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In vitro Antibacterial Potential and Phytochemical Screening of *Mentha piperita* L. Leaves Extracts

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Abstract: This research was aimed to study the phytochemical composition and determine antibacterial potential of *Mentha piperita* L. (Peppermint) leaves extracts. The leaves of *Mentha piperita* were collected, processed for extraction in ethanol for two weeks and fractionated using chloroform, n-hexane and methanol. The result of the phytochemical screening showed the presence of alkaloids, glycosides, steroids, flavonoids and tannins but absence of saponins. Sensitivity test carried out revealed that, chloroform extracts have highest zones of inhibition of 15.21mm and 14.93mm at 100mg/ml against *P. aeruginosa* and *S. epidermidis* respectively. While the least activity was observed in ethanolic extracts against *S. pneumoniae and K. pneumoniae* with zones of inhibition of 8.00mm and 8.02mm respectively at 25mg/ml concentration. However, the susceptibility of the bacteria to the extracts was in the order of *P. aeruginosa* > *S. epidermidis* > *S. pneumoniae* > *K. pneumoniae* and the activity of the extracts was solvent wise ranked in the order of; Chloroform > methanol > ethanol > N-Hexane. Hence the leave extract of *Mentha piperita* were found to have significant antibacterial activity which may be due to the presence of the detected phytochemicals.

Key words: Antibacterial Activity · *Mentha piperita* · Phytochemicals · Sensitivity Test

INTRODUCTION

The development of medicinal plants in the domain of nutrition has unveiled their additional therapeutic potentials [1]. Plants are rich in nutrients and are also the main source of food for humans. They are equally rich in compounds which have pain relieving and healing potentials. This medicinal property of plants is attributable to the active principles which are often obtained from the plants extracts [2]. In the same era, terms like functional foods, nutraceutical and pharma foods have taken hold of the nutrition market, mainly aiming to provide nutrition and healthy diets [3]. The use of vegetables in controlling bacterial infections has continued despite advances in modern pharmaceutical products and dominance of synthetic drugs across the globe [4].

Mint leaf is botanically known as *Mentha piperita* L. (Peppermint), it belongs to the family lamiaceae. Mentha belong to the order *lamiales* [5]. It is a perennial glabrous and strongly scented herbs and native to the temperate region of Europe, western and central Asia. It is herbaceous perennial plant growing to height of 60-75cm. Mint are propagated by vegetative means through underground part called suckers [5]. The leaves, fresh or dried are the culinary source of mint. The leaves have a warm, fresh, aromatic, sweet flavour with a cool after taste and are used in teas, beverages, jellies, syrups candies and ice cream. Sometimes mint is used in alcoholic drinks as a flavourant as a garnishing agent such as the mint julep and the majito. Crème de manthe is a mint flavour liqueur used in drink [6]. Mint was originally used as a medicinal herb to treat stomach aches and chest pain. Other reported uses of *M. piperita* include; treating bowel

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syndrome, act as a stimulant, tonic, antiviral, anti-fungal and anti-bacterial agent [7]. The oils obtained from peppermint are found to possess astringent, antiseptic, antipyretic, antispasmodic, antimicrobial rubefacient, stimulant, emmenagogue and anti-aging properties [8]. Harmful side effects associated with use of synthetic drugs and high costs associated with drug development are gradually over time shifting the focus of alternative medicine to plant derived phytochemicals of medicinal significance [9]. Therefore, there is need to search for drug with less/no side effect and low-cost alternative medicine. The aim of this research was to study the phytochemical composition and determine the antibacterial potential of *Mentha piperita L.* (Peppermint) leaves extracts.

MATERIALS AND METHODS

Sample Collection: Fresh matured leaves of *Mentha piperita* L. (Peppermint) were collected from Kachia Local Government Area, Kaduna state, Nigeria and identified by Mr Zakariya Sani, Biological Sciences Department, Sule Lamido University Kafin Hausa. The sample was allocated a voucher number 0259. The leaves were rinsed thoroughly under a running tap water and ultimately dried under shade and then ground into fine powder using clean mortar and pestle [10].

Extraction and Fractionation: The powdered sample (200g) was soaked in 800ml of ethanol for two weeks with frequent agitation on a rotary shaker and filtered using Whatman No. 1 filter paper. The crude extract was further fractionated between chloroform, n-hexane and methanol respectively. These fractions were allowed to dried and stored in refrigerator for subsequent use [11].

Phytochemical Analysis: Phytochemical analysis was carried out to ascertain the presence of the following; alkaloids, tannins, flavonoids, saponins, glycosides and steroids. The test was conducted following the standard procedure described by Isah, *et al*. [12].

Preparation of Stock Solution: Four different concentration (12.5, 25, 50 and 100mg/ml) of ethanolic extracts, chloroform, n-hexane and methanolic fractions were prepared in sterile sample bottles by dissolving 1g of each extracts and fractions in 10ml of Dimethyl Sulfoxide (DMSO) [13].

Bacterial Isolates and Biochemical Tests: Bacterial isolates consisting of *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were collected from Microbiology Department, Kano University of Science and Technology Wudil. The isolates identity was confirmed using various standard biochemical tests as described by Cheesbrough [14].

Antibacterial Assay: The antibacterial activities were assessed by using agar well diffusion method as demonstrated by Kirby and Bauer [15]. The prepared Mueller Hinton ager media was poured on to well labeled Petri dishes under aseptic condition and allowed to solidify. A sterile cork borer was used to make four holes on the ager plates at fairly equidistant position with another hole at middle as control. The plates were inoculated with standardized inocula containing the test bacteria. Each of the four concentrations was dispensed in their respective holes while the control (Ciprofloxacin) at the middle of the plate [15]. After 24 hours incubation period, zones of inhibition were observed by clear zones of inhibition, measured and recorded in millimeter using a meter rule [16].

Determination of Minimum Inhibitory Concentration (MIC): Of nutrient broth 6.5g was prepared by dissolution in 500ml of distilled water. 5ml of the agar were subjected in to different test tubes and covered with cotton wool. Agar was autoclaved at 121°C for 15 minutes. For each test organism one control isolate and control extracts is used against four different concentration (12.5, 25, 50 and 100mg/ml) to make serial dilution by adding 1ml of the extracts to each test tubes and 0.5 ml of control isolate is diluted with 3 ml of distilled water and dispensed to each test tubes. The same procedure was applied for chloroform extracts, n-hexane extracts and methanol extracts and incubated at 37°Cfor 24 hours [17].

RESULTS AND DISCUSSION

The result of phytochemical screening of *Mentha piperita* as shown in (Table 1) revealed the presence of alkaloids, glycosides, steroids, flavonoids and tannins in at least three or more of the extracts but absence of saponins. According to Singh and Bat [18] these secondary metabolites have been reported for antimicrobial activity. Likewise, Vaghasiya *et al.* [19] reported that most plants produce secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, steroids and saponins that are used in pharmaceuticals, cosmetics and pesticide industries. Simple phenols and phenolic acids, quinones, flavones,

Table 1: Phytochemical Screening Result from *Mentha piperita* leaves Extracts

Extracts	Alkaloids	Flavonoids	Steroids	Tannins	Saponins	Glycosides
Ethanol	+	+	+	+	-	-
Chloroform	-	+	+	+	-	+
N-Hexane	+	+	+	+	-	+
Methanol	-	+	+	+	-	-

Key: Positive (+) = presence of compounds, Negative (-) = absence of compounds

Table 2: Degree of Sensitivity of Bacteria against *Mentha piperita* leaves Extracts

Extracts	Concentration(mg/ml)	*S. epidermidis*	*S. pneumoniae*	*P. aeruginosa*	*K. pneumonia*
Ethanol	100	13.33	12.67	14.67	12.38
	50	11.60	10.33	12.67	9.93
	25	8.67	8.00	9.67	8.20
	12.5	-	-	-	-
Chloroform	100	14.93	13.13	15.21	14.28
	50	12.41	11.12	13.37	11.37
	25	9.95	9.58	10.87	9.31
	12.5	-	-	-	-
N-Hexane	100	12.50	12.34	10.73	9.87
	50	10.32	10.70	8.56	8.01
	25	7.89	8.39	7.34	-
	12.5	-	-	-	-
Methanol	100	13.82	14.47	14.21	12.78
	50	12.10	10.24	12.38	9.52
	25	8.79	8.04	8.78	8.80
	12.5	-	-	-	-
Ciprofloxacin	250	26.52	24.23	23.78	21.86

Table 3: Minimum of Inhibitory Concentration of *Mentha piperita* leaves Extracts

Extracts	Concentration (mg/ml)	*S. epidermidis*	*S. pneumoniae*	*P. aeruginosa*	*K. pneumonia*
Ethanol	50	+	+	-	+
	25	+	+	+	+
	12.5	+	+	+	+
Chloroform	50	-	-	-	+
	25	+	+	-	+
	12.5	+	+	+	+
N-Hexane	50	+	+	+	+
	25	+	+	+	+
	12.5	+	+	+	+
Methanol	50	-	+	-	+
	25	+	+	+	+
	12.5	+	+	+	+

Key: Positive (+) = extract has no activity, Negative (-) = extract has shown activity

flavonoids and flavonols, tannins, coumarins, alkaloids, terpenoids and essential oils, lectins and polypeptides are the major groups of antimicrobial compounds [20]. In a similar research work Sowjanya *et al.* [21] showed the presence of alkaloids, glycosides, flavonoids and tannins in the leaf extacts of *Mentha piperita* L.

Table 2 shows antibacterial activity of *Mentha piperita* leaves extracts against certain group of bacteria. The chloroform extracts produced highest zones of inhibition of 15.21 mm 14.93 mm at 100 mg/ml against *P. aeruginosa* and *S. epidermidis* respectively. While the least activity was observed in ethanolic extracts against *S. pneumoniae and K. pneumoniae* with zones of inhibition of 8.00 mm and 8.02 mm respectively at 25 mg/ml concentration. However, the susceptibility of the bacteria to the extracts was in the order of *P. aeruginosa* >*S. epidermidis* > *S. pneumoniae* > *K. pneumoniae.* However, the activity of the extracts is solvent wise and could be ranked in the order of; Chloroform > methanol > ethanol > n- hexane. But not withstanding all the extracts showed degree of activity against the tested bacteria at a varying concentration. All the tested extracts showed no

inhibition at 12.5 mg/ml and this may be because of the little concentration as clear inhibition was observed at higher concentration. The general trend observed was increase in antibacterial activity with increase in concentration of the extracts. Sowjanya *et al.* [21] reported that Hexane and petroleum ether of *Mentha piperita* L. produced less significant antimicrobial activity compared to methanolic extracts. The Table 3 showed the result of minimum inhibitory concentration (MIC) which indicates that chloroform was found have more significant inhibition than other extracts and this extract inhibited *S. epidermidis*, *S. pneumonia* and *P. aeruginosa* at 50 mg/ml while methanol inhibited *S. epidermidis* and *P. aeruginosa* at 50 mg/ml and ethanolic extracts inhibited only *P. aeruginosa* at 50 mg/ml whereas, N-Hexane did not inhibit any bacteria.

CONCLUSION

The results obtained from this study confirmed the presence of some bioactive phytochemicals which may be an evident for the antibacterial activity of the plant leaves. This has proved the claim of the traditional healers for the use of the treatment of some health ailment related to bacterial infection. However, toxicity study needs to be carried out to ascertain the safety of the plant for human consumption.

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