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COMPARISON OF ANTIMICROBIAL ACTIVITY OF SEED OIL OF GARLIC AND *MORINGA OLEIFERA* AGAINST SOME FOOD-BORNE MICROORGANISMS

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ABSTRACT

This study was aimed at evaluating the phytochemical constituents and the antimicrobial activity of the seed oil of Moringa oleifera and garlic against some selected food-borne microorganisms (Staphylococcus aureus, Escherichia coli, Salmonella spp and Pseudomonas aeruginosa) using disc diffusion method. The results of the phytochemical screening revealed differences in the presence of the phytochemicals among the extracts. Saponins were detected in both Moringa oleifera and garlic seed oil, while alkaloid and tannins were observed in seed oil of garlic. Furthermore, the antibacterial assay results shows that the seed oil of Moringa oleifera was inactive against all the tested organisms, even at 100 % concentration. In contrast, garlic oil was found to be active against all the tested organisms. The highest inhibition was observed in E. coli (12 mm) at 100 % concentration, while at 20 % concentration, Salmonella Sp and P. aeruginosa showed resistance (00 mm). The antimicrobial activity of the seed oil of garlic may be attributed to its phytochemicals components which were not detected in the seed oil of Moringa oleifera. The results of this study have shown the potentials of the seed oil of garlic as an antimicrobial agent more especially in foods, by inhibiting the growth of the test organisms, which range from food-borne pathogens to food spoilage organisms.

Keywords: Antimicrobial, garlic, Moringa oleifera, and food borne pathogens

INTRODUCTION

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is diminished. This type of bacterial resistance to the antimicrobial agents poses a very serious threat to public health, and for all kinds of antibiotics, including the major last-resort drugs, the frequencies of resistance are increasing worldwide. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products (Kingston, 2008). Medicinal plant is any plant in which one or more of its parts contains substance (phytochemical) that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. About 80% of the world medicines are originally derived from plants sources especially those found in tropical regions. Phytochemicals are bioactive chemicals of plant origin. They are thus regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently in vogue in parts of the world (Solomon. *et al.*, 2013). In addition, medicinal plants have been identified and used throughout human history. Plants

have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Recently, considerable attention has been paid to eco-friendly and bio-friendly plants, which can prevent and cure different human diseases (Dubey *et al.*, 2004). World Health Organization reported that the use of traditional medicine in the first world countries is on the rise due to failure of conventional medicine that can cure chronic diseases, emergence of multi-drug resistant pathogens and parasites, adverse effects of chemical drugs, increasing cost and information of herbal medicine. Antimicrobial potentiality of different medicinal plants is extensively studied all over the world (Ahmed *et al.*, 1998). However, only a few studies have been carried out in a systematic manner. *Moringa oleifera* is a medicinal plant species, belonging to monogeneric family Moringaceae (order Brassicales). Almost all the parts of this plant: root, bark, gum, leaf, pods, flowers, seeds and seeds oil have been used for the various ailments in the indigenous medicine (Odebiyi and Sofowora, 1999). It is also known for its anti-helminthic activity, antimicrobial activity, detoxifier, immune booster and anti parasitic activity (Thilza *et al.*, 2010), among others. *Moringa oleifera* is an important food commodity which has had enormous attention as the 'natural nutrition of the tropics'.

The leaves, fruit, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa (Anwar and Bhangar, 2003; Anwar *et al.*, 2005). *Moringa* leaves have been reported to be a rich source of β -carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Dillard and German, 2000; Siddhuraju and Becker, 2003).

Garlic (*Allium sativum* L.) is among the oldest cultivated plant which is used for therapeutic purposes. Garlic has played one of the most important dietary and medicinal roles in human bodies for centuries and is used as a spice as well as medicinal herb. There are over 300 varieties of garlic grown world wide. In addition to the well known garlic and numerous other species are extensively grown for cooking purpose, such as leek (*Allium porrum* L.), scallion (*Allium fistulosum* L.), and Chinese chive (*Allium tuberosum* L.) (Nuutila *et al.*, 2002). The biological and medicinal functions of members of Alliaceae family are mainly due to their high organosulphur compound contents. It also contains many other sulfur containing compounds such as allin, ajoene, diallylsulfide, dithin, S-allylcysteine, and enzymes, vitamin B, proteins, minerals, saponins, flavonoids, and mallard reaction products, which are non-sulfur containing compounds (Kojuri *et al.*, 2007).

The plant which is of great medicinal importance takes place inside many foods especially meat ones due to its sharp odour, appetizer property and its calorie value is 140, has 63.8 g water, 28.2 g carbohydrate, 5.3 g protein, 0.2 g oil and 11 g cellulose in it 100 g (Baytop, 1999). Garlic can be consumed as fresh and has also its pills, capsules and extracts. While it is safe, when taken in careful amounts, it can lacerate stomach, when consumed in excessive amounts (Ayaz and Alpsoy, 2007). There is a wide range of reported therapeutic effects, such as hypolipidaemic, antiatherosclerotic, hypoglycaemic, anticoagulant, antihypertensive, antimicrobial, antidote (heavy metal poisoning) and hepatoprotective, preventing cold and flu symptoms through immune enhancement and exhibit anticancer and chemopreventive activities (Amagase, 2006). The antimicrobial of crushed garlic have been known for a long time. A wide range of effects antimicrobial properties including antimicrobial activity has been reported for crushed garlic (Bakri and Douglas, 2005). Recent chemical characterisation of sulphur compounds has shown that they are the main active antimicrobial agents (Rose *et al.*, 2005). Therefore, keeping in view the importance of these plants as important medicinal foods, this research was therefore, aimed at evaluating the phytochemical constituents and the antimicrobial activity of seed oils of garlic and *Moringa oleifera* against some food-borne microorganisms.

MATERIALS AND METHODS

(a) Collection of the plants oil

Factory extracted seed oils of *Moringa oleifera* and garlic with Batch no. 0034, B001, Manufacture date; 23/02/2013, 02/08/2013, and Expire date; 22/02/2016 and 01/08/2016 respectively were purchased from herbalist in Gombe main market, and were authenticated by a botanist at the Biological Science Department, Gombe State University.

(b) Sample processing

Strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* Sp., Previously isolated from dried sliced beef (Kilishi), at the Microbiology laboratory of Gombe State University, were collected and inoculated onto freshly prepared Eosin Methylene Blue Agar (EMBA), Mannitol Salt Agar (MSA), Cystine Lactose Electrolyte Deficient Agar (CLEDA) and *Salmonella Shigella* Agar (SSA) respectively. And were then incubated at 35 °C – 37 °C for 24 hours (Greenwood, 2002). Plates with hundred or more were regarded as significant growth. Colonies with different morphological characteristics were subcultured onto freshly prepared nutrient agar plates, and incubated aerobically at 37 °C for 24 hours to obtain pure form (Cheesbrough, 2006). All the media were prepared according to manufacturers instruction. Isolated strains were Gram stained and then subjected to biochemical test which include; coagulase, catalase test, motility test, Lysine decarboxylase (LDC), Indole, urease, oxidase, citrate test and kligler iron agar (KIA) test, as described by Cheesbrough (2006), for confirmation.

(c) Phytochemical Screening

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins and saponins was performed on each of the oils, as described (Solomon *et al.*, 2013).

(i) Test for Alkaloids (Wagner's reagent)

1 ml of each of the oils in separate tubes was treated with 3-5 drops of Wagner's reagent. Formation of reddish brown precipitate (or colouration) indicates the presence of alkaloids.

(ii) Test for Flavonoids (Alkaline reagent test)

1 ml of each of the oils in separate tubes was treated with few drops of 20 % sodium hydroxide solution, and observed for the formation of intense yellow colouration, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

(iii) Test for Saponins (Foam test). About 3 ml of water was added to 1 ml of each of the oils in separate tubes and shaken vigorously, formation of persistent foam, confirms the presence of saponins.

(iv) Test for Tannins (Braymer's test)

1 ml of each of the oils in separate tubes was treated with 10 % alcoholic ferric chloride solution and observed for the formation of blue or greenish coloured solution.

e) Standardization of Inoculum

The inocula were prepared from stock cultures, which were maintained on nutrient agar slant at 4°C and subcultured onto nutrient broth using a sterilized wire loop.

The density of suspension inoculated onto the nutrient broth was determined by comparison with 0.5 McFarland standard of barium sulphate solution (Cheesbrough, 2006).

(f) Antimicrobial susceptibility test

Mueller Hinton agar was prepared and sterilized as instructed by the manufacturer, the medium was poured into different petri dishes and allowed to cool. Using sterile swab sticks, each of the isolates was streaked across different plates. The inoculated plates were then allowed to stay for about 3-5 minutes. Prepared discs of the four different concentrations; 20 % (v/v), 40 % (v/v), 60 % (v/v) and 100 % of each of the oils were placed on the organism inoculated plates, with each of the plates containing different concentrations of one type of oil. Gentamicin (10µg) (Rapid labs, UK) was used as positive control while discs impregnated with DMSO was used as the negative control. Within 30 minutes of discs application, the plates were incubated aerobically in an inverted position at 37 °C for 24 hours. The diameter

of the zone of inhibition was measured to the nearest millimeter (mm) using a millimeter rule, as modified by Kirby-Bauer technique (Cheesbrough, 2006). For each test organism, two replicates were performed.

RESULT AND DISCUSSION

Table I: Shows the phytochemical screening of seed oil of *Moringa oleifera* and Garlic. The result revealed the presence of alkaloid, saponins, and tannins in seed oil of Garlic. Whereas only saponins were detected in seed oil of *Moringa oleifera*.

Table II: Shows the efficacy of various concentrations of the seed oil of *Moringa oleifera* and Garlic against the bacterial isolates. The result indicated that seed oil of *Moringa oleifera* was inactive against the tested organisms, even at 100 % (v/v) concentration. In contrast, Garlic oil was active against all the tested microorganisms. The result further revealed that the higher the concentration of the seed oil of garlic, the higher its efficacy. Highest sensitivity was observed at 100 % (v/v) concentration and the least at 20 % (v/v) concentration.

Table I: Shows the phytochemical characteristics of seed oil of *Moringa oleifera* and Garlic

Phytochemicals	<i>M. Oleifera</i> oil	Garlic oil
Alkaloids	--	+
Flavonoids	--	--
Saponins	+	+
Tannins	--	+

Key: + = Present, -- = Absent.

Table II: Shows the antibacterial activity of seed oil of *Moringa oleifera* and Garlic

Oil sample	Conc. %(V/V)	Zone of Inhibition (mm)			
		STA	EC	SS	PA
<i>M. oleifera</i> oil	20	0	0	0	0
	40	0	0	0	0
	60	0	0	0	0
	80	0	0	0	0
	100	0	0	0	0
	+V	28	28	27	23
Garlic oil	20	07	07	00	00
	40	08	08	07	07
	60	08	09	08	07
	80	09	09	09	08
	100	11	12	11	10
	+V	28	29	26	24

Key: STA = *Staphylococcus aureus*, EC = *Escherichia coli*, SS = *Salmonella sp*, PA = *Pseudomonas aeruginosa*, +v= Positive control (Gentamicin) -- = No inhibition.

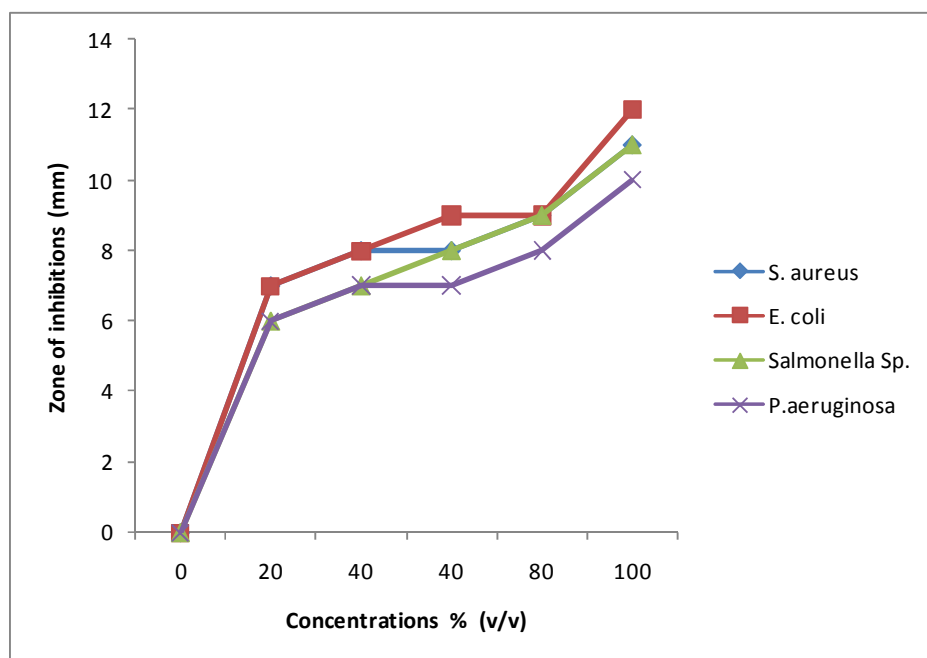


Fig. 1 Antimicrobial activity of seed oil of Garlic against *E.coli*, *S. aureus*, *Salmonella* Sp. and *Pseudomonas aeruginosa*.

This research was aimed at evaluating the phytochemical constituents and the antimicrobial activity of seed oils of garlic and *Moringa oleifera* against some food-borne microorganisms. Phytochemical screening of the seed oil of *Moringa oleifera* and Garlic revealed the presence of saponins, alkaloids, and tannins in Garlic oil, whereas, only saponins were observed in seed oil of *Moringa oleifera*. This result is in accordance with the work carried out by Bukar *et al.* (2010) on antimicrobial profile of *moringa oleifera* lam extracts against some food-borne microorganisms, which shows the absence of alkaloids, flavonoids, and tannins in *M. oleifera* seed chloroform extract, only saponins were detected. Similarly, active constituents like, alkaloid, saponins, and tannins with exception of flavonoids were detected in garlic oil. Interestingly, the findings in this work is in agreement with the work of Muhammad *et al.* (2014), who reported that tannins and alkaloids were present in garlic seed extract. But in contrast to this research, flavonoids was reported to be present in the garlic seed extract. However, saponins were observed in garlic oil in this work, which was not reported by Muhammad *et al.* (2014). Furthermore, Farooq *et al.* (2007) reported that plants occur in varying habitats, and a great magnitude of variation in the concentration and composition of phytochemical ingredients in the different parts of such plant is expected. In addition, Waller and Nowacki (1978), reported that phytochemicals are produced in response to perceived threats by the plants, as such variation exist in the production of these phytochemicals depending on the type and amount of threat encountered by the plant. The antibacterial activity of various concentrations of the seed oil of *Moringa oleifera* and Garlic against the food-borne bacterial isolates, revealed that the seed oil of *Moringa oleifera* was inactive against all tested

organisms, even at 100 % (v/v) concentration. This findings is in accordance with the work carried out by Spiliotis *et al.* (1997), on the antimicrobial activity of water seed extracts and seed oil of three *Moringa oleifera* varieties, tested against various microorganisms (including *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli* and *C. Albicans*), which documented that seed oil of *M.oleifera* has no antimicrobial activity. The lack of antibacterial activity in *M. oleifera* may be attributed to the absence of important phytochemicals such as tannins, flavonoids, and alkaloids. This active phytochemicals have been reported to confer antibacterial property on medicinal plants (Solomon *et al.*, 2013). Furthermore, garlic oil was observed to be effective against all the tested microorganisms. The efficacy of the oil increased with an increase in concentration. At 100 % (v/v) concentration, the highest sensitivity was observed in *Staphylococcus aureus* (11 mm), *Escherichia coli* (12 mm), *Salmonella* Sp. (11 mm), and *Pseudomonas aeruginosa* (10 mm). While moderate inhibition was observed at the lowest concentration 20 % (v/v), in *Staphylococcus aureus* (07 mm), *Escherichia coli* (07 mm), *Salmonella* sp. (00 mm), and *Pseudomonas aeruginosa* (00 mm) were resistance. The result of this research, agreed with the research conducted by Ross *et al.* (2001), which showed that Garlic oil possesses substantial and broad-spectrum antibacterial activity against both gram-positive and negative bacteria (including *Vibrio metschnikovii*, *S. aureus* and *L. Monocytogenes*). Also, in agreement with the work carried out by Ali and Blunde (2003), which reported that the antibacterial activity of different extracts (n-hexane, chloroform, acetone, butanol, ethanol and methanol) of Garlic against *E. coli*, *S. aureus*, *S. epidermidis* and *K. pneumonia*.

In addition, Ross *et al.* (2001), reported that Garlic oil contains diallylsulfide which contribute significantly to its antimicrobial activity. Also, the antibacterial activity of seed oil of Garlic can be attributed to its phytochemicals content, which are known to play a vital role in the antimicrobial activity of plants. In conclusion, the seeds oil of garlic presented activity against all the microorganisms tested, while seed oil of *Moringa oleifera* was found to be inactive against the tested organisms. Phytochemical screening shows the presence of alkaloids, tannins and saponins in seeds oil of garlic, while only saponin was detected in the seeds oil of *Moringa oleifera*.

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CONCLUSION

The results of this study have shown the potentials of seed oil of garlic as sanitizers/preservatives. This is due to the fact that it was found to possess antimicrobial activities against some food-borne microorganisms that are often implicated in the spoilage of foods and food-borne illnesses.

RECOMMENDATION

On the other hand, seed oil of *Moringa oleifera* can not be used for the above purpose. This is due to its inability to inhibit the growth of the tested food-borne microorganisms. Further research should be conducted to test the sanitizing and preservative effect of the oils on some foods.

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