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Inhibition Potential of *Zingiber officinale*, *Curcuma longa* and *Thymus vulgaris* Extracts on Pathogens Isolated from Food Samples

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Abstract: **Medicinal plants contain substances that can be used for treatment of infections and have the ability to inhibit resistant microorganisms. In this study the inhibition potential of ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) and thyme (*Thymus vulgaris*) extracts on pathogens isolated from food samples were determined. The food borne pathogens were isolated from milk and meat using mannitol salt agar, macconkey agar and salmonella shigella agar respectively. The air dried and sun dried samples were extracted with water and methanol respectively. The antimicrobial assays of the extracts were carried out using agar well method. The result revealed all the assayed plants as good antibacterial agent on all the test pathogens (≤28mm). The methanol extracts gave significant inhibition than aqueous extracts in all the samples ($p < 0.05$). The inhibition rate of the air dried sample was higher than that of sun dried one ($p < 0.05$). The obtained results revealed all the assayed plants with great antibacterial potential, air drying the best method for drying these plants and methanol, best solvent for their extraction.**

Keywords: **Antibacterial, aqueous extract, medicinal plants, food Borne Pathogens, methanol extract.**

1. INTRODUCTION

Foodborne pathogens are biological agents that can cause foodborne illness as a result of ingestion of a contaminated food or drink (Bintsis, 2017). Infectious diseases caused by these foodborne pathogens are life threatening all over the world (Li *et al.*, 2017). The most common foodborne pathogens are *Norovirus, Salmonella, Clostridium perfringens, Campylobacter, Escherichia coli and Staphylococcus aureus.*

Foodborne pathogens may be isolated from many sources such as meat product, drinking water, dairy product, eggs, vegetables and fruits etc. The prevalence of multi drug resistant (MDR) foodborne pathogens may be increased by the consumption of these contaminated foods (Gohari *et al*., 2021). The use of antibiotics to treat infections caused by this food borne pathogens is ineffective due to their bacterial resistance, thus leading to the progression of the illness (Hemalata and Virupakshaiah, 2016). Several plants known as medicinal plants can be used in the treatment of these foodborne infections because it contains substances that can be used for therapeutic purposes or exert beneficial pharmacological effect on the human or animal body. These plants have enormous potential for the discovery of new bioactive compounds that have the ability to inhibit resistant microorganisms. Plants such as ginger, thyme, turmeric, clove, oregano, thyme, cinnamon and cumin possessed significant antibacterial and antifungal activities against food spoilage bacteria like *Bacillus subtilis* and *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*, harmful fungi like *Aspergillus flavus*, even antibiotic resistant microorganisms such as methicillin resistant *Staphylococcus aureus* (Li *et al.*,2017; Mahmood and Mamood,2013;Sofowora *et al*.,2013; Gul and Bakht,2015).

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The whole plant or extracts of these plants can be used in the treatment of infections but the extracts are commonly used (Karen *et al.*, 2003). The solvents used in extraction of these plants are polar solvent (e.g., water, alcohols, acetone, acetonitrile, dimethylformamide (DMF), dimelthylsulfoxide (DMSO), isopropanol, and methanol) and nonpolar (e.g., n-hexane, ether, chloroform)(Abubakar and Haque, 2020)

Therefore, the present study focused on determination of the inhibition potential of ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) and thyme (*Thymus vulgaris*) extracts on pathogens isolated from food samples.

2. MATERIALS AND METHODS

Isolation of the Food Borne Pathogens

The food borne pathogens were isolated from milk and meat. The serially diluted samples (10^3) were inoculated into mannitol salt agar(MSA),Salmonella shigella agar (SSA) and macConkey agar respectively and incubated at 37^{o}C for 24h. Colonial appearances were observed and were subjected to other identification tests.

Collection and Preparation of the test samples

The plants assessed (*Zingiber officinale*, *Curcuma longa* and *Thymus vulgaris*) were bought from Garriki market, Enugu State, Nigeria.

The samples, ginger and turmeric were peeled, washed and sliced into pieces. Then all the spices were air dried and sun dried respectively. They were homogenized using home blender, sieved with muslin cloth and stored in sterile containers prior to use (George-Okafor *et al*., 2019).

Extraction of the samples

For aqueous extracts, 10g of the samples (air dried and sun dried) were mixed with 200ml sterile water and were agitated for 24h at room temperature respectively. Thereafter the recovered filtrates were concentrated by evaporation in water bath at 60^{o}C for 8h. The same were carried out on methanol extract except that the filtrates were allowed to evaporate naturally at room temperature. All samples were stored at 4^{o}C prior to use.

Inhibition Potential Assay

The inhibition potential assay was carried out using agar well method as described by Erhimu *et al*. (2019). The broth culture of the test organisms were inoculated into nutrient agar and were spread out using a swab stick. Thereafter, three wells were made on the culture using a sterile cork borer. Each of the extracts (0.1ml) was added into the wells and incubated at 37^{o}C for 24h. The zones of inhibition were measured using a meter rule (mm).

Statistical Analysis

The statistical analysis of data obtained were analyzed using IBM Statistical Product and Service Solutions (SPSS), version 18. One-way analysis of variance (ANOVA) with Duncan test for multiple comparism was used to compare means across the groups.

3. RESULTS AND DISCUSSION

The inhibition rate of air dried ginger extract as stipulated in Figure 1a, methanol extract gave the highest inhibition rate (≥25mm) in all the test organisms ($p< 0.05$). Both the aqueous and methanol extracts inhibited *Escherichia coli* than the other test organisms but there was no significant different in the inhibition rate ($p> 0.05$).

The result of the inhibition rate of the sun dried ginger extract was stipulated in Figure 1b. Methanol ginger extract had highest significant inhibition rate (≥22mm) in all the test organisms. The inhibition rate of air dried ginger extracts was higher than the sun dried ginger extract in all the test organisms with a significant difference. Karuppia and Rajaram (2012) obtained similar result (20 mm) on same pathogens. Lesser inhibition rate of ≥ 12mm was observed with methanol ginger extract against *Salmonella* species, *E. coli* , *Shigella* spp and *Citrobacter* (Yadufashije *et al.*, 2020).

The decrease in the inhibitory rate of sun dried food spices extract in all the samples could be that the functionality of the food spices was affected due to the increase of temperature during the drying process as reported by Zubair *et al*. (2020).

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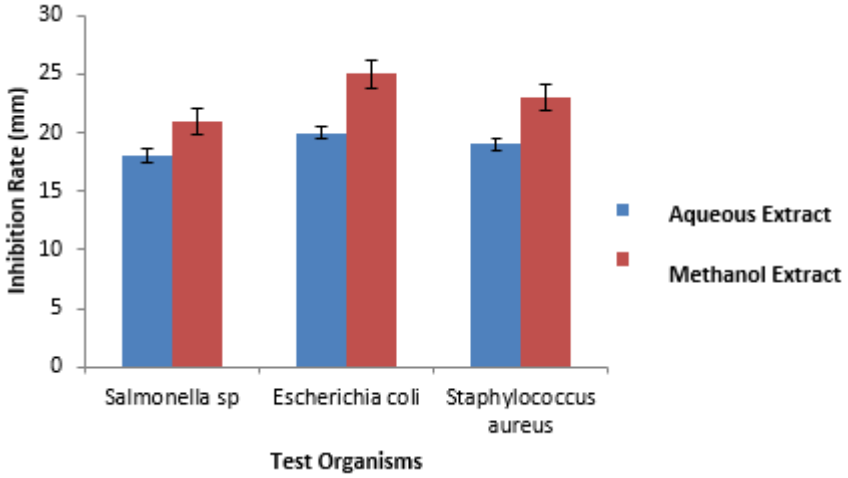

Figure 1a: Inhibition Rate of the Air dried Ginger Extracts on the Food Borne Pathogens

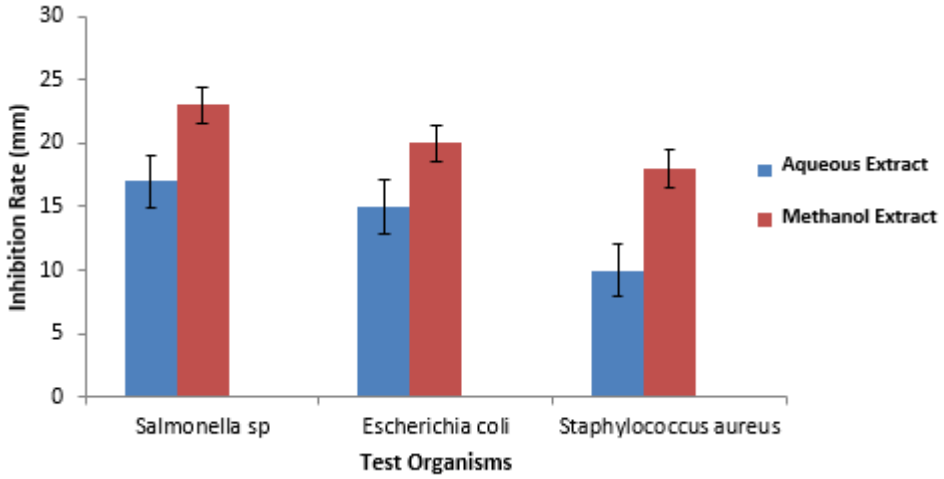

Figure 1b: Inhibition Rate of Sun Dried Ginger Extracts on the Food Borne Pathogens

The air dried and sun dried methanol tumeric extracts had higher inhibition rate of ≥24mm and ≥19mm respectively(Figure 2a & b). But air dried extract gave highest inhibition at $p > .05$ in all the test organisms but higher in *Escherichia coli* (Figure 2a). Turmeric aqueous extracts inhibited the growth of only *Escherichia coli* and *Salmonella typhi* while methanol extracts inhibited the growth of all microbes under study(≥13.5 mm) (Gul and Bakht, 2015). Inhibition of methanol turmeric extract against Escherichia coli, Staphylococcus aureus and Shigella dysenteriae was also obtained by Azhari *et al.*(2018). The inhibition rate (17mm) on *Escherichia coli* was reported by Ebrahimi *et al.* (2023) using turmeric aqueous extracts.

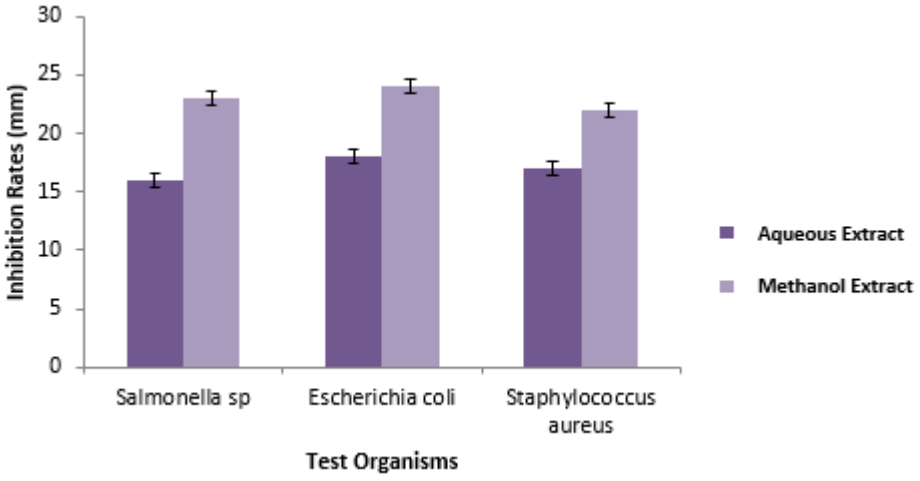

Figure 2a: Inhibition Rate of the Air Dried Tumeric Extracts on the Food Borne Pathogens

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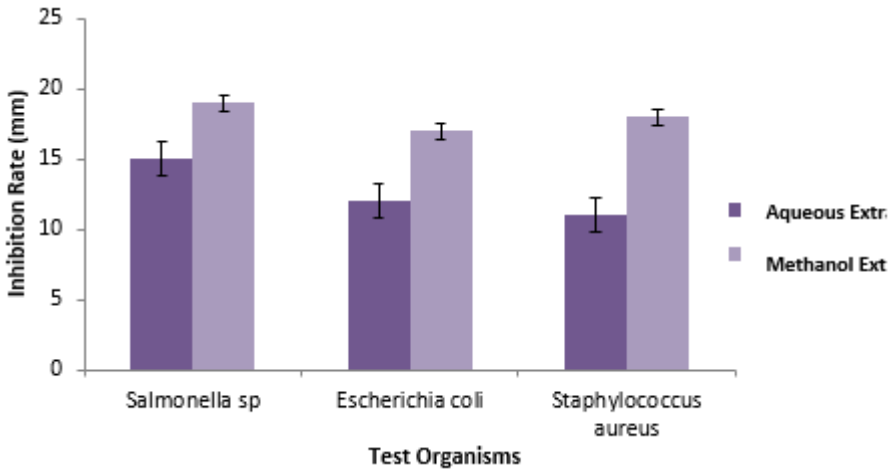

Figure 2b: Inhibition Rate of Sun Dried Tumeric Extracts on the Food Borne Pathogens

Air dried thyme methanol extract had the highest significant inhibition rate (≥28mm) among the plants assayed ($p< 0.05$) (Figure 3a). The sun dried sample gave lesser inhibition (22mm) than air dried sample (Figure 3b). A similar result (≥31mm) was reported by Mrabti *et al.* (2020) on same test organisms. The inhibition rate (≥16mm) of these organisms was also reported by Zarringhalam *et al.* (2013) using alcohol thyme extracts. The Inhibition Effect of thyme extracts on of *Campylobacter Jejuni* was also reported (Elsharawy, 2018). Highest inhibition by methanol extracts observed in all the samples could be that methanol is a good solvent for extraction as it has high polarity which could produce high extraction yields and can extract both lipophilic and hydrophilic molecules or substances.

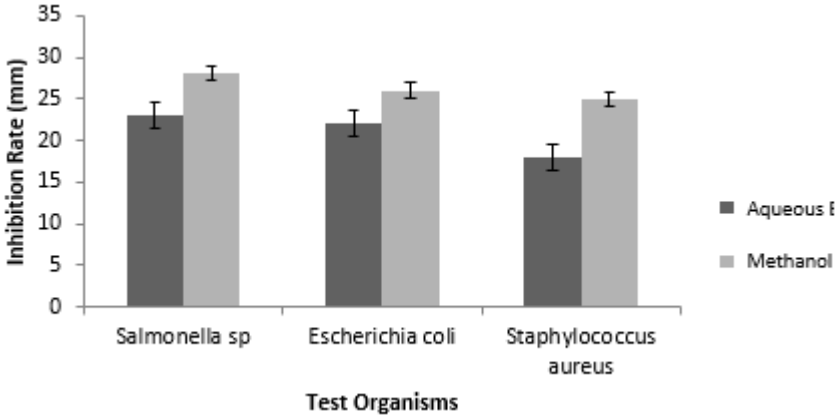

Figure 3a: Inhibition Rate of the Air Dried Thyme Extracts on the Food Borne Pathogens

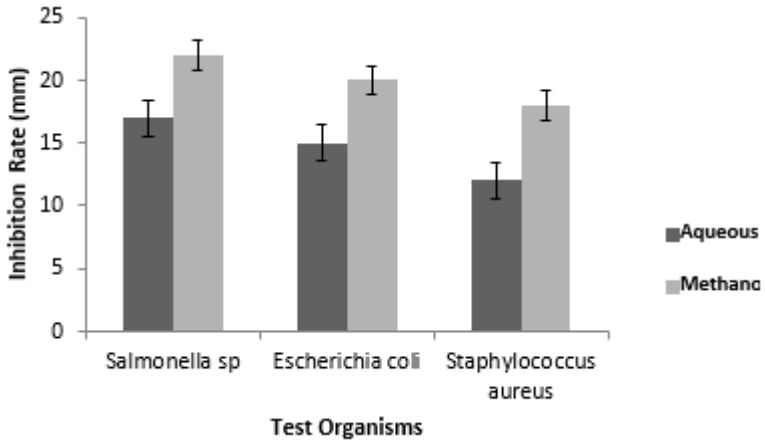

Figure 3b: Inhibition Rate of Sun Dried Thyme Extracts on the Food Borne Pathogen

The identification scheme of the assayed test organisms (Table 1) revealed their Gram reactions, biochemical test and colonial appearance on their differential media. The result revealed the food pathogens as *Salmonella*, *Escherichia coli* and *Staphylococcus aureus.* Similar results were also recorded by Hakkani *et al.*(2016) and Geletu *et al.*(2022) on same organisms .

Table 1: Identification Scheme of the Test Organisms

Test	***Salmonella***	***Escherichia coli***	***Staphylococcus aureus***
Gram reaction	- rod shaped	-rod shaped	+ **cocci** shaped
Catalase	+	+	+
Indole	-	+	-
Oxidase	-	-	-
Methyl red	+	+	+
Citrate	-	-	+
VP	-	-	+
Urease	-	-	+
Growth on MSA	NA	NA	Yellow colonies
Growth on SSA	Black colonies	NA	NA
Growth on Mac	NA	Pink colonies	NA

KEY: MSA= Mannitol Salt Agar, SSA= Salmonella Salt Agar, MAC = MacConkey Agar, NA= Not Applicable

4. CONCLUSION

The obtained results revealed air drying the best method of drying, the assayed food spices with great antibacterial potential and methanol, best solvent for their extractions.

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