Extrinsic Factors Influencing Antibacterial Activities of *Tapinanthus bangwensis* Against Diarrhoeal Causing Organsims

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ABSTRACT: *Tapinanthus bangwensis* is a parasitic plant with wide distribution and documented antimicrobial efficacies. Inconsistence in its activities has however, been suggestively linked with some factors. Hence, this study was carried out to establish the effect of some extrinsic factors on the antibacterial activities of *T. bangwensis* against some diarhoeal causing bacteria. Antibacterial activity of *T. bangwensis*, collected from some parts of Nigeria was determined by standard agar-diffusion method. Results from this study showed a significantly higher zone of bacterial inhibition with chloroform extract when compared with ethanol and aqueous extracts ($P<0.05$). Higher antibacterial activities was also observed with extracts obtained from air dried plants than those obtained from sun dried and oven dried plants ($P<0.05$). Steaming method of extraction produced a significantly higher zone of bacterial inhibition than cold and hot methods of extraction ($P<0.05$). Percentage weight yield of active crude compound s of *T. bangwensis* was highest in chloroform (4.63±1.99%) than methanol (2.83±2.06%) and water (2.28±1.90%) ($P<0.05$). A significant positive correlation ($r = +0.91$, $P<0.05$) was observed between percentage weight yield and zone of antibacterial inhibition, exhibited by *T. bangwensis*. Weight yield accounted for 70% of antibacterial activities with a linear relationship of $y = 3x + 5.6$. Antibacterial activities of *T. bangwensis* were however, not affected by its host plants and varying concentrations of its crude extracts ($P>0.05$). Conclusion from this study has shown that solvents and methods of extraction such as mode of plant drying and means of concentrating extracts as important and influential extrinsic factors that determines the antibacterial activities of *T. bangwensis*.

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1. Introduction

Tapinanthus bangwensis (order Santalales) belong to the family of Loranthaceae and constitute the largest group of parasitic plants which has about 950 species distributed in 77 genera(Engone and Salle,2006). Loranthaceae; including *Tapinanthus* constitute a great deal of pestilence in the natural forests, plantations, cultivated fruit trees and ornamental plants; causing damages to the host plants(Sonke et al., 2000., Boussim et al., 2004). *Tapinanthus bangwensis* is widely distributed in Nigeria. The plant exists as xerophytes on many host trees. Its leaves and young twigs have in the past been used in folklore treatment of diseases and sterility in cow.Documentations have been made about the antimicrobial efficacy of *T. bangwensis* (Adegbolahun and Olukemi,2010) but its activity tends to vary among investigators. Also, there have been controversies over the hypothesis that the antimicrobial efficacies of *T. bangwensis* is host plant dependent. Hence, we have decided to investigate the impact of host plant factor and other extrinsic factors on the antibacterial activities of *T. bangwensis* against some bacterial pathogens causing diarrhoea.

2. Materials and Methods

2.1. Collection of Plants

Fresh leaves and twigs of *Tapinanthus bangwensis* were collected from Iseyin, Southern part of Oyo State and Chagas village in Abuja, Nigeria.The plants were authenticated by Dr A. E. Ayodele at the department of Botany and Microbiology, University of Ibadan, Nigeria and were designated T1 – T4 on the basis of the host plants from which they were collected. T1 was from Triclisia gilletii (De wild) stainer, T2 from *Parkia biglobosa* (Jacq.) Benth,T3 from *Citrus aurantifolia* (Christm.) swingle and T4 from phyllanthus muellerianus.

2.2.Preparation of Plant Extracts

Fresh Leaves were divided into three groups. Group one were air-dried, Group two were sun-dried and Group three were oven-dried. After drying the leaves were shredded and separately preserved in air-tight cellophane bags.The shredded leaves were milled into powder. Cold extraction of the plant was made by soaking 100g of powdered plant into 400ml each of methanol, chloroform and water respectively in flasks. The flasks were manually agitated at intervals for 5 days. All extracts were then filtered with whatman no 1 filter paper into flask. The filtrates were later concentrated to dryness with the aid of a rotary evaporator. The steam extraction was carried out as described by Adeolu and Oladimeji(Adebolu and Oladimeji, 2005). The hot extraction involves placing the leaves and twigs of *T. bangwensis* in a pot and boiling for 30 minutes. This was then allowed to cool and the leaves were then squeezed to obtain the extract that was later concentrated by rotary evaporator. The yield of concentrates from the various extracts was then calculated using the following formula:

Percentage weight yield (%) = $W_2 / W_1 \times 100$.
Where
W_1 = Weight of herbal powder before extraction.
W_2 = Weight of concentrate after extraction.

2.3.Statistical analysis

All data were analyzed by SPSS package version 15. Comparison of mean zones of inhibition were determined by ANOVA (Analysis of Variance) while regression analysis was used to determine the linearity between percentage weight yields (%) of plant and their proportional antibacterial activities.

3. Results

The effect of host plants on the antibacterial activities of *T. bangwensis* was determined in table1. Although, the zones of inhibition (mm) varieds with different host plants, but the difference was insignificant (F=1.06, P>0.05). In table 2, when the mean of zones of inhibition of *T. bangwensis* concentrates from different solvents were compared, a significant difference was observed (F=10.13, P<0.05); with highest antibacterial activity being recorded with chloroform. Table 3 displayed various mean of zones of inhibition of *T. bangwensis* extracts processed by different methods of drying. Analyzed data showed that extracts prepared from air-dried plants of *T. bangwensis* hads the highest zone of inhibition of 13.50 ± 3.50mm (F= 6.47, P<0.05). Comparison between antibacterial activities among concentrates of *T. bangwensis* yielded by different extraction techniques was made in table 4. The significantly higher zone of inhibition was observed with steam (13.13±1.59mm) when compared with cold (9.58 ± 0.68) and hot (7.42 ± 0.85) methods of extraction (F=6.66, P<0.05). When means of zone of inhibition of *T. bangwensis* were compared at different concentrations that rangeds from 50–200mg/ml, no significant difference was observed (F=2.23, P>0.05) in table 5. Comparison of percentage weight yields (%) of crude *T. bangwensis* concentrate regarding the type of solvents of extraction demonstrated that the highest yield of 4.63±1.99% was with chloroform (F = 448.17, P<0.05) (Table 6). A significant correlation (r = +0.91, P<0.05) was observed between the percentage weight yields of *T. bangwensis* and its antibacterial activities, with a linear equation: y = 3x + 5.60. Regression determinants showed that percentage weight yield was accountable for 70% of the antibacterial activities (figure 1).

Table 1. Effect of host plant on the antibacterial activity of *Tapinanthus bangwensis*

Host plants	n	Zones of inhibition Mean±SEM(mm)
Triclisia gilletii	10	8.43 ± 1.65
Parkia biglobosa	10	6.45 ± 1.94
Citrus aurantifolia	10	8.34 ± 2.63
Phyllanthus muellerianus	10	6.42 ± 2.03

F = 1.06, P > 0.05.

Table 2. Effect of solvent of extraction on the antibacterial activity of *Tapinanthus bangwensis*

Solvents of extraction	n	Zones of inhibition Mean±SEM(mm)
Methanol	10	15.64 ± 1.20
Chloroform	10	19.00 ± 1.50
Water	10	11.20 ± 0.85

F = 10.13, P < 0.05.

Table 3. Effect of drying methods on the antibacterial activity of *Tapinanthus bangwensis*

Drying methods	n	Zones of inhibition Mean ± SEM (mm)
Air drying	10	13.50 ± 3.50
Sun drying	10	8.88 ± 2.03
Oven drying	10	9.00 ± 1.63

F = 6.47, P < 0.05.

Table 4. Effect of methods of extraction on the antibacterial activity of *Tapinanthus bangwensis*

Methods of extraction	n	Zones of inhibition Mean ± SEM(mm)
Steam extraction	10	13.13 ± 1.59
Cold extraction	10	9.58 ± 0.68
Hot extraction	10	7.42 ± 0.85

F = 6.66, P < 0.05.

Table 5. Effect of concentrations on the antibacterial activity of *Tapinanthus bangwensis*

Concentrations	n	Zones of inhibition Mean±SEM(mm)
100mg/ml	10	12.05 ± 2.06
150mg/ml	10	14.30 ± 1.99
200mg/ml	10	16.00 ± 1.90
50mg/ml	10	19.50 ± 2.45

F = 2.23, P > 0.05.

Table 6. Effect of Solvents on weight yield of the active ingredients in *Tapinanthus bangwensis*

Solvents	n	Weight yield (%) Mean ± SEM (mm)
Methanol	3	2.83 ± 2.06
Chloroform	3	4.63 ± 1.99
Water	3	2.28 ± 1.90

F = 448.17, P< 0.05.

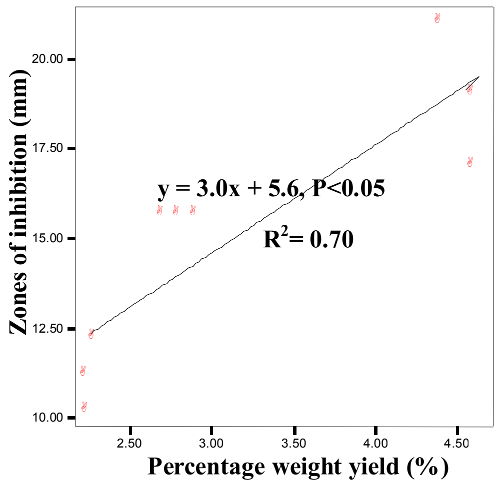

Figure 1. Relationship between inhibitory activities and percentage weight yield of active chloroform extract of *T. bangwensis.*

4. Discussion

Result of this study have shown that the antibacterial activities of *T. bangwensis* were independent of host plant factor. This observation supported the null hypothesis which says that host plant does not influence the antibacterial activities of *T. bangwensis.* The significantly higher percentage yield of crude concentrate of *T. bangwensis* by chloroform in comparison with other solvents demonstrated higher extraction strength with chloroform ($P<0.05$). Also, significantly the highest antibacterial activity was found in the crude chloroform extract which strongly suggests that the active antibacterial compounds might be organic and less polar in origin.

After processing of *T. bangwensis* leaves by different methods of drying, the highest antibacterial activity was observed in air dried extract while less activity was observed with oven-dried extract and least activity was observed with sun dried extract ($P<0.05$). Lesser antibacterial activity in sun dried extract may be due to photochemical degradation of the active compounds which in turn may result in structural modification of functional groups required for active antimicrobial activity(Adegbolahun and Olukemi,2010). Also, lesser antibacterial activity in oven-dried extract indicated that the active antibacterial compound of *T. bangwensis* might be heat-labile. This observation was similar to that of Olaniyi et al. (2010), Harnischfeger (2005) and Niggermann and Gruber(2003). Furthermore,the lesser antimicrobial activities in oven-dried extract might also be linked with loss of volatile contents of *T. bangwensis* such as phenols and essentials oils (Rajendran et al., 2007).

Steam method of extraction of *T. bangwensis* has been shown to produce bioactive compound with better antibacterial activities than cold and hot methods of extraction ($P<0.05$). The weakest antibacterial activity in concentrate of *T. bangwensis* derived from hot method of extraction further re-emphasize the volatile nature of active antibacterial constituents of *T. bangwensis* while lower antibacterial activity in cold method of extraction is a reflection of the decreased solubility of active plant constituent at lower temperature (SOT,2009). Comparison of the antibacterial activity of crude chloroform extract of *T. bangwensis* at concentrations such as 50, 100, 150, and 200mg/ml showed no significant difference ($P>0.05$). This indicated that *T. bangwensis* exhibited antibacterial activities almost at equal level at concentration range of 50-200mg/ml. Equilibrium of antibacterial activities between the lesser and higher concentrations of *T. bangwensis* clearly shows that the antibacterial quality of the active constituents of the plant is largely dependent on its molecular weight and diffusion rates through agar rather than its concentration. Since higher zones of inhibition in agar diffusion antibiotic susceptibility tests is an attribute of faster rates of drug diffusion and low molecular weight, it can then be inferred that the active antibacterial constituent of *T. bangwensis* might be among compounds with lower molecular weight.

In conclusion, the outcome of this study has demonstrated that antibacterial activities of concentrates from *T. bangwensis* could be best enhanced by methods that include air-drying and chloroform extraction of the plant via steaming.

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