International Journal of Pharmaceutical Research and Development

ISSN Print: 2664-6862
ISSN Online: 2664-6870
Impact Factor: RJIF 8
IJPRD 2024; 6(2): 118-125
www.pharmaceuticaljournal.net
Received: 02-07-2024
Accepted: 11-08-2024

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Investigating the bioactive characteristics of *Zingiber officinale* and *Citrus medica* with emphasis on their antioxidant, antimicrobial, and anthelmintic properties

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DOI: https://doi.org/10.33545/26646862.2024.v6.i2b.66

Abstract
Citrus medica and *Zingiber officinale* has a variety of bioactive components that have several therapeutic uses. This study evaluated the bioactive properties of aqueous and ethanolic extracts derived from the fruit peel of *Citrus medica* and the rhizome of *Zingiber officinale*, assessing their antioxidant, antibacterial, and anthelmintic effects. Dried, ground rhizomes of *Z. officinale* and fresh fruits peel of *C. medica* were extracted by maceration and Soxhlet extraction method with ethanol and water, respectively. DPPH assay and total antioxidant status (TAS) by a colorimetric method was performed to analyses the antioxidant properties. Total phenolic content from ethanol extracts were found the highest 13.5 ± 2.26 and 66±0.14 µg GAE/g dry weight, while total flavonoid content ethanol extracts were found 183.17±8.12 and 464±0.30 of the *Z. officinale* and *C. medica* respectively. Disc diffusion method, with minor modifications was used for the evaluation of *in-vitro* antimicrobial activity against *S. aureus*, and *E. coli*. S. aureus was affectively controlled by the *Z. officinale* and *C. medica* aqueous and ethanol extract at 10 µl/ml and 5 µl/ml. The mean diameters of inhibition zone of *Z. officinale* and *C. medica* extracts for 10 µl/ml and 5 µl/ml were 14.0±1.0, 12.67±0.58, and 15.2±0.66, 13.2±1.8 mm, respectively. Anthelmintic study was conducted using *Eisenia fetida* as a test organism, revealing a significant anthelmintic effect in the *C. medica* and *Z. officinale* extract compared to the standard drug. Paralysis of earth-worms occurred within 26 and 16 min when exposed to concentrations of 30 mg/ml while 66 and 46 min for 10 mg/ml of *Z. officinale* and *C. medica* extracts respectively. This study suggests that *Z. officinale* rhizomes and *C. medica* fruit peel extract may use as anthelmintic, antibacterial, and antioxidant agents. As a result, they highlight the natural source's potential uses in a variety of applications.

Keywords: *Zingiber officinale*, *Citrus medica*, antioxidants, antimicrobial, anthelmintic, phenolic compounds

Introduction
Medicinal plants have been used extensively for therapeutic purposes since ancient times. Fruits and vegetables provide numerous health benefits, which are largely attributed to their rich content of antioxidant compounds. These include phenolic acids, flavonoids, and tannins, as well as nitrogen containing compounds like alkaloids and amines. Additionally, they contain vitamins, terpenoids, and other metabolites that exhibit potent antioxidant properties [1]. The reason for this activity is that these materials can neutralize or scavenge reactive species by hydrogen donation, so reducing oxidative stress before they harm cells and other biological components [2]. Reactive oxygen species and reactive nitrogen species are byproducts of standard cellular metabolic processes. However, at high concentrations, these substances can be important mediators of damage to cellular structures such as proteins, lipids, and nucleic acids. If the oxidation of any of these substrates is not controlled, it may lead to the development of chronic diseases such as cancer, hypertension, diabetes, cardiovascular and neurodegenerative diseases [3]. In this sense, there has been a great interest in the search for natural antioxidants from plant materials, and the antioxidant activities of various extracts and isolated compounds have been investigated using different methodologies. Ginger (*Zingiber officinale*) belongs *Zingiberaceae* family, characterized as a horizontal, branched, fleshy, and aromatic perennial herb that can reach petiole lengths of up to 60 cm.

The rhizome of this plant is rich in a variety of secondary metabolites, including phenolic compounds like gingerols and shogaols, volatile sesquiterpenes such as zingiberene and bisabolene, and monoterpenes including curcumene and citral [4]. Ginger is used in Ayurvedic medicine throughout the world to treat a variety of ailments, including arthritis, cramps, rheumatism, sprains, sore throats, muscle aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever, and infectious diseases [5]. Ginger possesses antimicrobial properties, making it useful for treating bacterial infections [6]. Due to its easy availability, ginger is relatively inexpensive, widely accepted, and well tolerated by most people [7].

Citron, scientifically classified as *Citrus medica*, belongs to the *Rutaceae* family (Fig 1) that encompasses over 1,300 species across 140 genera [8]. The tree is diminutive and produces round or elongated fruits that are yellowish-orange in color, measuring between 8 to 12 centimeters in length. Citrus fruits are generally rich in pectin, sugars, minerals, and vitamins A, B, and C, along with carotenoid pigments and organic acids such as citric and ascorbic acid. They also contain a variety of active phytochemicals, including coumarins and flavonoids like naringin, naringenin, hesperidin, neohesperidin, rutin, hesperetin, nairutin, and tangeretin. This study highlights callus cultures of the plant as a promising source for the efficient and scalable production of compounds believed to possess antioxidant properties [9]. *C. medica* have been reported to show antifungal activity against some fungi, additionally, treatment of diabetes and Alzheimer's disease [10]. Many microbes and parasitic worms are contagious or harmful to humans. Juvenile parasites, for example, infect people through the skin or gastrointestinal tract (GIT) and then spread to other organs where adult worms display distinctive tissue distribution characteristics.

Using the natural products made from medicinal plants antibacterial properties to treat a range of microbial illnesses is one use for them. Across the globe, microbial infections are a leading cause of illnesses, physical disabilities, and deaths [11]. People are generally growing more conscious of problems, but the overuse of traditional antibiotics is also leading to misconceptions. Scientists have come to the conclusion that microbial resistance limits the useful life of antibiotics. Hence, it's become necessary to extract new compounds with bioactive properties from various sources, including plants. Medicinal plant extracts have been developed and used in a range of forms, including pills, tablets, liquid extracts, powders, and the creation of numerous value-added food products in recent years, thanks to developments in the functional food and nutraceutical industries. Traditionally, *Zingiber officinale* and *C. medica* L. has been claimed to possess various properties, but this has not yet been scientifically demonstrated. Thus, the present study was designed to evaluate the *in-vitro* capacity of antioxidant, antimicrobial and anthelmintic of the aqueous and ethanol extracts of *Zingiber officinale R.* and *C. medica* L. fruit peel. The findings from this study will be essential for advancing the understanding and development of plant-based treatments for diseases.

Materials and Methods

Z. officinale (rhizomes) and *C. medica* fruits were obtained commercially. Folin-Ciocalteu's phenol reagent, sodium carbonate, sodium nitrite, aluminum trichloride, copper (II) chloride, ammonium acetate, sodium bicarbonate, sodium hydroxide, ethanol, hydrochloric acid (37%), nutrient broth, nutrient agar, potato dextrose agar obtained from Merck. Ascorbic acid, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), and all other reagents used to prepare simulated gastric fluid and simulated intestinal fluid were obtained from Sigma-Aldrich. All other remaining chemicals and reagents employed were of analytical grade.

Extraction and analysis of phenolic compounds

Dried, ground rhizomes were extracted with ethanol and water by maceration. 12.5 g of plant material was soaked with 50 ml of ethanol/water for 24 h at room temperature. The resulting extracts were filtered through Whatman No. 1 filter paper. The filtrates was then evaporated to dryness by placing it in a hot air oven at 40 °C for 24 hours. Fresh fruits peel of *C. medica* was removed and kept for drying for one week at 50 ºC. Following this, 30 g of dried fruit peel was weighed, and then extracted successively using Soxhlet extraction method with 300 ml of ethanol and water. The extract was concentrated using a rotary evaporator at 50ºC (150 rpm) and stored at 4 °C.

The phytochemicals were analyzed for total phenolic content (TPC) and total flavonoid content (TFC). The TPC and TFC were established using method Singleton *et al*. (1965), Chang *et al*. (2002), respectively [12-13]. 5 mg of dried *Z. officinale* (rhizomes) and *C. medica* fruit extract was dissolved in a 10 ml mixture of acetone and water (6:4 v/v). 0.2 ml aliquot of the extract was combined with 1.0 ml of Folin-Ciocalteu reagent and incubated for 5 minutes at 25°C. Then, 0.8 ml of 15% sodium carbonate solution was added to the reaction mixture and kept for 90 min at room temperature. Similarly, extracts of *Z. officinale* (rhizomes) and *C. medica* fruit peel (0.5 ml) was mixed with 1.5 ml methanol, 0.1 ml 10% aluminum chloride, 0.1 ml 1 M potassium acetate and 2.8 ml distilled water. The mixture was vortexed and the reaction was kept at room temperature for 30 minutes.

The absorbance at 560 nm for TPC, 415 nm for TFC were measured using a Shimadzu 150 UV-1800 spectrophotometer (Japan). For TPC, the results were given as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW). The standard curve exhibited linearity from 0.01 to 0.1 mg/ml (r^2=0.997). TFC was reported in milligrams of rutin equivalent per gram of dry weight (mg RE/g DW). The standard curve exhibited linearity from 0.01 to 0.35 mg/ml, with r^2 of 0.999.

Radical scavenging assay for *in-vitro* antioxidant activity

The antioxidant properties of the both extracts from *Z. officinale* (rhizomes) and *C. medica* fruit peel were analyzed using the DPPH assay, following the method established by Blois in 1958 with some modifications [14]. The different concentrations (50-300 µg/ml) of extracts were tested for their free radical scavenging activity (RSA) against the standard antioxidant, ascorbic acid. Various concentrations of the extract and standard were prepared in methanol. 1 ml of each of these test concentrations was mixed with 1 ml of 1 mmol of DPPH reagent (prepared in methanol) and kept for incubation at room temperature for 30 min under dark conditions. Following this, the absorbance was noted at a wavelength of 517 nm and RSA was calculated based on the following formula:

RSA (%) = Abs. (control) - Abs. (sample)/ Abs. (control) X100

The extracts were examined in terms of total antioxidant status (TAS) by a colorimetric method developed by Erel [15]. In this method, Fenton reaction is used to produce hydroxyl radical and bright yellowish-brown dianisyl radical is produced by reacting with colorless substrate o-dianisidine. The results were demonstrated in micromolar Trolox equivalents per liter (µmol Trolox Eq/l).

***In-vitro* antimicrobial assay**

Antimicrobial activity of the extracts was determined by using the standard disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*. Petri dishes plated with Muller Hinton Agar (MHA) growth media were inoculated by adjusting with saline water to obtain a suspension at concentration of 1×10^6 CFU/ml using spectrophotometer, then 200 µl suspension of an overnight culture of test bacteria were spread plated to obtain uniform microbial growth using a sterile glass rod. Sterile Whatman filter paper discs (6 mm diameter) were soaked with 10 µl of extracts diluted in dimethyl sulfoxide (DMSO). This resulted in concentrations ranging from 5 to 15 µl/ml, which were then placed at intervals on the surface of the agar test plate. The positive control discs were saturated with 10 µl of ampicillin solution (15 µg/ml per disc), while the negative control used DMSO buffer. The plates were incubation at 37°C for 24 h and subsequently observed for the diameters of the zone of inhibition which were measured as millimetres and analyzes performed in triplicate. The mean values of the zone of inhibition were calculated to ascertain the antimicrobial activity of extracts [16-17].

***In-vitro* anthelmintic activity**

Earthworms (*Eisenia fetida*) were taken and carefully washed using normal saline to remove all faecal matter. Different concentrations (50 and 100 mg/ml) of *Z. officinale* (rhizomes) and *C. medica* fruit peel extracts were prepared using DMSO as the suspending agent and the final volume was adjusted to 10 ml. Albendazole (10 and 30 mg/ml) was used as the positive control. Normal saline served as the negative control and DMSO was used as the vehicle control. Two groups, each containing two equal-sized earthworms, were released into 10 ml of the desired concentrations of both extracts. Then, observed and recorded the time it took for the earthworms to become paralyzed and death. When the worms failed to be revived in normal saline, they were considered paralysed. The time of death for worms was recorded when their body colours began to fade and they stopped moving when shaken vigorously or submerged in warm water (50 °C) [18-19].

Statistical analysis

All experiments were conducted in triplicates, and the data obtained was statistically analysed, expressed as mean values ± SD.

Results and Discussion: Natural medicine has garnered significant interest, particularly due to recent global health crises and the rising occurrence of lifestyle-related conditions. It is considered a powerful, lower-risk, and safer substitute for conventional medicine, deriving medicinal elements from organic and naturally existing sources. Fruits and rhizomes play a crucial role in the human diet due to their abundance of beneficial phytochemicals. Extracts from various fruits, including citrus, berries, and pomegranates, have demonstrated wide-ranging medicinal benefits. The phytochemicals contained in fruits and rhizomes are particularly important due to their antioxidant properties, as they fight various pathogenic bacteria through different mechanisms. These phytochemicals exhibit antioxidant properties through several mechanisms, including scavenging free radicals and chelating or complexing metal ions. Phytochemicals found in fruits and rhizomes can have diverse effects on bacteria. They can disrupt the bacterial cell envelope, interfere with cell-cell communication and gene regulation, and inhibit metabolic and enzymatic functions. These phytochemicals have the ability to either directly or indirectly decrease microbial virulence by controlling the expression of factors that are associated with pathogenicity [20]. The phytochemicals present in the peel of *Citrus medica* fruit exhibit various medicinal properties, as our study has found. For example, n-hexadecanoic acid is a versatile compound that can act as a nematicide, antioxidant, pesticide, flavor enhancer, antifibrinolytic, hemolytic, lubricant, and anti-alopecic agent. Similarly, octadecanoic acid demonstrates hypocholesterolemic, nematicidal, antiarthritic, hepatoprotective, antiandrogenic, antihistaminic, anti-coronary, insectifuge, antieczema, and antiacne properties [21-22]. Palmitic acid, a type of essential fatty acid, is crucial in the diets of birds, invertebrates, and mammals.

Total phenolic and total flavonoid contents of ethanol and aqueous extract of the *Z. officinale* (rhizomes) and *Citrus medica* L. fruit peel

Phenolic compounds are aromatic plant derived molecules that contain one or more hydroxyl (OH) groups. These phenols exhibit a diverse array of beneficial biochemical activities, including antioxidant, antimutagenic, and anticancer properties, as well as the capacity to modulate gene expression. As the largest class of phytochemicals, phenols account for the majority of antioxidant activity found in plants and plant-based products [23-24]. Flavonoids, the largest class of naturally occurring phenolic compounds, are found in diverse plant tissues in both free and glycoside forms. These versatile molecules exhibit a wide range of biological activities, including antibacterial, anti-ulcer, anti-arthritis, anti-angiogenesis, and anticancer effects, as well as the ability to inhibit mitochondrial adhesion and protein kinases [25]. Total phenolic content (TPC) from ethanol extracts were found to have the highest TPC, i.e. 13.5±2.26 and 66±0.14 µg GAE/g dry weight, while total flavonoid content (TFC) ethanol extracts were found 183.17±8.12 and 464±0.30 of the *Z. officinale* (rhizomes) and *C. medica L.* fruit peel respectively (Table 1). Among the solvents, the yields of ethanolic *C. medica L.* fruit peel and *Z. officinale* (rhizomes) extracts was higher yields than aqueous extracts, i.e. 22.0 and 16.5% yield of ethanolic and aqueous *C. medica L.* fruit peel extracts, while 17 and 14% ethanolic and aqueous *Z. officinale* (rhizomes) extracts respectively. Ethanolic extracts of *Z. officinale* (rhizomes) were found to have higher amounts of TPC and TFC than aqueous extracts. The aqueous peel extracts of *C. medica* L. fruit had significantly higher total phenolic content (TPC) and total flavonoid content (TFC) than the ethanolic extracts. The

major proportion of TPC was found to be TFC in all peel and callus extracts. Phenolic compounds play a significant role in enhancing quality and nutritional value by influencing color, taste, aroma, and flavor, while also offering various health benefits. The results indicate that the portion with the greatest antioxidant capacity does not match the portion with the highest phenolic content. This discrepancy may stem from the presence of other antioxidant compounds in the fruit, including vitamins C, A, B, and E, as well as anthocyanins and carotenoids [26]. As expected, the total phenolic content and total flavonoid content of the aqueous extract were lower than the ethanol extract, due to the extraction procedure used. The decrease in phenolic acids and flavonoids found in water extraction could be due to oxidation of this chemical in the presence of water at high temperatures. Flavonoids are known to breakdown in the water at temperatures of 100 °C and above. The thermal stability of flavonoids varies based on the number, type, and positioning of substituents and hydroxyl groups. Compounds with fewer substituents tend to be less heat-stable [27]. As a result, the higher bioactive compounds in the ethanol extract than in the aqueous extract can be explained by the fact that the rhizomes are not ground and boiled water is used in the extraction process. Similarly, Martins *et al*. (2014) discovered that the hydro alcoholic extract of *Origanum vulgare* has higher antioxidant activity than the aqueous extract [28].

Determination of antioxidant activity as DPPH radical scavenging and total antioxidant status

The antioxidant properties were determined based on DPPH free radicals scavenging effects and total antioxidant status. DPPH radical scavenging activity in terms of percent inhibition of peel and rhizomes extracts in 80% (50-300 µg/ml) is presented in (Table 1). In the DPPH assay, the extracts act against scavenging activity to determine the IC_{50}. Therefore, a low IC_{50} value indicates greater scavenging capacity and increased antioxidant activity. The current findings showed that the DPPH scavenging activity of ginger samples increased significantly, especially in ethanol solvent. Fig 2 shows the percentage of ethanol extract's DPPH scavenging activity in comparison to the aqueous extract of *Z. officinale* (rhizomes) and *C. medica* fruit peel. All samples showed excellent DPPH inhibition. The ethanolic extracts of *Z. officinale* and *C. medica* demonstrated the most potent free radical scavenging activity. Furthermore, *C. medica* fruit peel (ethanol) samples revealed the highest radical inhibition (The lowest IC_{50}=42.5±0.17µg/ml), followed by *Z. officinale* (ethanol) (IC_{50}=48.5±0.12 µg/ml). The increasing order of DPPH scavenging activity in extracts was as follows: *C. medica L.* (ethanol) > *Z. officinale* (ethanol) > *C. medica L.* (aqueous)> *Zingiber officinale* (aqueous)> AA (control). Meanwhile, the samples extracted with water exhibited the lowest DPPH scavenging activity and the highest IC_{50} values ranging from 86.4±0.57 to 92.4±0.45 µg/ml, indicating low antioxidant capacity. Similarly, the DPPH inhibition activity depends on the extractable phenolic contents, such as reducing power activity. For ethanol extracts of *Z. officinale*, TAS increased significantly, showing the highest increment at 1.73 mg TE g-1, followed by *C. medica* fruit peel (1.02 mg TE g-1). The effect of antioxidants on DPPH derives from their hydrogen donating ability [29]. The aqueous extracts of *C. medica* fruit peel exhibited radical scavenging abilities comparable to that of ascorbic acid. The lower IC_{50} value is, the higher radical scavenging effect is; therefore, peal was found to have higher scavenging ability than ginger. Khalaf *et al*. found similar results in their own study when comparing the radical scavenging effect of *Zingiber officinale* with ascorbic acid [30]. Scavenging free radicals are proposed to be used for therapeutic expectations [31]. When comparing the DPPH radical scavenging values of water, water-ethanol, and ethanol extracts of galangal to those of BHA, BHT, trolox, and alpha tocopherol, Kose *et al*. discovered that the results were high but varied [32]. The studies show that the extract chemical and the compared material had differing effects on the results. Additionally, the antioxidant capacity values of any compound can vary depending on the measurement method used. *C. medica L.* fruit peel exhibited the maximum percent inhibition. The percentage of DPPH radical inhibition exhibited by different extracts of Stevia leaves and callus was observed to vary between 33.17% and 56.82% [33]. The antioxidant activities may be attributed to the phenolic and flavonoid contents, as it is reported that phenolic rings due to resonance stabilization of phenoxide ion contribute to antioxidant activities [34]. The aqueous extract demonstrated lower antioxidant capacity compared to the ethanol extract in our study. Ethanol is commonly used to extract tannins, polyphenols, and flavonols, producing extracts with potent antioxidant properties. The water extract process only leaches a small amount of flavonols into the polar solvent, resulting in preparations with inferior antiradical-scavenging capabilities. *C. medica* fruit peel extracts displayed notable antioxidant properties, comparable to the standard ascorbic acid. The radical scavenging activity (RSA) increased with the rising concentrations of *C. medica L.* fruit peel extract. It was observed that at the highest concentration of the fruit peel extract (900 µg/ml), a maximum RSA of 79% was achieved, while at its lowest concentration (30 µg/ml), the least RSA of 68% was observed. The lowest tested concentration of 50 µg/ml exhibited the highest antioxidant activity, outperforming the higher concentrations. Conversely, all the tested extracts demonstrated the least antioxidant activity at a concentration of 300 µg.

Antimicrobial activity assays of *Z. officinale (rhizomes)* and *Citrus medica L.* fruit extract

The *Z. officinale* (rhizomes) and *C. medica* fruit peel extract showed different degrees of growth inhibition of the assayed against the two bacterial species. The growth of the tested pathogens was affected by the concentration which ranged from 5 to 15 µl/ml. In this present study, strain A (*S. aureus*) was affectively controlled by the *Z. officinale* (rhizomes) and *C. medica* fruit aqueous and ethanol extract at 10 µl/ml and 5 µl/ml. The mean diameters of inhibition zone of *Z. officinale* and *C. medica* extracts for 10 µl/ml and 5 µl/ml were 14.0±1.0, 12.67±0.58, and 15.2±0.66, 13.2±1.8 mm, respectively. The inhibition areas of *E. coli*-strain-B for *Z. officinale* and *C. medica* aqueous and ethanol extract at 15 µl/ml similar concentration were 9.33±1.15, 11.25±0.76 and 13.0±0.5, 10.80±0.50 mm, respectively (Table 2). The *E. coli* species showed the smallest inhibition zone, with a mean diameter of 7.1 ± 0.9 mm at a concentration of 5 µl/ml. The minimum inhibitory concentration (MIC) is reported in Table 2. The MIC value of *Z. officinale* and *C. medica* aqueous and ethanol extract was 5-15 µl/ml.

Between 5 and 10 µl/ml of extract concentration, the differences in the inhibition against *S. aureus* and *Escherichia coli* were not significant ($p>0.05$). Peel extract of *C. medica* fruit prepared in 80% ethanol by Soxhlet extraction showed the highest antibacterial potential against *S. aureus* and *E. coli* (Table 2). Numerous studies have consistently identified fruit juice as the most potent antibacterial and antifungal agent, with juiceless pulp extract following closely. The varying sensitivity of test organisms to these antimicrobial compounds can be attributed to factors such as the specific nature and combination of phytocompounds present in the phytochemical crude extracts, as well as the inherent tolerance levels of the test microorganisms. Many phytochemicals, including phenols, flavonoids, alkaloids, glycosides, tannins, and acids, are known to be present in these extracts and to exhibit antimicrobial properties [35-37]. According to a study by Wang *et al*., ginger exhibits significant inhibitory effects against the bacteria *S. aureus* and *E. coli* [38]. A separate study found that ginger exhibits direct antibacterial properties, suggesting it could be used to treat a variety of bacterial infections. The agar well diffusion technique was used in the Ayla *et al*. study to determine the antimicrobial activity of methanol extracts of *L. officinalis* against *S. aureus* and *E. coli* [39]. In this investigation, inhibition zones measuring 12.67±0.58 and 10.33±0.58 against *S. aureus* and *E. coli* were obtained when the rhizomes, the part of *L. officinale*, were used at 10 µl/ml of extract concentration. The strong and weak antimicrobial activity was found for *S. aureus* (34 mm), and *E. coli* (23 mm), respectively when the extract concentration was 25%. The antimicrobial effect is considered as week (<12 mm), moderate (12 to ≤20 mm) and strong (≥20 mm) according to Pillai *et al* [40]. Ethanol extracts showed significant antibacterial activity against all tested bacterial strains ($p<0.05$). The aqueous extract of *Z. officinale* (rhizomes) and *C. medica* fruit exhibited a weak antimicrobial effect against *E. coli*, while the ethanol extracts of both plants demonstrated a moderate antimicrobial effect against *S. aureus* ($p<0.05$). Ethanolic extract of ginger was prepared by using dry ginger which was commercially available. Although, ethanol by itself has antimicrobial properties, this study is justified as the ethanol was evaporated when heated 40°C for 24 hrs. The tested ethanolic extract of ginger showed marked antibacterial activity against *Staphylococcus aureus*. The strongest inhibition activity of the ginger extract was observed against *Staphylococcus aureus* (14.0±1.0 mm zone) when diluted upto 10 µl when compared to *E. coli*. Though there is not much difference comparing the zone of inhibition of *Staphylococcus aureus* and *E. coli*, it is clear that the ethanolic extract of ginger is more efficient in gram positive organisms. It showed stronger inhibitory effects against *Staphylococcus aureus* when compared to *E. coli*. Previous studies have shown that soya beam extract of ginger has shown good antimicrobial activity against food borne pathogens-*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio cholera, Klebsiella spp*. and *Salmonella spp* [41]. Using the agar diffusion method, Sharef *et al*. investigated the antibacterial impact of ginger methanolic extract on *Escherichia coli, Pseudomonas aerogenosa, Proteus mirabilis, Staphylococcus aureus*, and *Klebsiella pneumonia* in comparison with the conventional antibiotics [42]. They have revealed that methanolic extracts of ginger showed stronger antimicrobial effect compared to Streptomycin, Rifambin and Cefotoxime. This study can clearly help interpreting the present results of ginger and *Citrus medica* extracts have effective antimicrobial properties than the standard antibiotics, though their moderate and week inhibition zones. The extracts' ability to inhibit bacterial growth is dose-dependent. The chemical characteristics of ginger may be responsible for the antibacterial and inhibitory effects of ginger extracts. Zingiberene is the primary component of sesquiterpenoids, which are the main components of ginger. Additional constituents consist of sesquiterpenoids such as β-sesquiphellandrene, bisabolene, and farnesene, as well as a trace amount of monoterpenoid fraction (β-sesquiphellandrene, cineol, and citral) [43].

Anthelmintic activity

The ethanol and aqueous extract of *Z. officinale* and *C. medica* exhibited varying degrees of anthelmintic activity against worms, causing paralysis followed by death at all tested concentrations. As shown in Table 3 and Fig 3, the extract of *Z. officinale* (rhizomes) demonstrated robust anthelmintic activity compared to the standard drug and *C. medica* fruit peel. Paralysis of earth-worms occurred within 26 and 16 min when exposed to concentrations of 30 mg/ml while 66 and 46 min for 10 mg/ml of *Z. officinale* (rhizomes) and *C. medica* fruit peel extracts respectively. The control treatments, DMSO (vehicle control) and normal saline (negative control), did not adversely affect the worms. When compared to conventional anthelmintic medications, the extracts of *Z. officinale* (rhizomes) and *C. medica* fruit peel demonstrated notable anthelmintic action in a dose-dependent manner. It was thus inferred that the *Z. officinale* (rhizomes) and *C. medica* peel comprised active constituents responsible for the anthelmintic activity of its aqueous and ethanol extract. The aqueous and ethanol extract of *C. medica* fruit peel demonstrated significant anthelmintic activity, including paralysis and death of worms at all tested concentrations. Both *in-vivo* and *in-vitro* studies on anthelmintic activity of various citrus fruits have consistently revealed their notable effects when compared to standard drugs [44]. Ethanol extracts from different citrus fruits, such as *Citrus auranti-folia*, *C. limon*, *C. reticulata* and *C. sinensis* have demonstrated substantial anthelmintic activities. Additionally, seed coats of *C. sinensis* fruit have been reported to exhibit significant anthelmintic activity [10, 45].

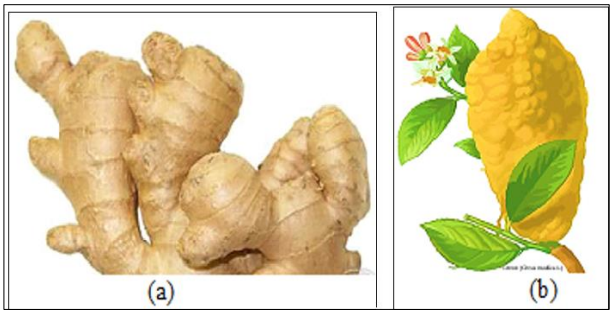

Fig 1: (a) Rhizome of *Zingiber officinale* (ginger), (b) Fruits of *Citrus medica* L. (citron)

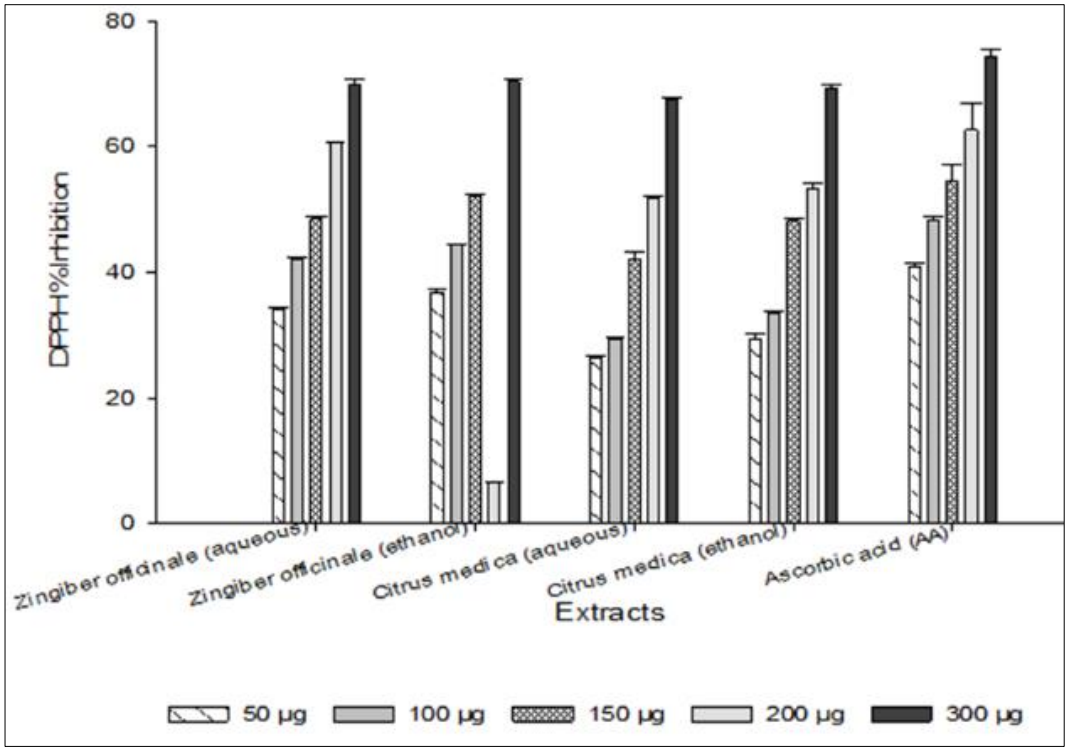

Fig 2: Graphical representation of DPPH radical scavenging activity in Ginger and *Citrus medica* L. peel extract

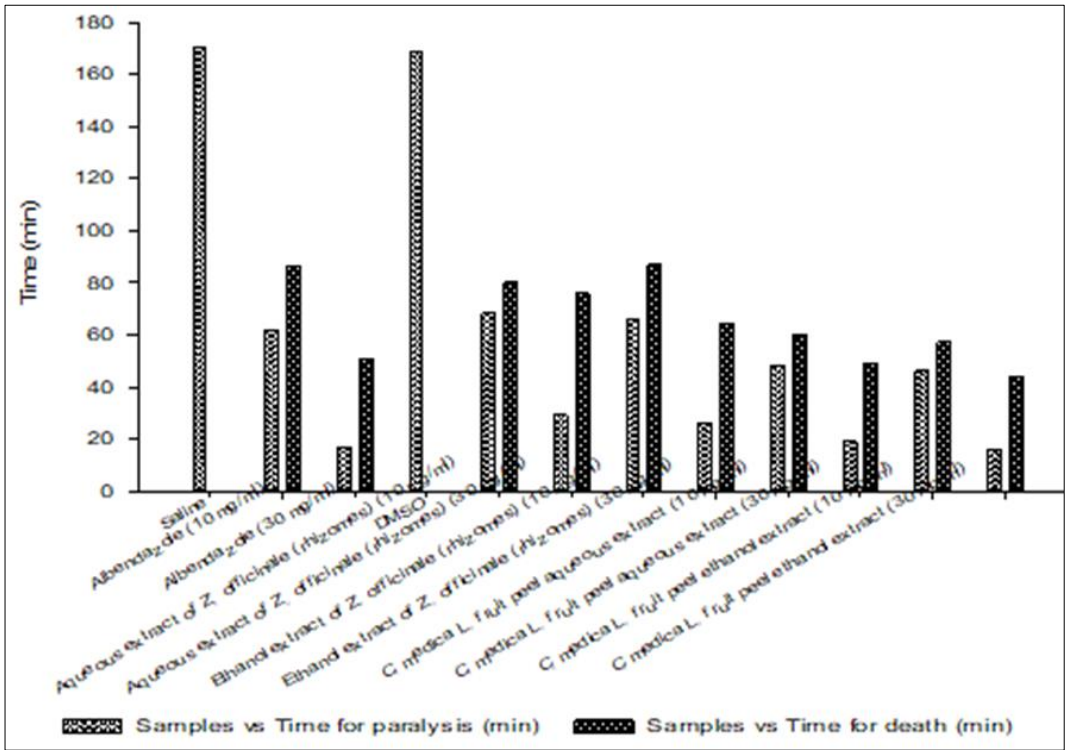

Fig 3: Anthelmintic activity of ethanol and aqueous extract of *Z. officinale* (rhizomes) and *C. medica* fruit peel

Table 1: DPPH radical scavenging and TAS activities of *Zingiber officinale* and *Citrus medica* extract

Antioxidant activities (Extracts/AA)	TPC (mg GAE.g^{-1})	TFC (µg CE.g^{-1})	DPPH (IC_{50} µg/ml)	TAS (mg TE.g^{-1})
Zingiber officinale (aqueous)	30.63± 0.22	133.20±4.16	86.4±0.57	4.1
Zingiber officinale (ethanol)	43.5 ± 2.26	183.17±8.12	48.5±0.12	1.03
C. medica L. (aqueous)	48.3±0.10	342±0.16	92.4±0.45	6.7
C. medica L. (ethanol)	57.6±0.14	464±0.30	42.5±0.17	1.72
Ascorbic acid (AA)	-	-	94.7±0.70	6.8

Mean ± standard deviation of triplicate measurements

Table 2: Antimicrobial activity of the ethanol and aqueous extract of *Z. officinale* (rhizomes) and *C. medica* fruit peel (inhibition zone, mm)

Strains/Bacteria/extract	Diameter of Inhibition Zone (mm)			MIC (µg/disc)
	5 µl/ml	10 µl/ml	15 µl/ml	
Aqueous extract of *Zingiber officinale* (Ginger)-strain A (*S. aureus*)	13.5±1.5	14.0±1.0	13.93±1.15	10.00
Aqueous extract of *Citrus medica* - strain A (*S. aureus*)	12.5±2.0	12.67±0.58	10.4±2.2	10.00
Aqueous extract of *Zingiber officinale* (Ginger) - strain B (*E. coli*)	8.4±0.6	9.31±1.6	9.33±1.15	15.00
Aqueous extract of *Citrus medica* - strain B (*E. coli*)	7.1±0.9	10.83±0.76	11.25±0.76	15.00
Ethanol extract of *Zingiber officinale* (Ginger) - strain A (*S. aureus*)	15.2±1.0	12.0±1.0	11.0±1.2	5.00
Ethanol extract of *Citrus medica* - strain A (*S. aureus*)	13.2±1.8	10.67±0.58	11.4±1.6	500
Ethanol extract of *Zingiber officinale* (Ginger) - strain B (*E. coli*)	10.2±1.4	12.4±1.5	13.0±0.5	15.00
Ethanol extract of *Citrus medica* - strain B (*E. coli*)	9.1±0.6	10.00±1.00	10.80±0.50	15.00
Aqueous- Control (Ampicillin)	11.05±1.0	12.80±0.5	16.82±0.5	15.00

Values are expressed as mean ± standard deviation.
Ethanol extracts showed significant antibacterial activity ($p<0.05$) against tested bacterial strains

Table 3: Anthelmintic activity of the ethanol and aqueous extract of *Z. officinale* (rhizomes) and *C. medica* fruit peel

Samples	Time for paralysis (min)	Time for death (min)
Saline	170	-
Albendazole (10 mg/ml)	62	86
Albendazole (30 mg/ml)	17	51
Dimethyl sulfoxide (DMSO)	169	-
Aqueous extract of *Z. officinale* (rhizomes) (10 mg/ml)	68	80
Aqueous extract of *Z. officinale* (rhizomes) (30 mg/ml)	29	76
Ethanol extract of *Z. officinale* (rhizomes) (10 mg/ml)	66	87
Ethanol extract of *Z. officinale* (rhizomes) (30 mg/ml)	26	64
C. medica L. fruit peel aqueous extract (10 mg/ml)	48	60
C. medica L. fruit peel aqueous extract (30 mg/ml)	19	49
C. medica L. fruit peel ethanol extract (10 mg/ml)	46	57
C. medica L. fruit peel ethanol extract (30 mg/ml)	16	44

Conclusion

Two distinct solvent extracts from *Zingiber officinale* (Rhizomes) and *Citrus medica* fruit peel were evaluated for their antioxidant activities and ethanol extracts demonstrated a superior antioxidant capacity, likely attributed to the presence of flavonoid compounds with hydroxyl groups that enhance hydrogen donating ability. Furthermore, the antimicrobial and anthelmintic properties of the selected plant materials were assessed against bacterial strains in the laboratory, revealing significant inhibition and paralysis effects on tested organisms. This study suggests that extracts from *Z. officinale* and *C. medica* possess notable antimicrobial, anthelmintic, and antioxidant activities. *Zingiber officinale* and *Citrus medica*, although widely recognized, presenting a valuable source of phytocompounds and highlighting their potential for future research and drug development.

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