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(RESEARCH ARTICLE)

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Effects of seed extracts of turmeric (*Curcuma longa linn)* on *Escherichia coli* and *Streptococcus species* isolated from urine of patients in Wukari, Taraba State, North East, Nigeria: Prospective antimicrobial alternative for Urinary Tracts Infections

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Abstract

Antimicrobial activity of different herbal extracts in different regions of the worlds has been established as a panacea to the advancement of effective chemotherapy against resistant bacteria. Seed extracts of turmeric as one of the antibacterial agents has been examined to this effect. Turmeric (*Curcuma Longa, Linn)*, is one of the most useful herbal medicinal plants owing to its biological active component Curcuminoids, which are natural phenols. Turmeric has shown to be effective as anti- inflammatory, anti-oxidant, antifungal, antiulcer, and anticancer, thus has a potential against various malignant diseases such as, diabetes, allergies, arthritis, Alzheimer's disease and other chronic diseases. This study examined the antibacterial activity of turmeric seed against *Streptococcus species* and *Escherichia coli* isolated from urine samples of patients. The antibacterial activities of aqueous and ethanolic extracts of dried and fresh turmeric seed was tested on the organisms using standard bacteriological technique. The zone of inhibition was more significant on the ethanolic extract of the fresh seeds and at a higher concentration (15g – 80ml and 20g – 100ml) on both organisms. Also, on both organisms, the result obtained revealed resistance of isolates to the aqueous extracts of the dried seed with heavy and moderate growth. In conclusion, the results obtained from the turmeric extracts shown considerable antimicrobial activity against *Streptococcus* species and *Escherichia coli* and can be used as a broad spectrum natural antimicrobial alternative for treating Urinary Tracts infections.

Keywords: Antimicrobial; Aqueous; Bacteria; Ethanolic; Patients; Turmeric; Urine

1. Introduction

Antimicrobial resistance (AMR) by many pathogenic microorganisms including uropathogenic bacterial has not only burdened Global health, it also has necessitated the instigation of many researchers to focus more on the dark aspects of folklore medicines particularly in the areas of herbal therapy [1, 2]. AMR is blamable for over 650,000 global deaths and is estimated to scale up to over 9 million deaths yearly by 2050 if deliberate action is not taken to curb the current situation [3]. A WHO report [4], has estimated that by the year 2030 over 20 million persons particularly in Africa and other developing Worlds could be plunged into extreme poverty as a direct consequence of AMR. AMR typically stems from microbial genetic mutation mainly due to drug overuse particularly on frequent infections such as Urinary Tract infections (UTI) [5]. UTIs are responsible for substantial clinical cases of morbidity across populations of all ages. Although, women are mostly affected as about 40% of them have had a UTI at a time in their lives [6]. *Escherichia coli* is reported to account for over 70% of all reported cases of UTIs making it the most commonly isolated

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uropathogen from clinical specimens [7]. The gram negative bacteria possess special virulence factors such as fimbriae, flagella and biofilm formation necessitating its seamless invasion into various host defence mechanisms [8]. Although, *Escherichia coli* are largely regarded as commensals in the lower intestines of warm-blooded organisms, some isolates however have the potential to also cause disease [9]. Apart from being mostly associated with UTIs, pathogenic *Escherichia coli* are also a leading cause of diarrhoea, often with high mortality rates, in developing countries [10]. *Streptococcus agalactiae*, also known as Group B streptococcus GBS is another bacteria involved with UTIs [11]. Although, GBS is a common commensal of the human gastrointestinal and genitourinary tracts however, it is able to cause other range of infections among immunocompromised individuals, the elderly, pregnant women and infants. GBS which is gram positive bacteria is responsible for over 2% of all reported cases of UTIs including asymptomatic bacteriuria (ABU), cystitis and pyelonephritis [12, 13]. In the United States of America alone, GBS is blamable for over approximately 159,000 cases of UTIs [14]. Plants and their products have repeatedly proven to be very effective in the treatment of diseases thereby forming the basis of sophisticated traditional medicine systems that have existence for a long time [14]. AMR has stimulated the necessity for plants to be explored as a dependable source to obtain a variety of drugs against diseases due their antimicrobial effects on a wide range of microorganisms even those with resistant genes [15, 16]. Seed extracts of turmeric as one of the antibacterial agents has been examined to this effect. Turmeric (*Curcuma longa*), belongs to the ginger family, *Zingaberaceae* [17]. It is reportedly been used extensively in the food production for its flavour preservative and colour characteristics [18]. Turmeric possess a wide range of pharmacological efficacy as it is used in Indian folk medicine for the treatment of various illnesses including but not limited to biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis [19]. Therefore, this research investigated the antimicrobial efficacy of extracts of Turmeric plant seed on both gram positive and negative uropathogens [20].

2. Material and methods

2.1. Collection of plant material

The harvested rhizomes of turmeric plant used were obtained from a farm at General Hospital Katsina-Ala, Benue State**.** The plant was identified to be turmeric by Botanists in the Department of Biological Sciences, Faculty of Pure and Applied Sciences, Federal University Wukari.

2.2. Preparation of plant material

The harvested rhizomes were carefully washed with clean water to remove debris. It was then peeled, and steamed for 10 minutes to remove the raw odour. The turmeric was later dried in the oven at a temperature of 65^{o}C two days. The dried rhizomes were polished to remove rough surfaces by handpicking before it was finally crushed into powder for analysis. Fresh turmeric was washed in distilled water and disinfected with 70% ethanol. It was then crushed into semi-solid form with a hand grater to aid easy extraction of the content of the plant. The dried and fresh turmeric were measured in weights of 5g, 10g, 15g and 20g. It was dissolved in 40ml, 60ml, 80ml and 100ml distilled water and 50% ethanol respectively

2.3. Sample collection

Mid-stream urine samples were collected from forty one (41) in-patients and out-patients at Kwararafa Hospital Wukari. The samples were collected aseptically with hand gloves, and in sterile urine containers. The samples were immediately labelled and taken to the laboratory for investigation.

2.4. Preparation of Media

Cysteine lactose-electrolyte deficient agar (CLED), MacConkey agar, and Nutrient agar prepared with strict adherence to the manufacturer's instructions, were used for culturing in this study.

2.5. Culture of samples

Petri dishes were labelled serially according to the three media prepared (Nutrient, MacConkey and CLED). 1ml of each urine sample was measured into the three different labelled petri dishes aseptically. About 20ml of the prepared media was poured and gently rotated to distribute the inoculums evenly in the plates. The plates were left to solidify under airflow. Each of the inoculated plate was incubated at 30 - 37^{o}C for 48hours in an inverted position to avoid condensation. Distinct colonies were sub-cultured with fresh MacConkey and Nutrient Agar by streak plate technique. Nutrient and CLED mixed cultures were sub-cultured on Nutrient and MacConkey agars. The sub-cultured plates were

incubated again at 30-37°C for 24 hours. The plates were examined after 24hrs for morphologically characteristics of the pathogens.

2.6. Isolation of Bacteria

The colonies of the sub-cultured plates were examined after 24hours for morphological characteristics in terms of Size (small, medium or large), Shape (spherical, irregular or rhizoid), Colour (Yellow, white or green), Elevation (elevated/raised, convex, concave, umbonate/umbilicate), Surface (Smooth, wavy, rough, granular, papillate or glistening).

2.7. Identification of bacteria

Confirmatory identification of bacteria isolates was done following biochemical charactization and gram staining of isolates. Catalase, Indole, Methyl Red, and Sugar fermentation tests were the biochemical tests carried out for identifying the isolates *Streptococcus* species and *Escherichia coli*.

2.8. Sensitivity test of turmeric extract on the isolates

1ml of solution of dried and fresh aqueous and ethanol extracts of turmeric seeds were measured aseptically in each of the labelled Petri dishes before dispensing the prepared nutrient agar swirling gently for proper mixing with the solution of the extracts.

The bacterial isolates, *Escherichia coli* and *Streptococcus* species were then inoculated on the sterile medium using the streak plate method before incubating them at 37°C for 24 hours. The plates were observed after 24 hours for growth or inhibition. Also, Control plates were prepared for *Escherichia coli* and *Streptococcus* species without the addition of extracts.

3. Results

Table 1 Isolation and identification of bacteria isolated from clinical (urine) samples

Sample	Morphological characteristics	Gram stain reaction	Bacterial Isolates								Biochemical Tests
			Cat.	Ind	MR	Lac	Suc	Fru	Glu	Gal	
A	Medium, spherical, yellow, dried and flat	+vecocci (chains)	-	-	-	+	+	+	+	+	*Streptococcus spp.*
B	Small, spherical, pink, mucoid and raised	-ve rods	+	+	+	+	+	+	+	+	*Escherichia coli*

Key: (-) = Negative, (+) = Positive, Cat= Catalase, Coa= Coagulase, Ind = Indole, MR = Methyl red, Lac = Lactose, Suc = Sucrose, Fru = Fructose, Glu=Glucose, Gal = galactose.

Table 2 Effects of aqueous and ethanolic extracts of dried turmeric seeds on *Escherichia coli* and *Streptococcus* spp

Concentration	*E. coli*		*Streptococcus spp.*	
	Aqueous	Ethanol	Aqueous	Ethanol
5g – 20ml	Heavy growth	Heavy growth	Heavy growth	Moderate growth
10g – 60ml	Moderate growth	Heavy growth	Moderate growth	Moderate growth
15g – 80ml	Moderate growth	Moderate growth	Moderate growth	Low growth
20g – 100ml	Moderate growth	Moderate growth	Low growth	Low growth

Heavy growth (10 and above number of colonies), Moderate growth (5–9 number of colonies), low growth (1– 4 number of colonies)

Table 3 Effects of aqueous and ethanolic extracts of fresh turmeric seeds on *Escherichia coli* and *Streptococcus* spp

Concentration	*E. coli*		*Streptococcus spp.*	
	Aqueous	**Ethanol**	**Aqueous**	**Ethanol**
5g – 20ml	Heavy growth	Moderate growth	Heavy growth	Moderate growth
10g – 60ml	Heavy growth	Moderate growth	Heavy growth	Moderate growth
15g – 80ml	Moderate growth	Low growth	Moderate growth	Low growth
20g – 100ml	Moderate growth	Low growth	Low growth	Low growth

Heavy growth (10 and above number of colonies), Moderate growth (5–9 number of colonies), low growth (1– 4 number of colonies)

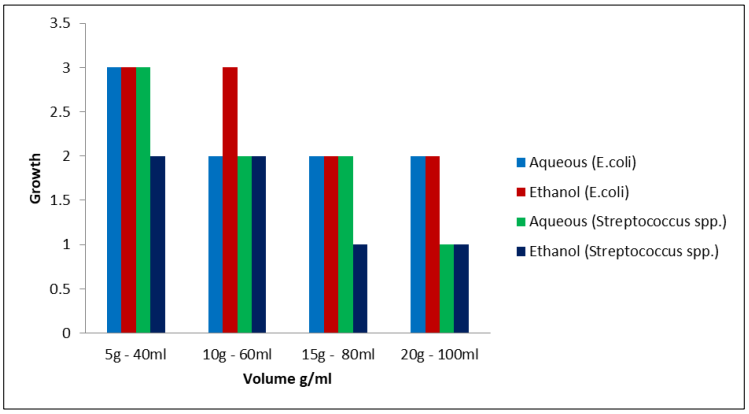

Figure 1 Effects of aqueous and ethanolic extracts of dried turmeric seed on *E.coli* and *Streptococcus spp*

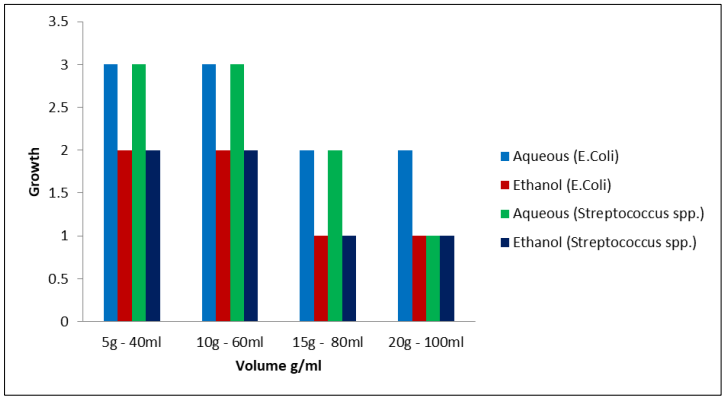

Figure 2 Effects of aqueous and ethanolic extracts of fresh turmeric seed on E.coli and Streptococcus spp

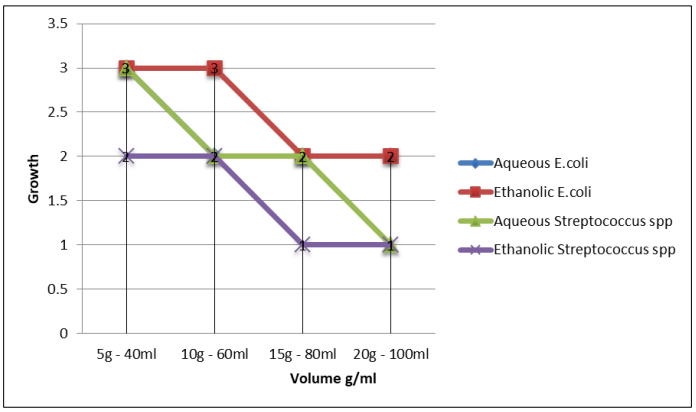

Figure 3 Effects of aqueous and ethanolic extracts of dried turmeric seed on *E. coli* and Streptococcus spp

Table 1, shows the identification of *Streptococcus* species and *Escherichia coli* isolated from collected urine samples. The table also represents their respective morphological and biochemical characteristics. The respective antimicrobial effectiveness of various ethanolic and aqueous concentrations of dried and fresh seed extracts of *Curcuma longa* against the test organisms is illustrated in Table 2 and 3 respectively. Figures 1 and 2 are histograms representing the results in Tables 2 and 3 while figures 3 and 4 are graphical presentation of histograms in figure 1 and 2.

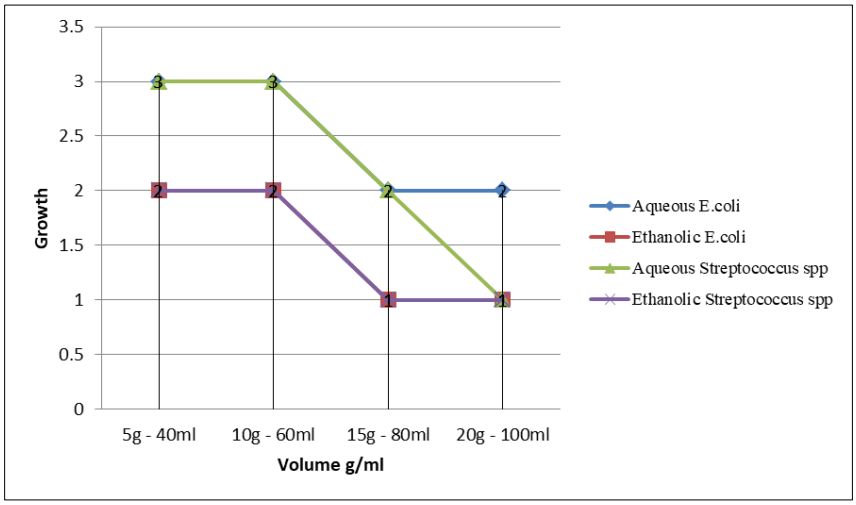

Figure 4 Effects of aqueous and ethanol extracts of fresh turmeric seed on *E. coli* and *Streptococcus spp*

4. Discussion

This research has been able to present distinctiveness in the antimicrobial effects of extracts of dried and fresh seed extracts of *Curcuma longa* against *Streptococcus* species and *Escherichia coli* at various concentration levels of 5g- 20ml, 10g-60ml, 15g-80ml, 20g-100ml. At low concentrations (5g- 20ml), aqueous and ethanolic extracts of dried seeds of *Curcuma longa* presented less antimicrobial effect on *Streptococcus* species and *Escherichia coli* as "heavy" growth was observed but, effective at higher concentration (10g-60ml, 15g-80ml, 20g-100ml) of both solutions as "low" growth of *Streptococcus* species and moderate growth of *Escherichia coli* were significant. At lower concentrations, observations on the fresh seed extract of the Turmeric plant is not dissimilar with that of the dried seed extract except that "moderate" growth of both test organisms was seen at high ethanolic concentrations (10g – 60ml) of the plant extracts. Similarly, "moderate" growth of *Escherichia coli* was seen at higher aqueous concentrations (15g-80ml, 20g-100ml) of the plant extract Nonetheless, low" growth of *Escherichia coli* was seen at higher concentration (15g-80ml, 20g-100ml) of ethanolic extract and low growth of *Streptococcus* species was significant at higher concentration (15g-80ml, 20g-100ml) of both aqueous and ethanolic extracts of the fresh seed. This implies has that at high concentrations, ethanolic extracts of both dried and fresh seed of *Curcuma longa* inhibit the growth of *Streptococcus* species and *Escherichia coli* more than the aqueous extracts. The efficacy of ethanol over other solvents in extracting phytochemicals with potential antimicrobials from plant has previously been established by [20] in the study which investigated the antimicrobial properties of *Vernonia amygdalina* on *Escherichia coli* and *Proteus species* isolated from urine samples. Similarly, a review by [21] on anti-bacterial potential of plants to combat resistance, ethanol revealed better potency in extracting phytochemicals with antibacterial properties such as saponins and alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, edotides and sesquiterpenes from plants. This current research noted the dissimilar pattern of growth inhibition of dried and fresh extract of *Curcuma longa*.

Fresh extracts showed more antimicrobial activity on the *Escherichia coli* organisms than the dried extracts but, no significant dissimilar growth inhibition pattern was observed for *Streptococcus* species on both dried and fresh Turmeric extracts. This finding again is inconsistent with [20] which concluded that the dried ethanolic and aqueous extracts of *Vernonia amygdalina* possess strong antimicrobial activities against the clinical isolates of *Proteus species* and *Escherichia coli* compared to the fresh extracts. Air-drying leaf of plants reduces the concentrations of toxic non-nutrients thereby improving its antimicrobial properties [22]. The distinction between findings of current research and that of [20] may not be unconnected with the fact that leafs and seeds of plants possess distinct phytochemical characteristics. Moreover, this current study involved both gram positive and negative organisms while the former focused only on gram negative bacteria. Gram negative organisms are known to be more resistant to antimicrobials due to their complex cell wall with thin peptidoglycan layer [23]. Consequently, this current research revealed that the extracts of Turmeric plant showed antimicrobial activity against gram positive *Streptococcus* species and gram negative

Escherichia coli. Turmeric plant contains Curcuminoids, (Curcumin) which is the most active phytochemicals of turmeric plant [24]. Curcuminoids are natural phenols and are responsible for the yellow colour of the turmeric [25]. The Curcumin has been shown to be effective not only as anti- inflammatory, anti-oxidant, antifungal, antiulcer, and anticancer but antibacterial [26].

5. Conclusion

Ultimately, this research has advanced the antimicrobial potentials of plants on microorganisms. This current study has been able make explicit that extracts of *Curcuma longa* inhibits the growth of gram positive *Streptococcus* species and gram negative *Escherichia coli.* Consequently this plant is therefore a possible broad spectrum natural antimicrobial candidate for treating uropathogenic bacterial.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors (Edobor Peter Kenneth Imarenezor, Onolunosen Abel Abhadionmhen, Samuel Tamunoiyowuna Cockeye Brown, Joyce Briska, Paula Paul Shinggu and Sunday Danya) declare no conflict of interest.

Statement of informed consent

All participants were adequately prepared for the study; were given health education, all necessary information provided and voluntarily signed consent form.

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