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In Vitro Evaluation of Antioxidant Activity of Galangal (*Alpinia Galanga*) And Ginger (*Zingiber Officinale*) Aqueous Extracts

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Abstract

Introduction: Antioxidants prevent free radical-induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. Natural antioxidants found in food, particularly fruits, vegetables, and other plant-based diets, play an important role in disease prevention.

Aim: The aim of this study was to evaluate antioxidative activities of aqueous extracts of ginger (*Zingiber officinale*) and galangal (*Alpinia galanga*) utilizing the DPPH assay method compared to that of ascorbic acid (Vit. C).

Methods: The research was conducted at the Faculty of Pharmacy, University of Kufa, from February to July 2021. The aqueous extracts of these plants were made using both hot and cold extraction methods. The antioxidative activities of both cold and hot aqueous extracts of these plants at concentrations of 500 and 1000 µg/mL were made utilizing the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay method.

Results: Both *A. galanga* and *Z. officinale* extracts demonstrated measurable activity comparable to ascorbic acid (vitamin C). The cold aqueous extract of *A. galanga* exhibited the highest antioxidant activity at a concentration of 500 µg/mL, while the hot aqueous extract of *Z. officinale* at 500 µg/mL showed the least activity. These findings suggest that the two plant extracts have different amounts of antioxidants at the same concentration. This indicates that *A. galanga* might be more effective at delivering antioxidant advantages than *Z. officinale,* and both the concentration and extraction method substantially affect antioxidant capacity, which is comparable to that of ascorbic acid.

Conclusion: the aqueous extracts of *Alpinia galanga* and *Zingiber officinale* demonstrated quantifiable antioxidant activity, with *A. galanga* exhibiting greater efficacy under cold extraction conditions. Furthermore, these extracts demonstrate effective antioxidant activity in the DPPH assay, which is comparable to that produced by ascorbic acid (vitamin C).

التقييم المختبري للنشاط المضاد للأكسدة للمستخلصات المائية من الخولنجان (*Alpinia galanga*) والزنجبيل (*Zingiber officinale*)

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الخلاصة

المقدمة: من خلال إزالة الجذور الحرة الضارة، تُعدّ مضادات الأكسدة ضرورية لحماية الجسم من الإجهاد التأكسدي. تُعزز مركبات مضادات الأكسدة الموجودة في الأطعمة الطبيعية كالفواكه والخضراوات والنباتات الطبية الصحة العامة.

الهدف: هدفت هذه الدراسة إلى استخدام طريقة اختبار DPPH لتقييم النشاط المضاد للأكسدة في المستخلصات المائية للزنجبيل (*Zingiber officinale*) والخولنجان (*Alpinia galanga*).

طرق العمل: أُجري البحث في كلية الصيدلة بجامعة الكوفة، خلال الفترة من فبراير إلى يوليو 2021. حضّرنا المستخلصات المائية للنباتات باستخدام الماء الساخن والبارد. استخدمنا طريقة اختبار DPPH (1,1-diphenyl-2-picrylhydrazyl) لإزالة الجذور الحرة بتركيزين (500 و1000 ميكروغرام/مل) لمعرفة مدى فعالية مضادات الأكسدة. النتائج: أظهر المستخلص المائي البارد لنبات *Alpinia galanga* أعلى نشاط مضاد للأكسدة عند تركيز 500 ميكروغرام/مل. من ناحية أخرى، أظهر المستخلص المائي الساخن لنبات *Zingiber officinale* عند تركيز 500 ميكروغرام/مل أقل نشاط. تُظهر هذه المقارنة أن مستخلصي النباتين يحتويان على كميات مختلفة من مضادات الأكسدة عند نفس التركيز. هذا يشير إلى أن *Alpinia galanga* قد يكون أكثر فعالية في تقديم فوائد مضادات الأكسدة من *Zingiber officinale* في هذه الظروف الخاصة. أظهر كل من مستخلصي *Alpinia galanga* و*officinale* Zingiber نشاطًا قابلًا للقياس يُضاهي حمض الأسكوربيك (فيتامين ج). يمكن أن تُثري هذه النتائج الأبحاث والتطبيقات اللاحقة في مجال الصحة والتغذية، مما يشير إلى أن كلاً من التركيز وطريقة الاستخلاص يؤثران بشكل كبير على قدرة مضادات الأكسدة، والتي تُشبه بشكل ملحوظ قدرة حمض الأسكوربيك. الاستنتاج: أظهر كلٌّ من مستخلصي الجلنجان (*Alpinia galanga*) والزنجبيل (*Zingiber officinale*) المائيين نشاطًا مضادًا للأكسدة قابلًا للقياس الكمي، حيث أظهر الجلنجان فعاليةً أكبر في ظروف الاستخلاص الباردة. تدعم هذه النتائج فكرة إمكانية كونهما مصدرين طبيعيين لمضادات الأكسدة.

1. Introduction

Extensive research has shown that free radicals contribute significantly to oxidative damage, affecting biomolecules in living organisms. To counteract this damage and reduce the risk of various diseases, the presence of antioxidants is crucial. Antioxidants are defined as compounds that, even at low concentrations relative to oxidizable substrates, can delay or inhibit oxidation processes. The human body has evolved defense mechanisms against reactive oxygen species, relying on both enzymatic and non-enzymatic systems that function to neutralize and detoxify these harmful agents (E.B. Kurutas, 2016; Kurutas, 2016). Antioxidants play a vital role in neutralizing excess free radicals and are generally required through dietary intake, except for certain endogenous compounds like glutathione, uric acid, and ubiquinol, which are naturally synthesized by the body. Due to the potential health risks associated with synthetic antioxidants, such as contamination with harmful chemicals and the generation of toxic byproducts, natural antioxidants have emerged as a safer and more appealing alternative. There has been an increasing interest in locating safe, natural sources of dietary antioxidants, particularly those derived from plants. Antioxidants are frequently incorporated into food products to disrupt oxidative chain reactions. They function by obstructing the initiation and propagation phases, resulting in reaction cessation and postponing oxidation (Liang et al., 2015). Numerous plant materials serve as natural sources of antioxidants, including herbs, spices, seeds, fruits, and vegetables. The interest in these natural components arises not only from their biological significance but also from their economic implications, as many can be derived from food by-products and underutilized plant species (Liang et al., 2015; Phaniendra et al., 2015). A multitude of phytochemicals has shown the ability to block the enzymes that produce free radicals and serve as innovative therapeutic agents for the treatment of oxidative stress-related disorders (Flieger, Flieger, & Baj, 2021; Flieger, Flieger, Baj, et al., 2021). The assessment of antioxidant capacity can be conducted through various methodologies; however, the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is a widely utilized, rapid, straightforward, and cost-effective technique for evaluating antioxidant properties. This method employs free radicals to determine the potential of substances to act as hydrogen donors or free-radical scavengers (FRS). The DPPH testing procedure involves the reduction of DPPH, a stable free radical. The free radical DPPH engages with an unpaired electron, resulting in a pronounced absorbance at 517 nm, characterized by a purple coloration. An FRS antioxidant, for instance, interacts with DPPH to produce DPPHH, which exhibits reduced absorbance compared to DPPH due to a diminished quantity of hydrogen. It is a radical relative to the DPPH-H form, since it induces decolorization or a yellow tint with an increase in the number of absorbed electrons. Decolorization substantially impacts the reduction capacity. Upon the amalgamation of DPPH solutions with the hydrogen atom source, the reduced form of diphenylpicrylhydrazine is generated, resulting in the loss of its violet hue (Flieger, Flieger, & Baj, 2021; Paterson, 1999; Richardson & Harborne, 1990). The target of this study was to evaluate the antioxidative ability of aqueous extracts from two locally prevalent medicinal plants, galangal (*Alpinia galanga*) and ginger (*Zingiber officinale*), in comparison to a standard antioxidant, ascorbic acid.

2. Materials and Methods

2.1. The Plant Sample Preparation

The fresh samples of ginger (*Zingiber officinale*) and galangal (*Alpinia galanga*) were purchased from local markets of Al-Najaf city and identified and verified by taxonomists. The chosen parts were cleaned to get rid of dust and then dried at room temperature in a shady place to keep them from being directly exposed to sunlight. This study was conducted at the Pharmacognosy Laboratory, Faculty of Pharmacy, University of Kufa.

2.2. Preparation of Plant Extracts

The cold extract method was made up by macerating 25 grams of plant powder with 250 mL of water for 3 days, while the hot extract method was made up by using a Soxhlet apparatus with the same weight of the powdered dried plant and 250 mL of water for 24 hours. After finishing both extract methods, filtration, and drying using a rotary evaporator, the extracts were weighed (Morabbi Najafabad & Jamei, 2014).

2.3. FT-IR Spectra

Determination of infrared spectra of the plant extracts was done and recorded as a KBr film using Shimadzu FT-IR 8400 (Japan), at the Faculty of Pharmacy/University of Kufa.

2.4. Antioxidant Activity Study of Aqueous Plant Extracts in The DPPH Assay Method

Antioxidant activity evaluation used an offline (DPPH) assay. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical cation technique was adapted to assess the ability of one hundred pure chemical compounds to scavenge free radicals. The DPPH reagent was DPPH 200 µl as a control in the first well of the microplate (Alothman et al., 2009). The DPPH assay method, used to measure the plant solutions' (extract and fractions) ability to get rid of free radicals, was done according to Alothman et al. (2009) (G et al., 2024). The desiccated plant samples combined with DMSO were allocated in test tubes at two concentrations (500 and 1000 µg/mL) in triplicate, while the DPPH solution (methanolic) was concurrently added to the test tubes with the plant samples. Distilled water was utilized as a control in the remaining wells on the plates. Subsequently, gently agitate the tubes for 2 minutes while they are incubated in the dark at room temperature for 30 min to measure scavenging activity. The scavenging % was assessed at 517 nm using an ELISA reader (TECAN, Grading, Austria). After incubation, 100 percent methanol was used as a blank.

The following formula was used to calculate the DPPH scavenging percent:

$$Radical\ scavenging\ (\%) = [(A)control - (A)sample/(A)control] \times 100.$$

3. Results and Discussion

3.1. FT-IR Spectra Results

The results showed absorption bands in the aqueous plant extracts, as shown in Fig.1, where it indicated that the infrared spectrum had an absorption band (3412, 3404 cm^{-1}) for *A. galangal* and *Z. officinale, respectively,* belonging to the hydroxyl group, as well as band 2927 cm^{-1} belonging to the C–H group of the phenyl ring. Table1 revealed the shown absorption bands in the aqueous plant extracts.

Table1: FT-IR spectrum data (cm^{-1}) of plant extracts

Plants	-OH	C-H	C=O	C=N	C=C	$-CH_3$
Alpinia galanga	3412	2929	1666	1409	1610	1444 1375
Zingiber officinale	3404	2929	1668 1633	1409	1523	1375

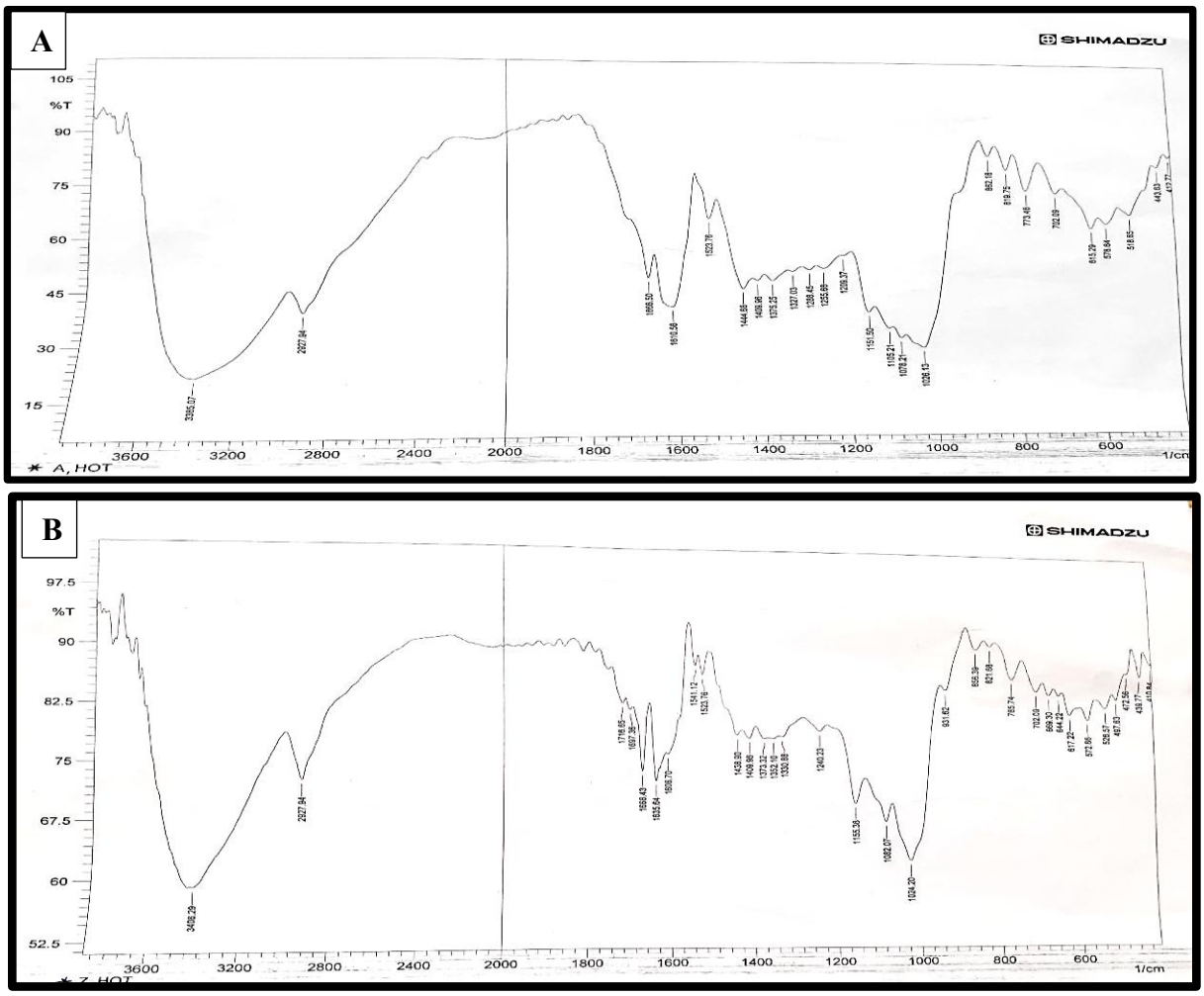

Figure1: FTIR spectra of samples (A) **and** (B) **showing the characteristic functional groups. The broad band around 3200–3500 cm⁻¹ corresponds to O–H stretching vibrations, indicating hydrogen bonding. Peaks observed at 2800–2950 cm⁻¹ are attributed to C–H stretching.** Absorption bands in the region of 1600–1650 cm^{-1} and 1400–1450 cm^{-1} correspond to asymmetric and symmetric stretching of carboxylate (COO^-) groups, while strong bands at 1000–1150 cm^{-1} are assigned to C–O–C and C–O stretching vibrations of the polysaccharide backbone. Minor shifts in peak positions indicate intermolecular interactions between components.

FT-IR spectroscopy is a valuable analytical tool for identifying unknown materials, assessing quality and stability, and determining the composition of complex mixtures through the detection of characteristic absorption bands. In this study, FT-IR analysis confirmed the presence of key phytochemical constituents in the plant extracts, as specific absorption peaks indicated the presence of glycosides, phenols, amino acids, and flavonoids. The antioxidant activity of the extracts can be attributed, at least in part, to these compounds—particularly phenols, flavonoids, and tannins, which are known to act as free radical scavengers and inhibit oxidative processes (Hernández-Fernández et al., 2023; Jerman Klen & Mozetič Vodopivec, 2011).

3.2. Antioxidant Activity Results of Aqueous Plant Extracts in The DPPH Assay Method

In the DPPH radical scavenging assay, antioxidants reduce DPPH, causing a color change from purple to yellow, with the extent of discoloration reflecting the sample's radical-scavenging capacity. The antioxidant activity of the tested plant extracts varied between samples, but both *A. galanga* and *Z. officinale* extracts demonstrated measurable activity comparable to ascorbic acid (Vit. C), as illustrated in Table2 and Fig.2 and Fig.3.

Table2: Antioxidant Effect of Plant Extracts and Ascorbic Acid (Vit. C) In DPPH Assay

Plant name	Type of extraction	Concentration µg/mL	Scavenging %
Galangal (*Alpinia galanga*)	Cold aqueous extract	500	.67085
		1000	.21380
	Hot aqueous extract	500	.08979
		1000	.45973
ginger (*Zingiber officinale*)	Cold aqueous extract	500	.25170
		1000	.07778
	Hot aqueous extract	500	.62845
		1000	.90268
Ascorbic acid (Vit. C)	-	500	85.047
		1000	94.031

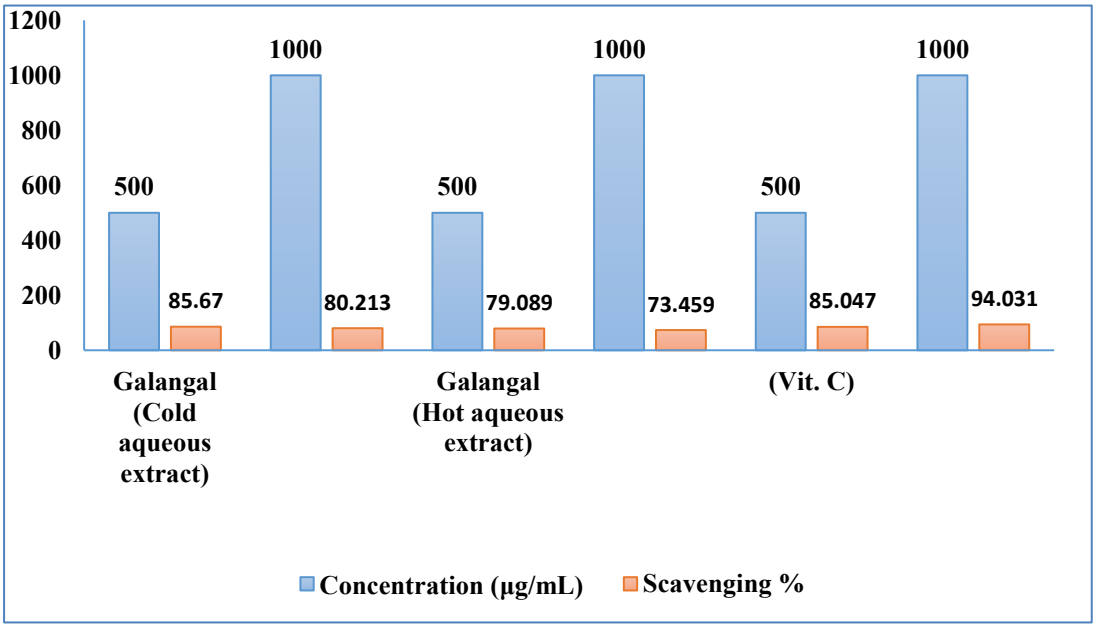

Figure2: Antioxidant effects of galangal extracts and ascorbic acid (Vit. C) in DPPH assay

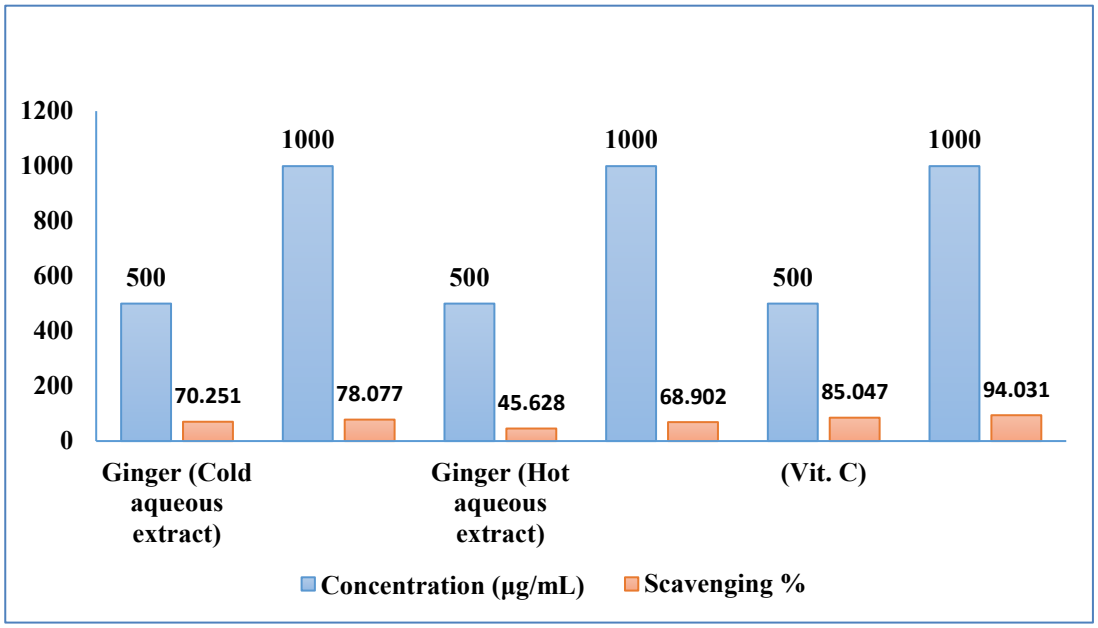

Figure3: Antioxidant effects of ginger extracts and ascorbic acid (Vit. C) in DPPH assay

The DPPH radical scavenging assay results demonstrate varying antioxidant capacities among the tested substances. Ascorbic acid (Vitamin C) exhibited strong activity, achieving 85.047% scavenging at 500 µg/mL and increasing to 94.031% at 1000 µg/mL. For galangal (*Alpinia galanga*), the cold aqueous extract showed scavenging percentages of 85.670% (500 µg/mL) and 80.213% (1000 µg/mL), while its hot aqueous extract yielded lower values of 79.089% (500 µg/mL) and 73.459% (1000 µg/mL). Ginger (*Zingiber officinale*) extracts displayed lower activity overall; its cold aqueous extract scavenged 70.251% (500 µg/mL) and 78.077% (1000 µg/mL), and its hot aqueous extract was notably weaker at 45.628% (500 µg/mL), increasing to 68.902% (1000 µg/mL). Concentration dependence was evident for most samples, though the direction varied (e.g., ascorbic acid increased with concentration, while Galangal hot extract decreased). The antioxidant activity of the plant extract is influenced not by the total amount or concentration of phenols and flavonoids, but by the specific forms and quality of the phenolic compounds (Juvekar et al., 2009; Sakat et al., 2010). Phenolic substances stabilize phenoxy radicals due to the presence of one or more hydroxyl groups that can neutralize free radicals (Sharififar et al., 2009). Flavonoid molecules significantly contribute to antioxidant activity and metal chelation, which are contingent upon the structure and arrangement of hydroxyl groups. These attributes mitigate free radical generation and, as a result, facilitate diverse biological functions (Sharififar et al., 2009).

4. Conclusion

Both galangal and ginger extracts possess significant antioxidant activity as measured by the DPPH assay; neither matched the potency of the reference standard ascorbic acid, particularly at the higher concentration (1000 µg/mL). The extraction method critically influenced efficacy: cold aqueous extraction consistently yielded extracts with superior scavenging activity compared to hot aqueous extraction for both plants. Galangal extracts generally outperformed ginger extracts, with the cold aqueous extract of galangal at 500 µg/mL (85.670%) demonstrating activity remarkably close to ascorbic acid (85.047%) at the same concentration. Ginger's hot aqueous extract, especially at 500 µg/mL (45.628%), showed the weakest activity. These findings highlight the

importance of plant species and extraction methodology in obtaining optimal antioxidant potential from natural sources.

5. Conflict of Interest

The authors declare there is no conflict of interest.

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