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In vitro antimicrobial evaluation of methanol extract of *Triumfetta rhomboidea* leaves against some clinical bacterial isolates

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ABSTRACT

The antimicrobial activity of methanol extract of *Triumfetta rhomboidea* leaves was evaluated against *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae*. Agar diffusion and agar dilution techniques were employed for the antimicrobial sensitivity and interaction studies. Phytochemical analysis of the crude extract was also carried out using standard procedures. The results obtained showed that the crude extract exhibits a substantial antimicrobial activity against *Klebsiella pneumoniae* (which recorded the greatest sensitivity), *Salmonella typhi* but not against *Staphylococcus aureus*. The result of the interactive studies between the crude extract and standard antibiotics showed relationship ranging from antagonism to synergy. Phytochemical analysis revealed the presence of terpenes, flavonoids and other phenolics and resins in the crude extract. Thus, this study shows that *Triumfetta rhomboidea* possesses promising antimicrobial activity especially against *Klebsiella pneumoniae*.

Keywords: *Triumfetta rhomboidea*, Antimicrobial activity, Microorganisms, Susceptibility, Resistance, Interaction.

INTRODUCTION

Many infective diseases are treated with chemotherapeutic agent, such as antibiotics, that selectively inhibit or kill the pathogen with little or no effect on the host. Ideally, antimicrobial agents disrupt microbial processes or structures that differ from those of the host. They may damage pathogen by hampering cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function or blocking metabolic pathways through the inhibition of key enzymes (Prescott et al., 2008). The conventional antimicrobial agents face a lot of resistant problems in recent times as microorganisms are loosing sensitivity to some of these drugs. Recently, concern has been expressed about the rising prevalence of pathogenic microorganisms which are resistant to the old generation, and to the newer or modern antibiotics that have been produced in the last decades (Cohen, 1992; Nascimento et al.,

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2000; Okesola and Makanjuola, 2009). Also the problem posed by the high cost; adulteration and increasing toxic side effects of these synthetic drugs coupled with their relative inadequacies in disease treatment especially in the developing countries portend serious limitations (Shariff, 2001). Consequently, the continuous acute need of novel and effective antibiotics for antimicrobial chemotherapy is clearly evident.

Phytochemicals derived from plants have shown great promise in the treatment of intractable infectious diseases (Nascimento et al., 2000; Rios and Recio, 2005) with lesser side effects compared to the synthetic drug agent (Iwu et al., 1999). In traditional herbal practice, indigenous medicinal plants have been employed in the treatment of several important infections (Fennell et al., 2004, Taylor et al., 2001). Also, plant-based extractives have equally served as source of lead compounds for further developments of future antimicrobial agents. Therefore, evaluation of a candidate medicinal plant may lead to identification of very effective herbal antimicrobial treatments or provide leads for further development into novel antimicrobial agents.

Triumfetta rhomboidea Jacq (Family: Tiliaceae) is an under shrub, widely distributed in tropical and subtropical India, Ceylon, Malay Peninsula, China, Africa and in America (Sivakumar et al., 2010). It is a perennial herb having important roles in ancient therapy. Various Parts of the plant used therapeutically are fruit, flower, leaves, bark and roots. The root is tonic styptic, galactogogue, aphrodisiac, cooling, useful in dysentery and as diuretic. Pounded roots are given in the treatment of intestinal ulcer. Leaves, flowers and fruit are mucilaginous demulcent, astringent, and also used in gonorrhoea and against leprosy (Barnes, 2002; Chattergee and Chandra, 1992; Chopra et al., 1986; Mukharjee, 2002).

Presently, there is very limited data on the scientific basis for antimicrobial utility of the leaf extract from *Triumfetta rhomboidea*. This study therefore, was carried out to evaluate the antimicrobial activity of its methanol extract against certain bacterial isolates.

MATERIALS AND METHODS

Collection of plant material

The leaves of *Triumfetta rhomboidea* were collected from Nsukka Enugu state. The plant was authenticated by Mr. H. Ozioko of the Center of Bioresearch (BDCP) Nsukka. A voucher specimen (UPH 570) was deposited in the herbarium of the Department of Botany, University of Port Harcourt, Port Harcourt.

Test microorganisms

The organisms used for this study included clinical isolates of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Salmonella typhi* obtained from the Pharmaceutical Microbiology Laboratory, University of Nigeria Nsukka. Identification of bacterial isolates was performed according to standard bacteriological techniques previously established (Baron and Finegold, 1990; Cowan et al., 1993).

Drugs and reagents

The following materials were used in the study, methanol (Fluka, Germany), nutrient agar (Oxoid), discs antibiotics: amoxicillin 25µg, augmentin 30µg, nitrofurantoin 300µg, gentamicin 10µg, nalidixic acid 30µg, ofloxacin 30µg, cotrimoxazole 25µg, tetracycline 30µg (Oxoid).

Antimicrobial activity of the crude extract of *Triumfetta rhomboidea* on the test bacteria

The antimicrobial sensitivity test was conducted using a modification of the agar disc diffusion technique (Okore, 2005). The extract was diluted in DMSO to obtain 200 mg/ml and 100 mg/ml of the extract. Thereafter, 0.02 ml amounts each were pipetted and spotted on a pre-made sterile filter paper discs. Nutrient agar in Petri- dish were prepared and dried in incubator for 30 minutes at 37 ^{o}C. The under of the plates were divided into portions. A standardized suspension of an 18 hour growing test

organism was spread on the surface of the plates using a sterile cotton swab. The plates were rotated while swabbing to ensure even application. Thereafter, the antibiotics discs were then aseptically applied on the surface of the agar plates. Control discs containing DMSO alone was employed. This was done for the 10 isolates of each test bacteria. The plates were incubated at 37 ^{o}C for 24 hour and the observed growth inhibition zones were recorded and mean calculated.

Antimicrobial sensitivity and minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) of *Triumfetta rhomboidea* extract against the different isolates employed in the study were determined using the agar diffusion technique (Okore, 2005). Briefly, serial concentrations of the methanol extract (12.5, 25, 50 and 100 mg/ml) were prepared in DMSO. This solution was introduced into equidistant wells of 6 mm bored on the surface of nutrient agar seeded with the laboratory isolates of test organisms. Blank DMSO were also placed in separate wells and served as controls. The plates were incubated at 37 °C for 24 h after a pre-diffusion period of 30 min at room temperature. Inhibition zone diameter were measured and recorded and MIC determined graphically.

Interactions of the crude extract with the antibiotics

A modified agar dilution and diffusion procedure was employed. Briefly, a 100 mg/ml in DMSO of the plant extract was prepared and mixed with 20 ml of molten nutrient agar. This was poured in an agar plate and allowed to solidify to form the base antimicrobial agar. The standardized test bacteria already prepared was streaked on the surface of this plant extract agar base using a swab stick. Antibiotic discs were also placed on the plate. This evaluation was done for the *Klebsiella pneumoniae* and *Salmonella typhi* isolates. Two controls, one containing only the base agar with the plant extract and streaked microorganisms, and the other containing the base agar (without the plant extract) with the streaked microorganisms and over-layed with antibiotics discs were also employed in the study. All these plates were incubated at 37 ^{o}C for 18 – 24 hours. Growth in control A is a confirmation that the organism is not inhibited in the presence of the plant extract. The formation of zone of inhibition around the antibiotic discs in control B was employed in determining the activities of the disc antibiotics (Okore, 2005).

Data treatment

All experiments were done in replicates and mean of triplicate determinations established. The standard error of mean (SEM) were determined.

RESULTS

One (1) isolate each of *S. aureus* and *S. typhi* were sensitive at 200 mg/ml. However, for *K. pneumoniae*, six (6) of its isolates were slightly sensitive to the crude extract (100 mg/ml) (Table 1). Result of the minimum inhibitory concentration (MIC) recorded for some of the isolates are displayed in Figure 1. The MICs were shown to be summarily less than or equal to 2 mg/ml except for isolate 1 where it was not possible to establish susceptibility to the extract at other concentrations below 100 mg/ml . Table 2 shows the basal susceptibility profile of *K. pneumoniae* towards commonly used standard antibiotics. All the isolates of *K. pneumoniae* showed susceptibility to one or more of the antibiotics except to amoxicillin, augmentin, and cotrimoxazole respectively. When *T. rhomboideae* was combined with the standard antibiotics against *K. pneumoniae* isolates, the effect ranged from antagonism to slight synergy (Table 3). The pattern of interaction was largely antimicrobial type-depended with variant behaviours displayed by individual antibiotics/crude extract cocktails. Phytochemical studies showed that the methanol extract contained terpenoids and flavonoids, carbohydrates, and other phenolics, but do not show positive for alkaloids, glycosides, and steroids (Table 4).

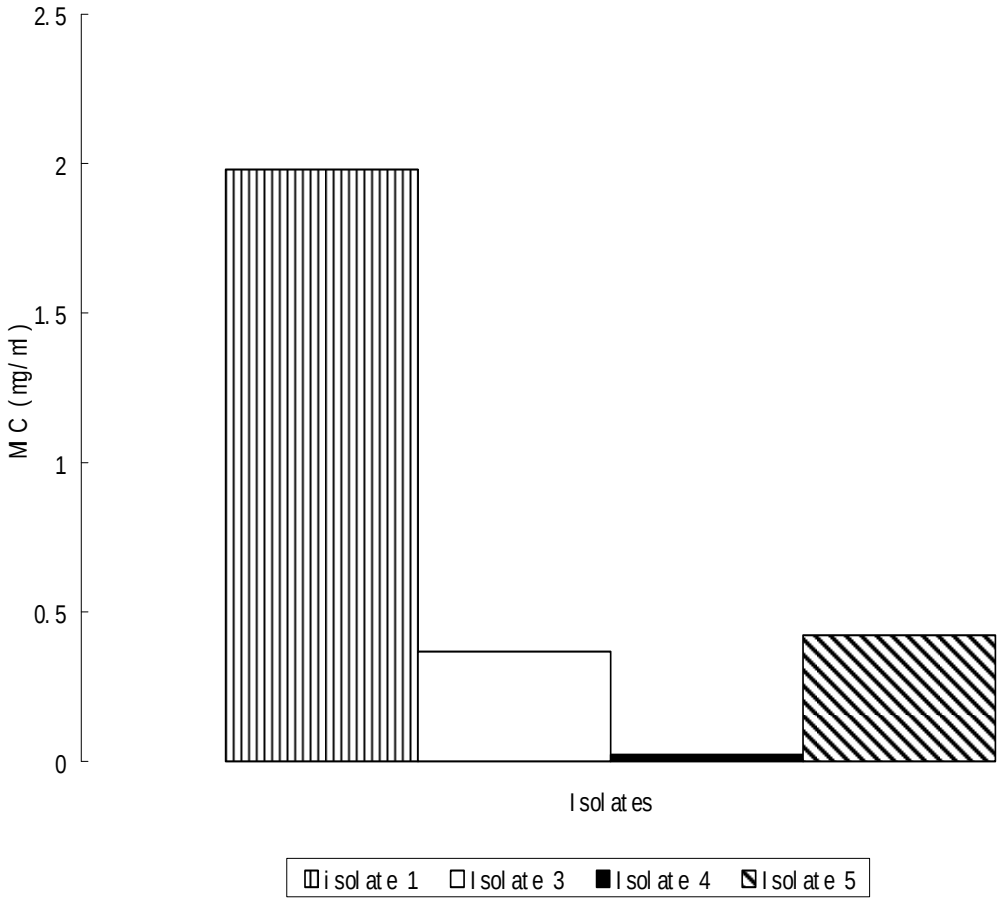

Figure 1: Minimum inhibitory concentration (MIC) of *T. rhomboidea* (TR) extract against *K. pneumoniae* isolates.

Table 1: Mean inhibition zone diameter (IZD) for crude extract against *Klebsiella pneumoniae.*

Isolates	**Inhibition zone diameter (mm)**
1	2 ± 0.00
2	7.5 ± 0.70
3	8 ± 0.00
4	9 ± 1.41
5	9 ± 1.41
6 - 10	0 ± 0.00

The extract was tested at 100 mg/ml; Tabulated values are Mean ± SEM.

Table 2: Mean inhibition zone diameter (IZD) for antibiotics against *Klebsiella pneumoniae.*

Antibiotics	**Inhibition zone diameter (mm) / isolates**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Nitrofurantoin 300 µg	11±0.00	21±1.41	24±0.00	22±1.41	21.5±0.70	20±0.00	15.5±0.70	20.5±0.70	21.5±2.12	-
Gentamycin 10 µg	17±2.82	17.5±0.70	16.5±1.76	16.5±0.70	16.5±0.70		13±1.41	16.5±0.70	17±1.41	16±1.41
Nalidixic acid 30 µg	22±0.00	28±0.00	20±0.00	23.5±0.70	31±1.41	+	16±0.00	+	24±1.41	20.5±0.70
Ofloxacin 30 µg	34.5±0.70	30±0.00	32±0.00	31±1.41	31.5±0.70	+	31±1.41	20.5±0.70	32.5±2.12	31±1.41
Tetracycline 30 µg	8.5±0.70	15.5±0.70	19±1.41	10.5±0.70	14.5±0.70	+	15±0.00	8±0.00	15±0.00	-

Tabulated values are Mean ± SEM.

Table 3: Mean inhibition zone diameter (IZD) for combined effect of crude extract and antibiotics against *Klebsiella pneumoniae.*

Antibiotics	**Inhibition zone diameter (mm) / isolates**					
	1	**3**	**4**	**5**	**6**	**7 – 10**
Nitrofurantoin 300 µg	11±0.00	21.5±0.70	22.5±0.2.12	16±0.00	-	0±00
Gentamycin 10 µg	10.5±0.70	-	7±0.70	7.5±0.70	7.5±0.70	0±00
Nalidixic acid 30 mg	16±1.41	-	22±0.00	15±1.41	15.5±0.70	0±00
Ofloxacin 30 µg	37±1.76	33.5±2.12	43±2.82	31±1.41	17±1.41	0±00
Tetracycline 30 µg	-	-	15±0.00	12.5±0.70	-	0±00

Tabulated values are Mean ± SEM.

Table 4: Phytochemical test of methanol extract of *T. rhomboideae.*

Compound	Results
Alkaloids	-
Saponins	-
Steroids	-
Terpenes	++
Phenolics	++
Flavonoids	++
Carbohydrates	+++

-, absent; +, presence with increasing amount

DISCUSSION

The search for novel antimicrobial agents is usually driven by the need to provide newer generation of antibiotics to combat the increasingly growing threats posed by microorganisms and infections worldwide in the face of rapid decline of efficacy of older antimicrobial agents (Makanjuola, 2009). This growing antimicrobial drug-resistant evolutionary concern is also partly responsible for the adopted approach of combining two or more antimicrobial agents possessing average or a below-average efficacy as a therapeutic strategy to stem the unfavourable trend (Aguwa, 1996). Research into newer antibiotics has involved several approaches including the evaluation of plant parts for possible antibiotics having previously yielded several useful antimicrobial agents (Cowan, 1999; Iwu et al., 1999). Sometimes, the active principles isolated from the medicinal plant occupy a pivotal position as lead molecules for the synthesis of more active and useful agents by the pharmaceutical chemist (Cowan, 1999).

Our initial evaluation of the methanol extract of *T. rhomboideae* has shown that the crude extract was moderately inhibitory against clinical isolates of *K. pneumoniae* (Table 1). This recorded activity was equally observed to be dose-dependent as MIC values for some of the isolates were equal to or less than 2 mg/ml (Figure 1). Crude extracts are usually complex mixtures of chemical/biological constituents whose overall activity is a result of possible interaction of antagonistic, indifferent, and synergistic constituents (Odimegwu et al., 2011). Further purification of crude extracts could possibly enhance the activity of individual constituents. On the other hand, given the potential benefit or non-benefit of administering combinations of two different antibiotics intentionally or unintentionally, we had the isolates profiled against the standard antibiotics (Table 2), and consequently proceeded to create interactive effect with the crude extract against the isolates. The result was a blend of relationships ranging from antagonism, indifference, and synergy (Table 3). It is noteworthy, that the activities of the quinolone antibiotics (Ofloxacin, Norfloxacin, Nalidixic acid) with the crude extract were largely indifferent to a first approximation, except for Nalidixic acid that displayed obvious generalised antagonism. However, a surprise candidate (isolate number 6) showing previously total resistances to the crude extract, nalidixic acid, and ofloxacin, gentamicin, and tetracycline respectively (Tables 2 and 3) suddenly became susceptible thereby defining an overt synergistic relationship. It is not known why this is so. A plausible explanation may be to assume that an inhibitory complex (possibly genetically disposed to by the isolate 6) possibly formed between the antibiotics and crude extract could be responsible for this. In the case of the aminoglycoside gentamicin, the outcome was consistent antagonism with some of the

isolates recording zero IZDs. Evaluation of antimicrobial combinations so as to define the nature of existing relationships apart from furnishing information on any possible modification of a potential plant-based antimicrobial lead molecule, could be of tremendous relevance in defining the pattern of biological effect of plant-based antimicrobial extracts and synthetic antibiotics when they are taken or administered concurrently especially in this era of herbal medicinal preparations boom. Another close observation of Table 3 could show that tetracycline recorded an overall antagonism with the crude extract. A bacteriostatic agent such as tetracycline, in the presence of multi-biological cocktail of the crude extract may possibly encounter biological barrier-induced attenuation.

So far, comparative analysis would show that while the widely utilised antibiotics cotrimoxazole, amoxicillin, and augmentin were totally ineffective against any single isolate, the crude extract recorded clear activity against six (6) of the ten (10) bacteria isolates. This outcome constitutes an encouraging boost for the crude extract, thus paving way for further studies on the plant. Moreover, the recorded antimicrobial activities against *K. pneumoniae* would equally demonstrate a good success-rating for the crude extract because of the evident crucial position occupied by the species as a biological compartment and an active disseminator of antimicrobial resistance determinants across various bacterial species and genera (Amita et al., 2003; Khadri et al., 2007). Therefore, a plant-based antimicrobial with promising activity against *Klebsiella spp* could translate into a useful therapeutic missile with an ultimate far reaching benefit to the antimicrobial world.

Finally, phytochemical analysis of the methanol extract could show the presence of terpenoids, phenolics, and flavonoids. It is therefore expected that one or all of these phytoconstituents could be responsible for the observed antimicrobial attributes.

Conclusion

At present, quite limited work has been carried on the antimicrobial potential of extracts of *Triumfetta rhomboideae*. It is expected that the useful outcome of this present study would consequently serve as a useful guide and basis for further evaluation of the active antimicrobial principles of the plant.

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