λ_{max} = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of quercetin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/mL) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of Q/g of extract).

Total Flavonoid Content = c $\frac{V}{m}$ where c = concentration from calibration curve,m = mass of the extract used, v = volume of the extract

Determination of Antioxidant Activity by DPPH Radical Scavenging Assay

The antioxidant activities of the extracts of two medicinal plants were determined by DPPH scavenging activity assay (Yamaguchi *et al*., 1998).500 µL of test solutions in various concentrations (100 µg/mL, 50 µg/mL and 10 µg/mL) and 500 µLof 0.2 M acetate buffer pH 5.5 solutions are mixed in a test tube. 250 µL of $5x10^{-4}$ M DPPH solution was added to the mixture in dark. The mixture was homogenized using a vortex mixer in a dark room (resistant to UV light) and stand for 30 minutes at room temperature. After that, the mixture was measured by a spectrophotometer UV absorbance at λ_{max} 517 nm. Vitamin C was used as a reference compound in the same concentration range as the test compounds. A control solution was prepared by mixing 500 µL of buffer (pH 5.5) solution, 500 µL of ethanol and 250 µL of 5×10^{-4} M DPPH solution in the test tube. Blank solution was prepared by mixing 500 µL of buffer (pH 5.5) solution with 750 µL of ethanol in the test tube. The mean values were obtained from triplicate experiments. The capability of scavenging DPPH radicals as a percentage of DPPH remaining in the resulting solution was determined using the following equation:

$$\text{DPPH (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}$$

where Abs control is absorbance of control and Abs sample is absorbance of sample.

Results and Discussions

The air dried samples were extracted with MeOH. After solvent removal by evaporation, the residue was used for the determination of total phenolic, total flavonoid, antimicrobial and antioxidant activities.

Figure 1.The plant of *Garciniaxanthochymus* Hook.f.

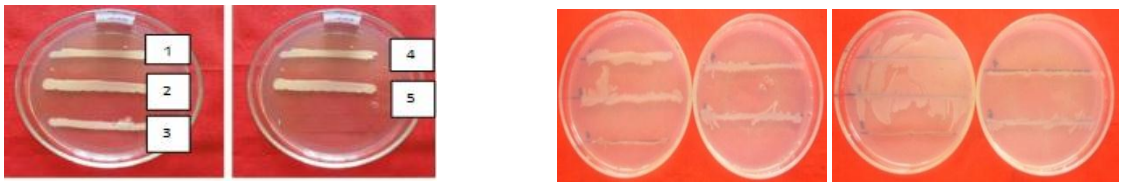
Figure 2.The plant of *Naucleasessilifolia*(Roxb.)Merr.

Preliminary Phytochemical Test of the Plant Extracts

Phytochemical anlaysis revealed that the extracts of *Garciniaxanthochymus* contained all tested phytochemical constituents except alkaloids. The extracts of *Nauclea sessilifolia*showed the presence of all tested phytochemical constituents except flavonoids.

Antimicrobial Activities of Selected Medicinal Plants

According to the antimicrobial experiments, *Garciniaxanthochymus* extract showed no antimicrobial activity on five tested microorganisms while *Naucleasessilifolia* extract revealed low antimicrobial sensitivity on *Bacillus pumilus* and *Escherichia coli*.



1. *Bacillus subtilis*, 2. *Staphylococcus aureus*, 3.*Pseudomonas aeruginosa*,
4. *Bacillus pumilus*5. *E. coli*

Figure 3.Antimicrobial activities of selected medicinal plants

Determination of Total Phenolic Contents in Plant Extracts

The total phenolic contents of the extracts of two medicinal plants were determined with Folin-Ciocalteu's reagent according to the (Singleton *et al*., 1999) method using gallic acid as a standard. The result of total phenolic content was found to be 169 mg GAE/g (*Garcinia xanthochymus*) and 123 mg GAE/g (*Nauclea sessilifolia*). These results were shown in figure (4).

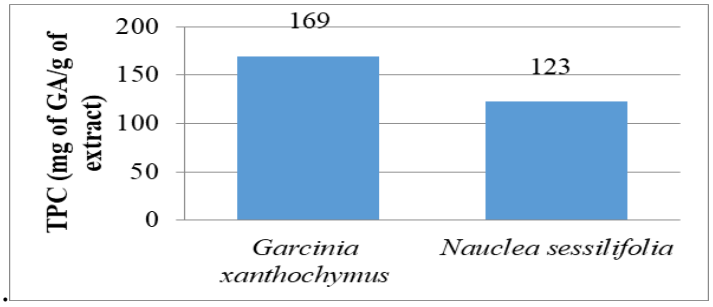


Figure 4. Total phenolic contents in the plant extracts expressed in terms of gallic acid equivalent (GAE) (mg of GA/g of extract)

Determination of Total Flavonoid Contents in Plant Extracts

The total flavonoid content in the extracts of two medicinal plants was determined using quercetin as a standard. The result of total flavonoid content was found to be 95.77 mg QE/ g (*Garcinia xanthochymus*) and 43.4 mg QE/g (*Nauclea sessilifolia*).

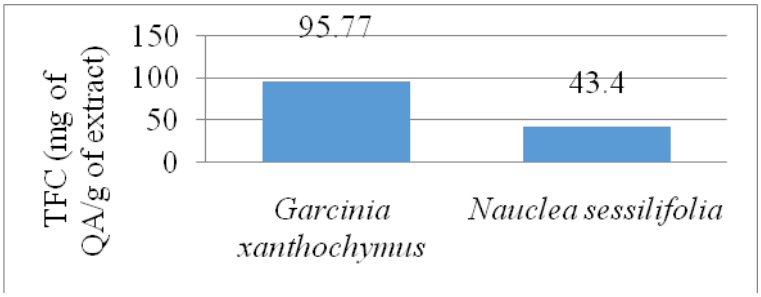


Figure 5. Total flavonoid contents in the plant extracts expressed in terms of quercetin equivalent (QE) (mg of Q/g of extract)

Antioxidant Activity by DPPH Scavenging Activity Assay

As shown in figure (6), DPPH radical scavenging activities of the methanolic extracts of *Garciniaxanthochymus* showed potent free radical scavenging activity with EC_{50} 22.45 21 µg/mL whereas that of *Naucleasessilifolia* exhibited the medium free radical scavenging activity with 40.21 µg/mL.

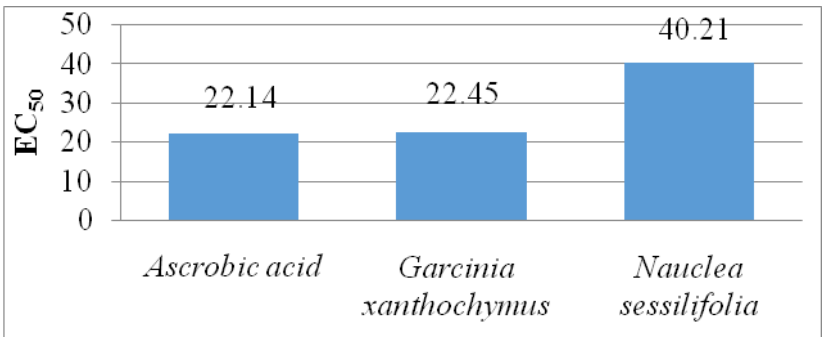


Figure 6.DPPHradical scavenging activity of ascrobic acid and methanolic extracts of ascorbic acid of two medicinal plants

Conclusion

In this study, two medicinal plants (*Garcinia xanthochymus*and *Nauclea sessilifolia*) were selected for phytochemical investigation, determination of total phenolic content, total flavonoid concentration, antimicrobial and antioxidant activities. The results of phytochemical analysis revealed that the extract of *Garcinia xanthochymus*contained all phytochemical constituents except alkaloids. The extracts of *Nauclea sessilifolia*showed the presence of all chemical constituents except flavonoids. According to the antimicrobial experiments, *Garciniaxanthochymus* showed the presence of all chemical constituents except alkaloids whereas *Naucleasessilifolia*contained all phytochemical constituents except flavonoids. *Garciniaxanthochymus* extract showed no antimicrobial activity on five tested microorganisms while *Naucleasessilifolia* extract revealed low antimicrobial sensitivity on *Bacillus pumilus* and *Escherichia coli*.The results of total phenolic content were found to be 169 mg GAE/ g (*Garciniaxanthochymus*) and 123 mg

GAE/g (*Naucleasessilifolia*). The flavonoid concentrations in two medicinal plants were found to be 95.77 mg QE/g (*Garciniaxanthochymus*) and 43.4 mg QE/g (*Naucleasessilifolia*). Among these two plants, the flavonoid concentration in *Garciniaxanthochymus* has the highest amount. According to antioxidant experimental data, the methanolic extract of *Garcinia xanthochymus*was found to be potent antioxidant activity with EC_{50} = 22.45 μg/mL. The methanolic extract of *Naucleasessilifolia* (EC_{50} = 40.21 μg/mL) was found to be medium antioxidant activity. There was a direct relation between antioxidant activity and the content of phenols in some extracts in this study. For example, *Garcinia xanthochymus*showed high phenolic and flavonoid contents and showed good antioxidant activity. These results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. The result of the present study suggests that selected plants can be used as a source of antioxidants for pharmacological preparations which is very well evidenced by the present work.

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