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Phytochemical Evaluation and Combination Effect of the Crude Methanol Extract of *Ochna rhizomatosa* with Standard drugs

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ABSTRACT

In our search for plants with antimicrobial activities, *Ochna rhizomatosa* a plant used in the treatment of typhoid fever in Nigerian folk medicine was investigated. The phytochemical investigation of the extract shows the presence of alkaloids, flavonoids, fatty acid and triterpenes. The results of antimicrobial assay on *Samonella typhi*, two resistant bacteria (Methicillin Resistance *Staphyloccocus aureous* (MRSA); Vancomycin Resistance *Staphyloccocus aureous* (VRSA)) and *Candida albican* expressed in terms of mean diameter of zones of inhibition (mm), shows that the leaves extract (LOS1) inhibited MRSA with a clear zone of 28.00 ± 0.20 mm. Whereas the stem bark (SOS1) shows a zone of 18.00 ± 0.50 mm at 100 mg/ml respectively. The combination of LOS1(100 mg/ml) with Ciprofloxacin (5 µg/ml), led to enhancement of the measured zone of inhibition (46.00± 0.25 mm). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results of the synergistic evaluation of LOS1 with Ciprofloxacin, reveals an enhancement of the inhibition (MRSA, MIC = 3.13 mg/ml) and bactericidal properties (MRSA, MIC = 3.13 mg/ml, MBC = 6.25 mg/ml). These showed that the standard drugs in combination with the extract from *Ochna rhimatoza* are potentials for the search for treatment for resistant bacteria.

Keywords: *Ochna rhizomatosa, Flavonoid, alkaloids,* Combination therapy, MRSA, VRSA.

INTRODUCTION

Natural products of plants with therapeutic ability, either as standard extracts or compounds, provide opportunities for development of new drug lead candidate due to the unrivalled availability of chemical variety (Cosa *et al*., 2006). Plants that have value medicinally have been known to gain their therapeutic power from materials which are active biologically, which could exist on some part of the plants. These active phytochemicals could include terpene, bioflavonoid, flavonoids, xanthenes, benzophenones, saponins, tannins, oxalate, alkaloid and so on (Duraipandujam *et al*., 2006).

Antibiotics have been widely used in the treatment of infections from bacterial origin and this has led to the rising and spread/stretch of resistant strains to drugs. Combining antibacterial agents and extract from plant was investigated in other works (Nascimento *et al*., 2000; Abu-Shanab *et al*., 2005). Few works have found out that an improvement can be made in the potentials for antimicrobials by their combination with active plant extract whose activity are against diverse/various pathogens which may include the following, *P aeruginosa, E. coil, S. aureus,* Vancomycin Resistant *Enterococci* (VRE) and so on (Chang *et al*., 2007; Horiuchi *et al.,* 2007; Adwan *et al*., 2008). *Ochna rhizomatosa* is a low shrub that may grow to a height of about 6 m high, mostly found in Savannah forest region of Mail to Nigeria (Burkill, 19985). *Ochna rhizomatosa* is used locally in the treatment of typhoid fever (Oliveria *et al*., 2002). In the present studies, we report our findings on the evaluation of the combination therapy between the alkaloid and flavonoid rich fraction of *Ochna rhizomatosa,* with Fluconazole and ciprofloxacin standard drugs.

MATERIALS AND METHODS

Plant collection

The leaves and stem bark of *Ochna rhizomatosa* was collected in the month of May, 2016 from Zaria, Kaduna State. The plant materials were properly authenticated by the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Voucher number 3421OC was assigned and deposited in the unit for referencing. The samples were air-dried under shade at room temperature for two weeks, afterward pulverized into coarse particles using mortar and pestle, then stored in dry container until needed for extraction.

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Microwave Assisted Extraction

The dried powered leaves (257.5 g) and stem bark (415.2 g) sample of *Ochna rhizomatosa*, were transferred to two different containers (1 litre capacity). 50 and 100 mL of H_2O were transferred to the measured samples each, to make the samples moist. The samples were then placed inside a domestic microwave oven (900 W) and microwaved at 3 mins pulses (x5) with intermittent cooling. The cooling was done at exactly room temperature, then poured into a conical flask (5 litres) containing water and methanol (analytical grade) in the ratio 1:4, and then kept for 30 minutes before filtration.

Phytochemical assay

The extract was checked if the following were present; flavonoid, alkaloid, triterpene and steroids in accordance with the methods described by Trease and Evans (1996) and Sofowora (2008).

Antimicrobial Assay

Antifungal and antibacterial activities of the alkaloid and flavonoid containing parts of the leaves and bark of stem were checked against three bacteria; *Methicillin Resistant Staphylococcus aureus (MRSA), Salmonella typhi, Vancomycin Resistant Staphylococcus aureus (VRSA)*) and a fungus (*Candida albicans*). After the period of incubation, examination of the plates for inhibited zones was done. The inhibited zones were measured by the use of a plastic rule (transparent one) and, the results expressed in millilitres (mm).The minimum inhibitory concentration (MIC) was investigated and no visible growth was recorded as the MIC in the test tubes (Bauer and Kirby, 1996).

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The MIC contents of the serial dilution were sub-cultured on the media which has been prepared. Incubation was done at 37°C for about 48 hrs for fungi and 24 hrs for bacteria, after which there was observance of plates for growth of colony. Those with no growth of colony were recorded as the MBC/MFC (Bauer and Kirby, 1996).

RESULTS AND DISCUSSION

The result of the phytochemical analysis (Table1) reveals that the leaves (LOS1) and stem bark (SOS1) of *Ochna rhizomosa* contain flavonoids, alkaloids, saponins and cardiac glycosides. Tannins were observed to be present in the stem bark only, while steroids/triterpenes and anthraquinones were absent in both leaves and stem bark. The result of the well-in-plate agar diffusion method (Table 2) shows a marked inhibitory zone (mm) for the leaves (LOS1) as compared to the stem bark (SOS1). The Methicillin Resistant *Staphylococcus aureus* (MRSA) was the most sensitive organism to LOS1, with an inhibitory zone of 28.00 ± 0.20 followed by VRSA (26.00 ± 0.15), *S. typhi* (22.00 ± 0.50), and *C. albicans* (20.00 ± 0.60). For the stem bark (SOS1), Vacomycin Resistant Staphyloccocus aureus was more sensitivity with inhibitory zone (mm) of 22.00 ± 0.45, followed by *S. typhi* (20.00 ± 0.30), MRSA (18.00 ± 0.50) and *C. albicans* (14.00 ± 0.50). The combination of LOS1(100 mg/mL) with Ciprofloxacin (5 µg/mL, Com1) and Fluconazole (5 µg/mL, Com2) led to enhancement in the diameter of zone of inhibition; MRSA (36.00± 0.25), VRSA (30.00± 0.50), *S. typhi* (32.00± .11).

Table 1: Results of Phytochemical screening of the crude methanol extracts of leaves (LOS1) and stem bark (SOS1)

Test	LOS1	SOS1
Flavanoids	+	+
Alkaloids	+	+
Steriod/triterpenes	-	-
Saponins	+	+
Tannins	-	+
Cardiac glycosides	+	+
Anthraquinones	-	-

Keys: + = present, - = absent

Key: *Staphylococus aureus, S. typhi = Salmonella typhi, C. albicans = Candida albicans*, Com1 = extracts (100 mg/mL)

Key: - = Not Determined, MSRA = Methicillin Resistant *Staphylococus aureus*, VRSA = Vacomycin Resistant and Ciprofloxacin (5µ g/mL), Com2 = extracts (100 mg/mL) and Fluconazole (5µg/mL)

Table 2: Results of Zone of inhibition (mm) of plant extracts and Standard antibiotics

	Zone of inhibition (mm)					
Organism	LOS1	SOS1	Cip	Flu	Com1	Com2
S. typhi	22.00 ± 0.50	20.00 ± 0.30	34.00 ± 0.15	-	32.00± .11	-
MRSA	28.00 ± 0.20	18.00 ± 0.50	25.00 ± 0.25	-	36.00± 0.25	-
VRSA	26.00 ± 0.15	22.00 ± 0.45	24.00 ± 0.40	-	30.00± 0.50	-
C. albican	20.00 ± 0.60	14.00 ± 0.50	-	22.00 ± 0.15	-	32.00 ± 0.15

Table 3: Minimum Inhibitory Concentration (MIC) of the Combination therapy (Concentration mg/ml)

	LOS1		SOS1		Com1	
Test organism	MIC	MBC	MIC	MBC	MIC	MBC
S. typhi	12.5	12.5	25	50	3.13	6.25
MRSA	12.5	6.25	12.5	12.5	3.13	6.25
VRSA	25	50	12.5	6.25	6.25	6.25

Key: Com1 = extract (mg/ml) + Ciprofloxacin (µg/ml)

Increased in the Zone of inhibition of Com2 was also recorded with a clear zone of 32.00 ± 0.15. The minimum inhibitory concentration (MIC) results (Table 3) revealed that a low concentration of 12.5 and 25 mg/mL of LOS1 inhibited the growth of MRSA, *S. typhi* and VRSA. The resistant bacteria were not only inhibited, the extract also demonstrated a bactericidal effect at concentration 6.25 to 50 mg/ml. The MIC and MBC results in the synergistic evaluation of LOS1 with Ciprofloxacin (Table 3), reveals inhibition and bactericidal properties at a much lower concentrations. The lowest MIC and MBC values of 3.13 and 6.25 mg/mL was observed for LOS1 against MRSA and *S. typhi* respectively, as compared to LOS1(MIC = 12.5 mg/mL) alone. A low MIC of 6.25 mg/mL inhibited the growth of VRSA, the leaves extract also showed a bactericidal (MBC) effect at 6.25 mg/mL. These findings showed that, Ciprofloxacin in combination with the extract of the leaves of *O. rhimatoza* are potentials for the search for treatment for resistant bacteria and typhoid fever.

CONCLUSION

From the results of this investigation, the plant *O. rhimatoza* in combination with Ciprofloxacin showed a more enhanced antibacterial activity, as evidence by the zone of inhibition. We therefore conclude that the plant possesses potentials, that can be explore in the emergence of diseases as a result of resistant bacteria.

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