ANTIBACTERIAL EFFICACY OF PIGMENTED AND DE-PIGMENTED EXTRACTS OF *Tridax procumbens* LINN (WHOLE PLANT)

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Abstract

This work was carried out to evaluate the antibacterial efficacy of pigmented and de-pigmented petroleum ether and methanolic extracts of Tridax procumbens (whole plant). Phytochemical screening of these extracts using standard methods revealed that the pigmented and de-pigmented petroleum ether extract showed the strong presence of steroidal nucleus only, while the pigmented and de-pigmented methanol extracts revealed the presence of alkaloids, saponins, tannins, flavonoids, steroidal nucleus and cardiac glycosides. The in-vitro antibacterial assay of these extracts revealed that the de-pigmented methanol extract showed appreciable activity against all the test organisms at 100mg/ml- a broad spectrum inhibitory effect that was quite similar to that produced by the standard drug, Tetracycline at 0.5mg/ml against Gram positive Staphylococcus aureus and Bacillus subtilis and was slightly better against Gram negative Pseudomonas aeruginosa. The minimum inhibitory (MIC), minimum bactericidal (MBC) concentrations and MBC/MIC ratio of the active extract ranged between 12.5-100mg/ml and 25-100mg/ml and 1.00 2.00 respectively. Purification of the active extract by preparative thin layer chromatography gave rise to polar fractions that exhibited similar/slightly better inhibitory activities against the test organisms at 50mg/ml than the crude de-pigmented methanolic extract at 100mg/ml. MIC, MBC and MBC/MIC ratios of the active fractions ranged between 12.5-50mg/ml and 25- 50mg/ml and 1.00 4.00 respectively. The above findings suggest that the de-pigmented methanol extract of A. boonei might be a valuable source of antibacterial substance(s) for the treatment of diarrhoea, typhoid fever, urinary and gastrointestinal infections.

Keywords: *Tridax Procumbens, petroleum ether, methanol, pigmented, de-pigmented, antibacterial.*

INTRODUCTION

Tridax procumbens L. (Family: *Asteraceae*) is a specie of flowering plant that occurs throughout the tropical and sub tropical region, locally known as Igbalode (Yoruba), Gogomasi (Hausa) and coat button (common name). It is a shrub with many lateral branches, leaves are simple and opposite, flowers are daisy-like yellow-centered white and fruit is a black seed covered with stiff hairs (Holms *et al.*, 1997; Sueseela *et al.*, 2002; Mann *et al.*, 2003). The plant has been reported useful as an anti hypertensive (Salahdeen *et al.*, 2004); anti-oxidant (Ravikumar *et al.*, 2005; Habila *et al.*, 2010); anti-coagulant, an antibacterial agent (Taddei and Rosas-Romero, 2000) an anti-fungal agent and insecticidal (Ali and Jahangir, 2001; Babu and Sanjeeva, 2003). The plant has been extensively used traditionally in the treatment of fever, typhoid fever, eye treatment and also in wound healing (Mann *et al.*, 2003). A review of the literature reveals no report on comparison of the class of secondary metabolites present in the pigmented and de-pigmented petroleum ether and methanolic extracts of *Tridax procumbens* (whole plant) and the antibacterial efficacy of these extracts against selected bacteria, as the development of microbial resistance to antibiotics makes it pertinent to constantly search for new, active and safe compounds effective against pathogenic bacteria.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Tridax procumbens (whole plant) was collected from a farmland in Bosso, Bosso LGA of Niger State, Nigeria in the month of July, 2009. The plant was duly identified and deposited at the Herbarium, Department of Biological Sciences, Faculty of science, Ahmadu Bello University, Samaru, Zaria, Nigeria.

Extraction Procedures

500g of air-dried *T. procumbens* was defatted by macerating it with 1.5L of petroleum ether (60-80°C) for a period of 6 days until the extracting solvent had become colourless. The resulting solution was combined, filtered and the filtrate concentrated in vacuo using a rotavapour. Dried extract was labelled 'Pp'. Dried marc was again macerated with 2.0L of methanol and subjected to same procedure as above. The dried extract was dried and labelled 'Mp'.

De-pigmentation of Crude Extracts

The method of Hostettmann *et al.* (1998) was adopted. 25g of petroleum ether extract of *T. procumbens* (Pp) was solubilized in 500ml of petroleum ether and thoroughly mixed with 125g of activated charcoal until a right consistency was achieved. This was tightly sealed and kept aside for 72h. The mixture was filtered and the residue washed severally with

petroleum ether to ensure a chlorophyll-free extract. Filtrate was concentrated in-vacuo, dried and labelled 'Pp-C'. For the methanolic-based extract, 50g of methanol leaf extract of *T. procumbens* (Mp) was solubilized in 800ml of methanol and mixed thoroughly with 250g of activated charcoal. The same procedure as above was repeated and the extract labelled 'Mp-C'.

Phytochemical Screening of the Extracts

All extracts (Pp, Pp-C, Mp and Mp-C) were screened for the presence of various phytoconstituents using standard methods (Sofowora, 1993; Evans, 1996).

Antibacterial Screening of the Extracts

Source of Bacteria: Five bacterial strains: *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* (clinical strain) in overnight cultures (at 37°C) in nutrient broth were used in this study. All organisms were obtained from Microbiology laboratory, Federal University of Technology, Minna.

Assay of Antibacterial Activity of the Extract

The agar-well diffusion method was employed (Perez *et al.*, 1990; Dall'Agnol *et al.*, 2003). Standardized inoculums containing 10^6 cfu/ml 0.5ml McFarland standards were evenly streaked onto the surface of sterile agar plates for each organism. 8mm wells were bored into the solidified agar using a sterile cork borer at equidistant. Samples were separately reconstituted to give concentrations of 100mg/ml (extracts), 50mg/ml (fractions) and 1mg/ml (La tetra-250, Mecure Nig. Ltd., Lagos). 0.5ml of each extract/fraction/drug was introduced into the wells with the aid of a Pasteur pipette individually. Plates were incubated aerobically at 37°C for 24hr and zones of inhibition around the wells were measured to the nearest millimetre using a meter rule. Experiments were carried out in triplicates. A plant extract is considered `active', when it has an inhibition zone of = 10mm (Zwadyk, 1972).

Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined using the broth dilution method (Sahm and Washington, 1990). To 0.5ml varying concentrations of the active extracts/fractions, 2ml of nutrient broth, followed by a loopful (0.5 McFarland turbidity standard) of the test organisms was added. A tube containing nutrient broth only seeded with the test organisms served as control. Tubes were incubated at 37°C for 24hr. The MIC was regarded as the lowest concentration showing no detectable growth/turbidity.

Determination of Minimum Bactericidal Concentration (MBC)

A loopful of broth was collected from those tubes showing no turbidity/ visible growth from the MIC tubes above and sub-cultured onto freshly prepared plates. Inoculated plates were incubated at 37°C for 24hr. The least concentration showing no visible growth after incubation was taken as the MBC.

MBC/MIC ratios of the Active Extract

The MBC/MIC ratio of the active extract was calculated by adopting the method of Agnese *et al* (2001).

Purification of De-pigmented methanol Extract (Mp-C)

Active de-pigmented methanol extract of *T. procumbens* (Mp-C) was further purified by preparative thin layer chromatography (1mm thickness) and petroleum ether: chloroform (1: 1) as mobile phase. Longitudinal bands were identified using UV lamp and scrapped. The resulting silica gel mixture for each band was separately triturated with acetone, filtered and the resulting filtrate for each band concentrated in-vacuo. Obtained fractions were subjected to antibacterial testing.

Determination of MICs, MBCs and MBC/MIC values of Active Fractions

This was determined for the active fractions of de-pigmented methanol extract of *T. procumbens* (Mp-C) using the earlier reported method.

RESULTS AND DISCUSSION

Table 1: Phytoconstituents of *T. Procumbens* (whole plant)

Constituents	Pp	Mp	Pp-C	Mp-C
Alkaloids	-	+++	-	+++
Saponins	-	+++	-	+++
Tannins	-	+++	-	++
Flavonoids	-	+++	-	+++
Steroidal nucleus	+++	+++	+++	+++
Steroidal cardioactive glycosides	+	+++	+	+++
Carbohydrates	-	+++	-	+++
Anthraquinones	-	+++	-	+++
Phlobatannins	-	-	-	-

Key: +++= Highly present; ++= moderately present; += fairly present; -= absent.

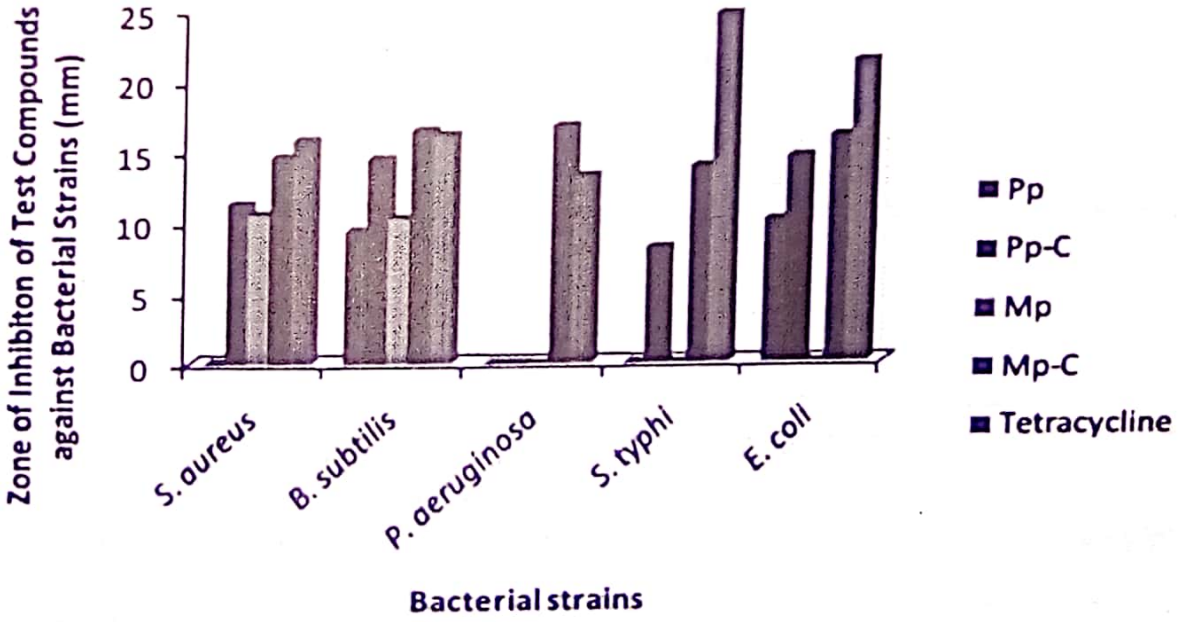

Figure 1: Antibacterial activity of the crude petroleum ether (Pp), methanol (Mp) and de-pigmented petroleum ether (Pp-C) and methanol (Mp-C) extracts of *T. procumbens* at 100mg/ml and Tetracycline at 1mg/ml against test organisms.

Table 2: MIC; MBC and MBC/MIC Values of Active Extracts of *T. procumbens* against some Bacterial Strains

Test compound	MIC (mg/ml); MBC (mg/ml) and MBC/MIC ratios of active extracts against test organisms				
	S. aureus	*B. subtilis*	*P. aeruginosa*	*S. typhi*	*E. coli*
Pp	-	-	-	-	50;100; 2.00
Pp-C	25; 25; 1.00	100; 100; 1.00	-	-	50; 50; 1.00
Mp	25; 25; 1.00	25; 50; 2.00	-	-	-
Mp-C	12.5; 25; 2.00	25; 25; 1.00	25; 25; 1.00	25; 25; 1.00	25; 25; 1.00

Key: – = Not determined.

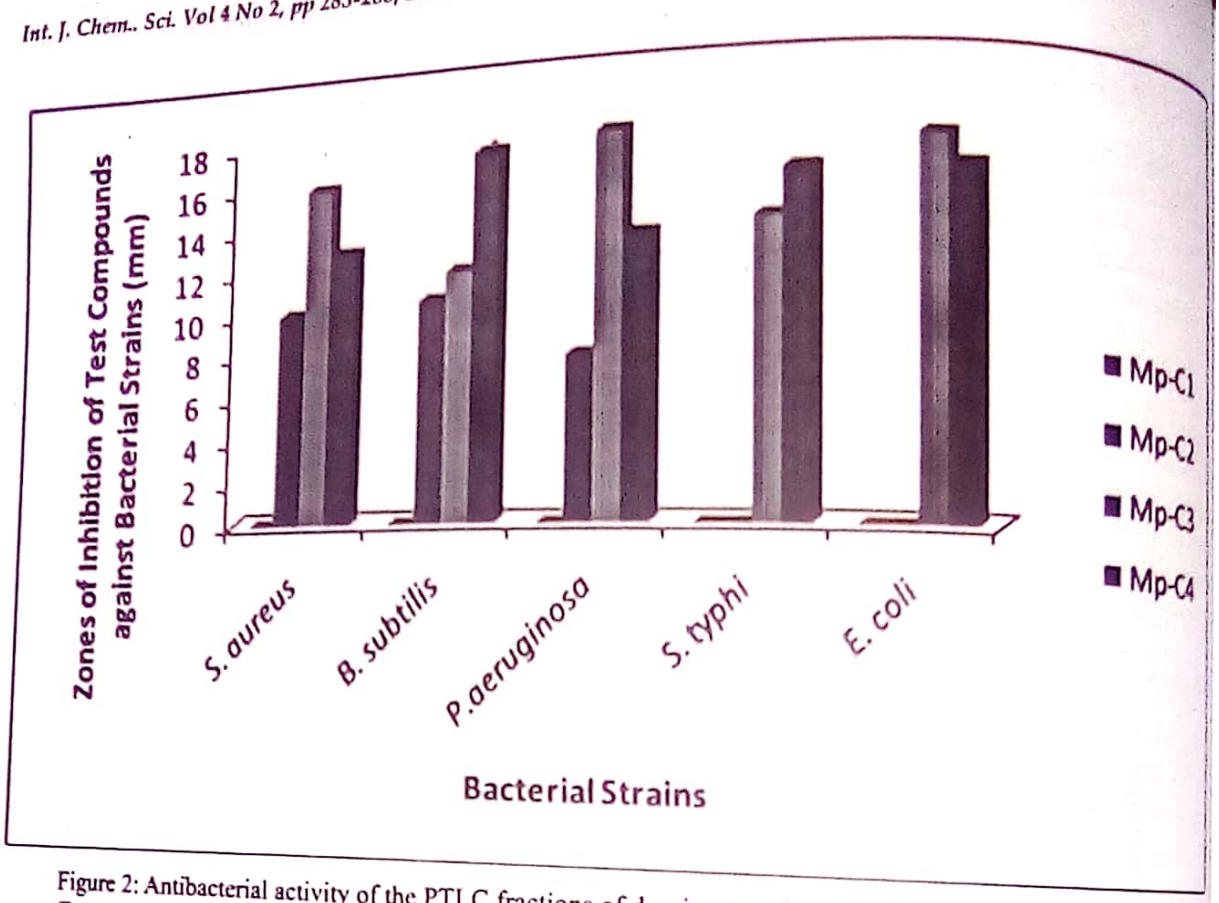

Figure 2: Antibacterial activity of the PTLC fractions of de-pigmented methanol extract of *T. procumbens* at 50mg/ml against test organisms.

Table 3: MIC; MBC and MBC/MIC Values of Active Fractions of *T. procumbens* against some Bacterial Strains

Test compound	MIC (mg/ml); MBC (mg/ml) and MBC/MIC ratios of active fractions against test organisms				
	S. aureus	*B. subtilis*	*P. aeruginosa*	*S. typhi*	*E. coli*
Mp-C3	25; 50; 2.00	25; 50; 2.00	12.5;25; 2.00	50; +++	12.5; 50; 4.00
Mp-C4	50; +++	25; 50; 2.00	25; 50; 2.00	50; 50; 1.00	50; 50; 1.00

Key: +++ = turbidity observed

Phytochemical screening of the crude pigmented and de-pigmented petroleum ether extracts (Pp and Pp-C) showed that both extracts were rich in steroidal nucleus only, while, the crude pigmented and de-pigmented methanol extracts (Mp and Mp-C) revealed a strong presence of alkaloids, saponins (sapogenins/triterpenoidal), tannins, flavonoids, steroidal cardioactive glycosides and anthraquinones as shown in Table 1. Often, low polarity solvents yield more of liphophilic components, whereas, alcohol extracts yield both apolar and polar components (Yrjonen, 2004).

Result of the antibacterial activity of the extra showed that the de-pigmented crude methanol extra (Mp-C) exhibited significant inhibitory activ against the test organisms. The extract exhibited broad spectrum activity against both *Gram* positi and *Gram* negative bacteria, while, the pigmente methanol extract (Mp) was only slightly active aga *Gram* positive *B. subtilis* and *Gram* negative *E. co* shown in Figure 1. Mp-C at 100mg/ml exhibited inhibitory activity that was similar and lower than produced by Tetracycline at 1mg/ml against

positive and *Gram* negative bacteria respectively. The observed appreciable broad spectrum activity of Mp-C as against Mp, although both extracts revealed the presence of similar phytoconstituents (Table 1), could probably be due to the removal of pigments such as chlorophyll from Mp-C, which is sometimes assumed to act as an inhibitory or masking substance (Khackik *et al.*, 1986) which sometimes interfere with the antibacterial property of some extracts (Khan and Saeed, 1998). However, chlorophyll (a green-coloured magnesium-containing pigment) present in plants, especially the leaves, has also been reported to possess lots of biological importance (Indrajith and Ravindran, 2009). This probably also accounts for why the pigmented crude petroleum ether extract (Pp) expressed practically no activity against the test organisms, while, the de-pigmented extract (Pp-C) was moderately active against *B. subtilis* and *E. coli*, although, both extracts revealed the presence of the same phytoconstituents. Generally, the medicinal values of medicinal plants reportedly lie in their phytoconstituents (Akinpelu *et al.*, 2008). Compounds like tannins, saponins, alkaloids and flavonoids have been linked to or suggested to be involved with antimicrobial activity (Palombo, 2006). The presence of some of these phytoconstituents in Mp-C as shown in Table 1, even in relatively low concentrations, could contribute to the observed antibacterial activity (Dall'Agnol *et al.*, 2003).

The efficacy of the de-pigmented methanol extract (Mp-C) was further supported by its low MIC and MBC values against the test organisms which ranged from 12.5-100mg/ml and 25-100mg/ml respectively (Table 3), while it had an MBC/MIC ratio that ranged from 1.0 - 2.0, an indication of a bacteriostatic effect. Extracts with MBC/MIC ratio ? 1.00, would indicate a bacteriostatic effect, while < 1.00, is indicative of a bacteriocidal effect. Calculated MBC/MIC ratio for an active extract is usually used to ascertain if the observed antibacterial effect was bacteriocidal or bacteriostatic in nature (Agnese *et al.*, 2001).

Antibacterial assay of the four major fractions collected from preparative thin layer chromatography of the active de-pigmented extract (Mp-C) at 50mg/ml, revealed that fractions Mp-C3 and Mp-C4 produced significant inhibitory effects against both Gram positive and *Gram* negative bacteria, while fractions Mp-C1 and Mp-C2 practically expressed no/insignificant activity against the test organisms as shown in Figure 2. Both fractions Mp-C3 and Mp-C4 produced inhibitory effects that were slightly better or similar to that exhibited by Mp-C against the test organisms. It is therefore likely that the antibacterial ty property of Mp-C is contained in fractions Mp-C3 and Mp-C4, which could be attributed to the presence of polar phytoconstituents in these fractions (since on PTLC both fractions had bands with R_f values of 0.42 and 0.34 respectively). Generally, polar compounds have been reported to exhibit significant antibacterial/antimicrobial activities against some pathogens (Muskhazli *et al.*, 2008; Nazemi *et al.*, 2010).

The MICs, MBCs and MBC/MIC values of active Mp-C3 and Mp-C4 fractions ranged from 12.5 - 50mg/ml, 50 - □ 50mg/ml and 1.00 - 4.00, respectively. An indication that the fractions were active and that the antibacterial effect produced by these active fractions, like its crude de-pigmented methanol extract, was bacteriostatic.

CONCLUSION

The presence of some phytoconstituents in the de-pigmented methanol extract of *Tridax procumbens* (whole plant) that were probably masked by pigments, could possibly be responsible for the promising significant inhibitory activity of the plant, suggesting that the removal of plant pigments, such as chlorophyll and further purification of such extracts, could make a medicinal plant an effective source of antibacterial substances. Further work will aim at isolation and characterization of the polar components obtained from preparative thin layer chromatography.

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