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# Assessment of the Phytochemical and Antibacterial Properties of the Synergy of *Murraya Koenigii* and *Telfaria Occidentalis* Ethanolic Leaf Extracts

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**Abstract:** *The phytochemical and antibacterial properties of ethanolic leaf extracts of Murraya koenigii and Telfaria occidentalis and their synergy were determined against five clinical bacterial isolates: Klebsiella pneumoniae, Esherichia coli, Bacillus cereus, Staphylococcus aureus and Shigella dysenteriae. Phytochemical analysis from both plants included saponins, flavonoids, sugar terpenoids, alkaloids, steroids, glycocides and tannins. The antibacterial activity of the extracts was determined by the disc diffusion method. Larger zones of inhibition were observed by M. Koenigii extract than T. occidentalis extract, and large zones of inhibition were observed by their synergy on the organisms than on their separate use. Synergistic antibacterial activity of the extract ranged from 6.0 ± 0.00mm to 25.0 ± 0.05mm, zone of inhibition of M. koenigii extract ranged from 0mm to 18 ± 0.03mm while that of T. occidentalis ranged from 0mm to13 ± 0.30. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the two separate extracts and their synergy were also evaluated. This ranged from 31.25mg/ml to 500mg/ml from M. koenigii, 31.25mg/ml to 250 mg/ml for T. occidentalis and 31.25 to 500mg/ml for their synergy (least MIC to highest MBC). The test organisms were assessed for their antibiotic susceptibility pattern and were observed to be multidrug resistant. The synergy of both plant extracts showed higher zone of inhibition against the test bacteria than the separate extracts and the standard antibiotics. It is therefore, recommended to use the synergy of both samples to treat infections caused by the test organisms.*

**Keywords:** *Telfaria occidentalis, Murraya koenigii, Antibacterial, Phytochemical property, Ethanolic extract.*

## 1. INTRODUCTION

Despite the impressive scientific progress in vaccination and chemotherapy, infectious diseases remain a serious health issue. Following the massive and inappropriate use of antibiotics, bacteria have developed various mechanism of resistance; consequently, infectious diseases remain one of the leading causes of morbidity worldwide (Ahluwalia and Sharma, 2007). 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. Therefore, the discovery of new antimicrobial agents is still relevant nowadays. Among the bacterial resistance mechanisms, efflux of antibiotics plays an important role; In fact it is widely recognized that the expression of efflux pumps encoded by housekeeping genes in bacteria is largely responsible for the phenomenon of intrinsic antibiotic resistance (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).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. At the same time, there is a growing demand among consumers for natural preservative or additives in processed foods (Gautierrez *et al*., 2008). In comparison to chemical or synthetic additives herbal additives from medicinal plants are preferred as these are safer, flavour enhancer and without any side effects (Brull and Coote, 1999).

A medicinal plant is any plant which is one or more of its organs contain substances that can be used for the synthesis of useful drugs (WHO, 1977). Medicinal plants contains biologically active

chemical substances such as saponins,tannins, essential oil flavonoids, alkaloids and other chemical (Sofowora,1996)which have curative properties. These complex chemical substances of different composition are found as secondary plant metabolite in one or more of these plants (Kayode and Kayode, 2011).

According to the World Health Organization, medicinal plants would 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). There are several published reports describing the antimicrobial activity of various crude plant extracts either in single or in combinations (Igoli *et al.*, 2005). It is estimated that there are about 2.5 million species of higher plants and the majority of these have not yet been examined for their pharmacological activities. Herbal extracts are fast becoming popular as natural antimicrobial preservatives or additives (Cox *et al.*, 2010).

The medicinal activities of *Telfaria occidentalis*, commonly called fluted pumpkin has been reported by many investigators. 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, vitamins; root contains cucubitacine, ses-quiterpene, 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). *Telfaria occidentals* is popularly used in soup and folk medicine preparation in the management of various diseases such as diabetics, anaemia and gastrointestinal disorder (Oboh*et al*., 2006). A study has shown that the ethanol root extract of *T. occidentalis* possess antiplasmodial potential (Okonko *et al*., 2007) and an inhibitory effects on some enterobacteriacae (Odoemena and Onyeneke, 1998) while Oluwole*et al*. (2003), reported *Telfaria occidentalis* anti-inflammatory activities (Kayode and Kayode, 2011).

*T. occidentalis* occurs in the forest zone of West and central Africa most frequently in Benin, Nigeria and Cameroon (Kayode and Kayode, 2011). It is a popular vegetable all over Nigeria and has been suggested that it originated in south-east Nigeria and was distributed by the Igbos, who have cultivated this crop since time immemorial (Badifu and Ogunsina, 1991). Studies have also reported the medicinal activities of curry leaf (*Murraya koenigii*).

The plant *Murraya koenigii* 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 chutreys (Gopalan *et al*., 1984). The green leaves were chewed raw for the cure of dysentery (Gopalan *et al*., 1984), while the leave 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). It has been reported to contain acytotoxic coumarin murraya compound and flavonoids which has constantly being screened for anti-tumour activity (Ruby *et al*., 1995). The main constituent reported were alkaloids volatile oil, amino acids, glycocides, proteins, monoterpenoids, cesquiterpenoids. Furthermore, the microbial mutations and appearance of new recombinant pathogenic microorganisms necessitate the continuous assessment of new antimicrobial activities of different medicinal plants. Currently, researchers have focused on the synergistic effects of various plant extracts such as *Glycyrrhizaglabra, Rehumpalmatumand Assiaangustifolia* (Dawoud *et al*., 2013). However, there are no study on the antimicrobial synergy of *Murraya koenigii* and *Telfaria occidentalis* leaves; hence the interest of the researcher in this study.

## 2. MATERIALS AND METHODS

### 2.1. 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, identification was confirmed with appropriate literature

(Hutchinson and Dalziel, 1954; Odugbemi and Akinsulire, 2006).The leaves were air dried, grinded and made into a fine powder using laboratory mortar and pestle. The powdered leave was kept in a sterile air-tight container to avoid contamination.

### 2.2. Preparation of Ethanolic Extract

Fifty grammes each of dried pulverized leaf powder was dissolved in 500ml each of ethano l (95% ethanol) for 24hrs and centrifuged at 3000rpm 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$^{o}$C. The extracts were now stored at 4$^{o}$Cin a refrigerator. Combination of both extracts was used in the synergistic assessment.

### 2.3. 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$^{o}$C.

### 2.4. 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).

### 2.5. Phytochemical Screening of the Extracts

Phytochemical screening of the extracts was carried out according to methods described by Odebiyi and Soforowa, (1978) and Trease and Esians, (1989). The component analysed for were: flavonoids, tanins, glycosides, reducing sugars, terpenoids, saponins, anthraquinones, alkaloids, steroids.

### 2.6. 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 hrs. 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 (6mm) and impregnated with the different concentration of the extracts (500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml 31.25mg/ml), 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 hrs. 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.

### 2.7. 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.

### 2.8. 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 unto 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.

### 2.9. Antibiotic Susceptibility Test

Antibiotic susceptibility tests of the isolates was performed according to the recommendations of the National Committee Laboratory Standards (NCCLS), (2002) using the following antibiotic discs: tetracycline (20µg), ampiclox (30µg), zinnacef (20µg), amoxicillin (30µg), rocephin (25µg), ciprofloxacin (l0µg), nitrofurantin (20µg), streptomycin (30µg), erythromycin (l0µg), gentamycin (I0µg), septrin (30µg), chloramphenicol (25ug), perfloxacin (l0µg), and ofloxacin (30µg). The antibiotics resistance was interpreted by diameter of inhibition zones around the antibiotic discs.

## 3. RESULTS AND DISCUSSION

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). But there are few reports on their synergistic effects, extract on some pathogenic organisms and comparing it with the activity of standard antibiotics.

Table 1 shows the phytochemical components of the extracts of *M. koenigii* and *T. occidentalis*. The results 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. These compounds 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).

**Table1.** *Phytochemical analysis of M. koenigii and T. occidentalis leaf extracts*

| Phytochemicals | *M. koenigii* | *T. occidentalis* |
|---|---|---|
| Flavonoids | + | + |
| Tannins | + | + |
| Glycosides | + | + |
| Reducing sugars | ++ | +++ |
| Terpenoids | + | + |
| Saponins | + | + |
| Anthraquinones | + | ++ |
| Alkaloids | + | + |
| Steroids | ++ | ++ |

*Key:*

*+ = less abundant*

*++ = abundant*

*+++ = highly abundant*

The antibacterial activity of the ethanolic leaf extract of *M. koenigii*, *T. occidentalis* and their synergy is shown in tables 2, 3 and 4 respectively. The extract showed varying antimicrobial activity against the test organisms as indicated in the tables. The highest concentration of 500mg/ml was the most effective is inhibiting the organisms, with *K. pneumonia* (18±0.03mm) being the most susceptible to *M. koenigii* and *S. dysenteriae* (15±0.10mm) was the least susceptible to *M. koenigii*. The antibacterial activity was measured as zones of inhibition in mm and it was shown 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 more growth inhibitions being both bacteriastatic and bacteriocidal.

**Table2.** *Antibacterial activity of M. koenigii leaf extract (zone of inhibition in mm)*

| | **Concentrations** | | | | |
|---|---|---|---|---|---|
| Test organisms | 500mg/ml | 250mg/ml | 125mg/ml | 62.5mg/ml | 31.25mg/ml |
| *S. aureus* | 17.0 ±0.02 | 15.0 ±0.05 | 12.5±0.01 | 15.0±0.06 | 7±0.04 |
| *K. pneumoniae* | 18.0 ±0.03 | 14.0 ±0.01 | 10.0 ±1.10 | 8.0±0.07 | 5.0 ±0.02 |
| *B. subtilis* | 7.0±0.10 | 0 | 0 | 0 | 0 |
| *E. coli* | 17.0±0.30 | 14.0±1.10 | 11.0±0.09 | 0 | 0 |
| *S. dysenteriae* | 15.0 ±0.10 | 12.0 ±0.25 | 11.0±0.30 | 10.0 ±0.02 | 6.0 ±0.01 |

**Table3.** *Antibacterial activity of T. occidentalisleaf extract (zone of inhibition in mm)*

| | | **Concentrations** | | | |
|---|---|---|---|---|---|
| Test organisms | 500mg/ml | 250mg/ml | 125mg/ml | 62.5mg/ml | 31.25mg/ml |
| *S. aureus* | 10.0 ±0.01 | 8.0 ±0.03 | 7.0 ±0.02 | 5.0±0.05 | 0 |
| *K. pneumoniae* | 12.0 ±0.03 | 8.0 ±0.01 | 7.0 ±0.10 | 5.0±0.04 | 0 |
| *B. subtilis* | 13.0 ±0.30 | 12.0 ±0.10 | 11.0 ±0.21 | 7.0±0.40 | 5.0±0.10 |
| *E. coli* | 12.0±0.10 | 10.0 ±0.10 | 9.0±0.02 | 5.0 ±0.01 | 0 |
| *S. dysenteriae* | 9.0 ±0.10 | 8.0 ±0.25 | 6.0 ±0.30 | 0 | 0 |

**Table4.** *Antibacterial activity of the synergy of M. koenigii and T. occidentalis leaf extract (zone of inhibition in mm).*

| | | **Concentration** | | | |
|---|---|---|---|---|---|
| Test organisms | 500mg/ml | 250mg/ml | 125mg/ml | 62.5mg/ml | 31.25mg/ml |
| *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 |

The antibacterial activity of T. occidetalis was less than that of *M. koenigii*, but it was also concentration dependent. At 500mg/ml, 12 ± 0.03mm inhibition zone was recorded against *K. pneumoniae*, from ethanolic leaf extract of *T. occidentalis*, compared to 18 ± 0.03mm against *K. pneumoniae* observed by *M. koenigii* leaf extract. Moreso, it was observed that the extracts were active against Gram positive and Gram negative organisms (Tables 2, 3 and 4). This indicates that the plant extracts contained active principle with broad antibacterial spectrum (Bankole, 1992).

The synergy of *M. koenigii* and *T. occidentalis* leaf extracts gave higher zones of inhibition (Table 4) neither *M. koenigii* extract nor *T. occidentalis* extract could give. This showed that both leaf extracts acted synergistically against the test isolates (Ates and Erdogrul, 2003; Adwan *et al.*, 2010). At 500mg/ml, the synergy extract gave a zone of inhibition of 25 ± 0.05mm against K. preumoniae, compared to 18 ± 0.03mm and 12 ± 0.03 recorded for *K. pneumoniae* by *M. koenigii* extract and *T. occidentalis* extract respectively. The results of this synergy is supported by Prekesh *et al.*, 2006a and Dawoud *et al.*, 2013.The additive and synergistic effects of phytochemicals enhanced the antibacterial effect of the synergy extract (combined) extract (Matchimuthu *et al.*, 2008) According to Cain *et al*. (2003), synergistic activity suggest different mode of action of the combining components.

The MIC and MBC of individual plant extract and their synergy were evaluated. The extract synergy (showed lower MIC and MBC values against the test organisms (Tables 5, 6 and 7). This observation was supported by Dawoud *et al.*, 2013). The MIC and MBC of *M. koenigii* (Table 5) on *K. pneumoniae* were 31.25mg/ml and 250mg/ml respectively, the MIC and MBC of *T. occidentalis* (Table 6) on *K. pneumoniae* were 62.5mg/ml and 250mg/ml. The other organisms had similar results and this further proved that *M. koenigii* was more potent than *T. occidentalis* agrees with those of Mohar *et al.*, 2011 and Akande and Yahaya, 2010.

**Table5.** *MIC and MBC of M. koenigii leaf extract*

| Test organisms | MIC(mg/ml) | MBC(mg/ml) |
|---|---|---|
| *S. aureus* | 31.25 | 125.00 |
| *K. pneumonia* | 31.25 | 250.00 |
| *B. subtilis* | 500.00 | ND |
| *E. coli* | 125.00 | 500.00 |
| *S. dysenteriae* | 31.25 | 250.00 |

*Key*

*ND= Not detected*

**Table6.** *MIC and MBC ofT. occidentalis leaf extract*

| Test organisms | MIC(mg/ml) | MBC (mg/ml) |
|---|---|---|
| *S. aureus* | 62.50 | 250.00 |
| *K. pneumonia* | 62.50 | 250.00 |
| *B. subtilis* | 62.50 | ND |
| *E. coli* | 31.25 | 125.00 |
| *S. dysenteriae* | 62.50 | 250.00 |

*Key*

*ND= Not determined*

**Table7.** *MIC and MBC of the synergy of M. koenigii and T. occidentalis leaf extracts*

| Test organisms | MIC(mg/ml) | MBC (mg/ml) |
|---|---|---|
| *S. aureus* | 31.25 | 125.00 |
| *K. pneumoniae* | 31.25 | 125.00 |
| *B. subtilis* | 62.50 | 500.00 |
| *E. coli* | 31.25 | 125.00 |
| *S. dysenteriae* | 31.25 | 250.00 |

Antibiotic susceptibility test was carried out on all the test bacteria employed in this work and the results have been displayed in table 8. The microorganisms were found to be resistant to many of the standard antibiotic 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. Multidrug resistance was observed for most of the test bacteria as they were resistant to more than one drug (Wasfy *et al.*, 2000; Akomie and Akpan, 2013). The worldwide escalation in both community and acquired antimicrobial resistant bacteria is threaten 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.*, 2009a). In comparison to the extracts, the synergy of both extracts was more potent than the standard antibiotics, since all the test organisms had higher zones of inhibition than the commonly used standard antibiotics. The susceptibility of antibiotic resistant bacterial strains to the plant extract is quite interesting and these plant extract can be used as an alternative in the treatment of diseases caused by these microorganisms (Cohen, 2002).

**Table8.** *Antibiotic susceptibility testing (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 |
| *Bacillus 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 | | | |

*Key:*

*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*

## 4. CONCLUSION

This study has shown that combinations of extracts demonstrated synergistic and additive effects on microorganisms. This synergy is performed 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 is therefore recommended that the synergistic use of medicinal plant extract be encouraged to prevent drug resistance and treat the emerging and re-emerging diseases caused by the organisms.

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