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Antimicrobial Effects of Tumeric and Neem Leaves on Clinical Isolates

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ABSTRACT

In vitro antibacterial activities of crude extract of Neem (Azadirachta indica) and Turmeric (Curcuma long Linn) were investigated against some bacterial isolates comprising of Salmonella typhi, Escherichia coli and Shigella dysenterae using the agar well diffusion and broth dilution techniques. The methanolic crude extract of both plants exhibited significant inhibitory action against the isolates tested at an initial concentration of 200mg/ml. The zones of inhibition exhibited by the extract against the tested organisms ranged between 10 and 28mm. There was a corresponding decrease in the zone of inhibition of the growth of the pathogens with decrease in extract concentration as was observed in the minimum inhibitory concentrations assay. The methanol extract of the leaves of Tumeric (Curcumin longa) exhibited a pronounced activity on Salmonella typhi by its wide zone of inhibition (28.0mm) when tested in its crude form. However, upon dilution at a minimum inhibitory concentration of 5%, a zone of inhibition of 8.0mm was observed against the same organism. Similarly, the same extract at its crude form was active against E. coli with a zone of 10.0mm and an MIC value of 0.1%. The potentials for developing antimicrobials from plants as revealed from this study seems rewarding as it will lead to the development of a phytomedicine against enteric pathogens.

Keywords: Neem, Tumeric, Plant, Antimicrobial, Resistance

INTRODUCTION

A variety of plants or materials derived from plants have been used for the prevention and treatment of diseases virtually in all cultures. Herbs have been used as sources of food and medicinal purposes for centuries and this knowledge have been passed from one generation to another [1].Medicinal plants also represent a rich source from which antimicrobial agents can be obtained [2].The antimicrobial properties of crude extracts prepared from plants have been reported [3, 4].This worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care [5].

Infections due to pathogenic bacteria and fungi represent a critical problem to human health [6].Despite the extensive use of antibiotics and vaccines programs, infectious diseases continue to be a leading cause of morbidity and mortality worldwide [7].Widespread antibiotic resistance, the emergence of new pathogens in addition to the resurgence of old ones and the lack of effective new therapeutics exacerbate the problems [7].

Turmeric (Curcuma longa L.) is a medicinal plant extensively used as home remedy for various diseases. C. Longa L, botanically related to ginger belongs to the Zingiberaceae family [8].Traditional Indian medicine uses tumeric powder for the treatment of biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Various sexquiterpenes and curcuminoids have been isolated from the rhizome of C. longa, attributing a wide array of biological activities such as antioxidant [9,10] anti-inflammatory [11],wound healing [12], anticancer and antibacterial activity [13].

In the last few decades there has been considerable interest in the active compounds in tumeric called curcuminoids. The anti-bacteria activity of curcumin biconjugates has been tested particularly for β-lactamase producing microorganisms [14].Tumeric oil has also demonstrated antibacterial activity against Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.

Clinical studies have shown that the neem leaves extract decreased the dental plague index caused by Streptococcus mutans [15,16]. An in vitro study has demonstrated that aqueous extract from Neem leaves inhibits biofilm formation and adhesion in composite resin by Candida albicans [17].On the other hand, the methanolic extract of neem has reported to have in vitro antiviral activity against group B coxsackie viruses, or against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans [17] .Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premises [18].

Due to the rapid emergence of antibiotic-resistant bacteria, there is a growing need to discover new antimicrobial agents with plants serving as frontlines. The decreasing development of new antibiotics is exacerbating this already dire problem. Without immediate action to discover and develop new antibiotics, it is projected that deaths attributable to resistant infections will reach 10 million per year by 2050.

The aim of this work was to investigate the anti-microbial properties of Neem leaf (Azadirachta indica) and tumeric leaf (Curcuma longa) extracts against clinical isolates including Salmonella typhi, Escherichia coli and Shigella dysenteriae.

METHODS

Preparation of plant extracts

Plant material included the dried leaves of Azadirachta indica and Curcuma longa. The samples were collected from the National Root crops Research Institute, Umudike Abia State Nigeria and identified by plant taxonomist at the Department of Botany, Michael Okpara University of Agriculture Umudike Abia State Nigeria. Plant material was ground to a powder in a mechanical grinder and was soaked into 95% methanol in a ratio of 1:10 (w/v) for 72 hours with vigorous shaking. The extract was subjected to evaporation and residual material was considered as source of methanol extract. Stock solutions were prepared in DMSO.

Screening of extracts and fractions for antibacterial activity

The antibacterial activities of the extracts were assessed by the agar well diffusion assay. Briefly, a stock solution of each of the plant extracts was made in dimethyl sulfoxide (DMSO). The test organisms were reactivated by streaking out on a freshly prepared nutrient agar plate. An aliquot of 100µl of suspension each isolate standardized to 0.5MacFarland standard was aseptically inoculated unto Muller Hinton agar plate using a cotton swab to create a lawn of the organisms. Wells were created on the agar surface using a flame sterilized cork-borer of 6mm diameter. An aliquot of 50µl of each of the plant extracts was loaded into each well. The plates were incubated at 37°C for 48 h. The diameter of the zone of inhibition was measured in mm using a transparent plastic ruler.

The minimum inhibitory concentration (MIC) was determined by preparing the extract into four different concentrations i.e., 5%, 1%, 0.1% and 0.01% respectively.

- Preparation of 5%: 5% of the raw extract was added to 95ml of water.
- Preparation of 1%: 1% of the raw extract was added to 99ml of water
- Preparation of 0.1%: 0.1% of the raw extract was added to 99.9ml of water.
- Preparation of 0.01%: 0.01% was added to 99.99ml of water.

Source of microorganisms

The isolates used in this study were isolated from stool samples on MacConkey agar and Salmonella Shigella agar and identified tentatively as Escherichia coli, Shigella dysenteriae and Salmonella species using standard biochemical characterization procedures.

RESULTS

The results of the antibacterial screening and determination of Minimum inhibitory concentration and Minimum bactericidal concentration are as depicted in tables 4.1 through 4.3 with the methanolic extracts of both plants showing appreciable inhibitory activity against the test isolates.

Table 1: Determination of MIC values of methanol extracts of Tumeric leaves against the isolates

Extract Concentration	E. coli	Shigella dysenteriae	Salmonella typhi
5%	10mm	10mm	8mm
1%	8mm	10mm	7mm
0.10%	8mm	12mm	8mm
0.01%	0	11	11mm
Control	23mm	25mm	28mm

Table 2: Determination of MIC values of methanol extracts of Neem leaves against the isolates

Extract Concentration	E. coli	Shigella dysenteriae	Salmonella typhi
5%	10mm	12mm	9mm
1%	11mm	0mm	9mm
0.10%	10mm	0	0
0.01%	8mm	0	0
Control	23mm	25mm	28mm

Table 3: Antibacterial activity of methanol extracts of Neem and Tumeric leafs against the isolates

Extract	Salmonella typhi	E. coli	Shigella dysenteriae
Turmeric	28mm	10mm	0mm
Neem	10mm	0mm	10mm
Control (Levofloxacin)	34mm	29mm	26mm

DISCUSSION

Drug development from plant-based compounds could be useful in meeting this demand for newer drugs with minimal side effects. The study was also conducted to obtain preliminary information on antibacterial activity of Neem (Azradrichia indica) and Tumeric (Curcumin longa) on clinical isolates (Escherichia coli, Shigella, dysenteriae, and Salmonella typhi). The result obtained in the present study provides a scientific support to the ethnomedical uses of the leaf extracts of turmeric and neem plants in the treatment of enteric disease.

The result showed that methanol extract of turmeric had more inhibitory effects on Salmonella typhi, and Escherichia coli, with a zone of 28mm and 10mm respectively, and no zones on Shigella dysenteriae. Methanol extract of neem leaf had inhibitory effect in Shigella dysenteriae and Salmonella typhi with a zone of 10mm and 10mm respectively, and no zone on Escherichia coli. Among the plant extracts used, neem demonstrated the least antimicrobial activities on the bacterial isolates. From the findings, the results of this research work could suggest that plants extract of neem (Azadirachta Indica) and Tumeric (Curcumin longa) possess antimicrobial properties highly effective in inhibiting the growth of some enteric pathogens in humans. The result obtained in this study is comparable to those recorded in similar studies by [19,20].

The examined plant extracts showed varying degrees of antimicrobial activities against the clinical pathogens tested. In this study, the methanol extract of the leaves of Tumeric (Curcumin longa) exhibited a pronounced activity on Salmonella typhi by its wide zone of inhibition (28.0mm) when tested in its crude form. However, upon dilution at a minimum inhibitory concentration of 5%, a zone of inhibition of 8.0mm was observed against the same organism. Similarly, the same extract at its crude form was active against E. coli with a zone of 10.0mm and an MIC value of 0.1%.

The antimicrobial activity of methanol extracts of the medicinal plants was observed to be concentration-dependent and the activity varied with concentration against the tested pathogens as shown in tables 4.1 and 4.2. The methanolic extract of Tumeric showed maximum zone of inhibition against Escherichia coli (10.0mm) at 5%. The diameter zone of inhibition reduced to 8.0mm as the concentration dropped to 1% and 0.1% respectively. A similar trend was observed for the other test isolates. This result is in agreement to similar studies carried out by [21,22].

For Azadirachta indica extracts, maximum zone of inhibition (10.0mm) was observed against Salmonella typhi and Shigella dysenteriae while Escherichia coli was resistant at the various concentration.

The ability of the extracts of the leaves of these plants to inhibit the growth of these pathogens confers medicinal value on them. The antimicrobial activity of the leaves of other plants has been document [22,23]. The presence of bioactive substances (alkaloids, flavonoids, phenols, saponins, steroids, tannins and others) in the leaves of these plants confers on the extracts the ability to inhibit the growth of the test pathogenic microorganisms [24,25].There are several reports of the inhibitory effect of turmeric in the form of extract against several bacteria. Moderate to good antimicrobial properties shown in several other studies validates the result of this study. The potentials for developing antimicrobials from plants seems rewarding as it will lead to the development of a phytomedicine against microbes.

CONCLUSION

Data from this study strongly suggest that the methanol extract of turmeric and neem leaves exhibits antibacterial activity against Escherichia coli, Salmonella typhi and Shigella dysenteriae. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized especially those related to control of antibiotic resistant microbes.

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