ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *Murraya koenigii* AND *Telfairia occidentalis* SYNERGY

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ABSTRACT

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The antibacterial activity of aqueous and ethanolic leaf extracts of Murraya koenigii and Telfairia occidentalis synergy were comparatively studied in this research, in order to determine the efficacy of both extracts in the treatment of infections caused by the test bacteria used in this study. Escherichia coli, Bacillus subtillis, Staphylococcus aureus, Klebsiella pneumoniae, and Shigella dysenteriae were used as test bacteria and the antibacterial activity of the extracts was determined by the Kirby-bauer disc diffusion method. Synergistic antibacterial activity of the aqueous extract ranged from 0.0 mm to 20.0±0.03 mm while the synergistic antibacterial activity of the ethanolic extract ranged from 5.0 mm to 25.0±0.05 mm. Larger zones of inhibition were observed in the ethanolic extract than the aqueous extract. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of both extracts were also evaluated. These ranged from 31.25 mg ml^{-1} to 250.00 mg ml^{-1} and 250.00 mg ml^{-1} to 500 mg ml^{-1} respectively for the aqueous extract, while the ethanolic extract had MIC and MBC values ranging from 31.25 mg ml^{-1} to 62.50 mg ml^{-1} and 125.00 mg ml^{-1} to 500.00 mg ml^{-1} respectively. The ethanolic extract was found to have lower MIC and MBC values than the aqueous extract. Their antibacterial activity compared well with the standard antibiotics used as positive control. The phytochemical analysis of the extract carried out revealed the presence of phytochemicals, which conferred antibacterial property on the plants. The phytochemicals were observed to be more prominent in the ethanolic extract than in the aqueous extract. The ethanolic extract therefore, was considered more effective in the treatment of infections caused by the test bacteria than the aqueous extract.

Keywords: *Phytochemicals, Test bacteria, Murraya koenigii, Telfairia occidentalis, Infections.*

INTRODUCTION

In the last two decades, antibiotic resistance has been an emerging problem worldwide (Walsh, 2000; Cohen, 2002). This has led to the search for new, safe and effective antimicrobial agents from alternative natural resources like plant products. Microbial infections constitute a major public health problem in developing countries (Adwan *et al*., 2010) where the high cost of antibiotics makes them unaffordable to the majority of the population. Despite the impressive scientific progress in vaccination and chemotherapy, infectious diseases remain a serious public health issue (Ahluwalia and Sharma, 2007; Poole, 2005). Also, the shortcomings of the drugs available today and the scarcity of novel antibiotics propel the discovery of new chemotherapeutic agents from medicinal plants (Ates and Erdogru, 2003; Gautierrez *et al*., 2008; Brull and Coote, 1999).

Medicinal plants contain biologically active chemical substances such as saponins, tannins, essential oil flavonoids, alkaloids and other chemicals (Sofowora, 1996) which have curative properties (Kayode and Kayode, 2011). Medicinal plants could be the best source to obtain a variety of drugs, therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al.,* 2000; Igoli *et al.,* 2005).

Telfairia occidentalis, commonly called fluted pumpkin has been reported by many investigators, as having many medicinal uses. In Nigeria, the herbal preparation of the plant has been employed in the treatment of anaemia, chronic fatigue and diabetes (Alada, 2000; Dina *et al*., 2006; Kayode and Kayode, 2011). The leaves contain essential oils and vitamins, root contains cucubitacine, ses-quiterpene and lactones (Iwu, 1983). The young leaves sliced and mixed with coconut water and salt are stored in a bottle and used for the treatment of convulsion in ethno medicine (Gbile, 1986). The leaf extract is useful in the management of cholesterolemia, liver problems and impaired defense immune systems (Eseyin *et al*., 2005; Oboh *et al*., 2006). A study has shown that the ethanol root extract of *T. occidentalis* possess antiplasmodial potential (Okonko *et al*., 2007) and inhibitory effects on some enterobacteriacae (Odoemena and Onyeneke, 1998) while Oluwole *et al*. (2003), reported *T. occidentalis* anti-inflammatory activities (Kayode and Kayode, 2011

Murraya koenigii commonly called curry leaf, belongs to the family *Rutaceae,* it is found throughout India and it is cultivated for its aromatic leaves. The leaves are pinnate, with 11-21cm broad and flowers are small, white with pleasant fragrance. The leaves are used extensively as a flavouring agent curries and chutneys (Gopalan *et al*., 1984). The green leaves were chewed raw for the cure of dysentery (Gopalan *et al*., 1984), while the leaf paste were used eternally to treat bites of poisonous animals (Kesari *et al*., 2005), bruises and eruption (Kumar *et al*., 1999). The plant had reported to possess positive inotropic effect (Rahman and Gray, 2005), antidiabetic, cholesterol reducing property, antibacterial and microbiological activity (Manfred *et al*., 1985), antiulcer activity (Xie *et al*., 2006), antioxidative property and cytotoxic activity (Shah and Juvekar, 2006; Ruby *et al*., 1995)). Antibacterial activities of extracts of different plants against various microorganisms have been reported by many scientists (Sagdic and Ozcar, 2003; Shan *et al*., 2007; Gautierrez *et al*., 2008). Currently, researchers have focused

on the synergistic effects of various plant extracts (Dawoud *et al*., 2013), with little or no comparative analysis, hence the interest of the authors in this research, with the aim of comparatively studying the aqueous and ethanolic leaf extracts of *Murraya koenigii* and *Telfairia occidentalis* synergy.

MATERIALS AND METHODS

Collection of plant materials

Fresh *M. koenigii* and *T. occidentalis* leaves were purchased from New Benin market in Benin City, Edo State, Nigeria and identified in Department of Plant Biology and Biotechnology of the University of Benin, Benin City. The leaves were air dried, grinded and made into a fine powder using laboratory blender. The ground leaf was kept in a sterile air-tight container to avoid contamination.

Preparation of aqueous and ethanolic extracts

Fifty grammes each of dried pulverized leaf powder was dissolved in 500 ml each of distilled water (for aqueous extract) and ethanol (for ethanolic extract) for 24 hours and centrifuged at 3000 rpm to enable paper diffusion of the active ingredients into the extraction medium. Filtration was later carried out using Whatman's (No. II) filter paper and the filtrate was evaporated to dryness using steam water bath at 100 °C. The extracts were now stored at 4 °C in a refrigerator. Combination (synergy) of both extracts, ratio 1:1 was used in the synergistic assessment.

Collection of test bacteria

The test bacteria were collected from the Department of Medical Microbiology, University of Benin Teaching Hospital, (UBTH), Benin City, Nigeria. Their identity was confirmed using standard biochemical tests as prescribed by Jolt *et al*. (1994) and Cheesbrough, (2006). The test bacteria were maintained on nutrient agar slants at 4 °C.

Description of research bacteria

The test organisms: *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis* and *Shigella dysenteriae*, have been previously described (Prescott *et al*., 2005; Akinnibosun *et al*., 2008a, 2008b).

Phytochemical screening of the extracts

Phytochemical screening of the extracts for flavonoids, tanins, glycosides, reducing sugars, terpenoids, saponins, anthraquinones, alkaloids and steroids was carried out according to methods described by Odebiyi and Sofowora, (1978) and Trease and Evans, (1989).

Determination of antimicrobial activity

The inocula were prepared by enriching the test organisms in nutrient broth and in incubating them at 37 ^{o}C for 24 hours. Antimicrobial activity of the extracts was evaluated against the test organisms using the disc diffusion method (Newman *et al*., 2003). Nutrient agar plates were seeded with the suspension (diluted cultures) of the test bacteria. Sterilized Whatman (No.I) filter paper was used to prepare the disc (6 mm) and impregnated with the different concentration of the extracts (500 mg ml^{-1}, 250 mg ml^{-1}, 125 mg ml^{-1}, 62.5 mg ml^{-1}, 31.25 mg ml^{-1}), dried and placed aseptically on seeded plates with the help of sterile forceps. The discs were spaced to prevent overlapping of zones of inhibition. The plates were incubated at 37 ^{o}C for 24 hours. The resulting zones of inhibition were measured and recorded. Standard antibiotics were used as positive control and for antibiotic susceptibility testing of the test organisms.

Determination of minimum inhibitory concentration (MIC)

The nutrient agar was prepared and sterilized, then poured into sterile Petri dishes and allowed to solidify. The surface of the medium was inoculated with the test isolates. The disks soaked in different concentrations of the extract were placed on the surface of the seeded nutrient agar. The plates were incubated at 37°C for 24 hours, after which they were examined for the presence of growth inhibition. The MIC was taken as the lowest concentration that prevented the growth of the test microorganisms.

Determination of minimum bactericidal concentration (MBC)

A loopful of the content of each plate in the MIC determination above, which did not show any visible growth after the period of incubation was streaked in freshly prepared nutrient agar to determine their MBC and then incubated at 37°C for 24 hours after which it was observed for visible growth. The lowest concentration of the subculture with no growth was considered as minimum bactericidal concentration.

Antibiotic susceptibility test

Antibiotic susceptibility tests of the isolates was performed according to the recommendations of the National Committee Laboratory Standards (NCCLS), (2002) using tetracycline (20 µg), ampiclox (30 µg), zinnacef (20 µg), amoxicillin (30 µg), rocephin (25 µg), ciprofloxacin (l0 µg), nitrofurantin (20 µg), streptomycin (30 µg), erythromycin (l0 µg), gentamycin (10 µg), septrin (30 µg), chloramphenicol (25 ug), perfloxacin (l0 µg), and ofloxacin (30 µg). The antibiotics resistance was interpreted by diameter of inhibition zones around the antibiotic discs.

RESULTS AND DISCUSSION

Antibacterial activity of aqueous and ethanolic leaf extracts of *M. koenigii* and *T. occidentalis* synergy was compared in this research. Results show the phytochemical components of the synergy of *M. koenigii* and *T.*

occidentalis (Table 1). This indicated the presence of flavonoids, tannins, glycosides, reducing sugars, terpenoids, saponins, alkaloids, anthraquinones and steroids. This agrees with the works of Akande and Yahaya, (2010) and Mohar *et al*. (2011), who isolated the compounds from the leaves. The phytochemicals were prominently expressed in the ethanolic extract synergy. This could possibly be due to the stronger extraction capacity of ethanol than water. Phytochemicals have been found to inhibit bacterial growth and are capable of protecting certain plants against bacterial infections (Oyewole and Abalaka, 2012; Clark, 1981; Gonzala and Matler, 1982).

Table 1: Phytochemical analysis of *M. koenigii* and *T. occidentalis* synergy

Phytochemicals	Aqueous synergy	Ethanolic synergy
Flavonoids	+	++
Tannins	+	++
Glycosides	+	++
Reducing sugars	+	++
Terpenoids	+	++
Saponins	+	++
Anthraquinones	+	++
Alkaloids	+	++
Steroids	+	++

Key: + = Present, ++ = Prominent

The antibacterial activity of the aqueous and ethanolic leaf extract of *M. koenigii,* and *T. occidentalis* synergy is shown in Tables 2 and 3 respectively. The extracts showed varying antimicrobial activity against the test organisms as indicated in the results. The highest concentration of 500 mg ml^{-1} was the most effective is inhibiting the organisms, with *E. coli* (20 ± 0.03 mm) and *S. aureus* (20 ± 0.03 mm) being the most susceptible to aqueous leaf extract of the synergy, while *S. dysenteriae* was the least susceptible to the extract at that concentration. *K. pneumoniae* (25 ± 0.05mm) was the most susceptible to the ethanolic leaf extract of the synergy while *S. dysenteriae* (17 ± 0.20 mm) was the least susceptible to the extract at that concentration. The antibacterial activity was measured as zones of inhibition in millimeters (mm) and it was revealed in this study, to be concentration-dependent. That is, increase in concentration of the extract resulted in increased antimicrobial activity. This agrees with the finding of Kurosaki and Nishi (1933) and Akinnibosun and Akinnibosun (2011) who reported that higher concentration of antimicrobial substances showed appreciably higher growth inhibitions being both bacteriostatic and bacteriocidal. It was observed that the extracts were active against Gram-positive and Gram-negative test organisms. This indicates that the plant extracts contained active principle with broad antibacterial spectrum (Bankole, 1992). Higher zones of inhibition were observed in the ethanolic leaf extract than in the aqueous leaf extract at the same concentrations. This observation may be due firstly, to the fact that the action of the active ingredients in the plants was enhanced by the ethanol used as solvent for extraction and secondly, the ethanol had stronger extraction capacity than the water hence produced more active ingredients (Akinnibosun and Akinnibosun, 2011; Ates and Erdogrul, 2003; Adwan *et al*., 2010). The additive and synergistic effects of phytochemicals enhanced the antibacterial activity of the synergy (Matchimuthu *et al*., 2008; Prekesh *et al*., 2006a and Dawoud *et al*., 2013). Synergistic activity suggests different mode of action of the combining components.

Table 2: Antibacterial activity of aqueous leaf extract of *M. koenigii* and *T. occidentalis* synergy (zone of inhibition in mm)

.	Concentration				
Test organisms	500 mg ml^{-1}	250 mg ml^{-1}	125 mg ml^{-1}	62.5 mg ml^{-1}	31.25 mg ml^{-1}
S. aureus	20.0 ± 0.03	15.0 ± 0.02	13.0 ± 0.20	10.0±0.03	7.0 ± 0.10
K. pneumoniae	15.0 ± 0.02	13.5 ± 0.01	12.0 ± 0.30	7.0 ±0.07	0
B. subtilis	14.0 ± 0.22	12.0 ± 0.10	8.0 ± 0.20	0	0
E. coli	20.0 ± 0.30	17.0 ± 0.20	13.0 ± 0.05	10.0±0.03	5.0±0.01
S. dysenteriae	10.0 ± 0.20	7.0 ± 0.10	0	0	0

The MIC and MBC were evaluated and results shown in tables 4 and 5. The aqueous MIC and MBC values ranged from 31.25 mg/ml to 250.00 mg/ml and 250.00 mg/ml to 500 mg/ml respectively, while the ethanolic extract had MIC and MBC values ranging from 31.25 to 62.50 mg/ml and 125.00 to 500.00 mg/ml respectively. The results revealed that the ethanolic extract had lower MIC and MBC values than the aqueous extract, indicating greater efficacy shown by the ethanolic extract. This was supported by Dawoud *et al*. (2013), Mohar *et al*. (2011) and Akande and Yahaya, (2010).

Results of the antibiotic susceptibility test (Table 6) also showed the effect of standard antibiotics against the test organisms employed in this work. The microorganisms were found to be resistant to many of the standard antibiotics used. The resistant nature of these microorganisms may have been acquired via plasmid transfer or

chromosomally mediated (Walsh, 2000; Cohen, 2002; Coutinho *et al.*, 2010). Drug abuse and indiscriminate misuse of antibiotics among the general population has favoured the emergence of resistant strains. The worldwide escalation in both community and acquired antimicrobial resistant bacteria has threatened the ability to effectively treat patients, emphasizing the need for continued surveillance, more appropriate antimicrobial prescription, prudent infection control and new treatment alternatives (Mulvey, 2014; Rhomberg *et al.*, 2006; Chikere *et al.*, 2008; Okonko *et al*., 2009). The synergy of the plants compared well with the standard antibiotics, as seen in their zones of inhibition. The susceptibility of antibiotic-resistant bacterial strains to the plants synergy suggests that the synergy can be used as alternative in the treatment of diseases caused by these microorganisms (Cohen, 2002).

Table 3: Antibacterial activity of ethanolic leaf extract of *M. koenigii* and *T. occidentalis* synergy (zone of inhibition in mm)

	Concentration				
Test organisms	500 mg ml^{-1}	250 mg ml^{-1}	125 mg ml^{-1}	62.5 mg ml^{-1}	31.25 mg ml^{-1}
S. aureus	23.0 ± 0.01	20 ± 0.02	18.0 ± 0.01	12.0 ± 0.03	6.0 ± 0.00
K. pneumoniae	25.0 ± 0.05	21.0 ± 0.03	17.0 ± 0.10	12.0 ± 0.07	9.0 ± 0.20
B. subtilis	18.0 ± 0.20	13.0 ± 0.10	8.0 ± 0.30	5.0 ± 0.00	6.0 ± 0.00
E. coli	19.0 ± 0.10	15.0 ± 0.20	12.0 ± 0.03	8.0 ± 0.03	6.0 ± 0.00
S. dysenteriae	17.0 ± 0.20	15.0 ± 0.15	13.0 ± 0.20	11.0 ± 0.02	7.0 ± 0.03

Table 4: MIC and MBC of aqueous leaf extract of *M. koenigii* and *T. occidentalis* synergy

Test organisms	MIC (mg ml^{-1})	MBC (mg ml^{-1})
S. aureus	31.25	250.00
K. pneumoniae	62.50	500.00
B. subtilis	125.00	ND
E. coli	31.25	250.00
S. dysenteriae	250.00	ND

ND= Not determined

Table 5: MIC and MBC of ethanolic leaf extract of *M. koenigii* and *T. occidentalis* synergy

Test organisms	MIC (mg ml^{-1})	MBC (mg ml^{-1})
S. aureus	31.25	125.00
K. pneumonia	31.25	125.00
B. subtilis	62.50	500.00
E. coli	31.25	125.00
S. dysenteriae	31.25	250.00

Table 6: Antibiotic susceptibility test (Positive control)

Organisms	CPX	S	SXT	E	PEF	CN	APX	Z	AM	R
S .aureus	13	0.0	0.0	10	15	13	0.0	0.0	0.0	0.0
B. subtilis	14	0.0	0.0	10	0.0	13	0.0	0.0	0.0	0.0
Gram –ve	TE	NB	OF	CPX	C	CN	AM			
E. coli	13.0	10.0	25	13	10	16	10			
K. pneumoniae	10.0	10	15	17	0.0	10	0.0			
S. dysenteriae	11.0	0.0	8.0	0.0	0.0	0.0	0.0			

CPX-Ciprofloxacin; R-Rocephin; S-Streptomycin; TE-tetracycline; SXT-Septrin; NB-Nitrofurantin; E-Erythromycin; C-Chloramphenicol; PEF-Pefloxacin; OF-Ofloxacin' CN-Gentamicin; AM-Amoxicillin; APX-Ampiclox; Z-Zinnacef

CONCLUSION

The synergy of plants is encouraged as microbial tolerance is less likely to develop against substances having more than one type of modes of action. Differential antimicrobial activity of the extract against different bacteria was due to the presence of different active phyto-compounds which made the antibiotic-resistant organisms to be susceptible. It has been observed in this research, that the synergy of the ethanol extract showed greater efficacy against the test bacteria than the aqueous extract and it is therefore recommended to treat the emerging and re-emerging diseases caused by the organisms and prevent multi-drug resistance among microorganisms.

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