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Evaluation of Antibacterial Properties, Acute Toxicity and Immuno-stimulatory Potential of *Scoparia dulcis*

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Abstract

Traditional herbal medicines still remain the basic health care means for a large majority of rural and urban dwellers in Nigeria. *Scoparia dulcis* is a medicinal plant widely used for the traditional remedy of upper respiratory tract infections including pneumonia. Methanolic extract of the plant was assessed for antibacterial activity against *Haemophilus influenzae and Klebsiella pneumonia* by agar-cup diffusion method. The extract was active against both pathogens with zones of inhibition measuring 23.0 and 20.5 mm for *H. influenzae and K. pneumonia* respectively. The minimum inhibitory concentration of the extract against the test organisms were 6.25 and 12.5 mg/ml respectively. The minimum bactericidal concentration was 25 mg/ml for both organisms. The extract was found to be non toxic with LD_{50} of 3807(mg/ /kg body weight). The plant extract also showed a good immunostimulatory activity with a significant increase ($p<0.05$) in white blood cell proliferation when administered into mice at doses of 100 and 1000mg/ml. The result of this investigation supports the popular use of this plant for the traditional remedy of pneumonia possibly caused by these test organisms and as blood tonic.

Key words: *Scoparia dulcis,* Methanolic extract, antibacterial activity, immune-stimulatory,

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Introduction

Traditional herbal medicines still remain the basic healthcare means for a large majority of rural and urban dwellers in Nigeria. The full integration of herbal medicine into the mainstream primary health care programmes will require rigorous scientific scrutiny of both their therapeutic potentials as well as the assessment of safety issues. *Scoparia dulcis* (sweet broom weed or licorice weed) is also known by various ethnic names such as ohinohine-sesere (Ebira), ungungbuhi(Gwari), roma fada(Hausa), aiya(Ibo), ufu-ija(Igede) and mesenmesen gogoro(Yoruba) to mention but a few in Nigeria. It is one of the important medicinal plants reported to have widespread traditional uses in the remedy and management of upper respiratory tract infections in Nigeria (Alli, 2011; Yisa, 2009; Edeoga et al, 2005 and Igoli et al, 2005). Other folkloric uses include treatment of bronchitis in Brazil, septicemia in Ecuador, and antiseptic for wounds and mouth-wash for infants among the indigenous tribes of Guyana (Latha et al, 2006). The scientific scrutiny of *S. dulcis* has become imperative because of the development of high resistance against antibiotics commonly prescribed for the treatment of upper respiratory infections most especially bacterial pneumonia and the need to look for other sources of novel antimicrobial agents particularly from medicinal plants, based on ethno-pharmacological information.

Apart from *Streptococcus pneumoniae,* two other important bacterial pathogens of pneumonia in infants and immuno-compromised patients are *Haemophilus influenzae* and *Klebsiella pneumonia*. They cause severe morbidity and mortality in intensive care units and peadiatric wards (Darwish and Hamouda, 2012). The high rate of antibiotic resistance among these pathogens poses serious problems for the treatment and management of pneumonia infections. Saikia et al (2010) reported that resistance to ampicillin, tetracycline, chloramphenicol and rifampicin by *H. influenza*e was as high as 81.27, 56.25, 38.75 and 22.5% respectively. Resistance to ampicillin, erythromycin, rifampicin and streptomycin by *K. pneumonia* was also observed to be as high as 100, 100, 100 and

96% respectively (Haryani et al, 2007). Emergence of extended spectrum beta-lactamase producing resistant strains of *Klebsiella pneumoniae* in clinical isolates in Nigeria was also reported (Iroha et al, 2009 and 2011).

There have been efforts to document the antibacterial activity of *S. dulcis* in Nigeria including those of Alli et al (2011) and Yisa (2009). None of these investigations assessed the antibacterial activity of *S. dulcis* against *Haemophilus influenzae* and *Klebsiella pneumonia* nor were the safety issues considered. The present study, was, therefore, conducted to evaluate the antibacterial susceptibility of *H. influenzae* and *K. pneumonia* to methanolic extract of *S. dulcis* and to assess its acute toxicity as well as its immunostimulatory potential.

Materials and Methods

Collection, Identification and Extraction of Plant Materials: Scoparia dulcis was collected from in and around farms at Federal College of Education, Otite-Okene Nigeria. The plant was earlier identified and authenticated at the herbarium of the Dept of Biological Sciences, A.B.U., Zaria, Nigeria with voucher number 555. The leaves were air-dried under shade at ambient temperature, pulverized into fine powder, packed into soxhlet extractor, defatted with n-hexane, and subsequently extracted with methanol. The extract was concentrated using rotary evaporator at 40^{O}C and transferred into a clean container. The weight of the crude extract was measured and recorded.

Test Microorganism: Clinical isolates of *Haemophilus influenzae* and *Klebsiella pneumonia* were obtained from the Dept. of Pharmaceutics and Pharmaceutical Microbiology, A.B.U., Zaria. The isolates were checked for purity and sub-cultured onto Chocolate Agar and Bismuth Sulphite Agar Plates respectively. The cultural characteristics were observed and compared to standard references (Murray et al, 2007). They were further subjected to various biochemical tests for confirmation.

Standardization of Inocula: The bacteria strains were tested for sterility and then grown in Nutrient broth at 37 ^{o}C for 24hr. The overnight cultures were subsequently diluted to give 0.5 McFarland standards (approximately 1.5 x 10^{7}cfu/ml.).

Antimicrobial Susceptibility Test: The agar- cup diffusion method according to NCCLS (2000) was adopted. Nutrient Agar plates were flooded with overnight culture of the standardized organisms and the excess drained off. After drying, wells measuring 7mm were bored aseptically into each inoculated plate using sterile cork borer. The wells were filled with the extract (100 µl of 100mg/ml) with the aid of Pasteur pipette. Standard antibiotic disc (Ofloxacin 5µg/disc) was used as positive control while sterile distilled water served as negative control. The plates were allowed to stand at room temperature for 1 h for the extracts to diffuse into the agar. Diameters of zones of inhibition were determined after incubating plates at 37^{o}C for 24 hr. The experiments were replicated and the zones of inhibition (mm) expressed as the mean and standard errors on means.

Determination of Minimum Inhibitory Concentration (M.I.C.): M.I.C. was determined by a modification of the agar dilution method (Ehinmidu, 1993). The extracts were sterilized using Corning sterile syringe filter (0.2µm pore size). 10ml of the double strength of the various extract concentrations (100, 50, 25.0, 12.5, 6.25, 3.125, 1.56, and 0.78mg/ml) were incorporated into 10ml of double strength molten agar at 50^{o}C and aseptically poured into Petri-plates. After setting, sterile paper discs (6mm) were aseptically applied to the surface of the set agar containing the various extract concentrations at equidistance in duplicates. 10µl of each standardized inoculum was then spot-inoculated onto each disc and allowed to diffuse for 20 mins before incubating at 37^{o}C for 18 hrs. The first lowest concentration that showed no visible growth of the inoculated test organism was recorded as the M.I.C. of the extract for the test organism.

Determination of Minimum Bactericidal Concentration (M.B.C.): All inoculated paper discs showing no visible growth from the M.I.C. determination were transferred to 5ml of sterile Nutrient Broth containing 5% Tween 80 (recovery medium) to neutralize the effect of the extract and incubated for another 18 hrs. The discs from the lowest concentration that showed no visible growth (cloudiness) in the recovery medium was taken as the M.B.C. for the test organism.

Acute toxicity studies: This was done using the method of Lorke (1983). The test was conducted in two phases. In the first phase, nine (9) mice (average weight of 20 g) were divided into 3 groups of three mice each. To each group, one of the three doses of 10, 100 and 1000 mg/kg body weight was administered intraperitonially. The animals were then observed over a period of 24 hours for mortality. The response of the animals was also noted. In the second phase three (3) mice were given the extract at doses of 1600, 2900 and 5000 mg/kg each (doses higher than doses where no

mortality was observed as directed in the Lorke, 1983 table). The LD_{50} was calculated using the formular:

$LD_{50} = \sqrt{C X D}$

Where C = the highest dose at which no death occurred in the second phase,

D = the least dose at which death occurred in the second phase

The extract was considered as extremely toxic, highly toxic, moderately toxic, slightly toxic, practically non toxic and harmless when the LD_{50} value ≤ 1 mg/kg, 1-50 mg/kg, 50-500 mg/kg, 500-5000 mg/kg and >15g/kg body weight respectively.

Immuno-stimulatory effect of S. dulcis extract on Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC): A modification of Chidume et al. (2002) method was adopted for this study. Nine (9) mice of average weight 25g were grouped into three, three in each group. The first group served as control, the second and third groups was administered with the extract at doses of 100 and 1000 mg/kg body weight intraperitonially respectively on the 1st, 5th and 9th days. On the 10^{th} day, blood samples were collected by applying pressure on their tails and then cutting off the tip of each tail. White cell diluting pipette (capillary tube) was used to collect blood directly from the tail of each mouse for TLC. The TLC was done by making 1:20 dilution of the blood samples with white cell diluting fluid and counting with the aid of Neubaver counting chamber under the microscope at x10 magnification. The DLC was determined by making a thin film of the blood samples on microscopic slides and staining with the Leishman's stain. The films were air dried at ambient temperature and examined microscopically under oil immersion(x100 magnification).

Results and Discussion

The study showed that methanolic extract of *S. dulcis* has antibacterial activity against *Haemophilus influenzae* and *Klebsiella pneumonia* as presented in Table 1. The observed zones of inhibition against the two organisms were 23.0 and 20.5 mm respectively. Ofloxacin (control) showed smaller zone of inhibition against *H.influenzae* compared to the extract. This probably indicates the emergence of resistant strain of *Haemophilus influenzae* in Nigeria according to NCCL (1993) standard. The minimum inhibitory concentration of the extract against *Haemophilus influenzae* and *Klebsiella pneumonia* were 6.25 and 12.5 mg/ml respectively while the minimum bactericidal concentrations for both test organisms was 25mg/ml. The MIC and MBC of the extract against the test organisms are far less than the control. This is expected because the extract is still in the crude form.

Table 1: Antibacterial activity of Methanolic extract of *S. dulcis* against the Test organisms.

	Test organism	Zone of inhibition (mm)	M.I.C (mg/ml)	M.B.C (mg/ml)
Extract (100mg/ml)	*H. influenza*	23.0±1.0	6.25	25.0
	K. pneumonia	20.5±0.5	12.50	25.0
Control	*H. influenza*	16.5±0.83	0.004	0.016
(Ofloxacin5µg/disc)	*K. pneumoniae*	23.0±0.86	0.002	0.004

The calculated LD_{50} for the extract was 3807mg/kg of body weight (Table 2). This implies that the extract is practically nontoxic. This result is at variance with that of Hasanat *et al*. (2011) that showed cytotoxicity against brine shrimp (L_{C50} value of 40.39µg/ml). However, brine shrimps differ significantly from mice in their physiology and metabolism. No report of *S.dulcis* poisoning in humans have been reported. This probably account for its wide spread use in the preparation of herbal medicines in Nigeria.

The immuno-stimulatory effect of the extract on the blood parameters (Table 3) showed significant increase ($p<0.05$) in the Total Leucocyte Count (WBC) at both 100 and 1000mg/ml. The extract also increased lymphocyte proliferation significantly in mice. This indicates that the extract stimulates the cellular immunity system in the experimental mice. These results support the traditional uses of the extract for blood cleansing and as general blood tonic earlier reported by Latha et al (2006).

Table 2: Acute toxicity studies of the methanolic extract of *S.dulcis*

	Weight of mice(g)	Concentration of Extract9mg/kg body weight)	Observation	Mortality	LD_{50} (mg/kg body weight)
Phase 1	21.3 19.8 20.5	1000	Extreme weakness of the animals	0/3	
	18.9 22.0 20.5	100	Scratching of the nose and restlessness	0/3	$\sqrt{C \times D}$
	21.1 19.5 18.8	10	Normal activities of eating and sleeping	0/3	
Phase 2	19.7	1600	Extreme weakness of the animal	0/1	$= \sqrt{5000 \times 2900}$ = 3807
	20.4	2900	Extreme weakness of the animal	0/1	
	19.4	5000	Comatose	1/1	

Table 3: Immuno-stimulatory effect of S. dulcis in mice.

Blood Parameters	Extract Concentration Control (0 mg/ml)	100 mg/ml	1000 mg/ml
Haemoglobin(g/dl)	10.7 ± 2.0	10.5 ± 4.3	10.5 ± 3.8
PCV (%)	56.5	56.1	57.1
White Blood cells ($x10^9$/l)	13.2 ± 5.8	13.1± 5.3	16.8 ± 5.6 **
Lymphocytes	9.0 ± 3.0	9.4 ± 0.7	12.2 ± 0.8 *
Neutrophiles	4.0 ± 2.6	3.5 ± 2.5	4.2 ± 2.5
Monocytes	0.2 ± 0.0	0.2 ± 2.5	0.4 ± 0.7
Eosinophiles	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
Basophiles	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

Average of three readings

** Highly significant ($P < 0.05$); * Significant ($P < 0.05$)

CONCLUSION

Methanolic extract of *S. dulcis* showed antibacterial activity against *Haemophilus influenzae* and *Klebsiella pneumoniae.* The diameters of zones of inhibition were 23.0 and 20.5 mm respectively. These zones of inhibition compare favourably with the sensitivity of the test organisms against standard antibiotic (Ofloxacin) disc which was 28.5 and 16.5 respectively. The inhibitory activity of the extract against the test organisms provides scientific support for the traditional uses of the plant for the treatment of upper respiratory infections particularly bacterial pneumonia caused by the test organisms. The study also showed that the plant is practically non toxic and that it has good immuno-stimulatory properties which explains its wide spread use in herbal preparations as blood tonic. Further investigation will be required on chronic toxicity profile of the plant.

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