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Research Article

Phytochemicals Screening and Evaluation of Antioxidants and Antibacterial Activities of Five Medicinal Plants

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ABSTRACT

Extracts of medicinal plants *Syzygium aromaticum, Mentha piperita, Cinnamomum verum, Pimpinella anisum* and *Zingiber purpurea* were investigated for their biological activity. The presences of phytochemicals, antibacterial and antioxidant activities were investigated. Parts of medicinal plants were extracted with acetone 50%. The extracts were evaluated for antibacterial activity using the disc diffusion method, while antioxidant activities were measured using ferric reducing/antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and phytochemical screening was performed using a standard method. *Syzygium aromaticum* fruit extract exhibited the most activity in the test antioxidants and antibacterial activity when compared with other medicinal plants. Phytochemical analysis revealed that alkaloids, flavonoids, triterpenoids, tannin, and carbohydrates were present in the extracts of *Syzygium aromaticum, Mentha piperita, Cinnamomum verum, Pimpinella anisum* L. and *Zingiber purpurea* while saponin and steroids was only present in the *Syzygium aromaticum* and *Zingiber purpurea* extracts. The studied medicinal plants have interesting antioxidant properties, antibacterial activity and a phytochemicals composition that could provide scientific evidence for some folk uses in the treatment of diseases and therefore it can provide natural source of antibacterial drugs and antioxidants and can be useful in preventing various diseases including cancer.

Key words: Medicinal Plants, Phytochemicals Screening, Antioxidants Activities, Antibacterial

INTRODUCTION

More and more chronic diseases are crippling the ageing population: cancers, arthritis and arthrosis, cardiovascular and neurodegenerative diseases bring more people to hospitals and retirement boarding houses[1]. Natural products represent a rich source of biologically active compounds and are an example of molecular diversity, with recognized potential in drug discovery and development[2]. Particularly, the plant kingdom offers a wide range of natural antioxidants. However, little is known about the practical usefulness of most of them. Many herbal and plant infusions frequently used in folk medicine have antioxidative and pharmacological properties connected with the presence of phenolic compounds, especially flavonoids[3]. The biological, pharmacological and medicinal properties of this group of compounds have been extensively reviewed[4]. Antioxidant is substance that can inhibit oxidation process by pressing the cell damage caused by free radical oxidation. Free radical is unstable because it has unpaired electron and usually look for pairs of electrons in biological macromolecules[5]. Presence of free radicals in the body can lead to various degenerative diseases such as cancer, diabetes and heart[6-7]. Using of the natural antioxidants in free radicals scavenging have also some advantages as the examples are being obtained easily, economically and have slight or no side effects[8]. Epidemiological studies have shown that high fruit and vegetable consumption has health benefits in the prevention of chronic dis-eases[9]. These foods are reported to contain a wide variety of antioxidant components, including phenolic compound[10]. The present study evaluates the efficacy of five medicinal plants used in traditional medicine for the treatment of chronic diseases. The plant extracts were screened for antioxidant. A preliminary phytochemical screening was done to determine the presence of different types of secondary metabolites.

MATERIALS AND METHODS

Chemicals Folin-Ciocalteu phenol reagent, ferric chloride ($FeCl_3 \cdot 6H_2O$), and HCl were obtained from Merck, (Darmstadt, Germany), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), gallic acid, 2,2'-azo-bis(2-amidinopropane) dihydrochloride, (AAPH), Trolox (6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid) and sodium acetate trihydrate, were purchased from Sigma (St Louis, MO, USA). Sodium carbonate was purchased from RDH (Seelze, Germany) and glacial acetic acid from Mallinckrodt Baker (Phillipsburg, USA). All chemicals and reagents used in this study were of analytical grade.

Preparation of Plant Extracts

Plant materials were extracted using the methods described previously [11]. Briefly, 0.1 g dried plant powder and 10 ml 50% aqueous acetone were stirred for 1 h in a 25-mL universal bottle at 1,000 rpm using a magnetic stirrer (IKA,

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Table 1: List of the investigated herbal samples

Samples No.	Common name	Scientific name	Family	Plant part used
1	Clove	Syzygium aromaticum	Myrtaceae	Fruit
2	Mint	Mentha piperita	Labiatae	Leaf
3	Cinnamon	Cinnamomum verum	Lauraceae	Bark
4	Anise	Pimpinella anisum L.	Apiaceae	Seed
5	Ginger	Zingiber purpurea	Zingiberaceae	Rhizome

Table 2: Antibacterial activity of the plant extracts and standard gentamicin discs

Test organism	Diameter of zone of inhibition (mm)					
	Clove	Mint	Cinnamon	Anise	Ginger	Gentamicin (10µg/disc)
Staphylococcus aureus	9	6	10	5	7	20
Bacillus cereus	12	9	13	8	10	18

Table 3: Phytochemicals analysis of plant extracts

Phytochemicals	Medicinal plants				
	Clove	Mint	Cinnamon	Anise	Ginger
Alkaloids	+	+	+	+	+
Saponins	+	-	-	-	+
Flavonoids	+	+	+	+	+
Steroids	+	-	-	-	+
Terpenoids	+	+	+	+	+
Tannins	+	+	+	+	+
Carbohydrates	+	+	+	+	+

Staufen, Germany). Samples were then centrifuged at 4,750 g for 10 min using a mini centrifuge (Thermo-line, China) and the supernatants were used for further analyses.

Total Phenol Content (TPC)

The determination of antioxidant activity through TPC was carried out according to the method of[11]. About 100 µL plant extract was added with 0.5 mL diluted Folin-Ciocalteu reagent. The samples extracts with Folin-Ciocalteu reagent) were left for 5 min before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength with spectrophotometer after 2 hours. Calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed as mg of gallic acid equivalents per 100 g of dry sample (mg GA/100 g of DW).

Ferric Reducing Antioxidant Power (FRAP)

The determination of antioxidant activity through FRAP was carried out according to the method of[11]. FRAP reagent was prepared fresh as using 300 mM acetate buffer, pH3.6 (3.1 g sodium acetate trihydrate, plus 16 mL glacial acid made up to 1:1 with distilled water); 10 mM TPTZ (2,4,6-tris (2-pyridyl)-*s*-triazine), in 40 mM HCLl; and 20 mM FeCl3•6H2O in the ratio of 10:1:1 to give the working reagent. About 1 ml FRAP reagent was added to 100 µL extracts and the absorbances were taken at 595 nm wavelength with spectrophotometer after 30 minutes. Calibration curve of Trolox was set up to estimate the activity capacity of samples. The result was expressed as mg of Trolox equivalents per 100 g of dry sample (mg TE/100 g of DW).

DPPH Radical Scavenging Activity

The determination of antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system was carried out according to the method of [11]. Stock solution was prepared by dissolving 40 mg DPPH in 100 ml methanol and kept at -20°C until used. About 350 mL stock solution was mixed with 350 ml methanol to obtain the absorbance of 0.70±0.01 unit at 516 nm wavelength by using spectrophotometer (Epoch, Biotek, USA). About 100 µL sample extract with 1 ml methanolic DPPH solution prepared were kept overnight for scavenging reaction in the dark. Percentage of DPPH scavenging activity was determined as follow: DPPH scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$. Where A is the absorbance.

Antibacterial Assay

Staphylococcus aureus and *Bacillus cereus* were used in experiment. Mueller Hinton agar was used in antibacterial assay. Plant extracts were dissolved in acetone to obtain a concentration of 40µg/10µL. Antibacterial assays were conducted using the disc diffusion method as previously described by [12]. Negative controls were prepared using the same solvent employed to dissolve the plant extract. Gentamicin discs (10 µg/ disc, Oxoid, UK) were used as control and positive controls. Zones of inhibition around the discs were measured in mm. The experiment was repeated in triplicate and the mean of diameter of the inhibition zones was calculated.

Phytochemical Analysis

One gram of the plant methanol extracts was dissolved in 100 mL methanol and subjected to preliminary phytochemical screening following standard methods[13-14].

Statistical Analysis

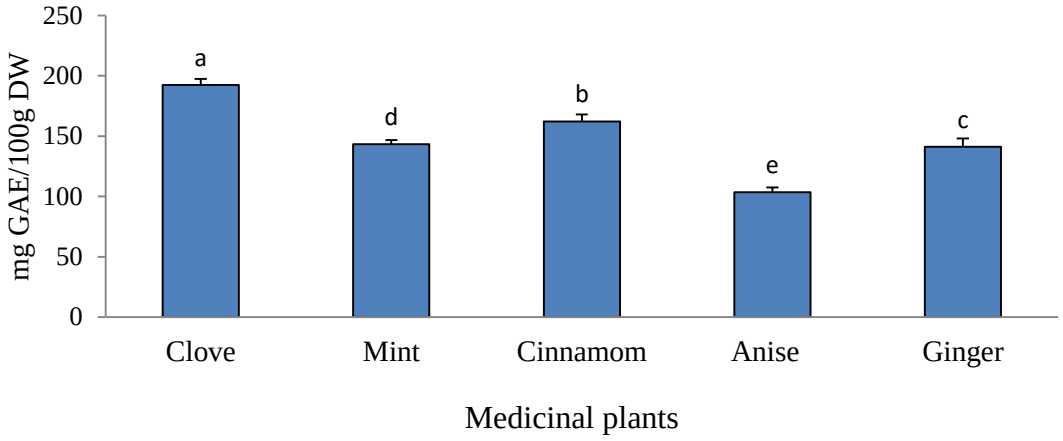

Figure 1: Total phenol content (TPC) of medicinal plants
[a-e] Mean with different letters are significantly different ($p<0.05$)

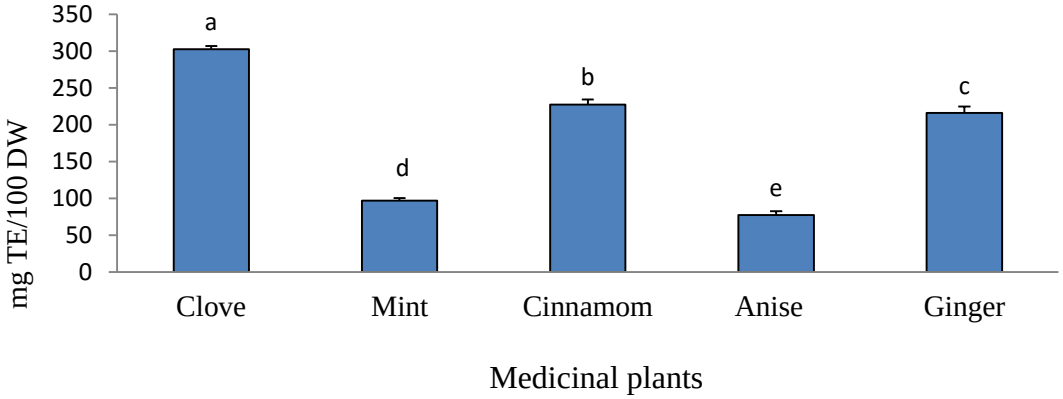

Figure 2: Ferric reducing antioxidant power (FRAP) of medicinal plants
[a-e] Mean with different letters are significantly different ($p<0.05$)

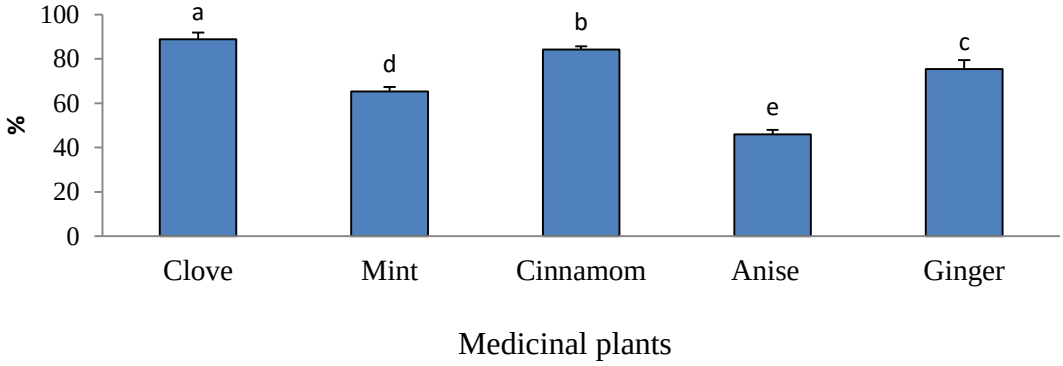

Figure 3: Radical-scavenging activity (DPPH) of medicinal plants
[a-e] Mean with different letters are significantly different ($p<0.05$)

Data were expressed as the means of three independent experiments. Statistical comparisons of the results were performed by one-way ANOVA using SPSS ver.19. Significant differences ($P<0.05$) among the medicinal plants were analyzed by Duncan 'triplicates range test.

RESULTS AND DISCUSSION

Antioxidants Activity

The TPC varied markedly among the tested plant extracts (Fig 1), ranging from 192.50 to 103.40 mg GAE /100 g DW, a variation of approximately 89-fold between the lowest and the highest TPC values. Two plants showed high phenolics contents (>160 mg GAE/100 g DW): *Syzygium aromaticum* and *Cinnamomum verum* (192.50and 162.10 mg GAE/100 g DW, respectively). Among the plants in this study, *Pimpinella anisum* showed the lowest TPC value (103.40 mg GAE/100 g DW). The antioxidant activity of the extracts determined using the FRAP assay are shown in Fig 2. The FRAP values ranged from 302.56 to 77.36 mg TE /100 g DW, representing a variation of approximately 225-fold between the lowest and the highest values. The trend for FRAP values of the five plant extracts was similar to that for their TPC values. The plants with high total phenolic contents, *Cinnamomum*

verum, Syzygium aromaticum and *Zingiber purpurea* also showed strong antioxidant activities in the FRAP assay (302.56, 227.33 and 215.93 mg TE/100 g DW, respectively). *Mentha piperita* and *Pimpinella anisum* extracts showed the weakest antioxidant activities in the FRAP assay (97.00 and 77.36 mg TE/100 g DW, respectively), reporters show that the high quantities of total phenole and flavonoids, which important correlation between their antioxidant activity was observed[15-16]. The radical scavenging activities of the plant extracts determined using the DPPH assay are shown in Fig 3. The DPPH radical scavenging activity varied from 88 to 46 % representing an approximately 42%-fold variation between the lowest and highest activities. *Cinnamomum verum* showed the highest antioxidant activity, followed by *Syzygium aromaticum* and *Zingiber purpurea* (88, 84 and 75 % respectively). *Pimpinella anisum* showed the lowest antioxidant activity (46 %) in the DPPH assay. Comparing antioxidant activity from this study and other published data is difficult due to the fact that content of antioxidant compounds can be influenced by extracting solvent, cultivar and location extraction methods used[17].

Antibacterial Activity

The antimicrobial activity of the *Syzygium aromaticum, Mentha piperita, Cinnamomum verum, Pimpinella anisum* and *Zingiber purpurea* varied depending on the bacterial species used. The diameter of the zone of inhibition varied ranging from 5.0 mm to 13.0 mm (Table 1). The antimicrobial activity of the *Cinnamomum verum* was found highest against *Bacillus cereus* while lowest activity was found against *Staphylococcus aureus*. *Staphylococcus aureus* showed lower sensitivity to *Mentha piperita* and *Pimpinella anisum* extract as compare to the *Bacillus cereus*. This result also indicated that soybean extract of *Cinnamomum verum, Syzygium aromaticum* and *Zingiber purpurea* were more effective as an antimicrobial agent compared to the *Mentha piperita* and *Pimpinella anisu*. This result also indicated that extract of *Cinnamomum verum* was more effective as an antibacterial agent compared to the other plant extracts. The result showed the good antibacterial activity of acetone extract of *Syzygium aromaticum* and *Zingiber purpurea* against *Staphylococcus aureus, Bacillus cereus.* The sensitivity of *S. aureus* to *Syzygium aromaticum, Mentha piperita, Cinnamomum verum, Pimpinella anisum* and *Zingiber purpurea* extracts is consistent with published data about eucalypt species, but the results are difficult to compare because literature assays were carried out at different conditions[18]. This is consistent with previous studies reporting that Gram-negative bacteria are more resistant to antimicrobials than Gram-positive microorganisms due to their outer lipopolysaccharide membrane[19].

Phytochemical Screening

Phytochemical screening of the plant extracts (Table 3) revealed that the crude extracts contained alkaloids, triterpenoids, tannins flavonoids and carbohydrates, while saponins and steroids were only present in *Syzygium aromaticum* and *Zingiber purpurea* extracts. The phytochemicals tested are known to exhibit medicinal activity and physiological activity. Flavonoids have been reported to possess antibacterial, antioxidant, anti-inflammatory, antiallergic, antimutagenic, and vasodilatory activity[20]. Saponins showed hypocholesterolemic and antidiabetic properties, while steroids and triterpenoids displayed analgesic properties[21-23]. The presence of biologically important phytochemicals in the *Syzygium aromaticum, Mentha piperita, Cinnamomum verum, Pimpinella anisum* and *Zingiber purpurea* extracts, as tested for in this study, contribute to their medicinal value, and therefore, point to potential sources for useful drugs.

CONCLUSION

These results highlight that different medicinal plants contained significantly different amount of antioxidant capacity and antibacterial activity. According to the results, *Cinnamomum verum Syzygium aromaticum* and *Zingiber purpurea* extracts brought about higher phenolic content, antioxidant capcity and antibacterial activity. Further study using bioassay guided of crude extracts of *Cinnamomum verum Syzygium aromaticum* and *Zingiber purpurea* extracts are needed to isolate and identify the active compounds. Their active constituents may be potential candidates with therapeutic value in the treatment of bacterial and oxidation.

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