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ANTI-BACTERIAL EFFECTS OF *AFRAMOMUM MELEGUETA* SEED EXTRACTS ON SOME ENTEROBACTERIACEAE ORGANISMS

Ukaegbu-Obi, K.M.[1], Odo, C.E.[2,3*] and Awa, C.[1]

[1]Department of Microbiology, College of Natural Science, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia, Abia State, Nigeria.

[2]Department of Biochemistry, College of Natural Science, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia, Abia State, Nigeria.

[3]Department of Biochemistry, Faculty of Biological Science, University of Nigeria, Nsukka, Enugu State, Nigeria.

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***Correspondence for Author**
Odo, C.E.
Department of Biochemistry, College of Natural Science, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia, Abia State, Nigeria.

ABSTRACT

The anti-bacterial effects of the methanol, ethanol and aqueous extracts of *A. melegueta* seeds were investigated on some enterobacteriaceae organisms (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella tyhi* and *Shigella* spp). The organisms tested showed varying degrees of sensitivity to the various extracts with the aqueous extract having the least activity when compared to the ethanol and methanol extracts which had closely inhibitory effects. The ethanol extract had higher zones of inhibition on the organisms than the methanol extract in the decreasing order: *E. coli* (21.5 mm), *Shigella* spp (17.0 mm), *S. tyhi* (11.5 mm) and *K. pneumoniae* (9.0 mm). The zones of inhibition exerted on the organisms by the methanol extract were in the decreasing order: *S. tyhi* (15.0 mm), *E. coli* (13.5 mm), *Shigella* spp (12.0 mm) and *K. pneumoniae* (0.0 mm). This study shows that the seeds of *A. melegueta* possess active ingredients that may be exploited
for the development of anti-microbials and alternative remedies for infections and diseases caused by the tested organisms.

KEYWORDS: *A. melegueta*, anti-bacterial, enterobacteriaceae, sensitivity and inhibition.

INTRODUCTION

Since prehistoric times, plants and their extracts have been used for their healing properties. Ancient Egyptians, for example, chewed willow bark to relieve fever and headaches. Thousands of years later, scientists discovered that the bark contains salicylic acid, the active ingredient used in making aspirin.[1] Medieval doctors believe that baldness could be cured by rubbing an onion on the scalp.[2] Even today, we are still using compounds derived from plants. Atropine employed in treating certain heart conditions and relaxing the muscles of the eyes was originally obtained from the deadly nightshade plant.[3] Plants with potent bioactive are regarded as components of phytomedicine.[4] Foxgloves contain a substance called digitoxin which was used to treat an irregular heart beat and drugs based on this substance are still currently in use.[5]

A. melegueta (of the family, Zingiberaceae) is a spice in the ginger family with the common name of "grains of paradise" or "alligator pepper". The spice is used in West Africa for the purpose of alleviating stomach ache and diarrhoea as well as hypertension with some limited reports on it being used for tuberculosis and as a remedy for snake bites and scorpion stings.[6] The seeds also tend to have general anti-microbial properties similar to many species. They have also been shown to have some molluscidal and repellant properties. They are one of the many pungents said to aid in sexuality and aphrodisia (although the class of pungent herbs appears to be mentioned more than *A. melegueta* seeds).[7] Despite the acclaimed anti-microbial potential of *A. melegueta* seeds, there is hitherto, dearth of available data regarding their anti-bacterial property and hence, the thrust of this study was to evaluate the anti-bacterial effects of the methanol, ethanol and aqueous extracts of *A. melegueta* seeds on some enterobacteriaceae organisms.

MATERIALS AND METHODS

Collection and Identification of the Plant Seeds

The seeds *of A. melegueta* were bought from Umuahia market in Umuahia North Local Government Area of Abia State, Nigeria. The seeds were identified and authenticated by a taxonomist at the Taxonomy Section of Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Preparation of the Plant Seeds

The seeds *of A. melegueta* were washed with distilled water, sun-dried for one week and ground into uniform powder using an industrial milling machine. The ground powder was stored in an air-tight container in readiness for the extraction process.

Extraction Procedure

The ground seeds were extracted using distilled water, 70% ethanol and 70% methanol as previously described.[8] The aqueous extract was prepared by soaking 20 g of the ground seeds in 100 ml of distilled water in a conical flask and stirring the mixture vigorously with a glass rod for proper extraction. The mixture was allowed to stand for 24 hours at room temperature after which it was filtered using Whatman No. 1 filter paper. The filtrate was concentrated at 45^{o}C under reduced pressure using a rotary vacuum evaporator and stored at 4^{o}C until used. The ethanol and methanol extracts were obtained using the same procedure.

Collection and Maintenance of the Test Organisms

The test organisms: *E. coli*, *K. pneumoniae*, *S. typhi* and *Shigella* spp were obtained from the stock cultures of the Microbiology Laboratory of Federal Medical Centre, Umuahia, Abia State, Nigeria. The identity of the test organisms were reconfirmed and stored at 4^{o}C in nutrient agar slants.

Reconstitution of the Extracts

One gram of each of the extracts was reconstituted with 2 ml of dimethyl sulfoxide (DMSO) to obtain a concentration of 500 mg/ml of each extract. The test tubes were labeled according to their content concentrations.

Anti-bacterial Activity

The anti-bacterial activities of the extracts were determined using the disc diffusion method as previously described.[9] In this method, the disc which had initially being impregnated with the extracts were aseptically picked using a pair of sterile forceps and placed on the surface of the different plates which had initially being inoculated with a pure culture of the test organisms using streak method. *E. coli* was cultured on a nutrient agar. *K. pneumoniae* was cultured using MacConkey agar while *S. typi* and *Shigella* spp were cultured on Salmonella Shigella Agar (SSA). The plates were incubated at 37^{o}C for 24 hours and the sensitivity of the organisms to the extracts was measured using a meter rule.

RESULTS

As shown in Table 1, the water extract had the least zones of inhibition on the test organisms. It slightly inhibited the growth of *S. typhi* and *E. coli* with inhibition zones of 9 and 11 mm respectively. However, it did not inhibit the growth of *K. pneumoniae* and *Shigella* ssp. The ethanol extract had the highest zones of inhibition on the test organisms. The highest zone of inhibition was 21.5 mm on *E. coli* while the least zone of inhibition was 9.0 mm on *K. pneumoniae*. *S. typhi* and *Shigella* ssp both had inhibition zones of 11.5 and 17 mm respectively. The methanol extract did not inhibit the growth of *K. pneumoniae*. *S. typhi* had the highest zone of inhibition (15 mm) while *Shigella* ssp and *E. coli* both had inhibition zones of 12 and 13.5 mm respectively. The ethanol and methanol extracts showed higher inhibitory zones than the water (aqueous) extract.

Table 1: Anti-bacterial activities of the extracts of the seeds *of A. melegueta* on the test organisms.

Organisms	Ethanol	Methanol	Water
E. coli	21.5	13.5	11
S. typhi	11.5	15	9
K. pneumoniae	9.0	0.0	0.0
Shigella spp	17	12	0.0

Values in the table indicate zones of inhibitions in millimetres (mm)

DISCUSSION

That the aqueous, methanol and ethanol extracts of the seeds of *A. melegueta* inhibited the growth of all bacteria tested as shown by the various zones of inhibition, indicates an anti-bacterial activity of the plant. Anti-bacterial activities of the extracts on the organisms were observed in the order: *E. coli* > *S. typhi* > *Shigella* ssp > *K. pneumoniae*. This is similar to the earlier report.[8]

The difference in the anti-microbial properties of a plant extract might be attributable to the age of the plant used, freshness of the plant materials, physical factors (temperature, light and water), contamination by field microbes, adulteration and substitution of the plant, incorrect preparation and dosage.[10] In the same vein,[11] reported that inactivity of plant extracts may be due to the age of the plant, extracting solvent, method of extraction and time of harvesting the plant material.

The anti-bacterial effect of the seeds of *A. melegueta* may be due to the phytochemical constituents in them. *A. melegueta* seeds are rich in phytonutrients such as flavonoids, phenolic compounds, tannins, saponins, terpenoids, cardiac glycosides and alkaloids.[12] The biological functions of flavonoids include: protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatotoxin, viruses and tumours. This may be the reason behind the use of the extracts of this plant in the treatment of intestinal troubles in herbal medicine.[13] The presence of phenolic compounds in the seeds of *A. melegueta* indicates that this plant might serve as an anti-microbial agent. This is because phenols and phenol compounds have been extensively used in disinfection and remain the standards with which other bactericides are compared. Phenolic compounds as electron donors are readily oxidised to form phenolate ion or guanine, an electron acceptor. Protonated phenol is used as a cleaning agent as it has potent anti-septic or bactericidal property.[14]

In conclusion, this study shows that the extracts of the seeds of *A. melegueta* possess anti-bacterial activities against the tested isolates. Also, this work indicates that ethanol and methanol are better solvents than water for the extraction of the active ingredients of the seeds of *A. melegueta*. Further studies on more effective method of extracting only the necessary constituents and standard reconstitution means as well as other processing, refining and purification measures are recommended.

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