Modern Journal of Health and Applied Sciences *(MJHAS)*

E-ISSN 3078-2791; P-ISSN 3078-2783

Journal homepage: https://mucjournals.muc.edu.ps

Evaluation of the phytochemicals and antimicrobial properties of *Tetrapleura Tetraptera* and *Piper Nigrum*

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ARTICLE INFO

Received: 25th May 2024
Received in revised form: 02nd July 2024
Accepted: 05th July 2024
Published: 15th September 2024

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Citation : Esoh, R. T., Davila, D. T., & Gyampoh, S. (2024). Evaluation of the phytochemicals and antimicrobial properties of Tetrapleura Tetraptera and Piper Nigrum. *Modern Journal of Health and Applied Sciences*, 1(1), 1-9.

DOI: https://doi.org/10.70411/MJHAS.1.1.2024127

ABSTRACT

The search for new substances with antibacterial activities has become an urgent necessity due to the resistance of many bacteria of medical importance to antibiotics. In an attempt to seek out new antibacterial agents from plants, *E. coli*, *S. aureus*, and *Salmonella spp* susceptibility to the seed extracts of *Tetrapleura tetraptera and Piper nigrum* were assessed. A Qualitative phytochemical screening was also done to establish the various phytochemicals found in the plants. To achieve these findings, the dried seeds of *Tetrapleura tetraptera and Piper nigrum* were collected in the Bamenda food market and ground. Further, the powder obtained was subjected to aqueous and alcoholic extractions separately in which the alcoholic and aqueous extracts of *Tetrapleura teraptera and Piper nigrum* seeds were evaluated for their antibacterial potential against *E. coli*, *S. aureus*, and *Salmonella spp* using agar diffusion. The obtained results indicated that *S. aureus and E. coli* were susceptible to the alcoholic and aqueous extract of *Tetrapleura tetraptera* (with a MIC of 50mg/ml; 100mg/ml), respectively, for *S.aureus* and (25mg/ml; 50mg/ml) for *E.coli.* For *S. aureus*, susceptibility to aqueous extract of *Piper nigrum* shows sensitivity (with MIC of 100mg/ml). Complete resistance was registered with the alcoholic extracts of *Piper nigrum*. Furthermore, the phytochemical screening results indicated the presence of resins, alkaloids, glycosides, tannins, flavonoids (present in all powder), and saponins (absent only in *Piper nigrum).* In conclusion, the alcoholic and aqueous extract of *Tetrapleura tetraptera* seeds has antibacterial activity against *S. aureus* and *E. coli*. It was proposed that further studies should be carried out on the susceptibility of *Tetrapleura tetraptera* to other bacteria.

Keywords : *Tetrapleura tetraptera, Piper nigrum,* Antimicrobial properties, *E. coli*.

1. Introduction

For centuries, people have recognized the health benefits of plants. This has become even more relevant today as the effectiveness of antibiotics declines in the face of increasingly resistant pathogens (Abdallah, 2017). Spices packed with bioactive compounds have a long history of use across cultures for various purposes beyond just flavoring food (Gupta et al., 2014; Jessica Elizabeth et al., 2017). They act as natural preservatives, extending shelf life and preventing food spoilage and foodborne illness (Liu et al., 2017). Additionally, some spices have applications in both food production and traditional medicine, where they can inhibit infections and eliminate harmful pathogens. The antibacterial efficacy of some spices and seasonings have been proved scientifically, such as black seed (*Nigella sativa*) (Abdallah & Koko, 2017), garlic bulb (*Allium sativum*) (Shafiur Rahman et al., 2006), onion (*Allium cepa*) (Kim, 1997), thyme (*Thymus vulgaris*) and clove (*Syzygium aromaticum*) (Nzeako et al., 2006), cinnamon bark (*Cinnamomum verum*) (Julianti et al., 2017), oregano (*Origanum vulgare*) (Saeed & Tariq, 2009), cumin (*Cuminum cyminum*) (Allahabri et al ., 2010) and many more. In light of the current worldwide focus on natural products, medicinal plants, and traditional medicine, it is necessary to reinvigorate research on spices to develop novel natural pharmaceuticals. It is worth noting that a significant percentage, up to 80%, of the global population continues to depend on medicinal plants and natural products as their primary source of healthcare (Beyene et al., 2016). This study highlights the medicinal and nutritional importance of black pepper (*Piper nigrum*) and *Tetrapleura tetraptera* fruits and their efficacy as antibacterial agents.

Figure 1. *Piper nigrum* seed and powder.

Figure 2. *Tetrapleura tetraptera* seed and powder.

2. Experimental part

2. 1. Materials

A good quantity of dry spices of *Tetrapleura tetraptera* and *Piper nigrum* were obtained from the Bamenda food market from randomly selected plants in February 2021. These spices were brought to the laboratory and passed in an incubator for 24 hours to dry. The surface of the working table was then disinfected with a 1:10 dilution of hypochlorite for 15 minutes. The spices were well ground on the grinding stone to obtain powder. The powder was then accurately weighed out using a balance, divided into three portions, and kept in two separate air-tight Containers for later use.

2. 2. Geographical distribution, medicinal importance, and classification of plants.

2. 2. 1. *Tetrapleura tetraptera*

- **Geographical distribution and medical importance**

Tetrapleura tetraptera is native to tropical Africa with a distribution range extending from Mauritania to Tanzania (Anyamele et al., 2023), commonly known as the “Aidan tree” in Nigeria, "Kikangabalimu" in Uganda, "Prekese" in Ghana, and “four cona” in Bamenda, Cameroon. The tree prefers humid and tropical climates, thriving in rainforests and regions with rich, well-drained soils. *Tetrapleura tetraptera* has significant medicinal value, particularly in traditional African medicine. Some of its key medical benefits include antimicrobial properties, Antioxidant Properties, Anti-inflammatory and Analgesic Effects**.** Its medicinal benefits are attributed to bioactive compounds such as alkaloids, flavonoids, saponins, tannins, phenols, and glycosides, which are essential for health (Adesina et al., 2016; Ekwenye & Okorie, 2010).

- **Botanical classification**

Kingdom: Plantae
Order: Fabales
Family: Fabaceae
Genes: Tetrapleura
Species: *Tetrapleura tetrapterae*

2. 2. 2. *Piper nigrum*

- **Geographical distribution and medical importance**

Black pepper (*Piper nigrum*) is one of the most widely used spices in the world spices and is considered “ The King of spices’’ among various spices (Damanhouri & Ahmad, 2014), which has a significant geographical distribution such as India, Indonesia, Malaysia, and Brazil (Ahmad et al., 2012). The peppercorns of *Piper nigrum*, along with their active constituents, are commonly utilized in a variety of culinary dishes. Additionally, various parts of *Piper nigrum*, including its secondary metabolites, serve multiple purposes in medicine, as preservatives, and as natural control agents. Biologically, *Piper nigrum* holds significant value. Research has demonstrated that both peppercorns and the secondary metabolites of *Piper nigrum* exhibit a range of biological activities, including anti-apoptotic, antibacterial, anti-colon toxin, antidepressant, antifungal, anti-diarrheal, anti-inflammatory, anti-mutagenic, anti-metastatic, and antioxidative effects (Ashokkumar et al., 2021; Zou et al., 2024).

- **Botanical classification**

  Kingdom: Plantae

  Order: Piperrales

  Family: Piperaceae

  Genus: Piper

  Species: *Piper nigrum*

2. 3. Aqueous extraction

In the first portion, 25 g of the powdered spices were placed in a clean and sterile 200 ml sterile container, and the appropriate amount of distilled water was added. The container was well mixed by shaking intermittently. After 48 hours, the content was filtered using Whatman No1 filter paper into another clean sterile container. This container and its contents were then evaporated to dryness in an incubator at 500 degrees Celsius.

2. 4. Alcoholic extraction

For the second portion, 25 g of the powdered spices were placed in a clean and sterile 200 ml container and the same\ appropriate amount of methanol was added. The container was shaken intermittently for 48 hours, after which its contents were filtered using Whatman No1 filter paper into another clean, sterile container. This container and its contents were then evaporated to dryness in an incubator at 60 degrees Celsius.

2. 5. Fractionation of the extract

1g of each extract was dissolved in two pre-sterilized vial bottles containing 5 ml of distilled water. This gives a concentration of 100 mg/ml.

Figure 3. Alcoholic and aqueous extraction of *Piper nigrium.*

Figure 4. Alcoholic and aqueous extraction of *Tetraplera tetraptera*

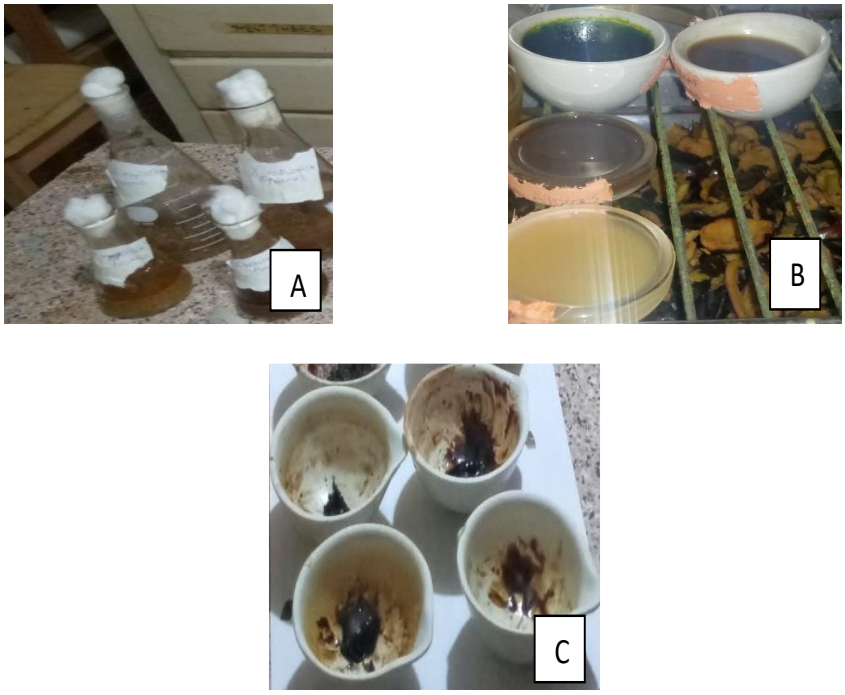

Figure 5. (A) Alcoholic and aqueous extraction, **(B)** removal process of solvent, and **(C)** Extract.

2. 6. Phytochemical screening

The methanolic and aqueous extraction of these spices (*Piper nigrum* and *Tetrapleura tetraptera*) were used as the samples for qualitative phytochemical screening of resins, saponins, flavonoids, alkaloids, and glycosides following the standard procedures described by (Evans, 1997).

- **Detection of resins**

0,5 g of the powdered spices were added to 5 ml of boiling ethanol. It was then filtered through Whatman No1 filter paper and the filtrate was diluted with 4 ml of 1% aqueous HCl. The formation of a heavy resinous precipitation indicated the presence of resins (Evans, 1997).

- **Detection of saponins**

0,5 g of each sample powder spice was stirred with 10 ml of distilled water in a test tube. The test tube and its contents were then placed in a water bath to heat up to boiling point. Frothing which persists in warming was taken as preliminary evidence for the presence of saponins.

- **Detection of flavonoids**

0,5 g of each sample of the powder spices was dissolved in 2 ml dilute NaOH solution. A few drops of concentrated H_2SO_4 were then added. The presence of flavonoids was indicated by the disappearance of color (Evans, 1997).

- **Detection of alkaloids**

0,5 g of each sample of the powder spices was stirred with 5 ml of 2 N HCl in a water bath. The content of the test tube was then filtered using Whatman No1 filter paper and 1ml of the filtrate was tested with a few drops of Dragendorff's reagent and a second 1ml portion treated similarly with Wagner Wagner's reagent.

- **Detection of glycosides**

0,5 g of each sample of the powdered spices was stirred with 10 ml of boiling distilled water. This was filtered and 2 ml of the filtrate was hydrolyzed with a few drops of concentrated HCl and the solution was rendered alkaline with a few drops of ammonia solution. 5 drops of this solution were added to 2 ml of Benedict's qualitative reagents and boiled. The appearance of a reddish-brown precipitate showed the presence of glycoside.

- **Detection of tannins**

0,5 g of each sample of the powdered spices was stirred with 10 ml of boiling distilled water. This was filtered, and a few drops of 6% ferric chloride were added to the filtrate. The second portion of the filtrate was treated with a few drops of iodine solution. The appearance of a faint bluish coloration confirms the presence of tannins.

2. 7. Identification of the test organisms

A pure culture of *Staphylococcus aureus*, *E. coli*, and *Salmonella* was obtained from the Bamenda Regional Hospital, they were all identified based on microscopical staining reactions and biochemical tests.

2. 7. 1. Identification of *S. aureus* on biochemical test

- **Catalase test**: This test was used to identify both gram-negative bacilli and gram-positive cocci isolated from MacConkey and blood agar.
- **Coagulase test**: this test was used to differentiate pathogenic *S. aureus* from nonpathogenic *Staphylococcus*. A drop of plasma was placed on a glass slide, using a wire loop, and a well-isolated colony was taken from the culture plate and emulsified with the drop of plasma. Agglutination was observed indicating *S. aureus* on motile bacteria.

2. 7. 2. Identification of *E. coli*

- **Indole test**: this test was carried out for all gram-negative bacilli isolated from MacConkey agar to differentiate indole-producing from non-indole-producing bacteria.
- **Motility test**: this test was done to differentiate motile from non-motile bacteria. A drop of the sub-cultured colony from peptone water using a pasture pipette was placed on a slide, covered with a cover slide, and observed under objective 10 after 2 minutes *E. coli* was also positive for catalase.

2. 7. 3. Identification of *Salmonella*

- *Salmonella* is the only test organism that was positive for catalase.

2. 8. Preparation of required media

3.8 g of the Muller Hinton agar powder was dissolved in 100 ml of distilled water and the solution was sterilized by autoclaving at 121 ℃ for 15 minutes and then waited to cool to 45 ℃. It was then poured into 5 sterile Petri dishes, where it cooled and solidified under sterile conditions. These petri dishes were dried and incubated for 24 hours to validate contamination by any microbe.

2. 9. Susceptibility testing

- **Preparation of isolate**

Susceptibility testing was done using the agar well diffusion technique.

- **Inoculum standardization**

A small piece of *staphylococcus aureus* pure culture was picked up from the plate with the aid of a sterile inoculating loop, diluted into 10 ml of pre-prepared alkaline peptone water in a test tube, and incubated for 24 hours. A small piece of *E. coli* and *Salmonella* pure culture was also picked up from the plate and diluted into 1 ml of sterile distilled water in a clean test tube. The test organisms were then adjusted to a turbidity of 0.5 McFarland by adding the pure culture progressively in 10 ml and 1 ml sterile distilled water respectively until it matched with the 0.5 McFarland standard.

- **Susceptibility testing against *staphylococcus aureus***

A plate of Muller Hinton agar was used and the standard inoculum of *Staphylococcus aureus* was poured on the plate. The excess inoculum was removed aseptically and the surface of the plate was allowed to dry. Three wells were dug on each section of the plate using a sterile radio antenna of about 4 mm; one well was filled with the aqueous extract, the second with the ethanol extract, the third with a solution of 1 mg/ml of chloramphenicol (positive control) and the fourth well was left empty (negative control).

- **Susceptibility testing against *E. coli.***

A plate of Muller Hinton agar was used, and the appropriate amount of inoculum of *E. coli* was poured on the plate. Excess inoculum was removed from the surface of the plate and allowed to dry. Three were dug with the help of a sterile radio antenna of about 4 mm. The plate was incubated at 37^0 C for 24 hours after which the antimicrobial sensitivity was evaluated.

- **Susceptibility testing against *Salmonella***

Not much work was done for *Salmonella* testing because the bacteria strain was resistant to all the extracts.

3. Results

3. 1. Phytochemical screening results

Table 1. Types of compounds identified in the phytochemical screening of *Tetrapleura tetraptera* and *Piper nigrum.*

Seed powder	Phytochemicals					
	Saponins	**Resins**	**Alkaloides**	**Tannins**	**Glycosides**	**Flavonoids**
Piper nigrum	-	++++	++	+	+	+
T. tetraptera	+	+++	+	+	+	++

(-): absent; (+): present, (++++) highly present

3. 2. Antimicrobial susceptibility results

Table 2. Evaluation of MIC (mg/ml) and antimicrobial susceptibility testing of *Tetrapleura tetraptera* and *Piper nigrum* on *S. aureus, E. coli,* and *Salmonella*.

Extract	*S. aureus*	*E. coli*	*Salmonella*
Alcoholic extract of *P nigrum*	100.00	100.00	00.00
Aqueous extract of *P nigrum*	100.00	100.00	00.00
Alcoholic extract of *T tetraptera*	50.00	25.00	00.00
Aqueous extract of *T tetraptera*	100.00	50.00	00.00

4. Discussion

Results of the phytochemical screening components (Saponin, resins, alkaloids, tannins, glycosides, flavonoids) of spices during this study indicated that *Tetrapleura tetraptera* contains all the six secondary metabolites studied, antibacterial susceptibility testing on *Staphylococcus aureus* and *E. coli* was sensitive to the aqueous, and alcoholic extract of *Tetrapleura tetraptera* at an MIC of 100 mg/ml for *S. aureus* and 50mg/ml for *E. coli* respectively at incubation temperature of 24 hours. On the other hand, the phytochemical screening of *Piper nigrum* revealed that the spice is highly rich in resins, alkaloids, flavonoids, glycosides, and tannins. The susceptibility testing on *Staphylococcus aureus* with the aqueous extract revealed it to be sensitive with an MIC of 100 mg/ml.

5. Conclusion

To conclude, the antibacterial susceptibility testing using an aqueous extract of *Tetrapleura tetraptera* and *Piper nigrum* on *Staphylococcus aureus* and *E. coli* revealed sensitivity, while the alcoholic extract of *Tetrapleura tetraptera* and *Piper nigru*m was also sensitive on *Staphylococcus aureus* and *E. coli*. On the other hand, the aqueous extract of *Piper nigrum* was sensitive only to Staphylococcus. Both the alcoholic and aqueous extracts of *Tetrapleura tetraptera* and *Piper nigrum* were resistant to *Staph* and *E. coli*. All these spices have phytochemical properties except for saponin which is absent in *Piper nigrum*. To the medical laboratory students; these plant extracts should be used to test for the susceptibility of other groups of bacteria.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data Availability

No data was used for the research described in the article.

References

Abdallah, E. M. (2017). Black Seed (Nigella sativa) as antimicrobial drug: a mini-review. *Novel Approches in Drug Designing and Develop*, *3*(2), 1-5.

Abdallah, E. M., & Koko, W. S. (2017). Medicinal plants of antimicrobial and immunomodulating properties. *Antimicrobial Research: Novelbioknowledge and Educational Programs; Formatex Research Center: Badajoz, Spain*, 127-139.

Adesina, S., Iwalewa, E., & Johnny, I. (2016). Tetrapleura tetraptera Taub-ethnopharmacology, chemistry, medicinal and nutritional values-a review. *British Journal of Pharmaceutical Research*, *12*(3), 1-22.

Ahmad, N., Fazal, H., Abbasi, B. H., Farooq, S., Ali, M., & Khan, M. A. (2012). Biological role of Piper nigrum L.(Black pepper): A review. *Asian Pacific Journal of Tropical Biomedicine*, *2*(3), S1945-S1953.

Anyamele, T., Onwuegbuchu, P. N., Ugbogu, E. A., & Ibe, C. (2023). Phytochemical composition, bioactive properties, and toxicological profile of Tetrapleura tetraptera. *Bioorganic Chemistry*, *131*, 106288.

Ashokkumar, K., Murugan, M., Dhanya, M., Pandian, A., & Warkentin, T. D. (2021). Phytochemistry and therapeutic potential of black pepper [Piper nigrum (L.)] essential oil and piperine: A review. *Clinical Phytoscience*, *7*(1), 52.

Beyene, B., Beyene, B., & Deribe, H. (2016). Review on application and management of medicinal plants for the livelihood of the local community. *Journal of Resources Development and Management*, *22*(1), 33-39.

Damanhouri, Z. A., & Ahmad, A. (2014). A review on therapeutic potential of Piper nigrum L. *Black Pepper): The King of Spices. Med. Aromat. Plants*, *3*(3), 161.

Ekwenye, U. N., & Okorie, C. F. (2010). Antibacterial activity of Tetrapleura tetraptera Taub. pod extracts. *Int J Pharma Bio Sci*, *1*(4), 734-741.

Evans, W. C. (1997). Trease and Evans' pharmacognosy. *General Pharmacology*, *2*(29), 291.

Gupta, N., Parashar, P., Mittal, M., Mehra, V., Khatri, M., & Rajguru, S. (2014). Antibacterial potential of Elletaria cardamomum, Syzygium aromaticum and Piper nigrum, their synergistic effects and phytochemical determination. *J. Pharm. Res*, *8*(8), 1091-1097.

Jessica Elizabeth, D. L. T., Gassara, F., Kouassi, A. P., Brar, S. K., & Belkacemi, K. (2017). Spice use in food: Properties and benefits. *Critical reviews in food science and nutrition*, *57*(6), 1078-1088.

Julianti, E., Rajah, K. K., & Fidrianny, I. (2017). Antibacterial activity of ethanolic extract of cinnamon bark, honey, and their combination effects against acne-causing bacteria. *Scientia pharmaceutica*, *85*(2), 19.

Kim, J.-H. (1997). Anti-bacterial action of onion (Allium cepa L.) extracts against oral pathogenic bacteria. *The Journal of Nihon University School of Dentistry*, *39*(3), 136-141.

Liu, Q., Meng, X., Li, Y., Zhao, C.-N., Tang, G.-Y., & Li, H.-B. (2017). Antibacterial and antifungal activities of spices. *International journal of molecular sciences*, *18*(6), 1283.

Nzeako, B., Al-Kharousi, Z. S., & Al-Mahrooqui, Z. (2006). Antimicrobial activities of clove and thyme extracts. *Sultan Qaboos University Medical Journal*, *6*(1), 33.

Saeed, S., & Tariq, P. (2009). Antibacterial activity of oregano (Origanum vulgare Linn.) against gram positive bacteria. *Pakistan journal of pharmaceutical sciences*, *22*(4), 421-425.

Shafiur Rahman, M., Ibrahim Al-Sheibani, H., Hamad Al-Riziqi, M., Mothershaw, A., Guizani, N., & Bengtsson, G. (2006). Assessment of the anti-microbial activity of dried garlic powders produced by different methods of drying. *International Journal of Food Properties*, *9*(3), 503-513.

Zou, R., Zhou, Y., Lu, Y., Zhao, Y., Zhang, N., Liu, J., Zhang, Y., & Fu, Y. (2024). Preparation, pungency and bioactivity transduction of piperine from black pepper (Piper nigrum L.): A comprehensive review. *Food Chemistry*, 139980.