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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *THYMUS VULGARIS* (THYME) ON *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Aim: This research was aimed at screening for the presence of active components of the plant leaf and to determine the antibacterial susceptibility of the extract on *Escherichia coli* and *Staphylococcus aureus.* **Methods***:* The study was conducted within Kaduna metropolis (Sheikh Abubakar Gumi market) in August, 2022. Phytochemicals were investigated using standard method. Microbial susceptibility was carriedout using disc diffusion and Cup-plate methods. **Results**: The phytochemical screening of the ethanolic extracts of *Thymus vulgaris* leave showed the presence of alkaloids, tannins, saponins, steroids and flavonoids. The extract showed activity at both 25mg/ml and 50mg/ml against *Escherichia coli* and *Staphylococcus aureus*. The zones of inhibition exhibited by the extract against these test isolates were observed to have direct relationship with the concentration of the extract. **Conclusion**: *Thymus vulgaris* leave extract has antimicrobial activity and showcased potential drug candidate against infections associated wiith the tests bacteria.

Keywords: *Thymus vulgaris;* Screening; Anti-bacteria.

1.0 INTRODUCTION

The name "thyme is derived from the Greek word "thymom" meaning to fumigate. The botanical suffix for wild thyme is derived from a Greek word to creep [1]. Thyme is indigenous to the Mediterranean with many species coming from an area that encompasses southern Europe, western Asia and North Africa and is one of the great commercially valuable herbs. The pleasant aroma of thyme already drew the attention of ancient Egyptians who used to make ointments for embalming and Ancient Greeks who employed it as a fumigant both appreciated the antiseptic properties of thyme. Besides, this plant has an adequate pungent aromatic, wherever flowers, leaves and oils extracted from the plant are widely used, special as a medicament for treating diseases or in food ingredients for a variety of cuisines, such is much appropriated to give a flavouring to soups for delightful savour. Moreover, historically, thyme uses was reported to extend back to ancient Rome and Egypt [2]; [3].Thyme contains many vital and effective components of secondary metabolic constituents which made it play a major role in prevention, treatment and other uses.

The plant contains 1-2% of essence rich in two isomers: thymol and carvacrol as well as other monoterpenes such as p-cymene, borned and geraniol. Thymol is additionally known by several names such as 2-isopropyl-5-methyl phenol (IPMP) or isopropyl- m-

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cresol, 1-methyl-3-hydroxy-4- isopropyl benzene is a natural monoterpenoid phenol derivative of cymene, $C_{10}H_{14}O$, isomeric with carvacrol, found in oil of thyme, and extracted from *Thymus vulgaris* (common thyme) and also found in other diversities of plants [4]; [5]. Thyme owes most of its properties to this essence, it contains flavonoids and phenolic acids which help enhance the properties of its essence. The phytochemicals in thyme include tannins, bitters essential oil, terpenes, flavonoids and saponins. The fresh, dried leaves and the essential oil extracted from the herb are medically potent. They are aromatic, antiseptic, diaphoretic (increase perspiration), analgesic, antispasmodic and diuretic. Thymus is used in the treatment of dry coughs (expectorant), eases rheumatic pain, bronchial mucus, exhaustion, expels worms especially hookworms and round worms, fungal infections, bacterial infections, gastritis, gingivitis (thyme in toothpaste), infected gums, improves digestion, indigestion, laryngitis, peptic ulcer, relaxes spasms, shingles, skın and scalp complaints, soothes digestive system, sore throat, support normal body functions/ counters effects of aging, tonsillitis upper respiratory tract infections and whooping cough [6]; [7].

Traditionally, thyme was used for healing several respiratory ailments such as bronchitis, sore throat, asthma and gastritis disorders also were used for the medication of other illnesses like antispasmodic, antiviral, antimicrobial and antiseptic. Additionally, fresh thyme contains many vital compounds such as flavonoids, tannins and phenolic antioxidants. Moreover, it is rich in vitamins, selenium, magnesium, manganese, iron, calcium and potassium. While the main phenolic antioxidant components are thymol which is for antioxidant activities. Thyme contains saponins, which interact with cholesterol in the human body to form insoluble complexes, as well as prevent the intestines from absorbing endogenous and exogenous cholesterol. The human body is infected with many diseases they may be caused by microorganisms such as bacteria, viruses, fungi, etc. Bacteria can live inside the human body or on his skin and some of them are good for human health, and it is referred to as healthy bacteria, some of which are damaging to the human body and are referred to as harmful or pathogenic bacteria. A proposal that structurally likes thymol and carvacrol already found in thyme enhances the permeability of the bacterial cell wall, therefore describing the antimicrobial effect of the thyme [5].

In a research conducted by Mukhtar and Okafor [8] using thymus extract against some clinical isolates of bacteria reported that the zones of inhibition <6mm in diameter is resistance and > 8 mm in diameter is susceptible. Carvacrol has antimicrobial activity against several diverse bacterial and strains. At present the bactericidal action of thyme essence on typhoid, diphtheria and tuberculosis causative microorganisms and especially on meningococcal (which cause meningitis), pneumococcus and streptococcus. It antiseptic action is mainly centered in the digestive respiratory genital and urinary System and especially in the pharyngeal and oral mucosa, as well as those of the genitalia. Moreover, to these incredible benefits, thyme also contains many vitamins (A, C, E, K, and B6) and minerals (calcium, iron, phosphorous, manganese and magnesium) as well as omega-3 and omega-6. On the other hand, for the therapeutic advantage of the thyme plant since ancient

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times, against bacteria and viruses that cause allergies, asthma, mouth and throat infections, which make it the focus of the attention of many scientists and the practice of using it to prove its effectiveness and great advantageousness [9].Therefore, to shield against pathogenic microorganisms, the immune system must be strengthened by eating dietary therapy foods containing active chemical ingredients, especially if the food is focalized on herbs and what is medicinal and to get prevention of diseases and eliminate them. The presence of the biochemical components differs from one plant to another and from one species to another; this depends on the genetic factors as well as on the place and conditions that the plant lives in. The functional groups, compositions and structures of chemical compounds play an important role in determining their activity against microbes, hence the distinction of each plant from another in its activity against one type of these microbes. For instance, the presence of phenolic groups exists in plant extract will cause an effect on microbes while the fractions of essential oil will stimulate the cell membrane, will alter permeability and leakage of vital intracellular components, also the impairment of the bacterial enzyme system and cell respiration [5].

This research was aimed at screening for the presence of active components of the plant leaf and to determine the antibacterial susceptibility of the extract on *Escherichia coli* and *Staphylococcus aureus.*

2.0 MATERIALS AND METHODS

2.1 Study Area/ Design

Thymus vulgaris (thyme) plant was purchased from Sheikh Abubakar Gumi central market situated in Kaduna North Local Government Area, Kaduna in August 2022.

2.2 Sample Collection and Handling

2.2.1 Extraction of Plant Material

50g of the ground thyme leaf was weighed accurately. The weighed sample was then wrapped very well using Whitman's No 1 filter paper and a wire. It was then placed into the thimble of the Soxhlet apparatus filled with 250cm^3 of di-ethyl ether and placed in a water bath. The Soxhlet apparatus was then assembled with sample in the extraction thimble. The preparation was allowed to flux for about 4 hours there after the thimble was carefully removed when the
boiling flask was almost free of the di-ethyl ether. It was then transferred into a conical flask placed on a water bath to concentrate and obtain the under extract. The crude extract was allowed to cool which was finally weighed and recorded. The crude extract was re-dissolved in ethanol to yield solutions containing 25 and 50mg/ml of leaf extract.

2.2.2 Phytochemical Sereening

The crude extract of *Thymus vulgaris* was screened for the presence of phytocompounds according to the method of Chessbrough [10].

2.2.3 Media Preparation

Nutrient agar and nutrient broth were prepared according to manufactures instructions.

2.2.4 Test Isolates

The clinical isolates of *Escherichia coli and Staphylococcus aureus* were collected from Yusuf Dantsoho Memorial Hospital. The isolates were gram stained. Portion or the sterile nutrient agar was separately poured into 2 sterile bijou bottles which were left to solidify in slants form. The slants were inoculated with the test organisms. The preparations were incubated overnight in an

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incubator at 37^0C. The cultures were stored in a refrigerator prior to standardization of inoculum.

2.2.4 Standardization of lnoculum

Direct colony suspension method was used to standardize the inoculum of the test 1solates. Therefore a 2ml volume of sterile distilled water was poured in a test tube to which a colony of the bacterial isolate was added directly and emulsified using sterile wire loop. The suspension was adjusted which has a similar appearance of an overnight broth culture by adding distilled water.

2.2.5Antibacterial Sensitivity Test

The sensitivity of the test organisms namely *Escherichia coli and Staphylococcus aureus* were carried out using the cup plate diffusion method and agar disc diffusion assay. Two different concentrations of the leaf extract 25mg/ml and 50mg/ml were tested for antibacterial activity.

2.2.6 Antibacterial Activity using cup-plate diffusion Method

Using a sterile cotton swab the standardized inoculums was spread all over the surface of the previously prepared sterile nutrient agar plates. It was allowed to dry for 5 minutes. Agar wells were prepared using a sterilized cork borer of 6 mm diameter using a syringe of 0.1ml of different concentrations. The plant extracts (25 and 50mg/ml) were added to the well in each plate separately. The plates were incubated in an upright position at 37^0C for 24 hours. The diameters of the zones of inhibition were measured in mm and the results recorded.

2.2.7 Antibacterial Activity using Agar Disc Diffusion Assay

The culture media prepared was aseptically and gently poured into the sterile petri-plate and allowed to solidify, using a sterile cotton swab, the standardized inoculum was spread all over the surface of the previously prepared sterile nutrient agar. It was allowed to dry for 5 minutes. Sterile disc of six millimeter width was then impregnated with 25 and 50 mg /ml or extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated in an upright position at 37^0c for 24 hours. The diameters of the zone of inhibition observed around the disc were measured in mm and the result was recorded.

2.2.8 Preparation of Erythromycin

0.3g of erythromycin was weighed and dissolved in 1m of distilled water in a test tube. The paper discs of 6 mm were embedded in same drops of the mixture and the other portion was kept for the preparation of the cup-plate diffusion method.

3.0 RESULTS AND DISCUSSION

After the extraction from the analysis of *Thymus vulgaris* extract, the weight of the crude extract was observed and recorded as (6.0g %). The results of phytochemical screening of *Thymus vulgaris* extract at different concentration are presented in Table 1. The result indicates the presence of tannins and saponins in the extracts while screened Alkaloids, Steroids and flavonoid were absent in the extracts. The result is similar with some findings of [11] whose phytochemical screening showed presence of some secondary metabolites which include stannins, saponnins with low amount of alkaloids, flavonoids.[5]also reported the presence of the phytochemical secondary metabolites in abundance amounts, which are

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tannins, phenols, Fats and Fixed oils, Amino acids, alkaloids, flavonoids and steroids and triterpenoids. *Thymus vulgaris* was chosen for this study because of their reputation in traditional medicine as antimicrobial agents and usage in many diseases.

Table 1: Phytochemical Screening of *Thymus vulgaris* Extract

Extract	Alkaloids	Tannins	Saponins	Flavonoid	Steroids
25mg/ml ethanol extract of thyme	-	+	+	-	-
25mg/ml ethanolic extract + water on bath	-	+	+	-	-
25mg/mlethanolic extract with chloroform	-	+	+	-	-
25mg/mlethanolic extract with NaOH	-	+	+	-	-

Keys: + Indicates presence of phytochemical compound - Indicates absence of phytochemical compound

The crude extracts were subjected to antimicrobial assays using cup-plate diffusion method and the inhibition zone was measured in mm. The result of antibacterial susceptibility test using cup – plate diffusion method at two different concentrations (25 and 50mg/ml) is presented in **Table 2**. At 25mg/ml concentration, *Escherichia coli* were resistant (6mm). However *Staphylococcus aureus* at the same concentration indicated higher susceptibility (9mm) because of the wide zone of inhibition. Accordingly, both *Escherichia coli* and *Staphylococcus aureus* were susceptible to the extract at 50mg/ml concentration. Greater susceptibility was recorded on *Staphylococcus aureus* because of the wide zone of inhibitions (12mm). The zones of inhibition observed and recorded for both isolates were greater than 8mm. this is in conformity with the work of Mukhtar and Okafor [8] who reported that the zone of inhibition < 6mm in diameter is resistance and > 8mm in diameter is susceptible.

However erythromycin was also tested against the two isolates as standard to compare the level of efficacy of the extract on the isolates. Both *Escherichia coli* and *Staphylococcus aureus* were susceptible to erythromycin with 26mm and 30mm zones of inhibitions respectively.

Table 2: Antibacterial Susceptibility Test using Cup-plate Diffusion Method at 25mg/ml and 50 mg/ml.

Concentration	*Escherichia coli*	*Staphylococcus aureus*	*P value*
25mg/ml	7.5±2.12	8.5±0.71	0.5918
50mg/ml	6.5±3.54	11.5±0.71	0.3002
Erythromycin 0.3g/ml	23.0±4.24	28.0±2.83	0.3977

Values are presented as mean ± Standard deviation, P< 0.05.

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The ethanol extracts leaf of *Thymus vulgaris* using cup-plate diffusion method inhibition (mm) of 7.5, 6.5 and 23.0 on *Escherichia coli* at concentrations (mg/ml) 25, 50 and Erythromycin 0.3respectively and 8.5, 11.5 and 28.0 on *Staphylococcus aureus* at concentrations (mg/ml) 25, 50 and Erythromycin 0.3respectively as shown in Table 2.There was significant difference in the zones of inhibition, since the P< 0.05.

Table 3: Antibacterial Susceptibility Test using disc diffusion method at 25mg/ml and 50mg/ml

Concentration	*Escherichia coli*	*Staphylococcus aureus*	P value
25mg/ml	9.5±0.71	12.5±0.71	0.0513
50mg/ml	15.0±1.41	18.5±0.71	0.0887
Erythromycin 0.3g/ml	25.5±0.71	29.0±1.41	0.0887

Values are presented as mean ± Standard deviation, P< 0.05.
Keys: Interpretation of zones of inhibition, < 6mm in diameter = Resistant
>8mm in diameter = Susceptible

The result of antibacterial susceptibility test using disc diffusion method at 25 and 50 mg/ml concentration is presented in **Table 3**. Both *Escherichia coli* and *Staphylococcus aureus* were susceptible to *Thymus vulgaris* extract at 25 and 50mg/ml concentration the zone of inhibition observed and recorded for both isolates were greater than 8mm. This result is in disagreement with the work of Wafaa and Mohammed [11] essential oil extract of *Thymus vulgaris* leaves was found effective against all tested Gram positive and Gram negative bacteria inhibition zone (range between 11-25mm).The ethanol extracts leaf of *Thymus vulgaris* using disc diffusion method inhibition (mm) of 9.5, 15.0 and 25.5 on *Escherichia coli* at concentrations(mg/ml) 25, 50 and Erythromycin 0.3respectively and 12.5, 18.5 and 29.0 on *Staphylococcus aureus* at concentrations(mg/ml) 25, 50 and Erythromycin 0.3respectivelyas shown in **Table 3.** There was significant difference in the zones of inhibition, since the P< 0.05 while the 25mg/ml is statistically significant (P value 0.0513).

CONCLUSION

Thymus Vulgaris extract contains saponins and tannins as the bioactive ingredients and it has antibacterial activity against *Escherichia coli* (6mm and 9mm) and *Staphylococcus aureus* (8mm and 12mm) at 25mg/ml and 50mg/ml concentration of the extract respectively. Both *Escherichia coli* and *Staphylococcus aureus* were susceptible to erythromycin with 26mm and 30mm zones of inhibitions respectively.

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Competing Interests
Authors have declared that no competing interests exist**.**

Authors' Contributions

Musa, F.M & Idris, H' designed the study and wrote the protocol, Muhammad, J. wrote the first draft of the manuscript and managed the literature searches; Alhassan, S & Namadina, M. M' managed the analyses of the study. All authors read and approved the final manuscript."

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