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Original Research Article

Antioxidant (Superoxide and Hydroxyl Radical Scavenging) and Antibacterial Activity and Bioactive Chemical Properties of Ginger (*Zingiber Officinale*)

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Abstract: Environmental studies have shown outstanding antibacterial properties of the *Zingiber officinale*. Ginger is antimicrobial in nature, and is known as scientifically belonging to Zingiber officinalis. It can be stated that its extracts, particularly ones containing a lot of gingerol and other chemicals, have been proven to be effective against multiple harmful pathogens. The findings support the possible application in ginger as a natural antibacterial as well as the historical uses in the treatment of bacterial diseases. The functional groups that reflect in the group recorded in the ginger powder samples on the entire mid-infrared range (4,000-650 cm -1) measured were =C -H, alkenes, C-F, alkyl halides, C=C, aromatic, and N-H, amide. Radical scavenging activities [Hydroxyl radical scavenging] of crude (methanolic extract), ethanol, hexane and water fractions of Zingiber officinale (ginger) compared with Mannitol (standard) were 126.95+3.97, 217.77+6.19, 149.01+4.73, 193.42+5.00, and 531.96+26.09 respectively. While recorded 31.05±1.07, 38.54±1.20, 30.45±1.18, 41.00±1.27 and 53.08±2.00 respectively comparison with Quercetin (standard). In *vitro* antimicrobial activity of Ginger (*Zingiber Officinale*) extracts on five microorganism: according to the type of extract (methanol, ethyl acetate fraction, ethanol fraction, comparison with standards AP-Ampicillin and CF-Cephalothin) recorded 18.00±0.38, 14.00±0.34, 23.00±0.41, 25.09±0.44 and 23.96±0.42 respectively for *Klebsiella pneumoniae*. While recorded 20.95±0.39, 16.00±0.35, 24.00±0.42, 27.08±0.47 and 25.00±0.43 respectively for *Escherichia coli*. At the same time record 23.00±0.41, 17.09±0.36, 22.00±0.40, 29.17±0.49 and 31.05±0.51 respectively for *Streptococcus pyogenes*. While recorded 17.09±0.38, 25.08±0.44, 20.81±0.39, 31.00±0.50 and 28.39±0.48 respectively for *Staphylococcus aureus* and recorded 19.65±0.40, 27.00±0.47, 30.07±0.50, 35.19±0.54 and 31.00±0.51 respectively for *Bacillus subtilis*.

Keywords: Superoxide, Hydroxyl Radical Scavenging, Bioactive Chemical Properties, Ginger, Antibacterial activity.

INTRODUCTION

A large number of ginger characteristics fall behind the scent, color, form and size of the ginger rhizome. The ginger comes in the form of little ginger, red ginger, and white ginger among other varieties. The fiber of smaller sized ginger is soft compared to that of bigger ginger types. This ginger with its pungent aroma and spiciness compensates the smallness of the rhizome [1, 2]. Small ginger rhizome contains a lot of nutrients as well, including protein, oleoresin, essential oils and starch. In an earlier study, ginger essential oil was found to have high percentage of hydrocarbon compounds especially sesquiterpenes (66.66%) and monoterpenes (17.28%). Nevertheless, the chemical composition may vary depending on the growing place of ginger [3-5]. To take an example, mineralization processes in soil can be retarded at elevated heights as a result of drops in soil pH as well as micronutrient levels. Plants that contain certain chemicals might vary in the amount they produce according to elevation to adapt to changing climatic changes. Thus, it would be safe to note that the biological effect of the essential oils depends greatly on their chemical composition. Today ginger is reported to have certain beneficial effects on a human body. The antioxidant can strengthen the body antioxidant in cases of acute

kidney injury. Other reported beneficial effects include xanthine oxidase inhibitory activity, potent anti-inflammatory effects with in vivo and in vitro efficiencies, and antimicrobial activity on some pathogenic microorganisms and chemo-preventive ability against carcinogens [6, 7]. These biological effects are related to its bioactive phytochemicals, mainly gingerols, shogaol and other phenolic constituents. The natural antioxidants can be found abundantly in the phenolic and flavonoid molecules. There is a need to standardize herbal components in the real sense since most events particularly on environment (during cultivation, harvesting, and the post-harvest process) make ginger to have varying concentrations of active components [8]. The herb standardization and quality control analytical tools have developed to the extent that large data files can be generated within a short time. This brought about the entry of chemometrics, a higher application of data management, in the major cross-examination of data. Quite many analytical tools exist that apply chemometrics software in construction of a trend of herbal identification relative to its intended purpose, including the classification identification, discrimination identification, geographical origins identification, and identification of herbal adulteration. PR is the most applied chemometric tool in the control of herbal quality and can be unsupervised or supervised. Unsupervised PR is applied in data visualization where correlation between samples or objects, and variables is determined without the existence of a preconceived class. Unsupervised PR uses techniques such as the principal component analysis and clustering. An example of supervised PR that is widely applicable to the standardization of herbs is soft-independent modeling of class analogies and linear discriminant analysis (LDA). The application of PR chemometrics in quality control of ginger and specifically to the HPLC setup has been already addressed in past literature. Fourier transforms infrared fingerprint experiments can be performed to detect the difference in the chemical composition of plant species. FTIR has a number of advantages, namely low cost [9, 10], short processing time, minimal sample preparation. However, chemometric analysis must be undertaken because of the complexity of the pattern in the fingerprint region. Chemometrics refers to the field of statistics and mathematics that utilizes absorbance data obtained out of IR spectra to effectively useful information. Spectra of FTIR used in coordination with chemometrics have made it possible to classify herbal ingredients. The literature review shows that studies have not been done to classify the Indonesian ginger using PR chemometrics. Hence, the purpose of this study was to bridge that gap by analyzing Indonesian ginger using operator of chemometrics method to distinguish and discriminate Indonesian ginger using FTIR spectra and antioxidant activities variables [12, 13]. Many organic chemicals that are prevalent in nature are mainly a property of the vegetable kingdom. Plants have an enormous ability to create various molecular structures that your body needs in all sorts of tasks. The primary metabolites play the role of ensuring cellular growth and well-being. The secondary metabolites contribute to plant defence and adaptability and are synthesized by a plant using these molecules by exceptionally complex biochemical routes. Essential oils are the mixture of low molecular weight liposoluble substances with the strong smell. They are characterized by being an amalgamation of a few secondary metabolites formed by plants. Due to their elevated bioscientific activity, essential oils are mainly reputed to be in the leading position of activities in the fields of applied pharmaceutical, cosmetic, and agri-food processes, and on whose account, they remain more notably of the greatest economic and therapeutic importance. The potential of plant chemicals as food preservatives and microbial diseases prevention has only recently begun gathering steam the fact the use of plants in medical and spice purposes has been in use since ancient times [14]. The ease of access and the ease of consumption of antimicrobials by people in general, predetermines the indiscriminate usage, and self-medication process that leads to the present-day crisis of bacteria resistance to the available commercialized antibiotics. Some factors have been identified to increase drug-resistant microbes, rendering the use of antibiotics ineffective. These are the failure to have an organized program in relation to use of antimicrobial, partial dose of the antimicrobial, and unclear diagnosis. With microbe resistance to synthetic antibiotics on the rise, the well-known side effects of the synthetic antimicrobials, and growing consumer interest in eco-friendly and health-conscious consumer products, natural products are being considered as a potential alternative to these unnatural products [15, 16]. Studies on the antimicrobial activity of widely used medical plants occupy a large part of the works in this direction. Ginger, whose botanical name is *Zingiber officinale* Roscoe, can be used in some ways in the kitchen, the pharmaceutical sector, traditional medicine. We aimed to identify the bioactive chemical properties of ginger, in the form of its antioxidant and antibacterial activities.

MATERIALS AND METHODS

At the Hillah market I purchased some five or six pounds of little ginger rhizome (*Zingiber officinale*). The rhizomes validity was checked by the Plant Systematics Laboratory. The rhizomes were dried and powdered fine and subjected to several tests.

The Production of Samples One Goes Through

The root rhizomes were washed and then cut in thin slices and once they had dried up they were harvested at 10 to 12 months. The next procedure was to dry the sliced rhizomes in an oven maintained at a temperature of 40C degrees lasting between three and five days. The dried rhizome was ground using professional blender. In order to obtain homogeneous granules, the ginger powder was sifted with the help of a 20-mesh filter.

Fourier Transform Infrared Spectrum Analysis

Running and scanning the FTIR instrument (Model/Make: IFS 25, Bruker, Germany) with a PC-based software enabled the acquisition of FTIR spectra of both defatted and native GLVs. In preparation of FTIR analysis, a minute amount

of powdered leaf samples was dissolved in KBr and compacted to thin film. A range of wave number 4000 cm-1-500 cm-1 was used in obtaining the infrared light transmittance data. Treated KBr pellets were used as a control and analytical analyses were performed three times. With the help of the comparison of spectral data to a reference it was possible to identify which functional groups were present in the sample.

Hydroxyl Radical Scavenging

This was determined unlike what Aruoma *et al*., (1989) [17], explained, in a slightly different manner. Two deoxyribose condensed in presence of TBA to provide a quantitative degradation product, which forms the basis of this experiment. After 1h incubation at 37 C, 0.5 ml of the product mixture is mixed with 1 ml of 2.8 percent TCA. Aqueous TBA was added 1 ml of 1 percent followed by incubation at 90 C. If desired color was achieved, the reaction with aqueous TBA was terminated by the addition of 500 ml of biuret-free water. A suitable blank solution was used to measure the absorbance against the 532 nm upon cooling. We repeated each test on six occasions to serve as a control, we used mannitol a conventional OH scavenger. We compared test solution and blank solution to figure the percentage inhibition.

Superoxide Radical Scavenging

The reduction of NBT was determined by a previously described method [18, 19]. The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates the superoxide radicals in the presence of which NBT is reduced to purple formazan. The reaction mixture was 1 ml consisting of 20 mM Tris-acetate buffer (pH 7.4), 73 mM NADH, 50 mM NBT, 15 mM PMS, and various amounts (0-20 mM/l) of the sample solution. The quantity of produced formazan was determined by recording the absorbance at 562 nm with respect to an appropriate blank following 5 minutes incubation at room temperature. We repeated each test six times A positive control was stipulated using quercetin.

Statistical Analysis

With a confidence level or interval of 95 or 99 percent, we used SPSS 19.0 (IBM, New York, NY, USA) to compare the means of the sample using the ANOVA. The statistical significance was calculated when the p-value was less than 0.05.

RESULTS AND DISCUSSION

Perennial herb Roscoe produces a fleshy, articulated rhizome with a rough, brownish -white surface. Ginger is one of the most popular and ancient medicinal plants on earth. Some of the benefits of ginger have been proven by experiments, most remarkable ones being its antibacterial property [20, 21]. The essay utilized numerous in vitro microbiological protocols to examine ginger essence oil and the findings were that most of the essential oils had antibacterial activities on all of the animal strains of bacteria cultivated. A range of phytochemicals such as camphene, zingiberene, phellandrene, and zingerone have been attributed to the antibacterial effect to a large extent. Characteristic of ginger is an herbaceous habit, perennial growth, rhizome-articulation, adventitious roots and leaves, reduced basilars, and atrophied floral bracts, and one flower to each bract. Ginger rhizome has a long body though rather flattened in shape and could be yellow or quite dark brown leather. It has a striated lengthwise and what is called fingers come off the rhizomes at an angle. The internal is yellow brown in color whereas the endoderm is yellowish also. Fibrovascular bundles are numerous and oil cells are many. The fragrance of the perfume is beautiful and aromatic and the taste is quite pungent. Ginger is both a medicine plant and a common one [22]. It can help in inflammation, rheumatic diseases and abdominal pains. Its root is filled with carminative, digestive, perspiration, anti-influenza and stimulating properties of herbs. Ginger is a pungent and fresh spice that finds use as a food seasoning and flavoring. It is used as a building block in the preparation of beverages, as well as breads, cakes, cookies, and jams. I employ it in my cosmetics on account of its fragrance. Ginger has demonstrated many biological effects, such as antifungal, anti-inflammatory, antiviral, antibacterial, antioxidant, and anticancer. The food and pharmaceutical industries have shown interest in ginger essential oils, concentrates and extracts made out of rhizomes due to these attributes [23, 24] . Plants Essential oils are very rich in the volatile and fragrant secondary metabolites of monoterpenes and sesquiterpenes. The oil obtained out of *Z. officinale* plant is reported to have antibacterial activity in various studies. On hydrodistillation of *Z. officinale* essential oil, it was found that among other bacteria, L. monocytogenes had the highest degree of sensitivity to the oil and the largest zone of inhibition (37 mm) [25-27]. The consideration that the tested oil has a stronger effect on Gram-negative in the location suggests that other microbial targets, such as plasma membrane, might exist; since the essential oil components are lipophilic and have the potential to change the fluidity and permeability of the plasma membrane. In vitro antimicrobial activity of Ginger (*Zingiber Officinale*) extracts on five microorganism: According to the type of extract (methanol, Ethyl acetate fraction, Ethanol fraction, comparison with standards AP-Ampicillin and CF-Cephalothin) recorded 18.00±0.38, 14.00±0.34, 23.00±0.41, 25.09±0.44 and 23.96±0.42 respectively for *Klebsiella pneumoniae*. While recorded 20.95±0.39, 16.00±0.35, 24.00±0.42, 27.08±0.47 and 25.00±0.43 respectively for *Escherichia coli*. At the same time record 23.00±0.41, 17.09±0.36, 22.00±0.40, 29.17±0.49 and 31.05±0.51 respectively for *Streptococcus pyogenes*. While recorded 17.09±0.38, 25.08±0.44, 20.81±0.39, 31.00±0.50 and 28.39±0.48 respectively for *Staphylococcus aureus* and recorded 19.65±0.40, 27.00±0.47, 30.07±0.50, 35.19±0.54 and 31.00±0.51 respectively for *Bacillus subtilis*.

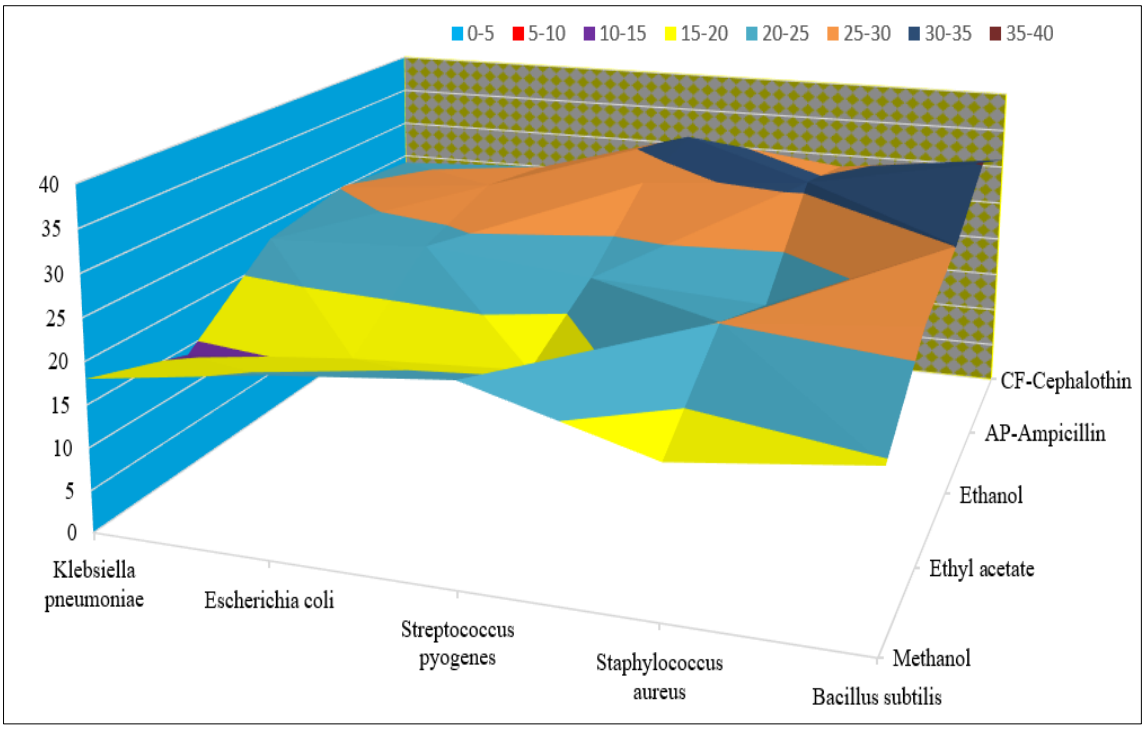

Table 1: Summary of functional groups corresponding to IR absorption in powder samples of Ginger (*Zingiber Officinale*) in whole range of mid-infrared (4,000–650 cm−1)

No.	Position (cm−1)	Intensity	Vibration modes of functional groups
1.	827.46	74.505	=C–H, Alkenes
2.	873.75	72.300	=C–H, Alkenes
3.	927.76	69.360	=C–H, Alkenes
4.	1010. 7	48.730	C-F, alkyl halides
5.	79.328	1236.37	C-F, alkyl halides
6.	79.285	1313.52	C-F, alkyl halides
7.	73.681	1417.68	C=C, Aromatic
8.	77.448	1604.77	N-H, Amide
9.	79. 466	2358.94	-
10.	81.850	2918.30	C-H, Alkane
11.	74.844	3269.34	N-H, Amide

Ginger characterization and separated forms differentiation can be favored using Fourier transform infrared spectra (FTIR) owing to its fingerprint analysis ability. Due to this, under optimal conditions chemometrics coupled with FTIR spectroscopy is popular to identify ginger in terms of the peak intensities along with wavenumbers. Some of the active constituents of ginger include gingerol, shogaol, gingerol and gingerol. The compounds also have alkanes, conjugated double bonds (C=C), carbonyls (C=O), and methoxys (OCH3). It was shown that ginger has some active chemicals as the functional groups that can be identified in the FTIR spectra were detected. With full wavenumbers as the variable of Abs values, a principal component analysis (PCA) was used after the optimization procedure. Radical scavenging activities [Hydroxyl radical scavenging] of Crude (methanolic extract), Ethanol, Hexane and Water fraction of *Zingiber officinale* (ginger) were compared to Standard Mannitol and were recorded at 126.95±3.97, 217.77±6.19, 149.01±4.73, 193.42±5.00, and 531.96± 26.09 respectively. While recorded 31.05 ± 1.07, 38.54 ± 1.20, 30.45 ± 1.18, 41.00 ± 1.27 and 53.08 ± 2.00 respectively comparison with Quercetin (standard) Figure 1 and 2.

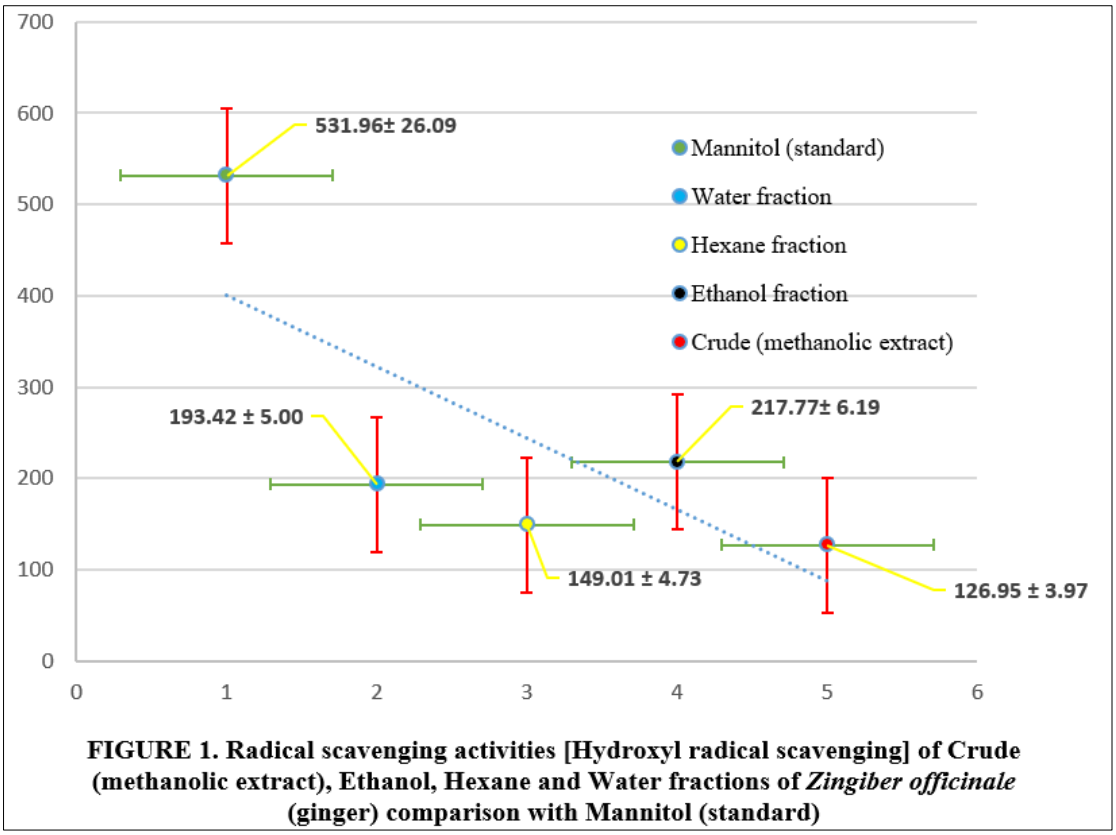

FIGURE 1. Radical scavenging activities [Hydroxyl radical scavenging] of Crude (methanolic extract), Ethanol, Hexane and Water fractions of *Zingiber officinale* (ginger) comparison with Mannitol (standard)

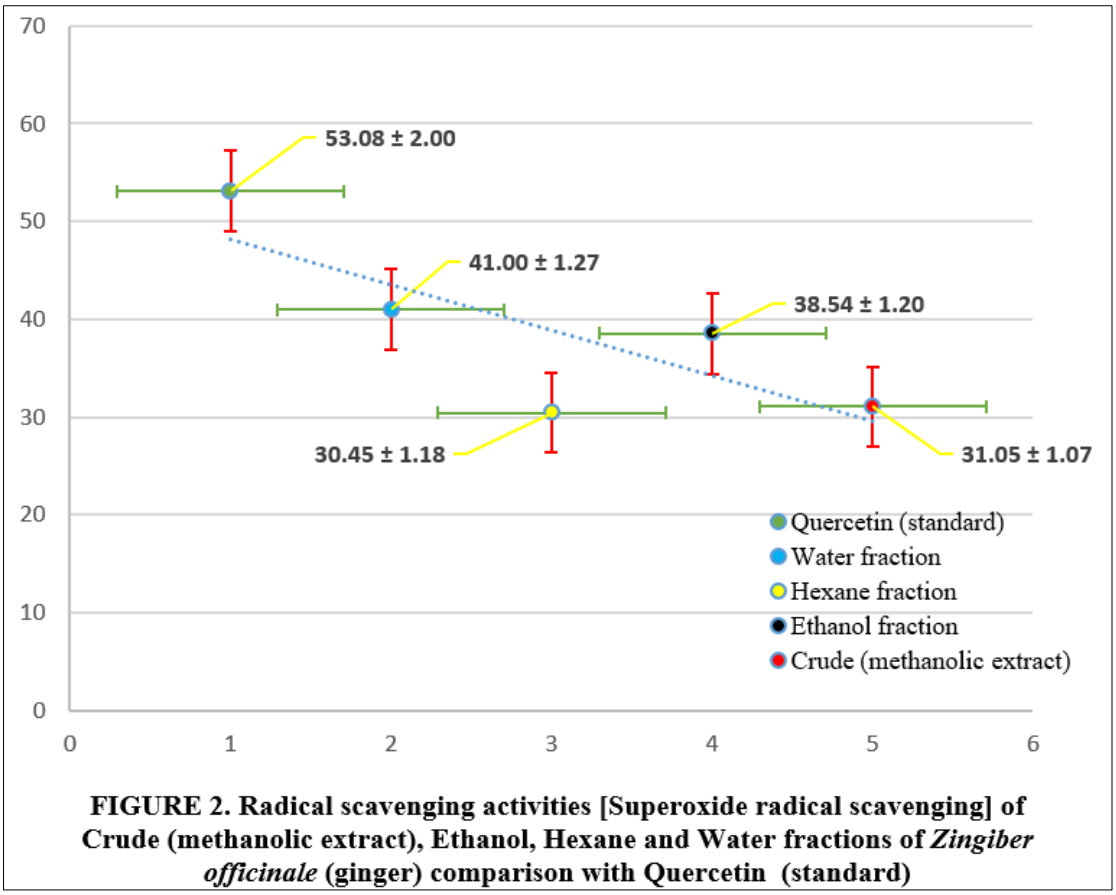

FIGURE 2. Radical scavenging activities [Superoxide radical scavenging] of Crude (methanolic extract), Ethanol, Hexane and Water fractions of *Zingiber officinale* (ginger) comparison with Quercetin (standard)

Moreover, ethanol ginger extracts showed stronger antioxidant activity in comparison with the ethanol water extract. However, the opposite is true as boiling water showed the least extraction potency. Ginger contains an abundance

of chemicals that are known antioxidants, and these are known as polyphenols. This has added popularity to ginger as a culinary ingredient especially within the Eastern parts of the United States. These are phytochemicals that are actively preventive against the severe oxidation-related diseases such as cancer, arthritis, cognitive disorder, diabetes and heart disease [28]. Bioactive components of ginger demonstrated the antioxidant effect of ginger. Carocho and Ferreira assert that antioxidant chemicals reverse the production of the free radicals and consequently inhibit oxidative stress. The other role played by antioxidants in foods is to extend the shelf life of the food products, besides having a number of medical applications. In addition, Tohma *et al*., stated that the SAC of phenolic compounds, acid-phenols, and flavonoids antioxidant activity of ginger have been studied the most. Not to mention that ginger essential oil, minerals, vitamins, and fibers are all renowned to maintain a healthy condition. The non-thermal drying system provided the advantage of affecting the flavonoid release owing to its characteristic of being heat-sensitive and its retention being temperature and drying treatment-dependent. Thus, the sensitive antioxidants in the freeze-dried samples (namely, the flavonoids) were preserved. When it comes to methods of processing that maintain intact plant quality, freeze-drying turns out to be the best of all. Though water is a polar substance, the hydrophilic compounds present in the plant tissue, including hydroxyl (OH-) or carboxyl (COO-) groups are likely to be washed away by surface tension action of high temperature water, hot water is therefore the least effective solvent to extract active principles in ginger tissue. Ginger extract using a mixture of water and ethanol (an 80 percent aqueous solvent) produced better antioxidant activity. The enhancement of the dissolving ability between the solute molecules and solvent water-ethanol, together with the comparative polarity of antioxidant chemicals could have produced this effect. Polar antioxidants can be extracted successfully using dilitrated mixtures of water and ethanol as suggested amounts. The effect of drying methods on antioxidant properties of gingers was analyzed since ginger is very highly antioxidant and abundant in natural antioxidants. Drying treatment increased the antioxidant abilities of ginger and reduced the moisture activity of the chestnut [29]. Further, drying results in the formation of new materials with antioxidant potential, and a rise in the antioxidant capacity is attributed to the release of previously bound phenolic compounds because of cell wall disintegration.

CONCLUSION

Therefore, the possible new therapy routines of combating the refractory infection diseases founded on the application of natural extracts of Zingiber Officinale should be also explored more, as our findings and the given research allow to suppose. There is the possibility of extending the technology developed to verify the geographical origin of gingers and fight adulteration. FTIR spectra are also advantageous in some respects as a fingerprint method. One of the faster and environmentally non-destructive methods to study herbal constituents, such as ginger, is to integrate FTIR spectra with chemometrics. Despite the above aspects, there are numerous reports of antibacterial activity in ginger essential oil and this phenomenon provides an indication that the oil has untapped antibacterial potential, although more research is mandated to prove this.

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