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# Evaluation of Antibacterial and Antioxidant Activity of Ginger Rhizome and Ziziphus Leaves

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**Abstract:** Methanol extract of ginger tubers and ziziphus leaves were assessed for its antioxidant and antimicrobial activity. The antibacterial efficacy was determined using paper disc method against different gram negative bacterial and sensitivity in terms of zones of inhibition of all extract were also determined(Strains of *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*, *Salmonella entristic* and *vibrio parahemolyticus)*. Gentamicin was used as a standard drug for the study of antibacterial activity. The antioxidant activity was determined by measuring total phenolic content (TPC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH). The result shows that the methanol extracts of ginger tubers and ziziphus leaves were effective against all the bacteria tested. The methanolic extracts of ziziphus leaves show the largest antioxidant TPC, FRAP and DPPH value 457.23mg GAE/100g DW, 281.65 TE/100g DW, 92.31% , whereas the ginger extracts showed the minimum antioxidant TPC, FRAP and DPPH value which were given as 136.82 GAE/100g DW, 192.52 TE/100g DW, 76.21%. From the result it is concluded that the leaves of ziziphus and tubers of ginger methanol extract showed the antioxidant whereas the ziziphus methanol extract exhibited the antibacterial and activity.
**Keywords:** Antibacterial, Antioxidants, ziziphus, ginger.

## Introduction

The increase in prevalence of multiple drug resistance has shown the development of new synthetic antibacterial, antioxidative and anti-inflammatory drugs; moreover, the new drug is necessary to search for new antimicrobial, antioxidant and anti-inflammatory sources from alternative sources. Phytochemicals from medicinal plants showing antimicrobial, antioxidant and anti-inflammatory activities have a potential of filling this need because their structures are different from those of the more studied plants[1].Ginger (*Zingiber officinale* Roscoe, fam. Zingiberaceae) is a perennial herb, with leafy stem up to 60 cm. The rhizome is horizontal, branched, fleshy, aromatic, white or yellowish to brown. Leaves are narrowly or linear-lanceolate, up to 20 cm long and 1.5-2 cm wide. Flowers are produced in a dense spike, yellow green with purple endings. This plant is widely distributed in South-Eastern Asia (ROSS, 2005). The rhizome is rich in the secondary metabolites such as phenolic compounds (gingerol, paradol and shogaoal), volatile sesquiterpenes (zingiberene and bisabolene) and monoterpenoids (curcumene and citral)[2]. Previous studies have demonstrated that plant extracts and isolated compounds from *Z. officinale* possess strong antioxidant[3], antibacterial, antifungal, anticancer and anti-inflammatory effects[4].

Among all the genus of the family Rhamnaceae members of the *Ziziphus* have been used for centuries in folk medicine. *Ziziphus*is a very common plant that is easily available all over the world. About 40 species of *Ziziphus*are available and one of which is, *Ziziphus mauritana* Lam., very common. Carbohydrates, starch, proteins, sugar, mucilage and vitamins are abundantly present in *Ziziphus* species,*Ziziphus mauritana*is generally grown in dry places[5]. The *Ziziphus mauritiana* leaves are eaten by cattle, camels, goats etc. by which they found minerals, useful for their health[6].*Ziziphusmauritana*fruits have highly useful contents quantity that is useful for human health[5]. This plant has various uses in traditional medicine for instance; the dried ripe fruit is a

mild laxative and fruits are applied on cuts and ulcers; are employed in pulmonary ailments and fevers. The leaves are helpful in liver trouble, asthma and fever. The powdered root is dusted on wounds [7].In present study was aimed to examine the methanolic extract of ginger and ziziphus for antimicrobial activity, antioxidant properties using standard methods. The findings from this work may add to the overall value of the plants.

## Materials and Methods

### Extract Preparation

5 grams of ginger powder was mixed with 10 ml methanol. All the three samples (ginger rhizome and ziziphus leaves) were air dried at room temperature. The dried parts were later grinded to power. The dried parts were used for extract using methanol. The extracts were filtered using Buckner funnel and Whatmann's No. 1 filter paper. Extracts was kept at 4°C to preserve the antibacterial property before they were used for disc diffusion assay.

### Determination of Antimicrobial Activity

Strains of *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli, Salmonella entristic* and *vibrio parahemolyticus*, bacteria were obtained from stock cultures preserved at 4 $^{o}$C at research laboratory of Universal Technology Malaysia,. All the bacteria tested were grown on Mueller Hinton Agar.

### Paper Disc Method

Filter paper discs (6mm diameter) were prepared using a punch machine. Filter paper discs were sterilized in a dry heat sterilizer and kept in the refrigerator for further use. A lawn of each bacterial isolate was prepared on MHA plates using a sterile cotton swab from the inoculum showing growth of 0.5 MacFerl and standard. MHA plates were dried for 15 minutes in the Laminar air flow cabinet. Three filter paper discs were placed one on top of other on dried MHA plates and extracts (30 µl) were added on each disc separately. Commercially available Gentamicin discs (10 µg, Oxoid, UK) were used as control.All plates were incubated at 37$^{0}$C for 18-24 hours and the zones of inhibition (diameter inmm) were measured on the agar surface.

### Extraction of antioxidant

The leaves of ziziphus and rhizome of ginger were cleaned and cut into small pieces, and then oven dried at 50$^{o}$C for 48 h. The dried sample was then pulverized using a mechanical grinder and passed through a 250 µm mesh and then stored at 4$^{o}$C until use. In the extraction process, about 1 g of ziziphus and gingerslurries were weighed in universal bottles and 10 mL solvent was added. Solvents used were 50% methanol; samples (ziziphus and ginger slurries with solvents) were then homogenized using homogenizer at 24,000 rpm for 1 min. All extracted samples were centrifuged by using tabletop centrifuge (MLX 210, Thermo-line, China) at 4750 *g* for 10 min. The supernatants were collected for further analysis.

### Total phenolic content (TPC)

The amount of total phenolics content (TPC) in pomegranate was determined with the Folin-Ciocalteu reagent base on[8]. About 0.5 mL of Folin-Ciocalteau (10%, v/v) was added to 0.1 mL of ginger rhizome and ziziphus leaves extract sample. The mixture was swirled and allowed to stand for 6 min followed by the addition of 1 mL 7.5% (w/v) of sodium carbonate (Na2CO3) and samples were mixed. Solutions were allowed to stand for 2 h at room temperature and the absorbance were read at 765 nm wavelength using spectrophotometer. The results were express as milligrams of gallic acid equivalents per 100 g of sample (mg GAE/100 g of DW).

### Ferric reducing antioxidant power (FRAP)

[10]method used to determine the antioxidant capacity of each sample. FRAP reagent was prepared by using 300 mM acetate buffer, (pH 3.6; 3.1 g of sodium acetate trihydrate, plus 16-mL glacial acetic acid and the distilled water made up to total volume of 1L) 10 mM TPTZ (2,4,6-tri (2-pyridyl)-striazine), in 40 mMHCl and 20 mM $FeCl_{3\cdot 6}H2O$ in the ratio of 10:1:1. Freshly prepared FRAP reagent (1000 µL), warmed at 37 °C, was mixed with 100 µl sample, standards. Samples were kept for 30 min and after that the mixture was transferred to micro plate plastic. The absorbance was measured at 595 nm wavelength using spectrophotometer. The result was express as milligrams of Trolox equivalents per 100 g of sample (mg TE/g of DW).

**Radical-scavenging activity (DPPH)**

The DPPH free radical scavenging assay will be measured using the method of [9] technique. The 2,2-diphenyl-1-picrylhydrazyl was dissolved in of methanol to prepare the DPPH solution. The DPPH solution was dilute several 42 times with methanol to obtain 0.9 absorbance at 516 nm, using spectrophotometer. 1 ml of DPPH solution was added to 100 µl of ginger rhizome and ziziphus leaves extract solution. The mixture was shaken in a vortex and kept for 2 h in dark place. After 2 h, the mixture was transferred to micro plate plastic and absorption of DPPH solution after the addition of the sample was measured at 516 nm using the spectrophotometer. The changing in absorption of each sample computed as difference between the plank and sample readings. The following equation (3.1) calculates the percentage of DPPH scavenging activity:The percentage of DPPH scavenging activity was calculated using the following equation: Radical scavenging (%) = $[(A_0 - A_1 / A_0) \times 100]$Where $A_0$is the absorbance of the control and $A_1$is the absorbance of the sample extracts

**Statistical analysis**

The experiment was carried out in triplicate. Statistical analysis of the data was performed by one-way ANOVA using (SPSS 19 software). Significant differences ($p<0.05$) between the two types of medicinal plants were analyzed by Duncan triplicates range test[11]

## Results and Discussion

**Antibacterial screening**

The antimicrobial activity of the ziziphus leaves and ginger rhizome varied depending on the bacterial species used. The diameter of the zone of inhibition varied ranging from (8.0 mm) to (14 mm) for ziziphus extract as compared to (7mm) to (11 mm) for ginger (Table 1). The antimicrobial activity of the ziziphus leaves was found highest against *Staphylococcus aureus*while lowest activity was found against *Vibrio para hemolyticus*. *Staphylococcus aureus* and *vibrio parahemolyticus* showed lower sensitivity to ginger extract as compare to the most other bacteria. This result also indicated that soybean extract of ziziphus leaves was more effective as an antimicrobial agent compared to the ginger rhizome. The present study was done to determine the antimicrobial activity of ziziphus leaves and ginger rhizome extract at methanol. In many countries like Malaysia, ginger is widely used in food preparation by cooking. The result showed the good antimicrobial activity of methanol extract of ziziphus against the tested food borne pathogens- *Staphylococcus aureus*, *Escherichia coli, Salmonella, Bacilluscereus* and *vibrio parahemolyticus*. 1[2]found the synergistic effect of ethanol extract of ginger and garlic against *Bacillus spp.* and *Staphylococcus aureus*. They also found the antimicrobial activity of the ethanol extract of ginger, lime and garlic against broad range of bacteria including *Bacillus spp., Staphylococcus aureus, Escherichia coli*, and *Salmonella spp*. The synergistic antimicrobial effect of soybean and ginger at boiling temperature against food borne pathogens indicates the thermostable antibacterial property of ginger extracts. Ginger extract was tested by KIM and PARK (2013) and the results against *P.aeruginosa*PA14 biofilm formation demonstrated positive effectiveness. [13]found out that the ethanolic extract of *Z. officinale* inhibited *P. aeruginosa* biofilm formation under both aerobic and anaerobic environments. Recent exploration came with the phenolic compounds isolated from *Z. officinale* being QSI (quorum sensing inhibitors). That was verified on *P. aeruginosa* MTCC 2297[14]

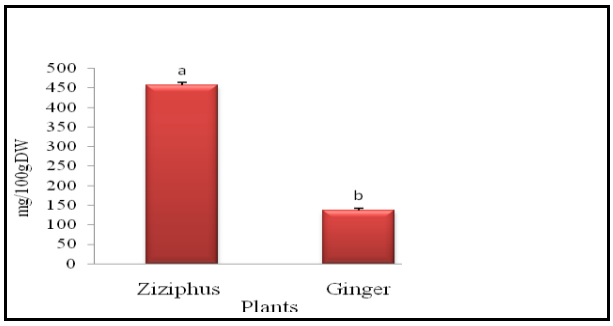
**Table 1: In vitro antibacterial activity of the extracts of ziziphus(leaves), ginger rhizome and standard kanamycin discs.**

| Test organism | Diameter of zone of inhibition (mm) | | |
|---|---|---|---|
| | Ziziphus extract | Ginger extract | Gentamicin(10µg/disc) |
| *Staphylococcus aureus* | 14 | 7 | 19 |
| *Bacillus cereus* | 12 | 9 | 18 |
| *Salmonella entristic* | 10 | 8 | 16 |
| *Vibrio parahemolyticus* | 8 | 7 | 20 |
| *Escherichia coli* | 11 | 8 | 17 |

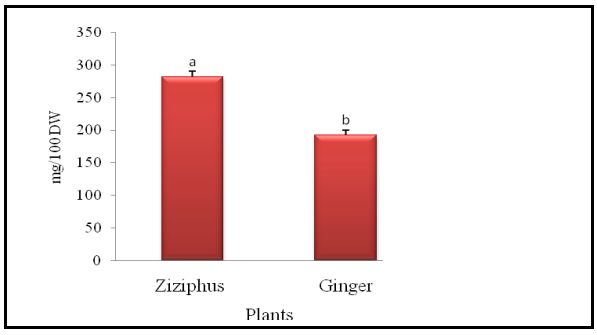
**Antioxidant activity**

A large number of methods have been developed to evaluate antioxidant capacity of medicinal herbs extracts or pure compounds. Nevertheless, few of them have been used widely due to the difficulty of measuring total antioxidant capacity owing to limitations associated with methodological issues and free radical sources [15]. A comparison between ziziphus leaves and ginger rhizome in terms of the total phenolic content (TPC) and antioxidant activity (FRAP, DPPH) is illustrated in Tables Figure (1, 2 and 3). The ziziphus leaves showed different trends with regard to total phenolic content. The TPC was higher in ziziphus leaves and ginger rhizome 457.23 mg GAE/100g DW and 136.82 mg QE/100g DW, respectively. The high contents of total phenolic compounds in this species contribute to important antioxidant activity of wormwood [16] Indeed, the phenolic fraction of plant extracts has been linked to their antioxidant capacity and antimicrobial activities [17] Flavonoids are a group of polyphenolic components synthesized by plants with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes, anti-inflammatory action, reduce blood-lipid and glucose and to enhance human immunity [18] .

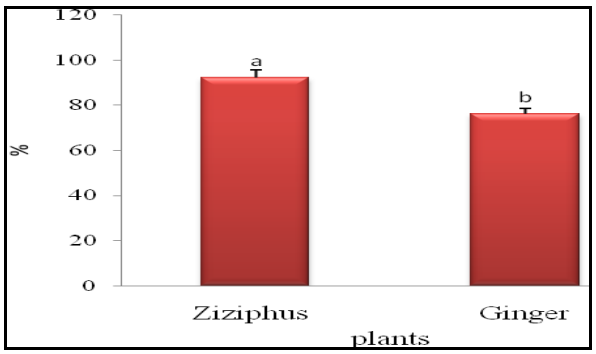
Antioxidant capacity is widely used as a parameter to characterize nutritional health food or plants and their bioactive components. Recently, interest has considerably increased in finding naturally occurring antioxidant to replace synthetic antioxidants, which were restricted due to their side effects such as carcinogenesis [19]. Two different and complementary assays: the DPPH• (2,2-di-phenyl-1-picrilhydrazyl) free radical scavenging and the FRAP (Ferric Reducing Antioxidant Power) were used to evaluate in vitro antioxidant activities of the obtained ziziphus leaves and ginger rhizome.Based on DPPH and FRAP tests, ziziphus leaves extracts present the higher antioxidant activities than ginger rhizome extracts. Furthermore ziziphus leaves exhibited the highest antioxidant activities according to both used tests, DPPH and FRAP (Figure 2 and 3). For methanol extract, the free radical scavenging varied 92.31% in ziziphus leaves to 76.21% in ginger rhizome extract. Concerning FRAP test, for ziziphus leaves and ginger rhizome extract the higher ferric reducing antioxidant power was observed inziziphus leaves and ginger rhizome extract 281.65 and 192.52 mg TE/100g DW, respectively. ziziphus leaves and ginger rhizome had relatively higher total phenolic than other medicinal plants such as *Alceakurdica*, *Stachys lavandulifolium*, *Valeriana officinalis*, *Lavandula officinalis* and *Melissa officinal* (0.22-10 mg CE/g DW),[20.] However, other studies have shown that phospholipids had the lower rate of antioxidant activity. 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.




**[a-b] Mean with different letters are significantly different (P<0.05)**
**Figure 1: Total phenol content (TPC) of ziziphus leaves and ginger rhizome**




**[a-b] Mean with different letters are significantly different (P<0.05)**
**Figure 2: Ferric reducing antioxidant power (FRAP) of ziziphus leaves and ginger rhizome**




**[a-b] Mean with different letters are significantly different (P<0.05)**
**Figure 3: Radical-scavenging activity (DPPH) of ziziphus leaves and ginger rhizome**

## Conclusion

These results highlight that different parts of ginger rhizome and ziziphus leaves contained significantly different amount of antibacterial activity and antioxidant capacity. According to the results, ginger rhizome and ziziphus leaves brought about higher phenolic compounds and higher antibacterial activity. It can be conducted that, ginger rhizome and ziziphus leaves can be considered as an excellence source of antibacterial activityand antioxidant compounds.

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