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In vitro antibacterial activity of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) aqueous extracts against isolates of *Brucella abortus*

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Abstract

Microbial resistance to antibiotics has become a problem plaguing the world. Currently, interest has been focused on exploring antimicrobial properties of plants and herbs. This work aim to evaluate the antibacterial activity of garlic (*Allium sativum*) bulbs and ginger (*Zingiber officinale*) rhizome on *Brucella abortus* isolates. Some concentrations of garlic and ginger extracts were tested for their antibacterial activity against *B. abortus* isolate brought from Central Veterinary Research Laboratory (CVRL), Soba, using well diffusion method. Moreover, minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of garlic and ginger were tested using broth dilution method. Sensitivity pattern of the conventional antibacterial against common pathogenic bacteria was tested using disc diffusion method. Aqueous extract of ginger produced dose-dependent increase in the zone of inhibition at a concentration of 15% and higher, whereas the garlic extract produced inhibition zone at a concentration of 5% and higher, i.e. *B. abortus* isolate showed relatively high sensitivity toward garlic extract than ginger which required a more concentrated extract to kill or inhibit *B. abortus* isolate that brought from (CVRL), Soba, Khartoum, Sudan. Further studies are needed to find out the efficacy, safety, and kinetic data of their active ingredients.

Keywords: Antibacterial Activity; Garlic; Ginger; *Brucella abortus*

1. Introduction

Brucellosis is a zoonotic disease with a worldwide distribution that is endemic in the world. *Brucella* has thirteen different recognized species, one being *B. abortus* (1). *B. abortus* remains a major cause of morbidity in humans and domestic animals (2). After invasion of the lymphoid system, the bacteria are developed within mononuclear phagocytes, and the infected cells play a crucial role in the spreading of the bacteria in specific locations of the body such as spleen, brain, heart, and bones (3). *Brucella* species virulence and chronic infections are thought to be due to their ability to escape killing mechanisms within macrophages, such as lysosomes enzymes and production of the oxidative burst (4). *B. abortus* is a zoonos is which causes abortion and infertility in adult cattle and is present worldwide (5). Humans usually infected when drinking unpasteurized milk from affected animals or when coming into contact with infected tissues and liquids (6). Temperature can be a limited factor for *B. abortus*. It can survive for a longer period at a cooler temperature (1).

Food and pharmaceutical industries still need to find new and improved antimicrobial agents effective against brucellosis. In spite of the improvements in food hygiene and production techniques, food safety is the most important

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public health issue (7). For this reason, to produce safe foods new methods are still needed, to reduce or inhibit foodborne pathogens (8). Because of increasing pressure from consumers and legal authorities, food industry has tended to reduce the use of chemicals or to adopt more natural alternatives for the maintenance or extension of product shelf life. Plants essential volatile oil and aqueous extracts are becoming more important due to their proved antimicrobial effects. One such botanical is garlic.

Garlic (*Allium sativum*), family Liliaceae, falls within the onion group. The bulb and cloves of the garlic are the most commonly used parts for medicinal purpose. It has a pungent odor, and antibacterial activity depends on the sulfur-containing compounds, the major one being allicin (9). Garlic is suggested to be originated in Central Asia and northeastern Iran. It has long been used worldwide, with a history of several thousand years of human trading activities (10). It has been used both in food processing and in some traditional medicine preparations. Garlic is usually used in food either raw or cooked. The garlic bulb is the only used part of the plant in most countries (11). Garlic contains essential compound such as saponins, glycosides, sterols, flavonoids (12).

Ginger (*Zingiber officinale*), family Zingiberaceae, is a flowering plant. Ginger root or rhizome, is worldwide used in food processing and in traditional medicine (13). It is a herbaceous perennial plant, and its inflorescences consisted of pale yellow and purple flowers that arise on separate shoots directly from the rhizome (14). Ginger is native to Southeast Asia Islands, and it was one of the oldest species exported from Asia to Europe (15). Ginger usually has a hot taste and fragrant flavor (16). The volatile oils of ginger rhizome compose 1-3% of the fresh weight, were responsible for the characteristic fragrance and flavor (17). Ginger contains essential compounds such as saponins, glycosides, sterols, flavonoids, and alkaloids (12).

Throughout history, many different cultures have recognized the potential use of garlic and ginger for prevention and treatment of different diseases. Recent researches have focused on four main areas: Heart disease, cancer, infectious disease, and antioxidant effects. Indeed, garlic is found to have antihypertensive (18), antioxidant (19), antiplatelet (20) antitumor (21), and lipid-lowering actions (22) Moreover, a recent study also points out towards the antimicrobial action of garlic (23, 24, 25).

This work aim to evaluate the antibacterial activity of the aqueous extracts of garlic (*A. sativum*) bulbs and ginger (*Z. officinale*) rhizome on *B. abortus* isolates

2. Material and methods

2.1. Collection of Plant Material

Both Ginger (*Z. officinale*) rhizomes and Garlic (*A. sativum*) bulbs that were used in this study to test their anti-*Brucella* activities were obtained from the big market in Wad Medani City, Gezira State, Sudan. The plant parts were left to dry in shade away from direct sunlight at room temperature. Each drought plant part was crush to a fine powder for the preparation of the aqueous extract for antibacterial tests.

2.2. Preparation of Aqueous Extracts

Each extract was prepared as a series of concentrations of 5, 10, 15 and 20% by dissolving 5, 10, 15 and 20 g of each powder in 100ml sterile distilled water, respectively, according to (Wattman) method for 24 hour. After shaking, the solids were removed by filtering the plant extracts through filter paper, then it was put in sterile bottles and kept in refrigerator until used.

2.3. Recovering of *B. abortus* Isolate

Hygroma sample collected from bovine was cultured, purified and identified according to criteria outlined by Alton *et al.*, (26), on the basis of cultural characteristics, colonial, morphology, gram reaction and biochemical tests as *B. abortus*. These tests were performed in Department of Bacteriology, Centre Veterinary Research Laboratory (CVRL), Soba, Khartoum.

2.4. Preparation of *B. abortus* Inoculum

Discrete colonies 3-4 from pure *B. abortus* culture were suspended in 3 ml normal saline and thoroughly mixed to provide homogenous suspension.

2.5. Seeding of Plates

By means of sterile cotton wood swab dipped into *B. abortus* suspension gently passed over the plate surface to spread evenly its suspension, left to dry in incubator for 10 minutes before used (never stored).

2.6. Purification of Cultures

This was obtained by sub culturing of typical and well isolated colony into nutrient agar plate. Growth was checked for purity by examining smears stained by Gram's method and under the microscope.

2.7. Staining Methods

The staining procedures were carried out according to Barrow and Felltham ⁽²⁷⁾. Gram-positive bacteria appeared purple, while Gram negative organisms stained red.

2.8. Preparation of Culture Media

All media used in this study were dispensed under aseptic condition in laminar air flow cabinet type 11 (Prettl R, Germany). Provided with fan, ultra violet lamp and flame. Media were obtained in dehydrated form.

The semi-solid media (Broth media (Tryptone Soya agar (TSA) Oxoid, CM3), Brain Heart infusion agar, Blood agar, Brain heart infusion broth (Oxoid, CM225), Trypose Soya Broth, were prepared according to manufacturer's instruction, and then stored at optimum (2-8°C) conditions before used.

Solutions, indicators (Andrade's indicator, Methylene blue, Rose Bengal Antigene) and sterilization, followed the standard methods.

2.9. Assay for Antibacterial Activity

Antibacterial was determined using the three methods (Tryptone Soya Agar, Brain Heart Infusion Agar, Blood Agar). Four concentrations of each extract were examined (5%, 10%, 15% and 20%).

2.10. Disc Diffusion Method

The tested organism was suspended in nutrient broth, 0.2 ml of the suspended broth was added to 2 ml of nutrient broth media, and shaken well. The mixture was flooded onto agar plates and left for 10 minutes, the excessive solution was aspirated completely. Four filter papers disc (5 mm diameter) were placed onto quarter of agar plate uniformly seeded with the tested organism and one disc embedded in distilled water was placed as control. This procedure was performed under strict aseptic condition. Four replicates were done. Then plates were incubated at 37°C for 48 hrs. The diameter of each zone of inhibition was measured in mm by transparent ruler, and the antibacterial activity was expressed as the mean diameter of zone inhibition (mm).

2.11. Well diffusion method

To 0.2 ml of nutrient broth culture of the tested organism, 2 ml of sterile nutrient broth medium were added, then shaken well and the mixture was poured on to the Petri dishes and left to 10 minutes for drying. Five wells (8 mm diameter) were made by means of sterile micropipette tips and the agar cut was removed. The extract volume of 0.2 ml from each prepared concentrate was carefully dispensed into the wells, and the plates were incubated for 72 hrs at 37°C. This procedure was performed under aseptic condition. Four replicates were done and the diameter of each zone of inhibition was measured by transparent ruler and recorded. The antibacterial activity was expressed as the mean diameter of inhibition zone (mm).

2.12. Broth Dilution Method

To 1.8 ml nutrient broth used as diluents, 0.2 ml of *B. abortus* culture was added to give 2.0 ml culture. 0.2 ml of each concentration (5, 10, 15, 20%) was added to the *B. abortus* suspension, incubated at 37°C aerobically for up to 48 hrs, and examined for bacterial inhibition (transparency of the medium).

2.13. Incubation Conditions

All cultures (plates and tubes) were incubated aerobically at 37°C for 72 hrs before reading of the results. The clear zones of the inhibition around the wells and discs were measured in (mm) and recorded, while the tubes showing

change of colour, the antibacterial activities were estimated as (+) adversely to depth of colour changes of the indicator used (Andrade's or methylene blue).

3. Results

3.1. Anti-*Brucella* Activities of Ginger Extract

3.1.1. Inhibition Zones of Well Diffusion Method

Concerning ginger aqueous extract (Table, 1), both concentrations 5% and 10% revealed 8 and 9.5 mm inhibition zone, respectively, to *B. abortus*. While 15% and 20% concentrations showed absolute inhibition as 12 and 14 mm diameter, respectively. The inhibition zone was clearly noticed in culture.

Table 1 Inhibition zone (mm) of *B. abortus* treated with ginger extract through Disc Diffusion Method

Concentration	Tryptone Soya Agar (TSA)	Blood Agar(BA)	Brain Heart Infusion Agar (BHIA)
5%	8.5	8	8
10%	9.5	9	9
15%	12	12	12
20%	14	13	14

3.1.2. Inhibition Zones of Disc Diffusion Method

Filter paper discs impregnated in 5% and 10% concentrations of ginger aqueous extract (Table, 2) gave no inhibition zone, while disc with 15% and 20%, showed cleared inhibition of 8 and 9 mm diameter, respectively.

Table 2 Inhibition zone (mm) of *B. abortus* treated with ginger extract through Well diffusion method.

Concentration	Tryptone Soya Agar (TSA)	Blood Agar(BA)	Brain Heart Infusion Agar (BHIA)
5%	-	-	-
10%	-	-	-
15%	8	8	8
20%	9	9	9

3.1.3. Inhibition Zones of Broth Method

Concentrations of 5% and 10% showed no anti-*Brucella* activity (Table, 3). The cultures noticed turbid and the indicator changed to pink (Andrade's indicator) or yellow (methylene blue), this means *B. abortus* was grown and multiplied, whereas 15% and 20% showed high anti-bacterial activity, since that, the colour was not changed.

Table 3 Characteristics of *B. abortus* to garlic and ginger aqueous extracts constituting Andrade's indicator

Concentration	Garlic	Ginger
5%	+	-
10%	++	+
15%	+++	+
20%	++++	++

(-) Mean absence of antimicrobial activities in extracts (deep red)

(+) Mean presence of antimicrobial activities in extracts (light red)

(++) Mean strong presence of antimicrobial activities in extracts (very light red)

(+++)

(++++)

3.2. Anti-*Brucella* activities of Garlic Extract

3.2.1. Inhibition Zones of Well Diffusion Method

Concentrations of (5, 10, 15, and 20%) garlic aqueous extract (Table, 4), appeared inhibition zone of 10, 12, 16, and 18 mm, respectively, on plates seeded with *B. abortus*.

Table 4 Inhibition zone (mm) of *B. abortus* treated with garlic extract through Well diffusion method.

Concentration	Tryptone Soya Agar (TSA)	Blood Agar (BA)	Brain Heart Infusion Agar (BHIA)
5%	10	10	10
10%	12	12	12
15%	16	16	16
20%	18	18	18

3.2.2. Inhibition Zones of Disc Diffusion Method

Clear zones of inhibitions were obtained and measured as 10, 13, 16, and 20 mm around the disc containing 5%, 10%, 15%, and 20% garlic extract concentration, respectively, on Broth plates of BHIA and TSA cultured with *B. abortus* (Table, 5).

Table 5 Inhibition zone (mm) of *B. abortus* treated with garlic extract through Disc Diffusion Method.

Concentration	Tryptone Soya Agar (TSA)	Blood Agar (BA)	Brain Heart Infusion Agar (BHIA)
5%	11	10	10
10%	13	13	13
15%	16	16	16
20%	20	20	20

3.2.3. Inhibition Zones of Broth Method

With regard to garlic extract that added to the broth culture of *B. abortus*, all concentrations showed relative anti-*Brucella* activity (Table, 6), all cultures noticed without any turbidity and the indicator did not change to pink or yellow, this mean *B. abortus* wasn't grown or multiplied i.e. the depth of culture colour adversely related to the garlic extract concentrations.

Table 6 Appearance of *B. abortus* to garlic and ginger aqueous extracts containing methylene blue indicator.

Concentration	Garlic	Ginger
5%	+	-
10%	++	+
15%	+++	+
20%	++++	++

(-) Means absence of antibacterial activity in extract (deep yellow).

(+) Indicated traces of antibacterial (Yellowish colour).

(++) Means the extract containing antibacterial activities (Slightly blue).

(+++ and +++) Confirm the presence of extract antibacterial activities.

4. Discussion

Since 1940s, the improvement of effective and safe drugs to deal with bacterial infections has revealed medical treatment, and the morbidity and mortality from microbial disease have been dramatically reduced. Regrettably, the development of effective antibacterial drugs has been accompanied by the emergence of drug-resistant organisms. The

phenomenon of resistance imposes serious constraints on the options available for the medical treatment of many bacterial infections.

The present study has demonstrated that garlic and ginger extracts effectively inhibited the growth of *B. abortus* though its sensitivity to the garlic and ginger, and this can be due to their chemical composition.

Khalifa and Kehail (12) running GC and phytochemical screening for garlic bulbs and ginger rhizome obtained from the same study area. The GC-MS database identified 7 different compounds from ginger rhizome, and only 4 different compounds from the garlic bulbs. The qualitative phytochemical screening of garlic bulb detected the presence of saponins, flavonoids, glycoside, sterols and tannins, whereas that of ginger rhizome contained the same classes in addition to alkaloids.

In other study, allicin exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action. The structural differences of the bacterial strains may also play a role in the bacterial susceptibility to garlic constituents. Apart from antimicrobial action, garlic is found to have antifungal activity (23, 24).

It is clear that garlic and ginger may be useful as an antimicrobial agent against to the *B. abortus*. The present study suggests that garlic and ginger are active against the organisms that are found to be resistant to conventional antibiotics, tables 1 and 2 demonstrate the activity of ginger extract in various concentrations in different media by using disc diffusion and wheel diffusion methods, also table 4 and 5 demonstrate the activity of garlic extract in different concentrations by using different media and different methods to assess the effectiveness. The obtained results are in agreement with that reported in some previous studies (24, 25, 28). Furthermore, studies also indicate that combination of garlic and ginger extracts with conventional antimicrobials leads to partial or total synergism (29, 30, 31, 32)

5. Conclusion

garlic bulbs and ginger rhizome aqueous extracts showed antibacterial activity on *Brucella abortus*, hence, TLC and GC-MS should be used to separate and identified the active components from garlic bulbs and ginger rhizome in order to biosynthesis of a promising selective treatment against brucellosis.

Compliance with ethical standards

The present research work does not contain any studies performed on animals/humans subjects by any of the authors'

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Disclosure of conflict of interest

All authors (Ayda, Ali, Firooz and Mutaman) declare no conflicts of interest regarding the publication of this paper.

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