Indigenous Myanmar medicinal plants and their *in vitro* antioxidant, antiglycation and antimicrobial activities

**Indigenous Myanmar Medicinal Plants (23 samples from 19 plants)**

- Ethanolic Extraction of Medicinal Plants
- Biological Activities of Plant Extracts

**Antioxidant Activity**
- DPPH free radical-scavenging assay
- Nitric oxide radical-scavenging assay
- Superoxide radical-scavenging assay
- Total phenolic content measurements

**Antiglycation Activity**
- BSA-fructose assay

**Antimicrobial Activity**
- Agar-well diffusion assay

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**PHARMACOLOGY | ORIGINAL ARTICLE**

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Indigenous Myanmar medicinal plants and comparison of their in vitro antioxidant, antiglycation, and antimicrobial activities

Htet Htet Win¹, The Su Moe¹*, Thin Thin Hlaing², Myint Myint San¹, Zar Kyi Win¹ and Khin Mar Mya³

Abstract:

Objective: The purpose of this study was to evaluate the potential biological activities of 23 ethanolic extracts from 19 traditional medicinal plants from Myanmar.

Methods: Antioxidant activity was determined using DPPH, nitric oxide and superoxide free radical scavenging assays. Antiglycation activity was studied with non-enzymatic protein glycation assay. Total phenolic content was measured compared with the gallic acid standard curve. The antimicrobial activity was evaluated against six clinically important bacteria by agar well-diffusion method.

Results: Among the studied plant extracts, AGE228 (Syzygium cumini (L.) Skeels. seed) was the promising medicinal plant for oxidative stress-related disease as it

ABOUT THE AUTHORS

The author is now working as a Senior Research Investigator at the Pharmaceutical Research Lab, Biotechnology Research Department under the Ministry of Education, Myanmar. The missions of the lab are investigation of the traditionally used medicinal plants for their bioactivities such as antioxidant, antiglycation, anti-diabetes, hepatoprotective and antimicrobial activities against the antibiotic resistant bacteria and bioassay guided isolation and identification of the active compounds from the most potent traditional medicinal plants. The present research report is the first step screening on the bioactivities of some medicinal plants in Myanmar. Based on the findings in this report, the author will be further exploring the in vivo anti-diabetes activity using mice model and compound isolation from the best plants.

Dr. Htet Htet Win has done all the in vitro antioxidant, antiglycation and antimicrobial assays used for this research and written the manuscript.

Dr. The Su Moe has contributed for the experimental design, has done all the assays together with Dr. Htet Htet Win and done the final approval of the manuscript.

Dr. Thin Thin Hlaing, Mrs. Myint Myint San and Ms. Zar Kyi Win have done all the work for the collection and extraction of medicinal plants for this research.

Dr. Myint Myint San has carried out the Total Phenolic Content Determination for this selected medicinal plant extracts.

PUBLIC INTEREST STATEMENT

Diabetes, a non-communicable and chronic disease, nowadays becomes a high threatening disease both in developed and developing countries although the death rate is higher in low- and middle-income countries. Medicinal plants having antioxidant and antiglycation activities are the interesting source for safe and effective medicine for diabetes. Moreover, many medicinal plants have wide spectrum of antimicrobial activity and most of the antibiotics used are of plant origin. The present study focuses on the in vitro antioxidant, antiglycation and antimicrobial activity of selected medicinal plants form Myanmar which are traditionally well known for the treatment of diabetes. The results found that the ethanolic extract of traditionally used medicinal plants could be effective not only for decreasing the oxidative stress and glycation end-products formation in glycation related diseases but also for treating the infectious diseases.
showed high antioxidant activities with the percent inhibition of 75.80 ± 1.02 and 98.45 ± 1.12 for DPPH and NO radical scavenging assays. The extract AGE223 (Tamarindus indica Linn. bark) also showed the high antioxidant activity for SO radical scavenging assay with the percent inhibition of 89.35 ± 5.72. These two extracts contained the highest phenolic content with 233.85 ± 0.04 and 214.47 ± 0.01 mgGAE/g of extract, respectively. AGE221 (Terminalia chebula Retz. branch) was the best AGE inhibitor with the percent inhibition of 79.06 ± 1.90. Most of the selected extracts showed antimicrobial activity. Among them, the antimicrobial activity of AGE232 (Phyllanthus distichus Muell. fruit) was comparable to the standard antibiotic, Chloramphenicol.

**Conclusion:** The results confirmed that the ethanolic extracts of traditionally used medicinal plants could be effective not only for decreasing the oxidative stress and glycation end-products formation in glycation related diseases but also for treating the infectious diseases.

**Subjects:** Biochemistry; Microbiology; Pharmaceutical Science

**Keywords:** 2; 2-Diphenyl-1-picrylhydrazyl; Nitric oxide; Superoxide; Total phenolic content; Anti-infective agents

1. **Introduction**

Glycation is a natural, non-enzymatic reaction between amino groups of proteins and carbonyl groups of reducing sugars which results in the generation of advanced glycation end-products (AGEs). AGEs are mainly involved in the pathogenesis of numerous diseases depending on their mode of interaction with target cells. Protein glycation and formation of AGEs is the key molecular basis of rheumatoid arthritis, osteoporosis, aging and chronic diabetic complications such as nephropathy, retinopathy, neuropathy, and cardiomyopathy (Singh, Bali, Singh, & Jaggi, 2014; Yap, Tan, Ng, Tan, & Fung, 2018).

Diabetes mellitus is associated with the oxidative stress which is developed by the imbalance of free radicals in the antioxidant systems of the body mechanisms. For instance, increased production of free radical could lead to hyperglycemia. The free radicals such as the reactive oxygen and nitrogen species have been known for their implications in the process of aging and many serious diseases such as diabetes mellitus, neurodegenerative disorders, cardiovascular diseases, respiratory diseases, cataract development, rheumatoid arthritis and various cancers. These free radicals, at high concentration, generate oxidative stress and nitrosative stress which can cause the damage to three important biological molecules including nucleic acids, proteins, and lipids (Phaniendra & Babu, 2015).

Inhibiting the process of non-enzymatic protein glycation and enhancing the antioxidant system is one of the strategic approaches to prevent glycation mediated diabetic complications. Although many AGE inhibitors have been identified for the treatment of glycation in chronic hyperglycemia, some showed strong activity but not safe for clinical use because of their undesirable side effects while some are safe but not efficient enough to inhibit the process of glycation. Therefore, safe drugs with strong activity are still needed to be explored for the treatment of glycation-mediated diabetic disorders (Rasheed, Sánchez, Yousuf, Honoré, & Iqbal Choudhary, 2018).

A number of plant species and plant products have been explored for their antiglycation and antioxidant activities. Some biologically important phytochemicals, particularly flavonoids, polyphenols, terpenoids, alkaloids and iridoids have been studied extensively for their antioxidant and anti-glycation activities (Amin, 2016). Medicinal plants possessing both antiglycation and antioxidant activities may have high protective and curative potential for diabetic mellitus (Perera, 2013).
Moreover, infectious diseases caused by multidrug-resistant bacteria become a major health problem challenging the commonly prescribed drugs (Srivastava, Chandra, Nautiyal, & Kalra, 2014) and hence the development of the highly effective drugs to fight these multidrug-resistant bacteria is also an important research field. Since most of the currently used antibiotics are of plant origin, there will still be many medicinal plants to be explored for their antimicrobial activity for these serious infectious diseases.

Myanmar is rich in medicinal plants according to its different climate zones and has a long tradition of using plant remedies for various medicinal purposes. However, there is little knowledge of scientific evidence of medicinal plants for their activities. Hence, this study aims to investigate the antioxidant, antiglycation and antimicrobial activity of selected medicinal plants form Myanmar which are traditionally well known for the treatment of diabetes.

2. Experimental

2.1. Plant materials

The plant samples were collected from Kyaukse district, Mandalay region, Myanmar. The collected samples were washed using distilled water, air dried and grounded into fine powder. Maceration method was used to extract the plant samples by soaking the samples in 95% ethanol for one month. They were then filtered and the solvent was removed by using a rotary evaporator. The solvent was evaporated to dryness, and the residues were put into screw-capped vials and stored in the refrigerator for further experiments. The botanical identification of plant species was done using Table 1. Selected Myanmar medicinal plants

| Extract code | Botanical name | Family name | Parts used |
|--------------|----------------|-------------|------------|
| AGE221       | Terminalia chebula Retz. | Combretaceae | Branch     |
| AGE222       | Punica granatum Linn. | Lythraceae  | Fruit      |
| AGE223       | Tamarindus indica Linn. | Caesalpiniaceae | Bark  |
| AGE224       | Tamarindus indica Linn. | Caesalpiniaceae | Pulp  |
| AGE225       | Annona squamosa Linn. | Annonaceae  | Fruit      |
| AGE226       | Aegle marmelos Corr. | Rutaceae    | Fruit      |
| AGE227       | Syzygium cumini (L.) Skeels. | Myrtaceae | Pulp      |
| AGE228       | Syzygium cumini (L.) Skeels. | Myrtaceae | Seed      |
| AGE229       | Alpinia officinarum Hance. | Zingiberaceae | Rhizome |
| AGE230       | Sesbania grandiflora (L.) Poir. | Fabaceae | Flower     |
| AGE231       | Citrus colocynthia Schred. | Cucurbitaceae | Leaves   |
| AGE232       | Phyllanthus distichus Muell. | Euphorbiaceae | Fruit |
| AGE233       | Caesalpinia bonducello Flem. | Caesalpiniaceae | Seed   |
| AGE234       | Persea gratissima C.F. Gaertn. | Lauraceae | Seed      |
| AGE235       | Persea gratissima C.F. Gaertn. | Lauraceae | Pulp      |
| AGE236       | Morinda angustifolia Roxb. | Rubiaceae | Fruit      |
| AGE237       | Tectona hamiltoniana Wall. | Verbenaceae | Bark     |
| AGE238       | Limonia acidissima Linn. | Rutaceae | Stern      |
| AGE239       | Gymura procumbens (Lour.) Merr. | Asteraceae | Leaves   |
| AGE240       | Jasminum grandiflourum Linn. | Oleaceae | Flower     |
| AGE241       | Crataeva religiosa Forst. | Capparidaeae | Bark    |
| AGE242       | Cassia auriculata Linn. | Caesalpiniaceae | Powder from local shop |
| AGE243       | Syzygium cumini (L.) Skeels | Myrtaceae | Flowers |
by the authorized botanist from Mandalay University, Mandalay, Myanmar. The selected Myanmar medicinal plants are shown in Table 1.

2.2. Chemicals
Analytical grade chemicals were used for all the experiments. 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH) was purchased from TCI Development Co.ltd (Shanghai, China). Folin & Ciocalteu’s phenol reagent, nitroblue tetrazolium chloride (NBT), bovine serum albumin, riboflavin and resazurin sodium salt were purchased from Himedia (Mumbai, India). Rutin hydrate and trypsin-EDTA solution were purchased from Sigma Chemicals Co. Ltd (St. Louis, USA).

2.3. In vitro DPPH free radical scavenging assay
The free radical scavenging activity of the extracts was determined with the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay as described by Lee et al. (Lee et al., 1998). The detailed experimental protocol was as mentioned by Moe et al. (Moe et al., 2018). The reaction mixture containing 5 µL of test sample (0.5 mg/mL) in dimethyl sulfoxide (DMSO) and 95 µL of DPPH (300 µmol/L) in ethanol, was placed in individual wells of a 96-well microtiter plate. After keeping the microplate in the darkness at 37°C for 30 min, the optical density (OD) of each well was read using a SPECTROstar Nano microplate reader (BMG, Labtech, Germany) at the wavelength 515 nm. DMSO was used as the blank, and ascorbic acid was used as the standard.

Inhibition rate was calculated using the following formula:

\[
\text{Inhibition rate} \% = \left(1 - \frac{\text{OD tested}}{\text{OD control}}\right) \times 100
\]

2.4. In vitro nitric oxide radical scavenging assay
The free radical scavenging activity of the extracts was determined with the nitric oxide (NO) radical scavenging assay as described by Michael et al. (Hertog, Hollman, & van de Putte, 1993). The detailed experimental protocol was as mentioned by Moe et al. (Moe et al., 2018). The reaction mixture, containing 10 µL of test sample (0.5 mg/mL) in DMSO, 20 µL of phosphate buffer saline (0.1 mol/L, pH 7.4) and 70 µL of sodium nitroprusside (10 mmol/L), was incubated at 25°C for 90–100 min to allow formation of nitrite ions. After incubation, 50 µL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added and allowed to stand for 5 min for completion of diazotization. Then, 50 µL of N-(1-naphthyl) ethylenediamine dihydrochloride (0.1% w/v) was added, stirred and allowed to stand for 20 min. A pink-colored chromophore was formed under the diffused light. The reduction of the pink-colored chromophore was measured at 540 nm against the corresponding blank solution. Gallic acid and ascorbic acid were used as the standards.

Inhibition rate was calculated using the following formula:

\[
\text{Inhibition rate} \% = \left|1 - \frac{\text{OD tested}}{\text{OD control}}\right| \times 100
\]

2.5. In vitro superoxide radical scavenging assay
The free radical scavenging activity of the extracts was determined with the superoxide (SO) radical scavenging assay modified from the protocol described by Patel Rajesh et al. (Rajesh & Natvar, 2011). The detailed experimental protocol was as mentioned by Moe et al. (Moe et al., 2018). The reaction mixture, containing 10 µL of test sample (0.5 mg/mL) in DMSO, 160 µL of potassium phosphate buffer (0.067 mol/L, pH 7.4), 15 µL of EDTA (4.5 mmol/L), 10 µL of NBT (1 mg/mL) and 5 µL of riboflavin (0.2 mg/mL), was incubated for 5 min under fluorescence light. The OD of the solution was measured at 560 nm against the corresponding blank solution. Gallic acid was used as the standard.

Inhibition rate was calculated using the following formula:
Inhibition rate (%) = \[ \left(1 - \frac{OD_{tested}}{OD_{control}}\right) \times 100 \]

2.6. Total phenolic content measurements

The total amount of phenolic compounds in the plant extracts was measured with the method described by Spanos and Wrolstad (Spanos & Wrolstad, 1990), with a few modifications. The detailed experimental protocol was as mentioned by Moe et al. (Moe et al., 2018). 100 µL of the extracts (1 mg/mL) was oxidized with 500 µL of freshly diluted 10% Folin–Ciocalteu’s reagent. Then, the reaction was neutralized by adding 500 µL of 7.5% (w/v) sodium carbonate, and samples were vortexed for 20 s. Next, the samples were incubated at 37°C for 1 h and the OD was measured with a digital photo colorimeter (Apel PD 303, Japan) at a wavelength of 765 nm. For each sample, three replicate assays were performed.

The total phenolic content (TPC) was calculated as Gallic acid equivalent (GAE) with the following equation:

\[ T = \frac{C \times V}{M} \]

\( T \) is the TPC in mg/g of the extracts as GAE; \( C \) is the concentration of gallic acid, established from the calibration curve in mg/mL; \( V \) is the volume of the extract solution in mL and \( M \) is the weight of the extract in g.

2.7. In vitro antiglycation assay

The in vitro antiglycation activity of the extracts was determined by measuring the ability of the extracts to inhibit the formation of AGEs, as described by Choudhary et al. (Choudhary et al., 2011). The detailed experimental protocol was as mentioned by et al. (Moe et al., 2018). In a 96-well black fluorescence plate, the reaction mixture, containing 10 µL of BSA (10 mg/mL), 70 µL of sodium phosphate buffer (0.1 mol/L, pH 7.4), 100 µL of fructose (500 mmol/L) and 20 µL of test sample (0.5 mg/mL) in DMSO, was incubated at 37°C for 7 days in the darkness. All the chemicals were dissolved in sodium phosphate buffer to get the desired concentration. To avoid microbial contamination, sodium azide (0.1 mmol/L) was added to the sodium phosphate buffer. After incubation, inhibition of AGE formation was measured at the fluorescence intensity of excitation (340 nm) and emission (440 nm) by using an Agilent Cary Eclipse Fluorescence spectrophotometer (G9800, US). Rutin (1 mmol/L) was used as a standard. The reaction mixture, without fructose, was used as the negative control and reaction mixture without extracts was used as a positive control.

Inhibition rate was calculated using the following formula:

\[ \text{Inhibition rate(%) = } 1 - \left(\frac{\text{OD tested}}{\text{OD control}}\right) \times 100 \]

2.8. Evaluation of antimicrobial activity by agar well-diffusion method

Two Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa), three Gram-positive bacteria (Staphylococcus aureus, Enterococcus faecalis and Bacillus cereus) and one strain of fungus (Candida albicans) were used as test microorganisms for this experiment. The agar well-diffusion method was used for evaluating antimicrobial activity, and followed a method modified from Schlegel et al. (Schlegel & Zaborosch, 1993). The detailed experimental protocol was as mentioned by Moe et al. (Moe et al., 2018). Muller Hinton Broth (Himedia, India) was inoculated with test micro-organisms, and incubated at 37°C overnight. The overnight broth culture was then diluted with normal saline to obtain the OD\(_{600\text{nm}}\) of 0.08-0.1. Muller Hinton agar plates were prepared and sterilized in an autoclave at 121°C for 15 min. The broth inculombs were evenly spread on the Muller Hinton agar plates with sterile cotton swabs to obtain a lawn of growth. After a plate was inoculated, 8-mm diameter wells were made on the agar medium with a sterile cork borer. The wells were then filled with 50 µL of extracts with a concentration of 25 mg per 50 µL. Ethanol (70%) was used to prepare the extracts and as a solvent control. Chloramphenical (30 µg/well) was used as positive control. The plates were
then incubated at 37°C for 24 h, and the diameter of the zone of growth inhibition was recorded and compared with the positive control.

2.9. Statistical analysis

All data were expressed as mean ± standard error mean of triplicate measurements. One-way analysis of variance (ANOVA) and Dunnett’s multiple comparison tests were performed to compare the differences between the plant extracts and standard control in antioxidant and antiglycation assays while Tukey’s multiple comparison test was done to compare the differences of the tested plant extracts from each other. In each analysis, \( P < 0.05 \) was considered to be statistically significant. Correlation and linear regression analyses were also performed to evaluate the

### Table 2. % Inhibition of various biological activities and total phenolic content of selected Myanmar medicinal plant extracts

| Extract code | In vitro DPPH free radical-scavenging activity (inhibition rate) | In vitro nitric oxide (NO) radical-scavenging activity | In vitro super oxide (SO) radical-scavenging activity | In vitro antiglycation activity | Total phenolic content (TPC) (mgGAE/g of extract) |
|--------------|---------------------------------------------------------------|------------------------------------------------------|---------------------------------------------------|-----------------------------|----------------------------------|
| AGE221       | 66.51 ± 2.28*                                                | 82.80 ± 3.12                                        | 76.98 ± 2.90                                     | 79.06 ± 1.90                | 78.60 ± 0.01*                     |
| AGE222       | 61.58 ± 1.78*                                                | 86.49 ± 3.73                                        | 60.53 ± 8.39                                     | 76.54 ± 2.18                | 35.85 ± 0.00*                     |
| AGE223       | 52.79 ± 4.99*                                                | 80.28 ± 7.92                                        | 89.35 ± 5.72                                     | 75.72 ± 2.49                | 233.85 ± 0.04*                    |
| AGE224       | 17.09 ± 0.99*                                                | 48.39 ± 2.27                                        | 16.62 ± 5.46                                     | 51.50 ± 3.89                | 11.23 ± 0.02*                      |
| AGE225       | 61.24 ± 1.89*                                                | 61.18 ± 5.01*                                       | 44.16 ± 5.97*                                    | 64.27 ± 4.05*               | 18.78 ± 0.00*                      |
| AGE226       | 48.62 ± 1.61*                                                | 62.96 ± 1.84*                                       | 49.19 ± 5.13*                                    | 28.78 ± 5.34*               | 27.63 ± 0.02*                      |
| AGE227       | 38.53 ± 0.11*                                                | 49.73 ± 5.64*                                       | 34.28 ± 6.29*                                    | 61.08 ± 3.04*               | 10.09 ± 0.02*                      |
| AGE228       | 75.80 ± 1.02                                                 | 83.84 ± 1.07                                        | 77.54 ± 8.14                                     | 59.72 ± 7.34*               | 214.47 ± 0.01                      |
| AGE229       | 60.55 ± 3.31*                                                | 63.44 ± 4.84*                                       | 71.39 ± 7.31                                     | 45.89 ± 5.58*               | 51.36 ± 0.00*                      |
| AGE230       | 30.28 ± 1.50*                                                | 59.17 ± 5.66*                                       | 42.61 ± 7.05*                                    | 44.26 ± 6.55*               | 17.29 ± 0.01*                      |
| AGE231       | 15.94 ± 3.40*                                                | 35.44 ± 6.30*                                       | 36.96 ± 3.37*                                    | 55.23 ± 5.28*               | 17.96 ± 0.00*                      |
| AGE232       | 44.95 ± 3.61*                                                | 58.50 ± 3.25*                                       | 21.86 ± 3.74*                                    | 30.81 ± 4.76*               | 28.69 ± 0.00*                      |
| AGE233       | 28.56 ± 2.11*                                                | 47.28 ± 5.88*                                       | 36.40 ± 6.04*                                    | 52.11 ± 3.18*               | 18.09 ± 0.00*                      |
| AGE234       | 32.00 ± 3.33*                                                | 88.52 ± 0.53                                        | 59.32 ± 5.39*                                    | 60.32 ± 2.96*               | 57.98 ± 0.00*                      |
| AGE235       | 13.88 ± 0.98*                                                | 16.56 ± 7.06*                                       | 8.67 ± 3.24*                                     | 35.52 ± 4.69*               | 8.03 ± 0.01*                       |
| AGE236       | 25.34 ± 0.60*                                                | 59.07 ± 3.99*                                       | 28.88 ± 3.87*                                    | 53.38 ± 3.92*               | 6.03 ± 0.00*                       |
| AGE237       | 59.86 ± 1.13*                                                | 64.70 ± 2.64                                        | 56.42 ± 5.78*                                    | 69.38 ± 2.76                | ND                                |
| AGE238       | 43.12 ± 0.92*                                                | 39.14 ± 7.27*                                       | 65.53 ± 4.58                                     | 11.99 ± 0.00*               | 10.83 ± 0.00*                      |
| AGE239       | 45.64 ± 2.10*                                                | 44.74 ± 3.81*                                       | 25.27 ± 4.59*                                    | 49.79 ± 3.76*               | 29.49 ± 0.01*                      |
| AGE240       | 57.22 ± 5.42*                                                | 31.07 ± 3.93*                                       | 40.04 ± 6.79*                                    | 7.84 ± 3.67*                | 48.60 ± 0.00*                      |
| AGE241       | ND                                                            | 88.92 ± 5.08                                        | 40.78 ± 7.96*                                    | 25.08 ± 3.11*               | ND                                |
| AGE242       | ND                                                            | 64.19 ± 9.71                                        | 67.31 ± 5.28                                     | 51.29 ± 2.07*               | ND                                |
| AGE243       | ND                                                            | 98.45 ± 1.12                                        | 71.60 ± 4.64                                     | 58.54 ± 4.22*               | ND                                |
| Ascorbic acid | 84.78 ± 0.47                                                 | 81.69 ± 1.13                                        | -                                                 | -                            | -                                 |
| Gallic acid  | -                                                             | 76.59 ± 0.38                                        | 83.24 ± 1.22                                     | -                            | -                                 |
| Rutin        | -                                                             | -                                                    | 87.68 ± 1.75                                     | -                            | -                                 |

Results were shown as the average of triplicates of experiments ± standard error of mean.*\( P < 0.05 \), vs standard in each group. Different letters (a to s) indicated descending order of the total phenolic contents of the tested extracts which are significantly different (\( P < 0.05 \)) from each other. DPPH: 2,2-diphenyl-1-picrylhydrazyl; GAE: gallic acid equivalent; ND: not detected.
correlations between antioxidant activity, antiglycation activity and TPC of extracts. Statistical analyses were performed using the Microsoft office 2010 and GraphPad Prism version 7.0.

3. Results

3.1. In vitro DPPH free radical scavenging assay
The antioxidant results of the DPPH free radical scavenging assay are shown in Table 2. According to the results, the extract **AGE228** (*Syzygium cumini* (L.) Skeels. seed extract) showed the best antioxidant activity with the percent inhibition of 75.80 ± 1.02 in scavenging the DPPH free radicals while the standard antioxidant compound, ascorbic acid, showed the percent inhibition of 84.78 ± 0.47. High antioxidant activity of above 50% inhibition to the DPPH free radicals was found in seven tested plant extracts (**AGE221**, **AGE222**, **AGE223**, **AGE225**, **AGE229**, **AGE237** and **AGE240**). Low antioxidant activity of below 50% inhibition was found in the remaining plant extracts. The DPPH free radical scavenging activity of **AGE228** was comparable to the activity of the standard ascorbic acid while all other plant extracts were significantly different (**P** < 0.05) from the standard.

3.2. In vitro nitric oxide (NO) radical scavenging assay
The antioxidant results of the NO radical scavenging assay are shown in Table 2. The extract **AGE243** (*Syzygium cumini* (L.) Skeels. flower extract) was the best plant extract with the percent inhibition of 98.45 ± 1.12 to the NO radicals and was found to be better than the standard ascorbic acid and gallic acid which showed the percent inhibition of 81.69 ± 1.13 and 76.59 ± 0.38. Strong radical scavenging activity of above 80% inhibition was found in six plant extracts (**AGE221**, **AGE222**, **AGE223**, **AGE228**, **AGE234** and **AGE241**). High antioxidant activity of above 50% inhibition was found in eight plant extracts and low activity of below 50% inhibition was found in the remaining plant extracts. NO free radical scavenging activities of 10 plant extracts (**AGE221**, **AGE222**, **AGE223**, **AGE228**, **AGE229**, **AGE234**, **AGE237**, **AGE241**, **AGE242** and **AGE243**) were comparable to the standards while other extracts were significantly different (**P** < 0.05) from the standard.

3.3. In vitro superoxide (SO) radical scavenging assay
The antioxidant results of the SO radical scavenging assay are shown in Table 2. The extract **AGE223** (*Tamarindus indica* Linn. bark extract) showed the best SO radical scavenging activity with the percent inhibition of 89.35 ± 5.72, which was better than that of the standard gallic acid with the percent inhibition of 83.24 ± 1.22. High radical scavenging activity of above 70% inhibition was found in four plant extracts (**AGE221**, **AGE228**, **AGE229** and **AGE243**). Among the rest tested plants, above 50% inhibition was found in five plant extracts and below 50% inhibition was found in ten plant extracts. Eight plant extracts (**AGE221**, **AGE222**, **AGE223**, **AGE228**, **AGE229**, **AGE238**, **AGE242** and **AGE243**) showed comparable superoxide radical scavenging activity with the standard gallic acid while other extracts were significantly different (**P** < 0.05) from the standard.
3.4. Total phenolic content (TPC) measurements

Total phenolic contents of tested medicinal plants are shown in Table 2. Standard calibration curve of gallic acid was prepared at the concentrations of 0, 1.7, 5, 15, 44, 131, 392 and 1176 mg/L (Figure 1) and total phenolic contents of the extracts were expressed as gallic acid equivalents. AGE223 (Tamarindus indica Linn. bark extract) and AGE228 (Syzygium cumini (L.) Skeels. seed extract) contained high phenolic content with 233.85 ± 0.04 and 214.47 ± 0.01 mgGAE/g of extract, respectively. AGE221, AGE229 and AGE234 showed considerable amount of total phenolic compounds with the concentration of above 50 mgGAE/g of extract while other extracts had low total phenolic content. When Turkey’s multiple comparison test was performed, the total phenolic contents of the tested plant extracts were found to be significantly different (P< 0.05) from each other.

3.5. In vitro antiglycation assay

The antiglycation activities of tested medicinal plants are shown in Table 2. AGE221 (Terminalia chebula Retz. Branch extract), AGE222 (Punica granatum Linn. fruit extract) and AGE223 (Tamarindus indica Linn. bark extract) were the best plant extracts which strongly inhibited the advanced glycation end-products with the percent inhibition of 79.06 ± 1.90, 76.54 ± 2.18 and 75.72 ± 2.49, respectively. The antiglycation activities of these plant extracts were comparable to the standard rutin with the percent inhibition of 87.68 ± 1.75. Eleven plant extracts were found to be active for antiglycation activity with the percent inhibition above 50 although other nine plant extracts did not show the remarkable activity with the percent inhibition below 50. The
antiglycation activity of AGE221, AGE222, AGE223 and AGE237 were comparable to the standard rutin while other plant extracts were significantly different ($P < 0.05$) from the standard.

3.6. Correlations between antioxidant and antiglycation activity with total phenolic content of tested extracts

The correlations of antioxidant and antiglycation activity with total phenolic content of tested medicinal plants were also studied and shown in Figure 2. The overall results revealed that the total phenolic content showed good correlation with NO radical scavenging activity ($r = 0.6$) and SO radical scavenging activity ($r = 0.7$), fair correlation with DPPH radical scavenging activity ($r = 0.5$) and poor correlation with antiglycation activity ($r = 0.4$).

3.7. Evaluation of antimicrobial activity

The antimicrobial activities of tested medicinal plants are shown in Table 3. In case of antibiotics, in general, 15 mm inhibition zone diameter is considered as the intermediate while below 15 mm as bacterial resistance and above 15 mm as sensitive to these antibiotics. In the antimicrobial test with the plant extracts, the greater the inhibition zones to the test bacteria, the better the antimicrobial activity of the extracts. Hence, inhibition zone diameter below 15 mm was considered as low activity and above 15 mm as high activity. In this study, AGE221, AGE222, AGE228 and AGE232 were found to be active against S. aureus. AGE224,
AGE224 and AGE232 showed the high antimicrobial activity against B. cereus. AGE224 and AGE232 were the only two plant extracts which showed the growth inhibition zone against E. coli. AGE229 and AGE232 exhibited the strong activity against E. faecalis. Only five plant extracts were tested for antimicrobial activity against P. aeruginosa. Among them, AGE222, AGE224 and AGE225 showed large growth inhibition zone of 20 mm, 17 mm and 20 mm in diameter, respectively. AGE221, AGE224, AGE225, AGE228, AGE232 and AGE240 showed the high antimicrobial activity against C. albicans. Among all tested plant extracts, AGE232 (Phyllanthus distichus Muell. fruit extract) was found as the most active extract with strong microbial growth inhibition activity on all tested microorganisms: S. aureus, B. cereus, E. coli, E. faecalis and C. albicans, having inhibition zone diameters of 17 mm, 20 mm, 21 mm, 23 mm and 18 mm, respectively. The antimicrobial activity of AGE232 was comparable to the activity of standard antibiotic, Chloramphenicol, which showed the inhibition zone diameters of 27 mm, 24 mm, 24 mm, 24 mm and 30 mm against S. aureus, B. cereus, E. coli, E. faecalis and C. albicans, respectively. 70% ethanol, the solvent used for the preparation of the plant extracts to get the desired concentration, showed no inhibition zone diameter against all tested bacteria.

4. Discussion

Naturally occurring antioxidant and antiglycation compounds originated from medicinal plants becomes an interesting research field in recent years as they show potential in reducing or preventing the risk of chronic complications for diabetic patients. In this study, 23 ethanolic extracts from different parts of 19 traditionally used medicinal plants in Myanmar were examined for their bioactivities. Three free radical scavenging assays were used to evaluate the antioxidant activity of the selected plant extracts. Then, their antioxidant activity was further established by measuring the total phenolic content by Folin–Ciocalteu method. Antiglycation activity was studied with non-enzymatic BSA-fructose assay to identify the AGE inhibitory activity of the extracts. The potential of antimicrobial activity of plant extracts was studied against six pathogenic bacteria by using agar well-diffusion method.

Out of 23 plant extracts, AGE228 (Syzygium cumini (L.) Skeels. seed), AGE223 (Tamarindus indica Linn. bark) and AGE221 (Terminalia chebula Retz. branch) were the most promising plant extracts for the antioxidant and antiglycation assays while AGE232 (Phyllanthus distichus Muell. fruit) was the most potent plant extract which showed the broad spectrum antimicrobial activity against the tested Gram-positive and -negative bacteria.

Syzygium cumini (L.) Skeels. was reported to be rich in alkaloids, glycosides, flavonoids, total phenolics, anthocyanins, ellagic acid, isoquercetin, kaemferol and myrecetin which are well known as the antioxidant compounds in scavenging of free radicals and having a protective effect on antioxidant enzymes (Ayyanar & Subash-Babu, 2012). In this study, when the ethanolic extracts of fruit pulps, seeds and flowers were compared for their antioxidant activities, the seed and flower extracts of Syzygium cumini (L.) Skeels. showed considerably higher antioxidant activity than the fruit pulp extract. This could be due to the higher content of the total phenols found in the seed which is in agreement with the previous study by Vats et. al. (Vats, Sehwag, & Das Seed, 2014).

Terminalia chebula Retz. contains gallic acid, ellagic acid, tannic acid, ethyl gallate, chebulic acid, chebulagic acid, corilagin, mannitol, ascorbic acid (vitamin C), and other compounds (Chang & Lin, 2012). In the present study, the branch extract showed the high antioxidant and antiglycation activity and thus this might be due to the high content of antioxidant compounds distributing the whole plant.

Nagarajan et al. described that Tamarindus indica Linn. bark contains several phytoconstituents like procyanidin, lupeol, saponins, sesquiterpenes, alkaloids, and phlobatannins. The major compounds are catechin and proanthocyanidins which are known as the gallic acid derivatives and having the antioxidant activity (Nagarajan et al., 2014). In our study, therefore, Tamarindus indica Linn. bark showed the highest activity in SO radical scavenging assay, and it was also found the high activity in other antioxidant assays and antiglycation assay.
**Phyllanthus distichus** Muell. fruits are used as astringent, laxative, and to heal ulcer, and have the antibacterial and antihyperlipidemic activity (D Andrianto et al., 2017). They contain ascorbic acid, tartaric acid, tannin, adenosine, kaempferol, and hypogallic acid (Sousa et al., 2007). In the present experiment, the crude ethanolic extract of the fruits was found to be the best among all tested extracts for antimicrobial screening.

Among the remaining tested extracts, **AGE222** (Punica granatum Linn. fruit extract) and **AGE229** (Alpinia officinarum Hance. rhizome extract) were moderately active in all antioxidant assays and **AGE222** was found one of the best extracts in antiglycation assay. Next, **AGE234** (Persea gratissima Gaertn.f. seed extract) and **AGE241** (Crataeva religiosa Forst. bark extract) showed high activity in NO radical scavenging assay. The remaining 14 plant extracts did not show significant antioxidant or antiglycation activity.

When the correlation analysis was carried out to determine the relationship between total phenolic content of the plants and their antioxidant and antiglycation activities, total phenolic content of the plant extracts had the good positive relationship with their antioxidant activity but small positive relationship with their antiglycation activity in general.

For all the antioxidant assays and antiglycation assay, the plant extracts and standard ascorbic acid/gallic acid were used the same final concentration at 500 μg/ml so that the activities of the plant extracts and the standards were able to be compared. According to the test results, although the tested plant extracts were crude ethanolic extracts, they showed the strong activities which were comparable to the standard antioxidant compounds. The result confirmed that the greater the percentage of inhibition to the free radicals, the better the antioxidant activity of the plant extract (Maw, Mon, & Oo, 2011; Meenatchi, Purushothaman, & Maneemegalai, 2017).

In case of agar well-diffusion assay, although **AGE232** (Phyllanthus distichus Muell. fruit extract) showed promising antimicrobial activity, other selected plant extracts exhibited low or moderate activity while they had no inhibition on the growth of *E. coli*. **AGE223** (Tamarindus indica Linn. bark) showed no inhibitory activity against all six tested pathogens. All the tested bacteria were sensitive to the standard antibiotic Chloramphenicol while 70% ethanol which was used as the solvent to dissolve the plant extracts showed no effect on the growth of bacteria.

**5. Conclusions**
We can conclude that the ethanolic extract of traditionally used medicinal plants could be effective not only for decreasing the oxidative stress and glycation end-products formation in glycation related diseases but also for treating the infectious diseases. Further experiments such as determination of effective dose, potential toxicity and side effects of the promising plant extracts should be carried out in more detail. The active constituents from the potential medicinal plants should be further isolated and studied for their biological activities so that the active compounds which can be used at the low concentration with high activity and low toxicity could be identified.

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**Competing interests**
We declare that we have no conflict of interest.

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