African Journal of Microbiology Research Vol. 5(31), pp. 5548-5553, 23 December, 2011
Available online at http://www.academicjournals.org/AJMR
ISSN 1996-0808 ©2011 Academic Journals
DOI: 10.5897/AJMR10.417

*Full Length Research Paper*

# Carrot (*Daucus carrota*), garlic (*Allium sativum*) and ginger (*Zingiber officinale*) extracts as bacteria selective agents in culture media

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Accepted 9 September, 2011

**Extracts of carrot, garlic and ginger as selective agents in basal bacteriological media were carried out on *Staphylococcus aureus* ATCC 15313*, Listeria monocytogenes* ATCC 2522, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 using a standard method. Ethanolic extracts, aqueous cold and hot extracts of the experimental plant products were made at the concentration of 200 mg/ml (2 g/10 ml). The ethanolic extracts inhibited the growth of all the test bacterial isolates. The cold aqueous extracts of garlic had inhibitory effects on the *S. aureus* and *L. monocytogenes* bacterial isolates but selectively allowed the growth of *E. coli*. The hot aqueous extract of ginger had no effect on any of the test bacteria. The hot garlic extract selectively allowed the growth of *L. monocytogenes* and *E. coli.* Phytochemical analysis of the carrots, garlic and ginger contained saponnin, resins, alkaloids, flavonoids, steroids and terpenes in varied proportions. We assume these products may have influenced the actions of the extracts on the test organisms. The results of this preliminary study suggest that aqueous extracts of carrots, garlic and ginger when incorporated in appropriate concentrations can serve as alternative selective agents in bacteriological culture media for bacterial isolation from highly contaminated biological specimens or separation of mixed cultures of bacteria in the laboratory.**

**Key words:** Carrot, garlic, ginger, selective agents, bacteria, culture media.

## INTRODUCTION

Spices from plants in addition to the impact of flavor have been used as preservatives and medicinal remedies (Kizil and Sougut, 2003). They are also antimicrobial agent for the control of the pathogenesis of infection (Liao, 2007). However, the need for new antimicrobial agents is closely associated with the problems of emergence of strains that are resistant to most present day's antibiotics (Ibekwe et al., 2000). On the other hand,the emergence and persistence of these organisms require that the frantic search for new and more effective antimicrobial agents should be a continuous process.

Medicinal plants are distributed worldwide, but they are most abundant in the tropical countries where we have the practice of herbal medicine in modernized form Rees et al. (1993) and Calixto (2000). This is also gaining momentum in Nigeria, with the various health officials and other persons coming to realize the potentials and efficacies of some of the indigenous plants (Nwaogu, 1997). This has led to the recognition of the value of traditional medical system. Although the antimicrobial activities of some spices including carrots, garlic, ginger and onions are documented, the required amounts added to foods for flavour is not sufficient to completely inhibit microbial growth. The antimicrobial activities vary widely depending on the type of spice, test medium and microorganism (Giese, 1994).

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Carrot (*Daucus carota*) is used to treat problems such as intestinal parasites, persistent diarrhea and different digestive problems (Dalby, 2003). Herbal remedies made from carrot are used in detoxifying the body (Dalby, 2003). Wild carrots seeds contain flavonoids, and a volatile oil including: asarone, carotol, pinene and limonene. Also cultivated carrot root contains sugar, pectin, carotene, minerals and asparagines (Mabey, 1997). Garlic (*Allium sativum*) contains many sulphur containing compounds. The most important of which in this case is alliin. This alliin comes in contact with an enzyme, allinase, and the allinase converts alliin to alliicin. Alliicin is responsible for the distinctive smell of garlic and its action against the growth of microorganisms. It interferes with RNA production and lipid synthesis, thus interfering with the phospholipids bilayer of the cell wall of Gram positive and Gram negative bacteria (Browning, 2000). Ginger (*Zingiber officinale*) has been used in ayurvedic medicine for the treatment of inflammation and rheumatism and is used in China and other countries as a treatment for nausea (Environs, 1999). Dietary ginger constituents, galanale A and B are potent apoptosis inducers in human T-cell lymphoma jurkat cells ginger. Specific constituents other than currcuminoids are potential anti-cancer agents (Cancerlett, 2003). Plants synthesize a large variety of chemical substance, known as secondary metabolites, which are of no apparent importance for the plant own life, but have prominent therapeutic effects on the animal system.

Antimicrobials are used in culture media as selective agents to restrict the growth of certain organisms and promote the growth of the desired ones. In this article, the utilization of carrots, garlic and ginger extracts as selective agents for the growth promotion of *Staphylococcus aureus* ATCC 15313, *Listeria monocytogenes* ATCC 2522, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 basal bacteriological media were evaluated.

## MATERIALS AND METHODS

### Sources of carrot, garlic and ginger

Carrot (*D. carrota*), Garlic (*A. sativum*) and Ginger (*Z. officinale*) where purchased from Bukuru Open market, Jos, Plateau state.

### Sources of test organisms

Typed culture of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 15313 and *L. monocytogenes* ATCC 2522 isolates from foods were obtained from Molecular Biology Laboratory, Federal College of Veterinary and Medical Laboratory Technology, Vom.

### Phytochemistry and extraction

Aqueous (hot and cold extraction) and solvent (ethanol) extraction methods were used. About 32 g of the washed fresh garlic, carrot and ginger were weighed using Mettler weighing balance. They were sliced into tiny pieces and blended in an electric blender with 160 mls sterile hot, cold distilled water and 160 mls of ethanol. These were then transferred into separate flask and shaken for one hour and filtered with Whatman No. 1 filter paper. The preparation was brought to 200 mg/ml using 2 g/10 ml (Sofowora 1982).

### Phytochemical screening

#### *Resin*

To 0.5 g of plants extract was added 5 ml of boiling ethanol and filtered through Whatman No. 1 filter paper and the filtrate was diluted with 4 ml of 1% aqueous HCl and formation of resinous precipitate indicate the presence of resins.

#### *Alkaloids*

To 0.5 g of the plant extracts was added 5 mls of 1% aqueous HCl on a steam bath. Few drops of Draggendroffs reagent were added to 1 ml of the filtrate. To 1 ml of the second portion of the filtrate was added few drops of the Wagner's reagent. The formation of precipitate was an indication of the presence of alkaloids.

#### *Tannins*

Ten millilitres of distilled water was added to 0.5 g of the plant extract and stirred and filtered band a few millilitres of 5% ferric chloride solution was added to a portion of the filtrate. A deep green coloration shows the presence of tannins. When a few drop of iodine solution was added to the second portion of the filtrate, a faint blue coloration was confirmatory of the presences of tannins.

#### *Glycosides*

Ten millilitres of boiling distilled water was added to 0.5 g of the plant extract. It was stirred thoroughly and filtered. Few drop of concentrated hydrochloric acid was added to digest 2 ml of the filtrate and few drop of ammonia solution was added to render alkaline. 2 ml of Benedict's qualitative reagent was further added to 5 drops of the filtrate solution and boiled. A reddish brown precipitate shows the presences of glycosides.

#### *Flavonoids*

A portion of 0.5 g of plant extract was dissolved in 2 ml of NaOH solution, a few drops of concentrated $H_2SO_4$ was then added. The solution became colourless which indicated the presence of flavonoids.

#### *Anthraquinone*

Five millilitres of chloroform was added to 0.5 g of plant extract and shake thoroughly for 5 min, it was filter and the filtrate shook with 100% ammonia solution. Pink, violet or red colours in the ammmoniacal layer (lower layer) indicate the presence of free anthraquinone.

#### *Steroids*

A portion of 0.5 g of plant extract was dissolved in 2 ml of chloroform and $H_2SO_4$ was carefully added from the side of the tubes to form a lower layer. A reddish brown colour at the interface

**Table 1.** Surface viable count showing effects of garlic extracts on the experimental organisms.

| Organisms | Media | Positive control (Gen.) | No extract | Cold extract | Hot extract | Ethanol extract | Negative control (NS) |
|---|---|---|---|---|---|---|---|
| *S. aureus* | Pw | NG | $1.2 \times 10^{13}$ | $2.2 \times 10^{13}$ | $8.5 \times 10^{18}$ | NG | G |
| | NB | NG | $2.1\ 10^{14}$ | $2 \times 10^{14}$ | $1.2 \times 10^{10}$ | NG | G |
| *L. monocytogenes* | PW | NG | $1.9 \times 10^{14}$ | $5.5 \times 10^{10}$ | NG | NG | G |
| | NB | NG | G | $1.4 \times 10^{12}$ | NG | NG | G |
| *E. coli* | PW | NG | $2.6 \times 10^{14}$ | NG | NG | NG | G |
| | NB | NG | G | NG | NG | NG | G |
| *P. aeruginosa* | PW | NG | $4.6 \times 10^{14}$ | $2.4 \times 10^{14}$ | $1.8 \times 10^{14}$ | NG | G |
| | NB | NG | G | $3.1 \times 10^{14}$ | $3.4 \times 10^{14}$ | NG | G |

PW- Peptone water, NB- Nutrient Broth, NG-No Growth, G -Growth, NS-Normal saline, Gen -Gentamycin.

indicates the presence of steroids.

***Terpenes***

Two millilitres of chloroform was added to 0.5 g of plant extract. 1 ml of acetic anhydride and 2 drops of concentrated sulphoric acid ($H_2SO_4$) was further added and a pink colour which changes to bluish green on standing indicated the presence of terpenes.

**Preparation of the test organisms**

Three colonies of fresh culture suspended in 5 mls of sterile nutrient broth and 5 mls of peptone water were incubated at 37℃ for 24 h. The suspension containing between $10^7$-$10^8$ cfu/ml of the organism suspension was adjusted to equal or match that of 0.5 McFarland standard ($10^5$cfu/ml) by making a dilution of 1:100 in sterile broth, both test and standard were held against a white background with contrasting black line to aid comparison (Cheesbrough, 2000).

**Determination of population viable count**

Using 10 fold serial dilutions, 9 mls of sterile nutrient broth and peptone water were put into two sets of test tubes 1 to 10 aseptically. 1 ml of each broth culture of the isolate was added to tube 1, these was mixed and 1 ml was taken from tube 1 to tube 2, the process was repeated serially up to $10^{10}$ and the last 1 ml was discarded from tube 10 (Miles and Misra, 1938).

Without any addition of extracts, each dilution of the inoculum were plated out on the nutrient agar plate and divided into sectors as drops from a calibrated pipette. Each drop was 0.02 ml in volume and approximately 50 drops of such gave a volume size of 1 ml, the drops were made to fall gently from a height of 2.4 cm onto the nutrient agar plate avoiding splashing from one sector to another. The plates were afterwards incubated at 37℃ for 24 h.

One ml of the extracts of 200 mg/ml was now added from tube 2 to tube 11 while tube 12 served as the positive control containing a known antibiotic (Gentamycin) and tube 1 served as the negative control containing 1 ml of sterile normal saline. These preparations were allowed to stand on the bench for 30 min for pre-incubation. After which the test tubes were placed in the incubator for 24 h at 37℃. After 24 h incubation, the test tubes content were gently mixed well and plated out onto nutrient agar plates. The plates were further incubated at 37℃ for 24 h.

After the incubation, discreet colonies were obtained at different dilutions for the different test isolates. Counts were made in the drop areas showing the largest number of colonies. Plates showing no growth of colonies indicated the inhibitory action of the extracts while a plate with growth shows resistance. The population density was calculated using the formula

Number of colonies × Number of drops/ml × dilution factor

## RESULTS

The results on the utilization of Carrot, Garlic and Ginger extracts as selective agents in bacteriological media showed the inhibition properties of the various extracts (Cold aqueous, Hot aqueous and Ethanolic extracts), against the stipulated typed bacteria which included *S.au*reus ATCC 15313, *E. coli* ATCC25922*, L. monocytogenes* ATCC 2522 and *P. aeruginosa* ATCC 27853 (Tables 1, 2 and 3). Hot aqueous garlic extract inhibited *L. monocytogenes* and *E. coli* in both Peptone water and Nutrient broth. However, cold garlic aqueous extract permitted the growth of *L. monocytogenes* in both media but showed inhibition of *E. coli* (Table 1*).* Positive selectivity of *S.aureus* and *P. aeruginosa* was also recorded in Garlic cold and hot extracts (Table 1). However, ethanolic extract of garlic showed no growth among the test organisms like the gentamycin used as positive control which inhibited all test organisms while sterile normal saline used as negative control supported the growth of all the organisms.

The effect of ginger extracts on the experimental bacteria at the concentration of 200 mg/ml showed that the cold and hot aqueous extracts permitted growth of all the isolates in peptone water and nutrient broth except for the ethanolic extract (Table 2).

On the effects of carrot extracts on the experimental

**Table 2.** Surface viable count showing effects of ginger extracts on the experimental organisms.

| Organisms | Media | Positive control (Gen.) | No extract | Cold extract | Hot extract | Ethanol extract | Negative control (NS) |
|---|---|---|---|---|---|---|---|
| *S. aureus* | PW | NG | G | $22 \times 10^{14}$ | G | NG | G |
| | NB | NG | G | $1.7 \times 10^{14}$ | G | NG | G |
| *L. monocytogenes* | PW | NG | G | $1.6 \times 0^{12}$ | G | NG | G |
| | NB | NG | G | $8.5 \times 10^{7}$ | G | NG | G |
| *E. coli* | PW | NG | G | $1 \times 10^{11}$ | G | NG | G |
| | NB | NG | G | G | G | NG | G |
| *P. aeruginosa* | PW | NG | G | $2 \times 10^{14}$ | G | NG | G |
| | NB | NG | G | $1.3 \times 0^{14}$ | G | NG | G |

PW- Peptone water, NB- Nutrient Broth, NG-No Growth, G -Growth, NS-Normal saline, Gen -Gentamycin.

**Table 3.** Surface viable count showing effects of carrot extracts on the experimental organisms.

| Organisms | Media | Positive control (Gen.) | No extract | Cold extract | Hot extract | Ethanol extract | Negative control (NS) |
|---|---|---|---|---|---|---|---|
| *S. aureus* | Pw | NG | G | $2.8 \times 10^{14}$ | $4.6 \times 10^{12}$ | NG | G |
| | NB | NG | G | $2 \times 10^{14}$ | $4.1 \times 10^{14}$ | NG | G |
| *L. monocytogenes* | PW | NG | $2.3 \times 10^{13}$ | $2.3 \times 10^{14}$ | NG | NG | G |
| | NB | NG | G | $8.5 \times 10^{7}$ | G | NG | G |
| *E. coli* | PW | NG | $2.1 \times 10^{13}$ | $1.4 \times 10^{13}$ | $1.7 \times 10^{12}$ | NG | G |
| | NB | NG | G | $3.2 \times 10^{14}$ | $2.2 \times 10^{12}$ | NG | G |
| *P. aeruginosa* | PW | NG | G | G | G | NG | G |
| | NB | NG | $3.9\times 10^{14}$ | $3.2 \times 10^{14}$ | $1.9 \times 10^{14}$ | NG | G |

PW- Peptone water, NB- Nutrient Broth, NG-No Growth, G -Growth, NS-Normal saline, Gen -Gentamycin.

bacteria when used at the concentration of 200 mg/ml of the extract inhibited *L. monocytogenes* in Peptone water, but allowed the growth of *S. aureus*, *E. coli* and *P. aeruginosa* in both media. The cold carrot extract supported the growth for all the test bacteria, while the ethanolic extract inhibition of all the bacteria as the gentamycin (Table 3). Phytochemical analysis of garlic, ginger and carrot extracts shows the presence of saponin, resins, alkaloids, flavonoids, steroids and terpenes in varied proportions (Table 4).

## DISCUSSION

The utilization of aqueous extracts of carrot, garlic and ginger as selective agent in bacteriological media Peptone water and Nutrient broths showed that the various methods of extraction-cold aqueous, hot aqueous and ethanolic had selective variables among test organisms, *S. aureus* ATCC 15313, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. All the ethanolic extracts of garlic, ginger and carrot used in this work inhibited all the test organisms. The reason for this action could possibly be due to the fact that ethanol possesses antibacterial properties which serves as its basis for use as a disinfectant in Laboratories. Also the inherent phytochemicals present in the spices could be readily depleted or neutralized by the ethanol. This is in agreement with earlier report of Sofowora, (1982) who concluded that ethanol is a good extraction solvent.

The hot aqueous extracts of carrot, garlic and ginger in peptone water supported selective growth for *S. aureus* and *P. aeruginosa* and *L. monocytogenes* but inhibited *E. coli* and *L. monocytogenes* in nutrient broth. This shows

**Table 4.** Phytochemical properties of carrot, garlic and ginger.

| Chemical compounds | Carrot extract | Garlic extract | Ginger extract |
|---|---|---|---|
| Saponins | ++ | +++ | + |
| Resins | + | + | + |
| Alkaloids | - | - | - |
| Draggendoff | ++ | ++ | ++ |
| Wagner's | ++ | ++ | ++ |
| Tannins | - | - | - |
| Ferric chloride | - | - | - |
| Iodine Solution | - | - | - |
| Glycosides | - | - | - |
| Flavonoids | + | + | + |
| Anthraquinone | - | - | - |
| Steriods | - | + | + |
| Terpenes | + | - | + |

+=Positive, -=Negative.

that in mixed cultures or clinical samples, food or environmental where *S. aureus, L. monocytogenes* and *P. aeruginosa* are desired, hot aqueous extracts of carrot, garlic and ginger in peptone water can be considered as most appropriate selective medium for their isolation. These findings are similar to the reports of Banerjee and Sarkar (2003), where they reported the growth of *S. aureus* and *P. aeruginosa* in the extracts.

However, this is the first report of using aqueous extracts of carrot, garlic and ginger in peptone water as selective agent in a medium for the isolation of *Listeria* organism in experimental samples. Junaid et al. (2006a) had reported the antimicrobial properties of *Ocinum gratissum* extracts on some selected bacteria of gastrointestinal isolates, but *L. monocytogenes* was not inclusive. This is therefore the preliminary report on antimicrobial properties of aqueous extracts of carrot, garlic and ginger on *L. monocytogenes* in Nigeria.

All the cold aqueous extracts in peptone water and nutrient broth used in this study exhibited positive selectivity growth for all the test isolates, except that of garlic which inhibited the growth of *E. coli*. Therefore, where *E. coli* is considered non-desirous pathogen in a mixed culture, cold aqueous extracts of garlic, can be used for its inhibition in peptone water and nutrient broth. However, the cold aqueous extracts of carrot and ginger in peptone water and nutrient broth can be used for the selective isolation of *E. coli*.

Phytochemical properties of the garlic, ginger and carrot extracts, it indicated the presence of saponnin, resins, alkaloids, flavonoids, steroids and terpenes in varied proportions. These phytochemicals are documented to be the major biologically active plant principles, as well as exhibiting physiological activity against the tested organisms (Sofowora, 1993). However, the concentration of these active principles of garlic, ginger and carrot used determines whether they can be used as selective or inhibitory agents when incorporated into bacteriological media. The MIC of extracts ranged from 0.0003 mg/ml to 0.7 ng/ml while MBC ranged 0.135 to 2.04 ng/ml. Results indicated that extracts of ginger and C*ercinia kola* roots may contain compounds with therapeutic activity (Akoachere et al., 2002).

Although garlic, ginger and carrot have long been documented for their antimicrobial activities against foodborne pathogens, Liao (2007), their selectivity for or against the test isolates as seen in this study was not significantly affected by the type of bacteriological broth used. Rather the presence or absence of phytochemicals (plant active principle) in these spices determines whether they will elicit positive or negative selectivity when incorporated into bacteriological media. It is concluded that the varied proportion of these phytochemicals present in the extracts influenced the extent of the selectivity as observed in this study. Thus, non- selectivity of ethanolic extracts of these spices are most appropriate, as the ethanolic must have inhibited the growth of the experimental organisms.

In conclusion, positive selectivity of these extracts is most effective on cold aqueous followed by hot and non-selective by the ethanolic extracts. Since the test isolates showed similar susceptibility pattern to the extracts in both peptone water and nutrient broth, either of the bacteriological media used in this study can serve for routine laboratory culturing and isolation of the test isolates.

## ACKNOWLEDGEMENTS

The authors are grateful to A. A. Chukwuedo for reading the manuscript and also, the staff of Biochemistry,

Federal College of Animal Health and Husbandry Technology, Vom and Molecular Biology Department Federal College of Veterinary and Medical Laboratory Technology, Vom for their assistance during the study.

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