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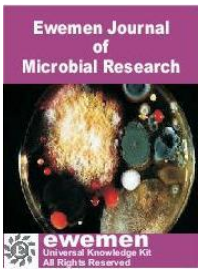

Full Length Research

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *MENTHA PIPERITA* (PEPPER MINT) LEAVES EXTRACTS ON UROPHATOGENIC *ESCHERICHIA COLI*

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ABSTRACT

Urinary tract infections (UTIs) are serious health problem affecting million of people every year. These are very common infections that occur when bacteria enter into urinary bladder and multiply any where along the normally sterile uninary tract. *Mentha piperita* (Lamiaceae), the peppermint plant, is an aromatic perennial herb growing 50-90 cm high, cultivated in most part of the world and have traditionally been used in folk medicine. In this study, the phytochemical constituents and antibacterial activitiy of this plant was investigated to justify its folkloric used on UTIs. A total of 130 urine samples were collected from patients suspected for urinary tract infection (UTIs) from Muhammad Abdullahi Wase Hospital using standard microbiological procedures. Agar well diffusion assay, minimum inhibitory concentration(MIC) and minimum bactericidal concentrations (MBC) were used to assess the antibacterial activity of aqueous, methanol and n-Hexane extracts of *Mentha piperita,* with ciprofloxacin used as positive control. The results showed zone of inhibition with methanol extract as highest, followed by aqueous extract, and then n-Hexane extract which gave the least zone diameter values. The zones of inhibition were 21.00±0.04, 20.66±0.15 and 19.66±0.57 mm for methanol, aqueous and n-hexane extracts, respectively. The zone of inhibition was 19.33±0.02 mm in ciprofloxacin. The minimum inhibitory concentrations were 0.78 mg/mL for methanol and aqueous extracts, and 3.13 mg/mL for n-Hexane extract. The minimum bactericidal concentrations were 0.78 mg/mL for methanol and aqueous extracts and 1.62 mg/mL for n-Hexane. The Phytochemical screening revealed the presence of the reducing sugar, saponins, phenols, flavonoids, alkaloids and tannins. *Mentha piperita* could be used for the treatment of urinary tract associated diseases due to its high antibacterial activity shown when compared with positive control. Therefore the ability of this plant extracts to inhibit these UTIs pathogens indicates the presence of entities capable of suppressing the growth of the test organisms.

Keywords: Antibacterial activity; *E. coli*; *Mentha piperita*; Phytochemicals; Urinary tract infection; UTI.

INTRODUCTION

Diseases due to pathogenic bacteria presents a critical problem to human health and being one of the main causes of morbidity and mortality worldwide (WHO, 1998). Urinary tract infections (UTIs) are serious health problem affecting million of people every year. These are very common in persons aged 20-50 years. Approximately 95% of infections occur when bacteria ascend through the urethra and the bladder (Harkins, 2000). Bethesda (2005) and David *et al* (2008), reported urinary track bacterial infections were more common in women than men becouse they have a shorter urethra. The common uropathogens identified in patients with UTI include 90% enteric gram negative bacilli bacteria, *E. coli* being the most common followed by the *Proteus* sp. *Klebsiella* sp. and *Enterococcus* sp. (Warren, 2005). Iroha *et al* (2009) reported *E. coli* as the most common bacteria capable of causing UTI infection in humans. UTIs can be cartegorized as symptomatic or asyptomatic infection based on the present or absent of the symptoms and hence symptoms enchance the diagnosis process among young healthy humans (Kunin, 1997).

The need for new antimicrobial agents is closely associated with the problems of emergence of strains that are resistance to most present day antibiotics (Finland *et al*., 1966). Nowadays, the major problem facing humans is the increase in the use of antibiotics that resulted in multiple drug resistance of medicinally important bacteria. This is probably due to the reported efficacies of these plant products which have been confirmed in different diseases situations in different parts of the world, and that their little or no known side effects which have made them succeed where most synthetic or conventional agents have failed. Scientific research has established that crude extracts from plants can potentiate the activity of antibiotics *in–vitro* (Marwquez, 2005).

The investigation of the efficacy of plant-based drugs used in traditional medicine have been paid great attention because they are mostly cheap and have little or no side effects (Dharmasiri *et al*., 2003). Plants have been reported to contained many bioactive compounds making them sources of different substances of medicinal uses. These substances include alkaloids, flavonoids, steroids, resins, fatty acid tannins and phenols (Balasaheb and Yogini, 2015; Sheeba, 2010; Nair *et al.,* 2005). Joshi *et al.,* (2011) reported use of plant materials in treatment of diarrhea, dysentery, cough, cold, fever, bronchitis, cholera etc.

This work aims to evaluate the phytochemical constituents and establish the antibacterial effects of *Mentha piperita* leaves extract on urophatogenic *E. coli*. *Mentha piperita* (Lamiaceae), the pepper mint plant is an aromatic perennial herb that grows to about 50-90 cm high, cultivated in most part of the world, and have been traditionally used in folk medicine. The leaves of the plant are frequently used as herbal tea and for culinary purpose to add flavour and aroma. The distinctive smell and flavour, a characteristic features of *Mentha piperita* are due to the naturally occuring cyclic terpene alcohol called menthol. Menthol is prescribed as a medication for gastrointestinal disorders, common cold and muscule skeletal pain (Patil *et al*., 2007). *Mentha piperita* is currently used to treat irritable bowel syndrome, Crohn's disease, ulcerative colitis, gall bladder and biliary tract disorders, and liver complaints (Robbers and Tyler, 1999; Fleming,1998; Tyler,1992).

MATERIALS AND METHODS

Materials

All the microbiological media and reagents used were of analytical grades and purchased from Ado Jones Scientific Supply and General Enterprises, Kano State. Equipment and glassware used were of the Department of Biological Sciences, Federal University Dutse, Jigawa, and the Department of Microbiology, Bayero University Kano.

Collection and extraction of plant material

The plant was collected from Sharada Garden along Dan'agundi, Kano, Nigeria, using the protocol of El-mahmood and Amen (2007). The collected plant was brought to the Herbarium section of Bayero University Kano in a polythene bag for taxonomic identification. A voucher specimen number: BUKHAN0337 was given to the specimen. The plant material was brought to the laboratory, rinsed with water to remove foreign material and dried (at a temperature of 40 °C) before examination (Parra *et al.,* 2001; Ahmed *et al.,* 2010; Ogugu *et al.,* 2012; Lalisan *et al.,* 2014). The leaves were removed from the dried sample and pulverized into powder using sterile electric blender (Lalisan *et al.,* 2014). The material was divided into three different bottles and separately extracted using water, methanol and n-Hexane. Percolation was carried out for over a period of seven days (Parra *et al.,* 2001). The extract was filtered using filter paper (Whatman no.1) and the

filtrate was concentrated to dryness under reduced pressure using rotary evaporator at 40-50 °C. The aqueous extract was dried by gentle heating at 45°C using water bath (Mayorga *et al.,* 2010). The dried extracts were then transferred into an air-tight container for further analysis.

Phytochemical tests

Mentha piperita extract was subjected to qualitative phytochemical screening according to standard procedure to test for the presence of the following secondary metabolites; alkaloids, flavonoids, saponins, tannins, Cardiac glycosides and steroids as described by (Odebiyi and Sofowora, 1984; Ogukwe *et al.,* 2004; Hassan *et al.,* 2005; El-mahmood and Doughari, 2008; William *et al*., 2015)

Microbiological Analysis

Collection of urine samples

A total number of 130 urine samples from UTIs suspected patients were collected from Muhammad Abdullahi Wase Specialist Hospital Kano, Nigeria, and the samples were transported immediately to laboratory in an ice box maintained at 4 °C (Cheesbrough, 2004).

Isolation and Identification

The urine samples were streaked onto sterile MacConkey agar, Eiosine Methylene Blue agar (EMB) and Cystine Lactose Electrolyte Deficient (CLED) agar, incubated at 37°C for 24 hrs (Betty *et al*., 1998). Colonies that showed lactose fermenting on MacConkey and CLED agar and green metallic sheen on EMB agar were identified. (Ochei and Kolhatkar, 2007; Chessbrough, 2004). The isolates were identified based on their gram stain and biochemical reactions e.g. indole production, citrate utilization, methyl red/Voges proskauer, catalase and triple sugar iron test (TSI) (Cheesbrough, 2004; Faddin, 2000). The identified isolates were preserved in nutrient agar slants, kept in refrigerator for further analysis.

Standardization of bacterial inoculums

The isolates were sub-cultured onto sterile nutrient agar plates incubated at 37°C for 18-24 hrs; the sub-cultured isolates were inoculated into a test-tube containing 5 mL of normal saline and compared with 0.5 MacFarlands turbidity standard that marched with 0.1×10^8 cfu/mL (Cheesbrough, 2004).

Preparation of concentrations

Di-methyl Sulfoxide (DMSO) was used for the preparation of methanol and n-hexane extracts, while sterile distilled water was used for aqueous extract. Four concentrations were made for each extract as follows; 100, 50, 25 and 12.5 mg/mL.

Antibacterial susceptibility testing

Antibacterial Susceptibility Testing was carried out using agar well diffusion method on Mueller-Hinton agar (MHA) as described by (Jahangirian *et al.,* 2013). Using the sterile cork borer, 5 wells of approximately 6 mm in diameter and 2.5 mm deep were bored on the agar surface; the wells were filled with 0.1 mL of the test samples, According to Boardi, *et al.,* (2015), the (MHA) plates were left for about one hour for complete diffusion of the extract. DMSO was used as negative control while ciprofloxacin was used as positive control (Abubakar, 2009). The plates were incubated at 37°C for 24 hrs. After the incubation period, the diameter of the growth inhibition zones (IZ) was measured in millimeter (mm).

Determination of Minimum Inhibitory Concentration (MIC)

Macro-tube dilution broth method was used as described by (Ochei and Kolhatkar, 2008). The lowest concentration of the extract that inhibits the growth of the test organism (bacteriostatic) was determined at 12.50 mg/mL, 6.25 mg/mL, 3.13 mg/mL, 1.56 mg/mL and 0.78 mg/mL respectively. The tubes were incubated aerobically at 37°C for 24 hrs; Clear suspensions shown after incubation were regarded as positive result, which has inhibited the growth (bacteriostatic) (Abubakar, 2009).

Determination of Minimum Bactericidal Concentration (MBC)

A loop full from the positive (MIC) tubes were streaked onto MHA plates and incubated at 37°C for 24 hrs. Absence of growth after incubation, determine positive result (bactericidal) (Olajuyigbe and Afolayan, 2012; Irkin and Koruhrogh, 2007).

Statistical Analysis

All the Experiments were carried out in triplicates. Data obtained was subjected to statistical analysis using one way Analysis of Variance (ANOVA) on SPSS version 16.0. Turkey-Kramer Multiple Comparisons Test was used to separate the means. $p>0.05$ was considered not significantly different.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis (Table 1) showed that saponins, phenols, flavonoids, tannins and reducing sugars were detected from methanol and aqueous leaves extract of *Mentha piperita.* This was in agreement with the findings of Naiman and Mazharuddin (2016) and Paramila *et al* (2012). Only saponins were detected in n-hexane, and this may be due to less polarity of the solvent.

Table 1: Preliminary phytochemical screening of extracts of *M. piperita*

Constituents	Methanol	n-Hexane	Aqueous
Reducing sugars	+	-	++
Glycosides	-	-	-
Flavonoids	-	-	+
Saponins	+	+	++
Tannins	+	-	+
Phenols	++	-	+
Alkaloids	+	-	+

Key: **+** = Positive (Slightly present**); ++** = Positive (Moderately); - = Negative (Absent).

The result of organism isolation and identification in the urine samples confirm the presence of *E. coli*. The isolation of *E. coli* from patients suspected with urinary tract infections corroborates with the findings of Naiman and Mazharuddin (2016) who isolated *Escherichia coli, Klebsiella pneumoniea, pseudomonas aeruginosa, Entrobacter feacalis and Proteus mirabilis*; Tabassum *et al* (2013) who isolated *Klebsiella pneumoniae, Pseudomonas aeriginosa, Entrobacter feacalis and Proteus mirabilis*; Fuad *et al* (2012) who isolated *Escherichia coli*; and Kumar *et al* (2012) who reported the isolation of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeriginosa, Shigella sp* and *Proteus sp*.

The leaves of *Mentha piperita* was extracted using solvents of different polarity and the profile of microbial inhibition was in accordance with the result of Para *et al* (2013) and Naiman and Khan (2016). Naiman and Mazharuddin (2016) earlier reported the inhibition to urinary tract infection pathogens using solvents of different polarity in the extraction process.

The results of antimicrobial susceptibility (Tables 2, 3 and 4) revealed the highest antibacterial susceptibility with inhibition zone of 21.00±0.04 mm from the methanolic extract. This agrees with the finding of Naiman and Mazharuddin (2016). The aqueous extract had moderate activity of 20.66±0.15 mm inhibition, and least activity was recorded in n-hexane extract with 19.66±0.17 mm inhibition zone. The antibacterial activity of methanolic extract of *M. piperita* against urinary tract infection pathogens had been reported earlier workers (Gupta, *et al.* 2009; Ayoola, *et al.,* 2008; Al-jiffir, *et al.,* 2011). The variation in the inhibition pattern of the different extracts may be as a result of the nature of chemical components which may be similar in methanol and aqueous extracts (Gosh, *et al.,* 2008). The use of ciprofloxacin as positive control in the study is in tandem with the reports of Naiman and Mazharuddin (2016), Al-jiffir *et al* (2011) and Rose *et al* (2001), who reported the use of this antibiotic in treatment of urinary tract infection and other gram negative bacilli.

Table 2: Antibacterial susceptibility of test organisms to methanolic extract of *M. piperita* (mm)

Isolate	Zones of Inhibition of at different concentration of extracts				Positive Control
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	
E006	21.00±0.04	12.66± 0.57	9.33± 0.57	7.33± 0.57	19.33±0.02
E009	10.66±0.18	10.00± 0.10	8.00±0.21	7.33±0.15	
E011	9.66±0.52	9.00±0.21	8.66±0.15	7.33±0.42	
E15	14.33±0.61	13.33±0.42	10.33±0.52	7.33±0.57	
E40	20.33±0.22	17.66±1.15	11.33±0.52	7.00±0.73	
E48	13.33±0.13	10.33±0.16	9.33±0.57	8.66±0.31	
E53	11.66±0.70	9.66±0.52	9.33±0.30	6.66±0.75	
E63	13.33±0.13	9.66±0.08	9.33±0.57	7.33±0.52	
E103	20.33±0.37	12.33±0.08	9.33±0.15	8.66±0.08	

Table 3: Antibacterial susceptibility of test organisms to n-hexane extract of *m. piperita* (mm)

Isolate	Zones of Inhibition of at different concentration of extracts				Positive Control
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	
E006	19.66±0.57	11.00±0.02	10.00±0.01	7.33±0.57	19.33±0.02
E009	12.00±0.28	12.00±0.75	10.0±0.01	7.0±0.020	
E011	12.66±0.07	10.00±0.06	9.00±0.73	7.33±0.57	
E15	12.66±0.57	10.00±0.04	9.00±0.73	7.33±0.57	
E40	10.00±0.11	9.66±0.08	7.00±0.32	0.00±0.00	
E48	16.66±0.51	13.33±0.51	12.66±0.57	9.66±2.08	
E53	8.66±0.88	7.33 ±0.88	6.33±0.57	0.00±0.00	
E63	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
E103	12.66±0.57	10.00±0.02	9.00±0.73	0.57±0.57	

Table 4: Antibacterial susceptibility of test organisms to aqueous extract of *M. piperita* (mm)

Isolate	Zones of Inhibition of at different concentration of extracts				Positive Control
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	
E006	12.00±0.60	10.00±0.03	9.66±0.57	7.33±0.57	19.33±0.02
E009	19.66±0.08	19.33±0.57	15.66±0.06	9.00±0.01	
E011	20.00±0.15	16.66±0.51	12.66±0.31	9.00±0.13	
E15	20.66±0.15	17.66±0.14	14.66±0.57	9.33±0.57	
E40	16.00±0.64	10.66±0.05	7.33±0.57	0.00±0.00	
E48	19.66±0.57	15.00±0.00	10.66±0.28	8.00±1.00	
E53	20.66±0.15	17.66±0.05	12.00±0.42	10.00±0.08	
E63	12.00±0.60	10.00±0.01	9.66±0.57	7.33±0.43	
E103	18.00±0.20	8.33±0.42	9.33±0.31	0.00±0.00	

The comparative analysis of leaves and stem of *Menthe piperita* was conducted by Saeed and Teriq (2015),

which showed high antibacterial activity of the leaves over stem extract. The result revealed a very strong antibacterial activity against the tested organisms, which shows superiority of *Mentha piperita* leaves extract over the standard positive control of ciprofloxacin.

The antibacterial activity of some plant spp. is related to the richness of phenolic compounds such as flavonoids and tannins (Hideyuki *et al*., 2002; Meng *et al*., 2001). Thus, the antibacterial activity of methanolic and aqueous leaves extract of *M. piperita* could be attributed to the presence of tannins and flavonoids, or saponins for the hexane extract.

Table 5: Minimum inhibitory concentration of *E. coli* using methanolic extract (mg/mL)

Isolates	MIC (mg/mL)				
	12.50	6.25	3.13	1.62	0.78
E006	+	+	+	+	β
E009	+	+	+	β	-
E011	+	+	β	-	-
E15	+	β	-	-	-
E40	+	+	β	-	-
E48	+	+	β	-	-
E53	+	β	-	-	-
E63	+	β	-	-	-
E103	+	β	-	-	-

Key: β = MIC Value; - = Turbidity Observed; + = No Turbidity Observed

Table 6: Minimum inhibitory concentration of *E. coli* using n-hexane extract (mg/mL)

Isolates	MIC (mg/mL)				
	12.50	6.25	3.13	1.62	0.78
E006	+	+	β	-	-
E009	β	-	-	-	-
E011	β	-	-	-	-
E15	β	-	-	-	-
E40	+	β	-	-	-
E48	+	β	-	-	-
E53	+	β	-	-	-
E63	β	-	-	-	-
E103	β	-	-	-	-

Key: β = MIC Value; - = Turbidity Observed; + = No Turbidity Observed

Table 7: Minimum inhibitory concentration of *E. coli* using aqueous extract (mg/mL)

Isolates	MIC (mg/mL)				
	12.50	6.25	3.13	1.62	0.78
E006	+	+	+	+	β
E009	β	-	-	-	-
E011	+	+	+	β	-
E15	+	+	β	-	-
E40	+	+	+	+	β
E48	+	β	-	-	-
E53	+	+	β	-	-
E63	+	+	β	-	-
E103	+	+	β	-	-

Key: β = MIC Value; - = Turbidity Observed; + = No Turbidity Observed

Tables 5, 6 and 7 shows the results of minimum inhibitory concentration (MIC). The results revealed an MIC of 0.78 mg/mL for methanol and aqueous extracts, and 3.13 mg/mL for n-hexane extract. The MIC values were lower than those reported by Piramila *et al* (2012), with MIC values of 3.13 µg/mL and 6.25 µg/mL for methanol and aqueous extracts. This phenomenon may be due to variability to site of isolation of tested organisms and strains of the organisms.

The results of minimum bactericidal concentration (MBC) (Tables 8, 9 and 10) showed activity of 0.78 mg/mL for methanol and aqueous extracts, while n-hexane extract showed 1.57 mg/mL. The result suggests that the methanol and aqueous extracts of the leaves of *Mentha piperita* exhibit similar MIC and MBC, while n-hexane exhibit different susceptibility. The bactericidal effect of this plant had earlier been reported by Brenda *et al* (2014) and Piramila *et al* (2012).

Table 8: Minimum Bactericidal Concentration of *E. coli* Using Methanolic Extract (mg/ml)

Isolates	MIC (mg/mL)				
	12.50	6.25	3.13	1.62	0.78
E006	+	+	+	+	β
E009	+	+	+	β	-
E011	+	+	β	-	-
E15	-	β	-	-	-
E40	-	-	-	-	-
E48	+	+	β	-	-
E53	+	β	-	-	-
E63	+	β	-	-	-
E103	+	β	-	-	-

Key: + = Absence of Growth, - = Presence of Growth, β = MBC Value

Table 9: Minimum bactericidal concentration of *E. coli* using n-hexane extract (mg/mL)

Isolates	MIC (mg/mL)				
	12.50	6.25	3.13	1.62	0.78
E006	+	+	β	-	-
E009	β	-	-	-	-
E011	-	-	-	-	-
E15	β	-	-	-	-
E40	β	-	-	-	-
E48	+	β	-	-	-
E53	-	β	-	-	-
E63	+	+	β	-	-
E103	β	-	-	-	-

Key: + = Absence of Growth, - = Presence of Growth, β = MBC Value

Table 10: Minimum bactericidal concentration of of *E. coli* using aqueous extract (mg/mL)

Isolates	MIC (mg/mL)				
	12.50	6.25	3.13	1.62	0.78
E006	+	+	+	+	β
E009	β	-	-	-	-
E011	+	+	+	β	-
E15	+	β	-	-	-
E40	+	β	-	-	-
E48	+	β	-	-	-
E53	+	+	β	-	-
E63	+	β	-	-	-
E103	+	+	β	-	-

Key: + = Turbidity observed, - = No Turbidity observed, β = MBC value

CONCLUSION

The extract of *Mentha piperita* leaves in this study showed antimicrobial activity against isolated *E. coli* from urinary tract infected patients, capable of suppressing the growth of the test organisms. This establishes the potential of the plant extracts to act as a alternative for preventing UTI. This study demonstrated that the extracts from the leaves of *Mentha piperita* is as effective as modern medicine. Biological and pharmacological screening of this medicinal plant using the modern tool may leads to some new interesting drug. Frequent and continued intake of mint leaves in daily diet may prove beneficial in keeping the pathogenic microbes below the threshold level. Further study is recommended to assess the presence of other bioactive compounds of chemotherapeutic potentials and also to ascertain the efficacy, toxicity and suitability of using the extracts in vivo.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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Authors HNG, TDW and AAI design the study. Authors AAI and HNG wrote the procedure and interprets the data. Authors HNG and AAI anchored the experiments. Authors AAI, HNG and TDW managed the literature searches and produced the initial draft. Authors YSM, AI and TDW revised the manuscript. Authors ASB and AI manage the financial activities.