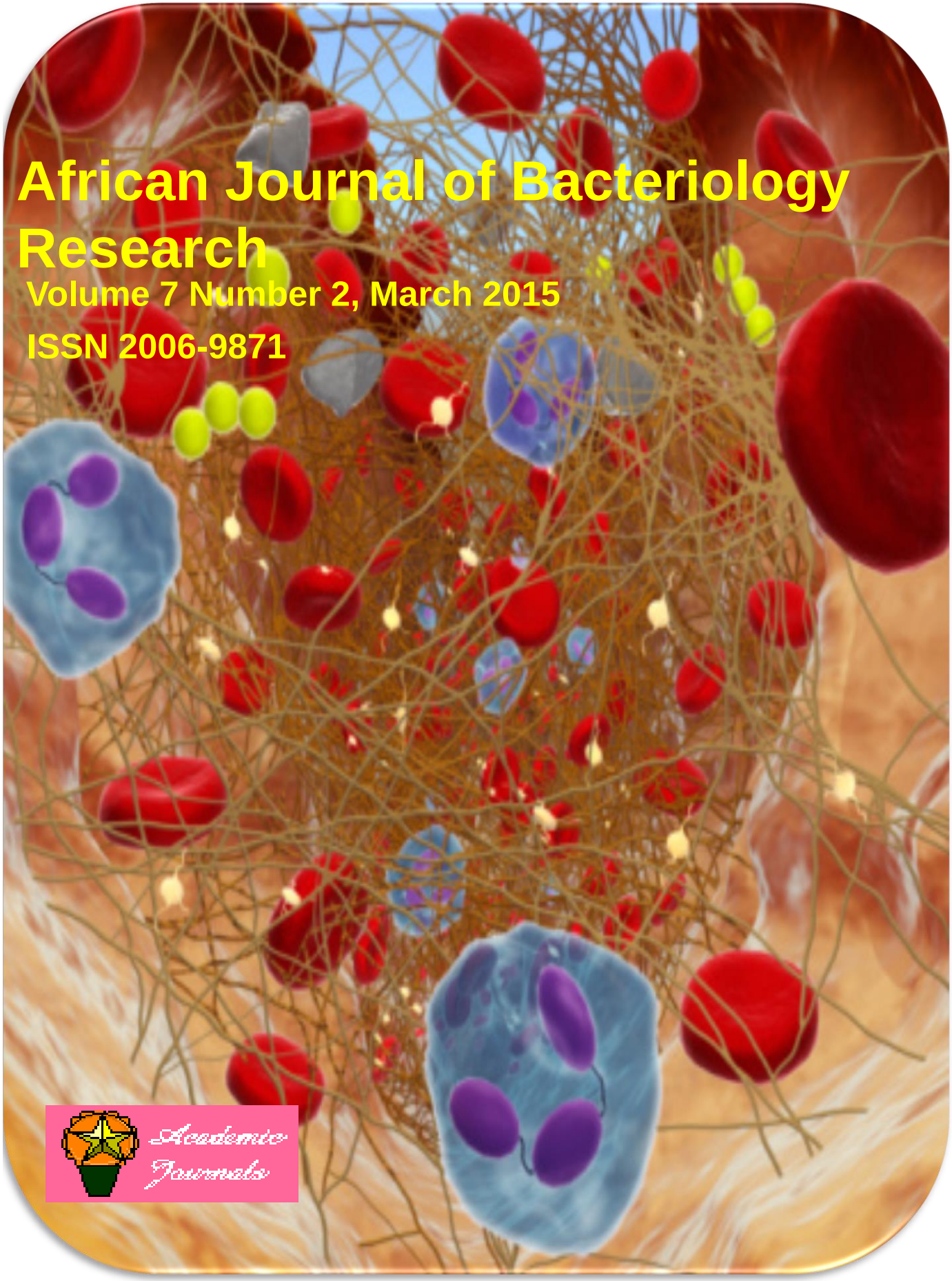

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Full Length Research Paper

Antibacterial activities of two medicinal herbs on *Salmonella typhi* isolates in Abakaliki, Ebonyi State, Nigeria: Improvement to herbal medicine

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The study on the antibacterial activities of some medicinal plants on *Salmonella typhi* isolates in Abakaliki Ebonyi State was conducted using agar diffusion method. The study shows that there is high level yield in the dried leaves than in the wet leaves of the experimental plants (W/D). The investigation reveals that phenol yield is 296% W and 67% D in bitter leaf while oil yield in Utazi is 1.900 W and 1.034 D; phenol is 276.00 W and 38.480 D. The phytochemical analysis showed the presence of alkaloids, tannins, glycosides, flavonoids, steroids, phenolic compounds, oil, proteins, terpenoids, fructose, glucose and essential oils. Antimicrobial test screening showed that the extract had a strong inhibitory effect against; *Staphylococcus aureus, Escherichia coli,* and *Klebsiella pneumonia.* The observation tends to confirm the medicinal value of these plant leaves and justify the claim of its local use in health remedies for diarrhea and kidney failure caused by *E. coli,* skin infection caused by *S. aureus* and pneumonia which is caused by *K. pneumonia*. However, this had proved inhibitory on *S. typhi* which could be synergistic or antagnostic in action and therefore suggest to be effective herbally when orally administered.

Key words: Antibacterial, medicinal herbs, *Salmonella species*, Ebonyi State.

INTRODUCTION

Medical herbs are plants which contain substance that can be used for therapeutic purpose or which are the precursor for the synthesis of useful drugs (Sato et al., 1997). Also herbs can be described as those plants which are valuable in medicinal production and could be used as food seasoned nutrition and other useful purpose to man (Byarygaba, 2004). Although, the practice of traditional medicine differs according to the locality, roots from which the certain geographical areas or climate makes traditional medicine a more specific indigenous

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cultural practices (Walker, 2001).

In Nigeria, the use of local herbs in the form of concoction and decortion treatment of various infections has been a common practice and about 75% of the people living in the rural areas depend on this practice (Yoshida et al., 1998). For instance, *Uvaria chamas* has been used in the South-East of Nigeria to treat feverish condition, malaria and body pains (Amadi et al., 2003). Other herbs such as the *Azadirachta indica, Aleovera* spp*., Psiduim guava, Carica papaya* and *Cymbopogen citrates* are used for various ailments such as malaria fever, headache, body pains, skin infections, stomach, diarrhea, etc simply by use of different methods of applications.

Moreover, it will serve as a cheaper means of treatment to the low income earner patients that cannot afford the cost of modern therapeutic used for treatment of typhoid fever. The germs *Salmonella* is among the most common cause of foods and water borne infectious disease in the world. A number of studies in Nigeria have shown that *Salmonella* infection is endemic in many parts of the country and its endemicity increases especially in areas with low environmental hygiene, and rural communities with without drinking water.

Garlic and onion is a common food for flavor and spice. Garlic contain a lot of health chemical compounds that are antioxidants, they reduce blood pressure, lower cholesterol, fight bacterial, fungal and viral infections, even detoxify cancer and protect liver from toxins including alcohol and synthetic drugs (Chen et al., 2002), (Aureli et al., 2006). *Allicin,* a penicillin like antibiotic hinders the growth of *Staphylococcus aureus and Salmonella typhi.* Another component of garlic called ajoene, has been found to thin the blood and contribute to lower cholesterol level (Mbata et al., 2006).

The onions bulb has been chemically analyzed and it is shown to contain an acrid, volatile oil, uncrystallizable sugar, gum, albumen, acetic and phosphoric acids, phosphate and citrate of calcium and water (Byargada et al., 1996). Onion possess properties allied to those of garlic, but in a milder degree, medically, it has been noted to be antihelminths, anti-inflammatory, antiseptic, antispasmodic, hypoglycaemic and tonic; sugar and onion juice form a syrup, much used in domestic practice for cough and other infections of the air-tube among children (Nweze et al., 2002). It is also useful in preventing oral infection and tooth decay. Fresh onion juice is a very useful first aid treatment for bee and wasp stings and bite of scorpion. Apart from all these usefulness of onion, it is commonly used for cooking of dishes, eating raw in making salad.

The bitter leaf, *Vernonia amygdalina* which is the type used in this research; contains the following chemical compound which include vernonioside which is said to have antimalaria effect, sesquiterpene and lactone that have anti tumor activities.

Traditionally, bitter leaf are commonly used for cooking of soup and porridge, but medicinally, it has been extensively used for the treatment of stomach ache, parasitic infection and has proven to be effective against drug resistant malaria parasites (Ohigashi et al., 1994).

Laboratory identification of salmonella can be typically isolated from stool of a diarrhea patient on MacConkey agar or moderately selective media for patient with enteric fever, this organism can be isolated from their blood, urine and bone narrow specimen. This study is aimed to determine the viability of some plants which have the ability to inhibit the growth of *S. typhi* and to determine the level at which these herbs can be used locally in prophylactic treatment.

MATERIALS AND METHODS

The herbs used include *V. amygdalina, Allium sativum and Allium cepa.* All these herbs except *A. sativum* were purchased from Abakpa Main Market Abakaliki, and *A. cepa* were also purchased at local market in Igbeagu Development Centre whereas *V. amygdalina,* the bitter species was collected from various home and farmland at Nkaliki Community in Abakaliki Local Government Area all in Ebonyi State. It was confirmed that the cultivation of *A. cepa* and *A. sativum* was common in the Northern part of Nigeria, from where the greater quantities were purchased from the sellers. The identification of these herbs was done by Professor S. S. C. Onyekwelu (taxonomist) of Applied Biology Department of Ebonyi State University, Abakaliki, Nigeria.

Preparation of materials

The herbs were washed with clean sterile distilled water and allowed to air dry for one hour before grinding them into powder using pestle and mortar. After this, blended herbs was collected and 40 g of each was measured and introduced into 200 ml of 95% ethanol, cold and hot water (Aureli et al., 2006), (Walker, 2000).

Ethanol extraction

40 g each of these herbs was weighed and introduced into 200 ml of 25% ethanol. This was allowed to stay for 24 h with intermittent shaking. After this period, they were filtered and the liquid extract was allowed to evaporate so that a concentrated extract will be obtained.

Cold water extraction

The same procedure described above was adopted for both cold and hot water extraction, but the extraction of *A. cepa* and *A. sativum* took the following processes.

Extraction of *A. cepa* (onion)

The onion were washed with distilled water and allowed to air dry for one hour. The outer covering of the onion were manually peeled off. The onion bulb being separated were washed and extracted in the following ways:

Table 1. The Antibacterial effect of the extracts against *Salmonella typhi.*

Botanical Names	Common names	Parts used	Results of the extracts		
			Cold H_2O	Hot H_2O	Ethanol
Allium sativum	Garlic	Bulbs	Sensitive	N.I	Sensitive
Allium cepa	Onion	Bulbs	Sensitive	N.I	Sensitive
Vernonia amygdalina	Bitter leaf	Leaves	N.I.	N.I.	N.I.

N.I. = No inhibition to the growth of the organism; sensitive means that extract inhibited growth of the organism.

1. Exactly 200 g of fresh onion bulbs were blended into fine powder and soaked in 100 ml of distilled water for 24 h. The pulp obtained was left in a clean sterile container and shaken vigorously to allow for proper extraction and it was filtered using a sterile muslin cloth after which the extract was obtained, air dried and stored below ambient temperature until required.
2. Exactly 200 g of fresh onion bulb were blended and soaked in100 ml of hot water for 24 hr; the resultant juice was extracted, air dried and stored.
3. Exactly 200 g of fresh onion bulb were blended and soaked in100 ml of 95% ethanol for 24 h and the extract was obtained, air dried and stored.
4. Exactly 200 g of fresh onion bulb were blended and the raw juice was extracted after standing in a clean glass for 25 h, it was extracted using a sterile muslin cloth and extract was air dried and stored.

Extraction of *A. staivum* (garlic)

The extraction of *A. sativum* is the same as the extraction of onion as stated above, whereas, the *V. amydalina* extract was obtained by squeezing the bitter leaf which released the pigment into a sterile bottles, respectively.

Media preparation

The media used includes Muller Hinton agar and deoxychocolate citrate agar (DCA). The media was prepared as instructed by the manufacturer. This was mixed very well before sterilizing it, using oven for 121°C for 15 min. It was allowed to cool for about 140°C before pouring about 20 ml into each of sterile petri dish and then allowed to gel before incubating them for 24 h to test for sterility.

Organism inoculum preparation

In the first eighteen hours, fresh culture of the organism *S. typhi* was used. The colony was collected from the media with sterile wire loop and introduced into the test tubes containing 2 ml of distilled water.

This was dissolved and the same turbidity with that of Macfarland solution standard (0.5) was obtained which made organism solution to yield 1 x 10^8 cells colony units.

Then about 0.2 ml of the diluted organism solution which is equivalent to two drops of the solution was added into the molten Muller Hinton. This was spread all over the media with the help of the glass spreader. After this, the sterile cork borer was used to make about five cups well (5 mm in diameter) in the prepared media, for the inoculation of the extract, which its procedure of preparation is described.

RESULTS

The sensitivity of the tested organism, *S. typhi* to the cold H_2O, hot H_2O and ethanol extract of these herbs, were evaluated by the cup plate agar diffusion method. The following plants and its parts were used for the sensitivity test against the organism. Table 1 shows result of the three herbs used, it was only two namely, garlic and onions that showed inhibitory effects on *S. typhi.*

The zone of inhibition of the control drug chloramphenicol differs depending on the herbs. In *A. sativum*: 29 mm with 0.4 g/ml while *A. cepa* is 23 mm. The result of the effect shows that *A. sativum* has the highest inhibitory effects on the tested organism followed by *A. cepa*. Also, the MIC of *A. sativum* is 0.4 g/ml, *A. cepa* also has 0.4 ml/ml (Table 2).

The combination of the extracts showed that when garlic was mixed with onion using the same concentration, 0.2 ml, its zone of inhibition was 28 mm which is higher in different zone of the concentration of the mixture. But when the three herbs, onion, garlic and bitter leaf were mixed, there was no reaction, the factor responsible for this susceptibility of *S. typhi* to the extracts are not exactly known but may be attributed to the presence of secondary plants metabolites. The diameter of the concentration of drugs CAC was 0.4 g/ml and it zones of inhibition was 34 mm (Table 3).

DISCUSSION

The sensitivity of the test organism to the cold water, hot water and ethanol extract of the three different herbs were evaluated using the cup plate agar diffusion method (Mbata et al., 2006). From the result obtained, it is shown that among the herbs used, it was only two, namely, *A. cepa* and *A. sativum* juice that shows inhibitory effect on the test organism *S. typhi,* whereas the remaining one herb were inactive to the organism. This has not only confirmed the facts that *S. typhi* possesses spores, unlike other species, and this peculiar feature has contributed to its stability to be highly resistant to many antibiotic such as carbenicillin and ciprofloxacin (De Pasquale et al., 2001; WHO, 2006).

Table 2. The MIC and inhibition zone diameter of the extracts on *S. typhi.*

Plant extract concentration (g/ml)	Inhibition zone/diameter (mm)	
	Allium sativum	***Allium cepa***
0.2	20 mm	11 mm
0.4	28 mm	13 mm
0.6	20 mm	10 mm
0.8	19 mm	9 mm
0.4. Drug control (CAC)	29 mm	23 mm

Table 3. Combination of extracts and its zone of inhibition.

Allium sativum + Allium cepa	
Zone of inhibition/diameter (mm)	**Different concentration (g/ml)**
28 mm	0.2 ml
11 mm	0.4
12 mm	0.6
34 mm	0.4 ml Drug control (CAC)

It was clear from this study that solvent extraction affected the degree of antibacterial activity of the extracts. It was also observed that the ethanolic extract of garlic gave the highest zone of inhibition (28 mm) using the concentration of 0.4 g/ml while ethanolic extract of onion gave 13 mm with 0.4 g/ml each against *S. typhi.* This credit to ethanol extraction was because it is an organic solvent and will dissolve organic compounds better, hence the active component required for antimicrobial activity.

It was also observed that raw onion and garlic had activity on *S. tyhpi.* The reason for this is not clear because the raw juice is thought to be more concentrated than the other extract since in some, like when *Escheichia coli* was used, there was no inhibition at all.

The hot water extracts of onions did not inhibit the growth of *S. typhi.* This may be explained by the fact that the antimicrobial substance in the onions extracts, which are mainly phenolic compound are destroyed by heat from the hot water which might have raised the temperature of the extracts inactivating them. In general, antibacterial components in the species plant are heat-labile; hence all spices lost their antibacterial activities by 20 min at 100°C.

The cold water extract inhibited the growth of *S. typhi* at 0.4 g/ml. It could be said that cold water as an extract could liberate the active constitution of garlic better as compared to onions. It is worthy to note that the antimicrobial activities of these plants extracts were dependent on the concentration of the extracts as reported early. Also, if the extract has high molecular weight, the rate of diffusion is always slow, reduced and also takes longer time, whereas of low molecular weight diffuses faster and at a quicker rate. The result obtained is evidence that garlic produced marked inhibitory effect on the tested organism.

Further, the allicin compound is an oily yellow liquid which was assumed to give garlic its characteristic odour. It also has a range of medical properties like synthesis when garlic is crushed this reaction is catalyzed by an enzymes alliinase. This allicin is responsible for the growth inhibition of *S. typhi, S. aureus* and other bacteria growth as reported by De Pasquale et al. (2001). It was observed that *A. sativum* in this study showed activity against the tested organism with minimum inhibition concentration (MIC) of 0.4 g/ml. This is an indication of possible high activity, since this tested organism is reported to be resistance to most antibiotic and herbs. Lastly, the *V. amydalina* recorded the least activity against this organism among the three herbs, since it show no zone of inhibition at the same concentration of 0.4 g/ml. Their individual zone of inhibition which has been recorded: 28 and 13 mm respectively to garlic and onion suggest an antagonistic effect.

Conclusion and recommendation

The effect of *A. cepa and A. sativum* in this study has shown that they really possess the same properties that are highly of antimicrobial importance. This study tends to collaborates the local use of these herbs in the treatment of diseases for instance, lime juice used to treat stomach upset, dairrhoea and skin related diseases, whereas garlic has been used to treat cancer, malaria, candidiasis and was able to inhibit the growth of *S. aureus,* and the

result of this research showed that these herbs have high activity against *S. typhi.*

Therefore, it is recommended that efforts should be geared towards identifying the active ingredient of these herbs and other unknown medicinal herbs, since they are common to obtain, cheap and available in our environment and has a lesser side effect when used for the treatment of various diseases.

However, people should increase the use of spices such as the garlic and onion in preparation of their dishes and eat them raw, likewise the use of *V. amydalina* should be encouraged in the family because of the role it plays in the liver. All these will help to prevent and equally inhibit the manifestation of *Salmonella* infection in the body system of an individual.

Conflict of interests

The authors have not declared any conflict of interests.

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