

PHENOLIC CONTENT AND ANTIMICROBIAL POTENTIALS OF *XYLOPIA AETHIOPICA* AND *MYRISTICA ARGENTEA*

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The Folin-Ciocalteu method was used to quantify the total phenolics in the aqueous extract of two spices, *Xylopi aethiopica* and *Myristica argentea*. A modified agar streak dilution method was used to assess the susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the aqueous extracts of the spices. The results showed that high levels of phenolics were present in the extracts. The extracts also showed high-modest antimicrobial activity against the tested bacteria.

Key words: *Myristica argentea*; *Xylopi aethiopica*; phenolics; antimicrobial activity; *Escherichia coli*; *Staphylococcus aureus*

СОДРЖИНА НА ФЕНОЛНИ СОЕДИНЕНИЈА И АНТИМИКРОБЕН ПОТЕНЦИЈАЛ НА *XYLOPIA AETHIOPICA* И *MYRISTICA ARGENTEA*

Употребен е методот на Folin-Ciocalteu за да се квантифициваат вкупните фенолни соединенија во воден екстракт од два вида, *Xylopi aethiopica* и *Myristica argentea*. За да се определи осетливоста на *Escherichia coli* и *Staphylococcus aureus* на водни екстракти од двата вида, користен е модифициран метод со разредување на агар. Резултатите покажаа дека во екстрактите е присутно високо ниво на фенолни соединенија. Освен тоа, екстрактите покажаа висока до умерена антимикробна активност во однос на тестираните бактерии.

Клучни зборови: *Myristica argentea*; *Xylopi aethiopica*; фенолни соединенија; антимикробна активност; *Escherichia coli*; *Staphylococcus aureus*

1. INTRODUCTION

Phytochemicals, such as phenolics, which are present in foods, have attracted a great deal of attention recently. This is due to reports of the role they play in preventing diseases [1]. Phenolics are secondary metabolites present in all plants and contribute to the development of color, taste and palatability, as well as the defense system of plants [2]. Reports abound on the multiple biological effects of phenolics, including their antimicrobial activity [3–5]. Phenolics are commonly found in both edible and inedible plants; extracts of spices

have been shown to be rich in phenolics [6]. Literature search reveals that spices are able to prevent or retard the growth of microorganisms [7–8] and phenolics have been implicated in this antimicrobial activity [9–11].

Historically some of these phenolics were considered as antinutrients due to their adverse effects in human metabolism [1]. But recent reports on the antioxidant and antimicrobial properties of phenolics lead to a rethinking among food scientists. The mechanisms responsible for the antimicrobial activity of phenolics include adsorption and disruption of microbial membranes, interac-

tion with enzymes and substrate and metal ion deprivation [1, 12]. The need to search for an antimicrobial agent among natural sources is justified by the increasing resistance of microorganisms to antibiotic [13] and the presence of chemical residues in foods [1].

*Xylopi*a *aethi*o*pica* and *Myristica argentea* are common spices in sub-Saharan Africa. While several studies are reported on the antimicrobial activity of *X. aethi*o*pica* extract [14–16], our literature search also reveals the presence of lignans and neolignans in *M. argentea* [17, 18]; in fact, Nakatami and coworkers reported on the antimicrobial action of these lignans [18]. The objectives of the present work are: (a) to determine the total phenolic content of *X. aethi*o*pica* and *M. argentea*, (b) to test the antimicrobial activity of these spices and (c) to establish a relationship between total phenolic content and antimicrobial activity of the spices.

2. MATERIALS AND METHODS

Samples. The spices were collected from a local farmer in Benin City, Nigeria. They were cleaned, freeze-dried and ground into fine powder by a laboratory mill.

Chemicals. All the reagents used were of analytical grade. Folin-Ciocalteu phenol, sodium bicarbonate, dimethyl sulfoxide (DMSO) and ampicillin were obtained from Sigma Chemical Co. (St. Louis, MO). Distilled deionized water (ddH₂O) was obtained with the aid of a Milli-Q water treatment apparatus (Millipore, Milan, Italy).

Extraction of Phenolics. The extraction was achieved as previously reported elsewhere [1] with some modification. Each powdered sample (10 g) was thoroughly mixed with 200 ml of ddH₂O. The mixture was sonicated for 1 h and centrifuged at 10000 g for 15 min at room temperature. The residue was re-extracted with ddH₂O until negative reaction with NaOH. The supernatants were collected, pooled and concentrated to dryness under vacuum. The residue was dissolved in a final volume of 10 ml of ddH₂O, vortexed for 5 min and filtered through a 0.45 µm Teflon membrane (Millipore). To prevent oxidation of the phenolics, extraction was achieved rapidly and extracts were immediately analyzed.

Determination of total phenolic content. The total phenolic content of the extracts was measured using the Folin-Ciocalteu method as described elsewhere [17]. Briefly, 1 ml of appropriately diluted samples and standard solution of gallic acid were added to 25 ml volumetric flask containing 9 ml of ddH₂O. A reagent blank using ddH₂O was also prepared. One milliliter of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of a 7% Na₂CO₃ solution was added with mixing. The solution was then immediately diluted to a volume of 25 ml with ddH₂O and mixed thoroughly. After incubation for 90 min at 23 °C, the absorbance relative to that of a prepared blank at 750 nm was measured using a Hewlett Packard 8453 diode array UV/vis spectrophotometer. The total phenolic contents of the samples are expressed in milligrams of gallic acid equivalents (GAE). All samples were analyzed in triplicate and the mean reported.

Antimicrobial activity

Microorganisms. *Escherichia coli* and *Staphylococcus aureus* were obtained from the Microbiology Department of the University of Benin, Nigeria. The bacteria were cultured aerobically at 37 °C in nutrient agar medium.

Extracts preparation. A powdered sample (ca 10 g) was extracted with 250 ml of boiling water for 1 h and filtered through whatman no. 4 filter paper. The extracts were freeze-dried and dissolved in ddH₂O at a concentration of 100 mg/ml.

Test assays for antimicrobial activity. Susceptibility of the test organisms to the extract was determined by employing a modified agar streak dilution method based on a radial diffusion [13]. Briefly, the bacterial suspension was prepared to contain approximately 10⁸ cfu/ml and the plates containing agar medium were inoculated (100 µl). A 50 µl of each sample was pipetted in a hole (depth 3 mm, diameter 4 mm) made in the center of the agar. The minimum inhibitory concentration (MIC) was considered to be the lowest concentration of the tested sample able to inhibit the growth of bacteria after 24 h. The diameters of the inhibitory zones corresponding to MICs were measured using a ruler, with an accuracy of 0.5 mm. The tests were carried out in triplicates and the mean reported. Controls using only inoculation and DMSO solution of ampicillin (standard) were also carried out.

3. RESULTS AND DISCUSSION

Figure 1 shows that the total phenolic content of *X. aethiopia* (375 mg of GAE) was lower than that of *M. argentea* (840 mg of GAE). The levels of total phenolics in both spices were higher than the levels of total phenolics in some vegetables and spices reported by previous workers [19]. The levels of total phenolic in *X. aethiopia* were surprisingly higher than the levels reported by Olukemi and co-workers [19]; Olukemi and co-workers in their studies of some Nigerian spices find the levels of *X. aethiopia* to be 152.24 mg/100mg tannic acid equivalents (TAE). To the best of our knowledge, some of the factors that may have contributed to the discrepancy in our results and that of Olukemi and his group may be the difference in the methods used in extraction and determination of phenolics. Phenolics are among the many phytochemicals found in foods that possess antioxidant and antimicrobial properties [3–5]. The levels of phenolics detected in the present studies suggest that *X. aethiopia* and *M. argentea* could contribute to potential health benefits.

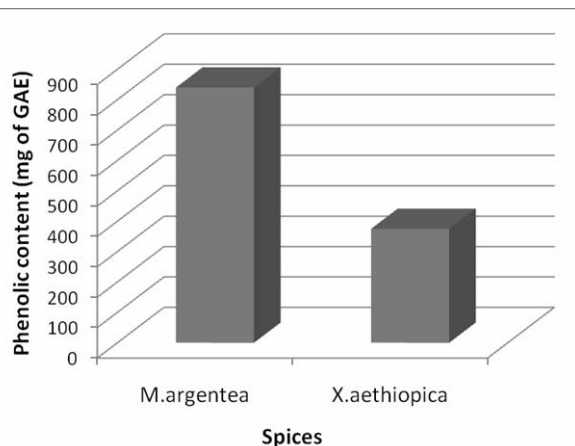


Fig. 1. Phenolic content of *Myristica argentea* and *Xylopi aethiopia*

The susceptibility of *E. coli* and *S. aureus* to *X. aethiopia* and *M. argentea* was also investigated. In general, the results revealed the antimicrobial potential of the extracts. Extracts from *M. argentea* were more effective in inhibiting the microorganisms than extracts from *X. aethiopia* (Table 1). The inhibition zones showed clear-cut antimicrobial potentials and the diameter of the inhibition zones increased with the levels of the phenolics in the extracts (Table 1). This was not surprising because phenolics have been implicated in the antimicrobial activity of spices, herbs, fruits

and vegetables [3 – 5]. Additionally, the relatively higher potency of *M. argentea* (total phenolic 840 mg of GAE) compared to *X. aethiopia* (total phenolic 375 mg of GAE) further suggests that phenolics certainly contribute to the antimicrobial effects observed.

Table 1

Antimicrobial activity of *Myristica argentea* and *Xylopi aethiopia**

Samples	MIC (mg/ml)	
	<i>E. coli</i>	<i>S. aureus</i>
<i>M. argentea</i>	9.80 (+++)	6.20 (+++)
<i>X. aethiopia</i>	28.3 (++)	17.8 (++)
Ampicillin	0.00633 (++++)	0.00633 (++++)

*No antimicrobial activity (-), inhibition zone < 1 mm. Slight antimicrobial activity (+), inhibition zone 2–3 mm. Moderate antimicrobial activity (++) , inhibition zone 4–5 mm. High antimicrobial activity (+++), inhibition zone 6–9 mm. Strong antimicrobial activity (++++), inhibition zone > 9 mm.

The results also showed that *S. aureus* was more sensitive to the extracts than *E. coli*. The antimicrobial activity of the extracts was modest, with MICs ranging between 6.20 and 28.3 mg/ml when compared with the standard ampicilline, which had MICs of 0.00633 mg/ml. Usually pure active compounds exhibit more potency than crude extracts. The present study confirms previous reports about the antimicrobial activity of spices and also suggests that phenolics contribute significantly to the antimicrobial activity of spices [1 – 3]. Though phenolics influence the antimicrobial effects, other chemical components of the extracts certainly would contribute.

In conclusion, the results obtained in the present work suggest that *X. aethiopia* and *M. argentea* are a good source of healthy phytochemicals, especially phenolics, indicating that inclusion of these spices in human diet could contribute to potential health benefits.

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