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**Research Article**

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# Phytochemical Screening and Assessment of Antibacterial Activity of Total Aqueous Extracts of *Afromomum melegeuta* and African Indigenous Salt

**Clément M. Mbadiko[1], Koto-te-Nyiwa Ngbolua[1*], Pius T. Mpiana[2], Mutambel ' Hity[3], Ruffin N. Kikakedimau[3], Gisèle K. Makengo[1], Aaron L. Pambu[1], Landry L. Kemfine[1], Gédéon N. Bongo[1], Théophile F. Mbemba[1]**

[1]Department of Biology, Faculty of Sciences, University of Kinshasa, P.O. Box 190, Kinshasa XI, Democratic Republic of the Congo
[2]Department of Chemistry, Faculty of Sciences, University of Kinshasa, P.O. Box 190, Kinshasa XI, Democratic Republic of the Congo
[3]General Commissariat on Atomic Energy /Nuclear Center of Kinshasa, University of Kinshasa, P.O Box 868, Kinshasa XI, Democratic Republic of the Congo

**Abstract** The present study was conducted in order to justify the use of *Afromomum melegueta* seeds intraditional medicine for the treatment of certain infectious diseases and to assess the influence of heat during the extraction of the active principles of the drug decoction on the antibacterial activity. Then, a bacteriological study was conducted to compare the antibacterial activity of the total aqueous and ethanolic extracts. In this study, it was also necessary question of testing the hypothesis that the addition of native salt in the total aqueous extracts of *A. melegueta* seeds in traditional medicine optimizes the antibacterial activity against three bacterial strains of which *Escherichia coli*, *Klebsiella sp.* and *Staphylococcus aureus*. A phytochemical screening was performed to identify the major chemical groups present in total extracts (aqueous and ethanolic) of *A. melegueta* seeds. The results showed that all tested samples have the antibacterial activity. However, the addition of native salt and aqueous extract mixture of *A. melegueta* seeds seems to have an antagonistic effect and showed a low activity compared to aqueous extracts tested individually. In addition, the aqueous extracts, in particular the macerated revealed a strong activity on the bacterial strains tested compared to other extracts. The phytochemical screening revealed the presence of alkaloids, tannins and total polyphenols in the aqueous extracts and the presence of quinones, steroids and triterpenoids in the ethanolic extracts. The seeds of *A. melegueta* have antibacterial molecules and can be used for the treatment of certain infectious diseases especially for Klebsiella infections.

**Keywords** *Afromomum melegueta*, Phytochemical screening, Antibacterial activity, native salt

## Introduction

Infectious diseases represent about half of the deaths in tropical countries. In developed nations, despite advances in the understanding of microbiology and their control, incidents of epidemics caused by resistant microorganisms to existing drugs and the emergence of unknown pathogens present enormous public health problems [1-8]. As a matter of fact, traditional medicine widely uses plants for the treatment of infectious diseases [3, 9]. Most of the plants used in traditional medicine are also used in food (such as condiments, spices, preservatives, etc.) [3, 10] and this is the case of *Afromomum melegueta* and an extract of plants: the native salt [1, 11-12]. The seeds of the *A. melegueta* and native salt are used individually or in combination for the treatment of certain infectious diseases

(tonsillitis, bronchopulmonary disorders, urinary tract infections, also like wound disinfectant, etc.) and/or in food (such as condiments, spices, preservatives, etc.) [12].

*Afromomum melegueta* is a plant that has both nutritional and medicinal values. It is an aromatic plant that is cultivated for its spicy fruit. The leaves are known for their medicinal properties as well as the seeds and these leaves are used in the treatment of measles and leprosy [1-2, 11, 13]. They are used to treat dysentery, bronchopulmonary disorders, sore throat, hay fever, ulcer Bruruli, sores, leprosy, measles, sexual asthenia, sexual sterility, sore teeth, rheumatism and migraine, as well as sedative, antipyretic, laxative, hemostatic agent and aphrodisiac [1-4, 11, 8, 9, 13-14]. The native salt is obtained from the different species of plants (palm oil, water hyacinth and banana). For each plant, the native preparation of this salt is performed in the same way. It begins with a harvest of mature leaves (water hyacinth, banana) or male inflorescences (palm) and these materials are dried in the oven for a few days and then burnt to ashes. The ashes are spread in water and filtered through a sieve or a clean cloth. The collected filtrate is subjected to boiling until we obtained a more concentrated solution, followed by cooling. The solution is finally dried in the open air till the obtaining of a residue which is the traditional salt [12]. Chemical studies on native salt or indigenous salt prepared with banana and oil palm male inflorescence show that this salt consists in major part of the chloride compounds, bicarbonate, potassium and to a lesser extent sodium salts, calcium and magnesium [12]. The bacteriological study performed by Uwera (1993) showed that the native salt prepared from oil palm male inflorescence is active on *Candida albicans*, *Staphylococcus spp.* and *Streptococcus spp.* Furthermore, several studies have shown that the seeds of *A. melegueta* also have antibacterial activity [12, 15-16].

The emergence of bacteria drug resistance increases the importance or the need to find new active molecules. Henceforth, the main aim of this study is to justify the use of *A. melegueta* seeds for the treatment of infectious diseases (such as tonsilitis, broncho-pulmonary disorders, urinary tract infections and so on.) and to assess the influence of heat (in the extraction of active ingredients by decoction) on the anti-bacterial activity of these extracts. This study aims also to scientifically verify the addition of native salt in the total aqueous extracts of *A. melegueta* seeds in traditional medicine or by the indigenous people to conserve food synergistically enhances the action of these two antimicrobials.

## Materials and Methods

### Preparation of Plant Material

The berries of the *A. melegueta* previously dried were purchased at Somba zikida (Kinshasa/DR Congo) from Kongo Central. In the laboratory, they were manually shelled in order to obtain the seeds and were sprayed using an electric grinder (Thomas Scientific USA). The powder served to the preparation of different extracts of *A. Melegueta* seeds. Moreover, the native salt used was purchased in the same market and was prepared from the male inflorescence of palm oil and was dried in an oven (65 °C) while in the laboratory and had a basic pH of 8.8.

### Bacterial Strains Used

The bacterial strains used namely *E. coli*, *S. aureus* and *Klebsiella spp.* were isolated in the bacteriological laboratory of Cliniques Universitaires de Kinshasa. These strains were obtained from patients with acute diarrhea, sore throat and urinary tract infections in hospitalized at the Cliniques Universitaires de Kinshasa.

### Bacteriological Assays

The research of the antibacterial activity was carried out using the agar dilution method. The extracted solutions were incorporated into an agar medium, sterilized and into petri dishes. After cooling, the surface of the agar was seeded with an inoculum of strains to be studied (in one or more parallel streak, star or flooding).After incubation, the minimum bactericidal concentration (MBC) is determined by the absence of bacterial colonies on the medium containing the lowest concentration of tested substances [5-7, 12].

**Preparation of extracts of *A. melegueta* seeds**

**Preparation of aqueous extracts**

**Maceration**

Ten grams of *A. melegueta* powder were macerated in 10 ml of distilled water for 48 h at laboratory temperature and filtered using Whatmann n°1 filter paper. Afterwards, the filtrate obtained was kept in the freezer and lyophilized after 24 h. The dry extract obtained after lyophilization was kept in a small glass jar.

**Decoction**

Ten grams of *A. melegueta* seed powder were boiled for 5 min in 10ml of distilled water in a microwave oven (House woldwaulovu model AM 57/MPWH); then filtered with filter paper. The filtrate was frozen and lyophilized and the obtained powder was kept in a small glass jar after lyophilization.

**Preparation of different concentrations of aqueous extracts**

500mg of dry extract was diluted in 10 ml of distilled water. From the stock solution, we took : 2ml; 1ml; 0.8 ml; 0.6 ml; 0.4ml; 0.32ml; 0.26ml; 0.24; 0.016; 0.08ml; 0.07ml; 0.06 ml; 0.05 ml; 0.04ml; 0.03ml; 0.02ml; 0.016 ml; 0.010 ml; 0.008ml; 0.004 ml which corresponds respectively to a concentration of 100µg, 50 µg; 40 µg; 30 µg; 20 µg; 16 µg; 13 µg; 12 µg; 8µg; 4 µg; 3.5 µg; 3 µg; 2.5 µg; 2 µg; 1.5 µg; 1 µg, 0.8 µg; 0.5 µg, 0.4 µg, 0.2 µg. These different concentrations of the aqueous extracts were put in test tubes each containing 2 ml of nutrient agar in order to obtain the final concentrations of dry extracts: 50 µg; 25 µg; 20 µg; 15 µg; 10 µg; 8 µg; 6.5 µg; 6 µg; 4 µg; 2 µg; 1.75 µg; 1.5 µg; 1.25 µg; 1µg; 0.75 µg; 0.5µg; 0.4 µg; 0.25 µg; 0.2 µg; 0.1 µg/ml respectively. The different mixtures of extracts were autoclaved and poured into petri dish. After solidification of the agar, we get a series of petri dish containing different concentrations of aqueous extract (decoction and maceration) ready for culture.

**Preparation of ethanolic extracts**

Ten grams of powder of *A. melegueta* seeds were macerated in 10 ml of ethanol for 48 h at laboratory temperature. The extract was then filtered and dried in an oven for 24 h (55 ° C). The ethanolic extracts obtained were not completely dried due to the presence of a large amount of oil in the seeds of *A. melegueta*.

**Preparation of different concentrations of ethanolic extracts of *A. melegueta* seeds to be tested**

The concentrations of ethanolic extracts were obtained in the same manner than the concentrations of the aqueous extract.

**Preparation of different concentrations of native salt**

The different concentrations of native salt were obtained in the same manner as the aqueous and ethanolic extracts.

**Preparation of different concentrations of native salt and aqueous extract mixture**

250 mg of the indigenous salt plus 250mg of aqueous extracts were diluted in 10ml of distilled water. Then from the stock solution, we took : 2ml; 1ml; 0.8 ml; 0.6 ml; 0.4ml; 0.32ml; 0.26ml; 0.24 ml; 0.016 ml; 0.08ml; 0.07ml; 0.06 ml, 0.05 ml; 0.04ml, 0.03ml, 0.02ml, 0.016 ml, 0.010 ml, 0.008ml, 0.004ml, which corresponds respectively to a concentration of 100 µg, 50 µg, 40µg, 30µg, 20µg, 16µg, 13µg, 12µg, 8µg; 4µg, 3.5 µg; 3µg, 2.5µg, 2 µg, 1.5µg, 1µg, 0.8µg, 0.5µg, 0.4µg, 0.2µg. The mixture of native salt and aqueous extract of *A. melegueta* seeds are incorporated into the test tubes containing 2ml of nutrient agar. Thus, the final concentrations obtained were of: 50 µg, 25µg, 20µg, 15µg, 10µg, 8 µg, 6.5µg, 6µg, 4µg, 2µg, 1.75mg, 1.5µg, 1.25mg, 1µg, 0.75 µg, 0.5µg, 0.4µg, 0.25µg, 0.2µg and 0.1µg/ml respectively. These homogeneous mixtures were sterilized and poured into Petri dish ready for culture.

**Preparation of inoculum and culture**

The bacterial strains used in this work were isolated from Cliniques Universitaires de Kinshasa. They were taken directly from patients with acute diarrhea, urinary tract infections and angina. To be sure of the purity of these strains stored in the laboratory, they were sub-cultured in petri dishes on nutrient agar. The purity of the strains was

verified by obtaining homogeneous colonies (after 24 hours of incubation at 36 °C); a loopful of colonies was then placed on pre-culture in 5 ml of nutrient broth for 3 hours (in an oven at 32 °C). At last, we cultured the bacterial strains by streaking on the surface of the agar containing different concentrations of the extracts (aqueous and ethanolic extracts of *A. Melegueta* seeds, native salt and native salt mixed with aqueous extracts from of *A. Melegueta* seeds).

**Phytochemical screening**

The research of different chemical groups present in total extracts of *A. melegueta* seeds was carried out following the method described by Tula [17] and Harborne [18]. The identification of these phytochemical groups was performed qualitatively based on the coloring reaction or precipitation which is occur in the test tubes by adding specific reagents.

## Results and Discussion

**Phytochemical Screening**

The phytochemical analysis carried out on the seeds of the *A. melegueta* is presented in Table 1.

**Table 1**: Phytochemical screening of *A. melegueta* seeds

| **Phytochemical groups** | **Extracts** | |
|---|---|---|
| | **Aqueous extracts** | **Ethanolic extracts** |
| Total polyphenols | + | - |
| Flavonoids | _ | - |
| Tannins | + | - |
| Alkaloids | + | - |
| Bound quinones | - | - |
| Free quinones | - | + |
| Steroids and triterpenoids | - | + |
| Saponines | - | - |
| Anthocyanins | + | - |

Legend: + : presence ; - : absence

From the table 1 above, the results reveal the presence of different compounds in *A. melegueta* seeds, namely: alkaloids, tanins, total polyphenols and anthocyanins from aqueous extracts. On the contrary, the ethanolic extracts reveal the presence of free quinones, steroids and triterpenoids.

**Antibacterial Activity Assay**

The antibacterial activity assay of aqueous (macerated and decocted) and ethanolic extracts of *A. melegueta* seeds is presented in table 2.

**Table 2**: Antibacterial activity of *A. melegueta* seed extracts

| **Tested bacterial strains** | **Minimum Bactericidal Concentration (MBC) in µg/ml** | | |
|---|---|---|---|
| | **Aqueous extracts** | | **Ethanolic extracts** |
| | **Decocted** | **Macerated** | |
| *E. coli* | 20 | 6 | 20 |
| *S. aureus* | 20 | 15 | 20 |
| *Klebsiella spp* | 6 | 0.4 | 4 |

Table 2 compares the results of the antibacterial activity of the total aqueous extracts (decoction, maceration) and the ethanolic extracts of *A. melegueta* seeds. We can notice that *Klebsiella spp*. is sensitive to the seed extracts than *S. aureu s*and *E. coli*. The aqueous extracts precisely the macerated presents a high activity compared to the ethanolic extracts. On the other hand, the decocted aqueous extracts show a low activity compared to the macerated one.

Table 3 compares the results of the antibacterial activity of aqueous and ethanolic extracts of of *A. melegueta* seeds to those of native salt tested individually and the mixture of native salt with aqueous extracts of *A. melegueta* seeds.

**Table 3:** The antibacterial activity of aqueous and ethanolic extracts, the indigenous salt and the mixture of indigenous salt with aqueous extract of *A. melegueta* seeds

| **Tested bacterial strains** | **Minimun Inhibition Concentration in µg/ml** | | | |
|---|---|---|---|---|
| | **Aqueous extracts** | **Aqueous extracts + native salt** | **Ethanolic extracts** | **Native salt** |
| *E. coli* | 6 | 10 | 20 | 20 |
| *S. aureus* | 15 | 20 | 20 | 20 |
| *Klebsiella spp.* | 0.4 | 0.8 | 4 | 15 |

The majority of the extracts tested showedthe antibacterial activity. This justifies their use in traditional medicine for the treatment of infectious diseases and as a preservative of food.

## Discussion

### Phytochemical Screening

While comparing the results of phytochemical screening to the antibacterial activity, we can deduce that the high activity of the aqueous extract, including macerated over the ethanolic extract should probably be caused by the fact that these two extracts do not have the same phytochemical profile. The active ingredients include, alkaloids, tannins and polyphenols must certainly be the cause of the high activity of macerated. The results of this phytochemical screening are quite different from other studies. This observation can be justified by the fact that the phytochemical composition depends on collection time, the organ, method of treatment and analysis, the nature of the used solvent and the origin of the sample (collection place) [1, 19, 15-16, 20].

### Antibacterial Activity Assay

The majority of the tested extracts showed the antibacterial activity. This justifies their use in traditional medicine for the treatment of infectious diseases and as a preservative of food. Our results meet those of Olaniran [21] which showed that the aqueous extracts and total ethanolic extracts of *A. melegueta* seeds have an activity on *Salmonella*, *E. coli* and *Klebsiella*. Souza et al., [15] reported that the total aqueous extracts of *A. melegueta* seeds are active against *S. aureus* and *Candida albicans*. The antimicrobial activity of methanol extracts of *A. melegueta* seeds was also reported by Sonibare *et al.* [16]. Uwera [12] showed that the solution of native salt prepared from the male inflorescence of the palm oil is active against *C. albicans* (3.75 µg/ml), *Staphylococcus spp.* (15 µg/ml) and *Spectrococcus spp.* (15µg/ml).The tested native salt and total ethanolic extract of *A. melegueta* seeds individually showed a low activity compared to the total aqueous extract. We believe that this may be due to the difference of the chemical composition of these extracts.

Olaniran [21] reported an antibacterial activity of the aqueous extracts and total ethanolic extracts of *A. melegueta* seeds on *Salmonella spp, Shigella spp*, *E. coli* and *Klebsiella spp*. The total ethanolic extracts of *A. melegueta* seeds exhibit high activity than the total aqueous extracts. These results are different from the results of the current study. This discrepancy could be due to the phytochemical profile of the plant extracts which often varies with respect to certain factors such as the place of collection of the drug, the harvest time, or in relation to the treatment that is inflicted on the drugs while packaging or in biological screening. Besides the phytochemical studies by N'guessant al., [19], Sonnibar et al., [16] , Echo et al., [14] and Okwu et al., [20] on the extracts of *A. melegueta* seeds collected in different regions showed different results. Another fact is that we used different bacterial strains than Olaniran. *Klebsiella spp.* was very sensitive to the extracts compared to other tested bacterial strains. The high sensitivity of *Klebsiella spp* in all the tested extracts justifies the use of *Afromomum melegueta* seeds to infections due to this microorganism; including urinary tract infections, bronchopulmonary disorders, angina, and infections of the digestive tract (diarrhea for instance ).

Contrary to our hypothesis, we observed in our study that the addition of native salt in the aqueous extract does synergistically enhance the activity of these two antimicrobials. However, the mixture of native salt and total

aqueous extracts of *A. melegueta* seeds had low activity compared to aqueous extract tested individually. It seems that the addition of native salt in the aqueous extract makes it less active (antagonist of the native salt). This could result from the instability of the active ingredients of *A. melegueta* in the presence of native salt. Moreover, the fact that the aqueous extract (decoction) showed low activity compared to the macerated can be explained by the negative influence of heat during the decoction which can cause degradation of the active ingredients heat-sensitive of *A. melegueta* seeds.

**Conclusion and Recommendation**

This study showed that *A. melegueta* seeds contain active bactericidal ingredients and demonstrate that some of these active compounds are heat labile, henceforth the requirement of avoiding extraction techniques that tend to boil the extracts of these seeds or perform other assays that would highlight the temperature scale susceptible of not denaturing the active principles of *A. melegueta*. The seeds of *A. melegueta* have to be recommended for the treatment of infections caused by *Klebsiella spp*. which showed a high sensitivity to the seed extracts. In fact, the native salt and aqueous extract mixture of *A. melegueta* seeds was 50% salt and 50% aqueous extract. Regarding these results, we believe that the salt concentration in the native salt mixture should be revised downwards. It would have also been better to apply the above extracts on foods in order to maintain and assess the duration conservation of these foods for assessing the effectiveness of native salt and aqueous extracts mixture of *A. melegueta*.

Further studies need to be performed in order to characterize and purify the active ingredients of *A. melegueta* seeds, to study the synergy between these different phytochemical groups and to identify the thermolabile active ingredients. We think that the bio-guided fractionation of these chemical groups could lead to the isolation of these active principles. It is appropriate to confirm the antagonistic effect of the native salt by performing these studies *in vivo* and/or to perform other studies on which the native salt and aqueous extract will be used in other proportions (i.e in a mixture on what we have low concentrations of native salt). It would have been also important to perform a study that will compare the antibacterial activity of native salts prepared with different plants (palm oil, water hyacinth and banana) or to study the stability of the active principles of *A. Melegueta* seeds in the presence of indigenous salt.

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