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Evaluation of Antibacterial Activity of *Ocimum basilicum* on Selected Enteric Pathogens

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ABSTRACT

Due to increased antibiotic resistance, it is necessary to source for new antimicrobial substances that will help curb antimicrobial resistance. The aim of this research was to evaluate the antibacterial activity of Ocimum basilicum in-vitro on some enteric pathogens. The phytochemicals present in the aqueous and ethanol extracts were analyzed using standard methods which reveal the presences of alkaloids, glycosides, saponins, phenols, flavonoids, tannins, proteins and carbohydrates. Three (3) clinical isolates of Escherichia coli, Salmonella typhi, and Shigella flexneri were used in this study. Wells of 4 mm were bored on inoculated plates of solidified Muller Hinton Agar containing the test organisms. The antimicrobial activity of the aqueous and ethanol extracts were analyzed on the test organisms using standard method at varying concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml and12.5 mg/ml, minimum inhibitory concentration was carried out using the broth dilution assay and the minimum bactericidal concentration was carried out by streaking method. Ciprofloxacin was used as a positive control. From the result the zones of inhibition ranged from 11mm to 5mm, in which Escherichia coli had zones of inhibition ranging from 11 mm-6 mm, Salmonella typhi had zones of inhibition of 8 mm-6 mm while Shigella flexneri had zones of 11 mm-7 mm. Escherichia coli and Shigella flexneri showed the highest zones of inhibition of 11 mm at 200 mg/ml compared to Salmonella typhi with a zone of inhibition of 9 mm at 200 mg/ml. This study revealed that the ethanol extract showed more antibacterial activity compared with the aqueous extract which had no activity on the test organisms. The minimum inhibitory concentration of the ethanol extract was at the concentration of 200 mg/ml for all the test organisms. The minimum bactericidal concentration at 200 mg/ml revealed the presences of growth on the plates for all the test organisms. Therefore the minimum bactericidal concentration of the extract against the test organisms will be at a concentration >200 mg/ml. The findings from this research revealed that Ocimum basilicum has the capability for treating diarrhea. Further research and purification of the plant extract both in-vivo and in-vitro assay is necessary.

Keywords: Evaluation, Antibacterial activity, *Ocimum basilicum*, Enteric pathogens.

INTRODUCTION

Enteric pathogens are commonly associated with nosocomial infections, particularly in environments where poor hygiene prevails [1]. According to WHO [2] though a global problem, drug resistance in bacteria poses a greater

threat in the developing world where morbidity of bacterial diseases is much higher and treatment options are limited. These organisms can live on inanimate surfaces for as long as seven days to months in the hospital environment, thereby, contaminating patients' food in the hospital [3]. According to Tetteh-Quarcoo et al. [4], insects particularly cockroaches could serve as vectors for the dissemination of enteric pathogens such as *Escherichia coli*, *Pseudomonas spp*, *Klebsiella spp* and *Salmonella spp* associated with nosocomial infection in the hospital environment [1]. Multidrug Resistant (MDR) strains of enteric bacteria such as *Salmonella*, *Acinetobacter*, and *Pseudomonas* have been implicated in outbreaks in both developed and developing countries [5,6]. Still in the same vain, Leopold et al. [7], stated that Multidrug Resistant *E. coli and Salmonella spp* often express extended-spectrum beta-lactamases that favor increased resistance to broad-spectrum beta-lactam antibiotics these genetically encoded traits are usually located on plasmids that are transferable between bacterial strains and species. Data on antibiotics Resistance for pathogens is generally limited in sub-Saharan Africa [7]. Due to the current burden of antimicrobial resistance, herbal medicines are an alternative means of treatment regimens [8].

The heterogeneous use of antibiotics has led to increasing problems of Multiple Drug Resistance (MDR) among pathogenic bacteria and this is of serious concern to both the clinicians and pharmaceutical industries which has made it significant to search for newer means of treatment that are highly effective, affordable, acceptable and available [9,10].

MATERIALS AND METHODS

Sample collection

Clinical isolates of *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri* were collected from culture banks of National Veterinary Institute of Research (NVIR) Vom. The cultures obtained were re-identified at the microbiology laboratory of University of Jos, based on cultural morphology and biochemical characterization following standard bacteriological procedures. Biochemical tests were used to confirm the isolates of *Escherichia coli*, *Salmonella typhi,* and *Shigella Flexner*. Fresh leaves of curry were purchased at Chorbe market in Jos North local government of plateau state and it was transported to the laboratory for washing and drying, a sample of the leaves were taken to college of forestry for identification and it was identified as *Ocimum basilicum*.

Preparation of plant extract: The leaves of *Ocimum basilicum* were air-dried at room conditions for 14 days, after which the dried leaves were ground using an electronic blender. Shredded plant materials were put in sterile bottles containing distilled water or 80% ethanol [11].

Ethanol extraction of plant materials and preparation of aqueous extract: A weight of 100 g of the powdered plant material was put into sterile conical flask containing 500 ml of 80% ethanol for 48 hours. The mixture was separated by sieving method using number one Whattsman filter paper. The resulting filtrate was properly air-dried to remove excess ethanol [12]. The aqueous extract was prepared by soaking 100 g of the curry powder into 500 ml of sterile distilled water for 48 hours. The extract was filtered using Whattsman No 1 filter paper. The resulting filtrate was evaporated using a water bath at 40°C to get the crude extract.

All extracts were stored at 4°C until required for phytochemical analysis.

Phytochemical screening: Screening of the phytochemical constituents of the extracts was carried out at the department of Pharmacy, University of Jos. The following chemicals were tested according to standard methods: Tannins, Flavanoids, Saponin, Alkaloids, Glycosides, and Terpenoids.

Determination of the antimicrobial sensitivity: The antimicrobial activity of *Ocimum basilicum* was carried out according to the method described by Joshi et al., [13]. In which 15ml of sterile Muller Hinton Agar was poured into various Petri dishes, they were allowed to solidify. The plates were inoculated with freshly prepared cultures of *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, which initially standardized to 0.5 McFarland standard, it was swabbed over the entire surface of the medium with the aid of a sterile cotton swab, the plates were left to dry for some minutes. A 4 mm diameter well was bored using a sterile borer and each well was filled with 0.2 ml of the various extracts (ethanol and aqueous extracts) at various concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml. The wells were far from each other to prevent overlapping of the zones of inhibition. The plates were left for 45minutes for proper diffusion of the extracts to the medium and they were incubated at 37°C for 24 hours. The zones of inhibition were measured in millimeters by measuring the diameter of the zones with a transparent meter rule. All the experiments were carried out in duplicates.

Determination of minimum inhibitory concentration (MIC): The minimum inhibitory concentration of the ethanol and aqueous extracts were determined for each of the test organisms in duplicates at varying concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml following adjustment to 0.5 McFarland turbidity standards. A tube containing nutrient broth was inoculated with the test organisms and this served as the control. All tubes were incubated at 37°C for 24 hours and then they were examined for growth by observing for turbidity.

Determination of minimum bactericidal concentration (MBC): The minimum bactericidal concentration of the various extracts on the test organisms was carried out in accordance with Ajaiyeoba et al., [14].

RESULTS

The tables shows the phytochemical constituents, zones of inhibition, minimum inhibitory and minimum bactericidal concentrations of the various extracts of *Ocimum basilicum* on the isolates of *Escherichia coli*, *Salmonella typhi,* and *Shigella flexneri*. Ciprofloxacin was used as a positive control.

Table 1: Phytochemical constituents of *Ocimum basilicum*

S. No	Phytochemical constituents	Ethanol	Water
1	Alkaloids	+	+
2	Glycosides	+	+
3	Saponins	+	+
4	Phenols	+	+
5	Steroids	-	+
6	Flavanoids	+	+
7	Terpanoids	-	-
8	Tannins	+	+
9	Proteins	+	+
10	Carbohydrate	+	+
Key: -: Absent +: Present			

Table 1 shows the phytochemical constituents present in the aqueous and ethanol extract of *Ocimum basilicum*, the result indicates the presence of secondary metabolites such as alkaloids, glycosides, saponins, phenols, flavonoids, tannins, proteins and carbohydrates. Steroids were absent in the ethanol extract while they were present in the aqueous extract. Terpanoids were absent in both the aqueous and ethanol extracts

Table 2: Antibacterial activity of *Ocimum basilicum* on test organisms

Test organisms	Plant extract	Diameter of zones of inhibition at different concentration (mg/mL)					Control Ciprofloxacin	
	Ethanol	200	100	50	25	12.5	+C (100)	-C
E. coli		11	8	6	-	-	44	-
Salmonella typhi		9	7	5	-	-	40	-
Shigella flexneri		11	9	7	-	-	40	-
	Aqueous Extract							
E. coli		-	-	-	-	-	44	-

Salmonella typhi		-	-	-	-	-	40	-
Shigella flexneri		-	-	-	-	-	40	-
Key: -: Negative								

Table 2 shows the antibacterial activity of the aqueous and ethanol extracts on the test organisms. The result indicates the effect of the extracts on the test organisms. The ethanol extract showed more antibacterial activity compared to the aqueous extract which had no activity on the test organisms at the various concentrations. *Escherichia coli* and *Shigella flexneri* showed the highest zones of inhibition of 11 mm at a concentration of 200 mg/ml while the lowest was *Salmonella typhi* with a zone of inhibition of 9 mm at 200 mg/ml.

Table 3: Minimum inhibitory concentration of ethanol extract of *Ocimum basilicum* on test organisms

Test Organisms	**Concentration of extract (mg/ml)**					
	200	**100**	**50**	**25**	**12.5**	**MIC**
Escherichia coli	-	+	+	+	+	200
Salmonella typhi	-	+	+	+	+	200
Shigella flexneri	-	+	+	+	+	200
Key: -: Absence of turbidity; +: Presence of turbidity						

Table 3 showed the minimum inhibitory concentrations of the ethanol extract to the test organisms and the result revealed that the minimum inhibitory concentration was at 200 mg/ml for all the test organisms.

Table 4: Minimum bactericidal concentration of ethanol extract of *Ocimum basilicum* on test organism

Test organisms	**Concentration in mg/ml** **200**	**MBC**
Escherichia coli	+	>200
Salmonella typhi	+	>200
Shigella flexneri	+	>200
Key: + Presence of growth		

Table 4 showed the minimum bactericidal concentrations of the ethanol extract to the test organisms and this result showed that the extract was not bactericidal at the concentration of 200 mg/ml.

Discussion

The indiscriminate use of antibiotics has led to the rising emergence of Multi-Drug Resistant pathogenic strains of bacterial causing diseases that challenge regular treatment. Antibiotics and chemically synthesized drugs, the trend to look out for alternative drugs in nature is increasing as the natural sources are less toxic [15]. The analysis on the phytochemicals constituent reveals that the ethanol extract and aqueous extract of *Ocimum basilicum* contains secondary metabolites such as Alkaloids, Glycosides, Saponins, Phenols, Proteins, Carbohydrates, Steroids, Flavonoids and Tannins. Steroids were absent in the ethanol extract while they were present in the aqueous extract, but Terpanoids were absent in both the ethanol and aqueous extract. Alkaloids, saponins, flavonoids, and tannins are known to have curative effect on pathogens [16]. Variation in the phytochemicals presents may be due to geographical factors as described by Sarti F et al. [17].

The antimicrobial activity of *Ocimum basilicum* varied greatly with solvents, due to some factors that influence the active ingredients present in the plant which include, extracting solvent, method of extraction, age of the plant, time of harvesting the plant [18]. Based on the findings it was observed that the ethanol extract has more antibacterial activity compared to the aqueous extract which has no activity on the test organisms [19]. Different concentrations of

200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml of ethanol extract of *Ocimum basilicum* were used in an agar well diffusion assay and this caused different degrees of zones of inhibition against *Escherichia coli*, *Salmonella typhi*, and *Shigella flexneri*. From the result, *Escherichia coli* and *Shigella flexneri* were more sensitive to the extract with the highest zone of inhibition of 11 mm at a concentration of 200 mg/ml while *Salmonella typhi* has a zone of inhibition of 9 mm at the same concentration. There was no activity of the ethanol extract at the concentrations of 25 mg/ml and 12.5 mg/ml on all the test organisms. All antimicrobial activities were observed to be concentration dependent which was similar with the findings of Tomar et al., [20]. The zones of inhibition of the ethanol extract which was low on the test organisms were similar with the findings of Nascimento et al., [21] who had a lesser zone of inhibition on the ethanol extract. It was observed that the minimum inhibitory concentration of the extract on *Escherichia coli*, *Salmonella typhi* and *Shigella flexneri* all had a minimum inhibitory concentration at 200 mg/ml which was the highest concentration [22]. This finding reveals that the extract has inhibitory effect at 200 mg/ml concentration, and it is similar with the findings of Tomar et al., [20]. Minimum inhibitory concentration was observed that after the period of incubation there was growth on the plates of the various test organisms at 200mg/ml the findings in this study was similar with the findings of Okigbo, [18] who said that the extract has no minimum inhibitory concentration at 200 mg/ml, this may be due to factors such as the extraction method, solvent used or intrinsic factors from the plant. Therefore the extract will be bactericidal at a concentration greater than 200 mg/ml.

Conclusion

The result from this study showed that *Ocimum basilicum* antimicrobial property that can potentially inhibit the growth of enteric pathogens. The ethanol extract was found to be effective against all tested bacterial strain, while the aqueous extract has no effect, this may be due to unforeseen factors. The bioactive components of plant this should be evaluated to determine the active ingredients. Toxicity study of the plant should be carried out so as to establish its safety and dosage especially in children, pregnant women, and lactating mothers. This research could be further extended to test the bioactive properties of the plants for therapeutic use. Extensive research on the plant should be done on broad range of microorganisms for both *in-vivo* and i*n-vitro.*

CONFLICT OF INTEREST

All authors have declared that no conflicts of interest exist.

AUTHORS' ROLE

All authors contributed to the manuscript.

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