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Gongronema latifolia Clones: Genetic Effects on some Phytochemical Composition and Anti-microbial Activity against *Salmonella typhi*

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ABSTRACT

The levels of some phytochemicals (alkaloids, phenols and tannins) and *in vitro* anti-microbial activity of different genetic resources and plant parts of *Gongronema latifolia* on *Salmonella typhi* were determined. There were significant ($P = 0.05$) differences in genetic effects and plant parts in phytochemical composition and anti-microbial activity against *S. typhi*. The leaves showed significantly higher phenolic contents (3.81 mg/100 g) than the stems (0.42 mg/100 g). Conversely, the stems contained higher alkaloid and tannin levels (12.50 and 4.67%, respectively) than the leaves (7.80 and 2.99%, respectively). Clone ABS-42-ABA had the highest phenol (4.02 mg/100 g) and alkaloid (13.75%) levels while clone ANS-38-AWKA had the lowest level of phenol (0.24 mg/100 g). The tannin level was significantly higher in clone IMS-20-NJIABA (6.03%) and lower in clone AKS-33-EKPENE EDIENE (2.11%). The leaves expressed significantly higher anti-microbial activity (2.4 mg/ml) against *S. typhi* than the other plant parts. The antimicrobial activity of the clones on *S. typhi* was significantly higher in ABS-42-ABA (2.15 mg/ml), and lowest in AKS-33-EKPENE EDIENE (6.43 mg/ml). The phenol concentration had a significantly higher negative correlation with minimum inhibitory concentration (MIC) than other phytochemicals. Similarly, path coefficient analysis indicated that phenol had a higher negative direct effect on MIC. Antibacterial properties shown by the clones and their parts provide a scientific basis and thus validate the use of the plant in treatment of typhoid fever and other bacterial diseases. Also, the varying efficacy by the different genetic resources and the plant parts with respect to phytochemical contents and anti-microbial activity against *S. typhi* could be the basis for further selection and improvement of the species for pharmacological purposes against typhoid fever.

Keywords: clonal variation, *Gongronema latifolia*, minimum inhibitory concentration, phenol, *Salmonella typhi*

INTRODUCTION

Uses of medicinal plants as drugs

Medicinal plants are undoubtedly relevant in both developing and developed nations as sources of drugs or herbal extracts for various chemotherapeutic purposes. It has been estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from plant substances (Tripathi and Tripathi 2003).

Food and medicinal uses of *G. latifolia*

Gongronema latifolia Benth. (Asclepiadaceae) is an important medicinal plant that has been widely utilized traditionally for medicinal purposes in Nigeria and other parts of sub-Saharan Africa (Agbo *et al.* 2005; Okafor 1997). It is native to West Africa (Nielsen 1965). The plant is eaten as a leafy vegetable and spice in Nigeria (Akpan 2004; Agbo *et al.* 2005). Okafor (1997) reported that the fresh leaf extracts of *G. latifolia* contain 62.7% protein. In a study with 46 clones of *G. latifolia*, Agbo *et al*. (2009) reported wide varying levels (16-58%) in protein content of the fresh leaves of clones. Separately, Eleyinmi (2007) showed that *G. latifolium* leaves contained 27% protein, but no range was provided nor was the number of clones or lines. The wide variability in the protein levels was attributed to genetic differences by Agbo *et al*. (2009).

The aqueous and ethanolic extracts of *G. latifolia* have hypoglycemic and anti-oxidative (Ugochukwu and Babady 2002; Ugochukwu *et al*. 2003) and anti-inflammatory (Morebise *et al*. 2002) properties. The consumption of leaves is believed to promote pregnancy (Okafor 1997). The leaves have also been used for the treatment of typhoid fever, dysentery, malaria, worm infection, cough, high blood pressure and in the management of diabetes mellitus (Agbo *et al.* 2005).

Phytochemical and anti-bacterial activities of medicinal plants

Gamaniel and Akah (1996) reported that the leaf extracts of *G. latifolia* contain five classes of phytochemical compounds: alkaloids, saponins, tannins, flavonoids and glycosides and suggested possible varied pharmacological effects. The results of anti-microbial activities of the leaf extracts of *G. latifolia* carried out by Eleyinmi (2007) showed positive activity against *Staphylococcus aureus*, *Salmonella enteritidis* and *Salmonella choleraesuis* serovar *typhimurium.* Phytochemical extracts of other medicinal plants have been reported to be sensitive against diverse microbial organisms associated with human diseases, including *Salmonella typhi* (Adegoke *et al*. 2009; Kaur and Arora 2009; Rankumar *et al*. 2009; Doherty *et al*. 2010). Substantial research efforts are presently being made on the medicinal potential of *G. latifolia*. However, there are no studies attributing the pharmacological effects of different *G. latifolia* clones to their chemical constituents. This research was then designed with the aim of testing the anti-microbial activity of different genetic sources of *G. latifolia* at varying levels of some im-

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portant phytochemicals associated with curative potentials against *S. typhi*. *S. typhi* was chosen because it has been identified as the most prevalent causal organism for typhoid fever in Southeastern Nigeria and cases of development of resistance to available antibiotics by *S. typhi* abound unlike other microbes that cause faecal oral infection. Hence, *S. typhi*, which is more prevalent and resistant, was selected for this investigation. The study also went further, through correlation and path analyses, to show the importance of the phytochemicals on the minimum inhibitory concentration (MIC) of *S. typhi* observed. The specific objectives of the research were: 1) to assess the levels of selective phytochemicals in selected clones of *G. latifolia*; 2) to determine the *in vitro* anti-microbial activity of different parts of *G. latifolia* resources on *S. typhi*; 3) to identify the direct effects of such phytochemicals on anti-microbial activity of the plants through path analysis.

MATERIALS AND METHODS

Materials

Test organism: The test microorganism used in this study was a clinical isolate of *S. typhi* because it is most prevalent and resistant to most antibiotics in use in the study area (Akinyemi *et al*. 2000)

Reagent: The reagents used for this study were all analytical grade chemicals and they comprised ethanol and dimethyl sulphoxide (DMSO). The culture media used were nutrient agar and nutrient broth product (Fluka, Biochemika, Spain).

***G. latifolia* clones:** Five heterozygous clones of *G. latifolia* (EBS-15-NKALAGU, IMS-20-NJIABA, AKS-33-EKPENE EDIENE, ANS-38-AWKA and ABS-42-ABA) were selected from the germplasm garden of the Department of Crop Science, University of Nigeria, Nsukka Research Farm. The clones were established more than six years ago from stem cuttings obtained from virgin forests in different localities in southeastern Nigeria. Roots, terminal stems and leaves from sample plants in each plot were harvested and used for the study. The level of phytochemicals in the crop species was determined in two years (2008 and 2009) to test for environmental effects on their availability. In 2009, the phytochemical contents of clones' leaves only were tested because of the higher levels of phytochemicals in leaves. The use of the crop species in determining the inhibition zone diameter of *S. typhi* was carried out in 2008.

Anti-microbial agents: The anti-microbial agents used for this study were the ethanolic extract of *G. latifolia* leaves and ciprofloxacin (500 mg tablet, source: Medibois Laboratories Pvt. Ltd., India).

Methods

Extraction of bio-active components

The *G. latifolia* samples were ground and the aliquot (100 g) was extracted at room temperature with ethanol (2 × 1000 ml, 24 h each). The ethanol extracts were concentrated in hot air oven (GallenKamp model O/H 300 MOS) at 60°C.

Determination of selected phytochemicals in *G. latifolia* extracts and experimental layout

The levels of phytochemicals in the crop species were determined in two years (2008 and 2009). Sampling of leaves of the test crop for determination of phytochemicals only in 2009 rather than determination of the inhibition zone diameter of *S. typhi* was to test for environmental influence on the availability of the phytochemicals responsible for the anti-bacterial effects observed. The repeated phytochemical determination gave an idea of their availability over time and enabled valid statements to be made on the genetic and environmental influence of the clones on the availability of these phytochemicals in plants. The alkaloid content of the plant parts (root, stem and leaves) of the five clones was determined as described by Harbone (1991). The level of tannin in the clones was determined using the method of Pearson (1976). The total phenol content was determined by a spectrophotometric method as described by Harbone (1973) and Obadori and Ochuko (2001). The treatments were arranged in a factorial experiment in a completely randomized design (CRD) with three replications. The five clones served as factor A and two plant parts were factor B.

Determination of inhibition zone diameter (IZD) of *G. latifolia* leaf extract on *S. typhi*

Inhibition zone diameter (IZD) was determined in 2008. The method used was the Agar Cup Diffusion Technique as described by Agboke *et al.* (2005). Nutrient agar was prepared, sterilized and allowed to cool to 45°C. About 0.5 ml of the suspension of *S. typhi* that was allowed to stabilize by incubating for 2 h at 37°C in the nutrient broth was pipetted into a sterile Petri dish. Prepared nutrient agar (20 ml) was poured into the dish and swirled three times in a clockwise direction and then three times in an anti-clockwise direction to ensure an even distribution of the test organism. It was then allowed to set and 0.5 g of each of the extracts (leaf, stem or root) was dissolved in 10 ml DMSO. Thereafter, three-two fold serial dilution was made from each stock solution. DMSO (2 ml each plant part) was measured into three other sterile test tubes, labeled 2, 3, 4. Then, 2 ml from the stock solution was aseptically introduced into test tube 2 and mixed thoroughly. Furthermore, 2 ml from solution 2 was introduced aseptically into test tube 3 and mixed thoroughly. Finally, 2 ml of solution 3 was aseptically introduced into test tube 4 and mixed thoroughly. The agar plate was divided into four sections using a marker and marked 1, 2, 3 and 4 representing the different concentrations of the serial dilution. Using an 8 mm cork borer, cups were made at the centre of each of the four sections. Then, 0.05 ml each of the dilution of the extract was aseptically dropped into the cups starting from the lowest concentration to the highest. The plate was labelled and incubated at 37°C for 24 h and the zones of inhibition were measured. This procedure was also used to evaluate the IZD of a standard drug (Ciprofloxacin 500 mg) used as a positive control and DMSO used as a negative control. The results were tabulated and graphs of IZD's square against the logarithm of concentrations were plotted. The MIC's were calculated from the graphs. The treatments were arranged in a factorial experiment in a CRD with three replications. There were five clones (EBS-15-NKALAGU, IMS-20-NJIABA, AKS-33-EKPENE EDIENE, ANS-38-AWKA and ABS-42-ABA) as factor A and three plant parts comprising leaves, stems and roots as factor B.

Statistical analysis

Data on phytochemical contents and antimicrobial activity of the clones and their plant parts on *S. typhi* were collected in triplicate. The data were subjected to analysis of variance following the procedure outlined for factorial experiments in CRD using Genstat Discovery Edition 3.0 (2007). Phenotypic and genotypic variances and broad sense heritability estimates of the clones were obtained using the procedure set by Uguru (2005). Pearson's correlation and path analysis were performed on the results of the phytochemical concentrations and MIC values of the different clones using the Dewey and Lu (1959) approach.

RESULTS

Phytochemical composition of *G. latifolia* clones and plant parts

The effects of the clones and their parts on the concentrations of the studied phytochemicals show that ABS-42-ABA had significantly ($P < 0.05$) higher levels of alkaloids and phenols (13.75% and 4.02 mg/100 g, respectively) (**Table 1**). The roots of all clones showed trace levels of all the phytochemicals and thus were not analysed. Clone IMS-20-NJIABA had a significantly lower level of alkaloids and higher level of tannins. Clones AKS-33-EKPENE EDIENE and ANS-38-AWKA contained the lowest levels of tannins (2.11%) and phenols (0.24 mg/100 g), respectively. The

Table 1 Effects of *Gongronema latifolia* clones and their plant parts on the concentration of the determined phytochemicals.

	2008[a]			2009[b]		
	Alkaloids (%)	**Phenols (mg/100 g)**	**Tannins (%)**	**Alkaloids (%)**	**Phenols (mg/100 g)**	**Tannins (%)**
Clones						
EBS-15-NKALAGU	9.00	2.03	2.54	8.50	2.10	2.13
IMS-20-NJIABA	5.25	3.21	6.03	5.12	3.02	6.15
AKS-33-EKPENE EDIENE	10.50	1.07	2.11	11.00	1.01	2.34
ANS-38-AWKA	10.25	0.24	5.36	10.62	0.32	4.98
ABS-42-ABA	13.75	4.02	3.11	12.82	4.11	3.00
LSD ($P < 0.05$)	0.29	0.10	0.11	0.24	0.11	0.12
Plant parts						
Leaves	7.80	3.81	2.99			
Stems	12.50	0.42	4.47			
t (0.05)	0.18	0.06	0.07			

[a], phytochemicals determined in leaves and stem plant parts.
[b], phytochemicals determined in leaves only.

Table 2 Interaction effects of *G. latifolia* clones and plant parts on the concentration of the determined phytochemicals in 2008.

Clones	**Plant part**	**Alkaloids (%)**	**Phenols (mg/100 g)**	**Tannins (%)**
EBS-15-NKALAGU	Leaves	7.50	3.57	2.64
EBS-15-NKALAGU	Stems	10.50	0.48	2.43
IMS-20-NJIABA	Leaves	4.00	5.95	4.50
IMS-20-NJIABA	Stems	6.50	0.48	7.56
AKS-33-EKPENE EDIENE	Leaves	9.00	2.14	1.79
AKS-33-EKPENE EDIENE	Stems	16.00	0.00	2.43
ANS-38-AWKA	Leaves	7.00	0.48	3.57
ANS-38-AWKA	Stems	13.50	0.00	7.14
ABS-42-ABA	Leaves	11.50	6.90	2.43
ABS-42-ABA	Stems	16.00	1.13	3.79
LSD ($P < 0.05$)		0.41	0.07	0.07

alkaloids and tannins were significantly concentrated in the stems while phenol levels were more abundant in the leaves. The interaction effect showed that IMS-20-NJIABA had the lowest level of alkaloids in both leaves and stems (4.00 and 6.50%) while ABS-42-ABA showed the highest level of phenols in both plant parts (**Table 2**). Conversely, AKS-33-EKPENE EDIENE and ANS-38-AWKA had no phenols in their stems, but had relatively high levels of alkaloids.

Anti-bacterial effects of the clones and their parts

The IZD of the clones increased progressively with increasing concentration of the extracts (**Table 3; Fig. 1**). Clone ABS-42-ABA had a significantly higher level of IZD at all concentrations while AKS-33-EKPENE EDIENE had the lowest. IZD was highest at all concentrations in the leaves followed by the stems but was zero in the roots of all clones at the lowest concentration of extracts (6.25 mg/ml). The MIC values of clones and plant parts showed that ABS-42-ABA had the highest IZD value as well as the lowest MIC value (1.66 mg/ml) from leaves (**Table 4**). However, ciprofloxacin tablets (control) had a lower MIC (0.83 mg/ml) than any of the clones. On the other hand, AKS-33-EKPENE EDIENE had the highest MIC value (6.43 mg/ml). In all the clones, the leaves showed lower MIC values than stems.

Correlation and path analysis of traits of *G. latifolia* related to the MIC factor

The correlation coefficients of the phytochemical contents and MIC of the *G. latifolia* clones indicated that phenols had a highly significant ($P < 0.01$) negative correlation with MIC (**Table 5**). On the other hand, alkaloids and tannins exhibited a significant ($P < 0.01$) and non-significant positive correlation with MIC, respectively. Alkaloids also had a negative correlation with phenols while phenols were nega-

Table 3 Inhibition zone diameter (IZD) mm of clones and parts of *G. latifolia* on *S. typhi* at different concentrations.

Clones at different concentrations	**Plant part**			
	Leaves	**Stems**	**Roots**	$\bar{X}$
6.25 mg/ml				
EBS-15-NKALAGU	6.00	3.00	0.00	3.00
IMS-20-NJIABA	8.00	3.00	0.00	3.67
AKS-33-EKPENE EDIENE	4.00	0.00	0.00	1.33
ANS-38-AWKA	4.00	0.00	0.00	1.33
ABS-42-ABA	9.00	5.00	0.00	5.00
$\bar{x}$	6.20	2.20	0.00	
12.5 mg/ml				
EBS-15-NKALAWU	8.00	5.00	0.00	4.33
IMS-20-NJIABA	10.00	4.00	2.00	5.33
AKS-33-EKPENE EDIENE	5.00	2.00	0.00	2.33
AVS-38-AWKA	5.00	4.00	3.00	4.00
ABS-42-ABA	10.00	6.00	3.00	6.33
$\bar{x}$	7.60	4.20	1.60	
25 mg/ml				
EBS-15-NKALAGU	10.00	8.00	2.00	6.67
IMS-20-NJIABA	11.00	6.00	3.00	6.67
AKS-33-EKPENE EDIENE	7.00	4.00	3.00	4.00
ANS-38-AWKA	7.00	5.00	4.00	5.33
ABS-42-ABA	11.00	8.00	5.00	8.00
$\bar{x}$	9.20	6.20	3.40	
50 mg/ml				
EBS-15-NKALAGU	11.00	9.00	3.00	7.67
IMS-20-NJIABA	13.00	8.00	7.00	9.33
AKS-33-EKPENE EDIENE	9.00	5.00	4.00	6.00
ANS-38-AWKA	10.00	7.00	6.00	7.67
ABS-42-ABA	13.00	10.00	8.00	10.23
$\bar{x}$	11.20	7.80	5.60	
	6.25 mg/ml	**12.5 mg/ml**	**25 mg/ml**	**50 mg/ml**
LSD ($P < 0.05$) for comparing 2 clones means	0.66	0.71	0.87	0.85
LSD ($P \leq 0.05$) for comparing 2 plant parts means	0.51	0.55	0.68	0.66
LSD ($P < 0.05$) for comparing 2 clones x plant parts	1.14	1.23	1.23	1.47

Table 4 Minimum inhibitory concentration (MIC) (mg/ml) values of *G. latifolia* clones, plant parts and control (ciprofloxacin tablet) on *S. typhi*.

Clones	**Leaves**	**Stems**	$\bar{X}$
EBS-15-NKALAGU	2.57	4.90	3.74
IMS-20-NJIABA	1.74	4.00	2.87
AKS-33-EKPENE EDIENE	3.31	9.55	6.43
ANS-38-AWKA	3.16	6.31	4.74
ABS-42-ABA	1.66	2.63	2.15
$\bar{X}$	2.49	5.48	
Ciprofloxacin (control)	0.83		

$LSD_{(p< 0.05)}$ for comparing 2 clones means = 0.06
$LSD_{(p< 0.05)}$ for comparing 2 plant parts = 0.04
$LSD_{(p< 0.05)}$ for comparing 2 clones x plant parts = 0.09

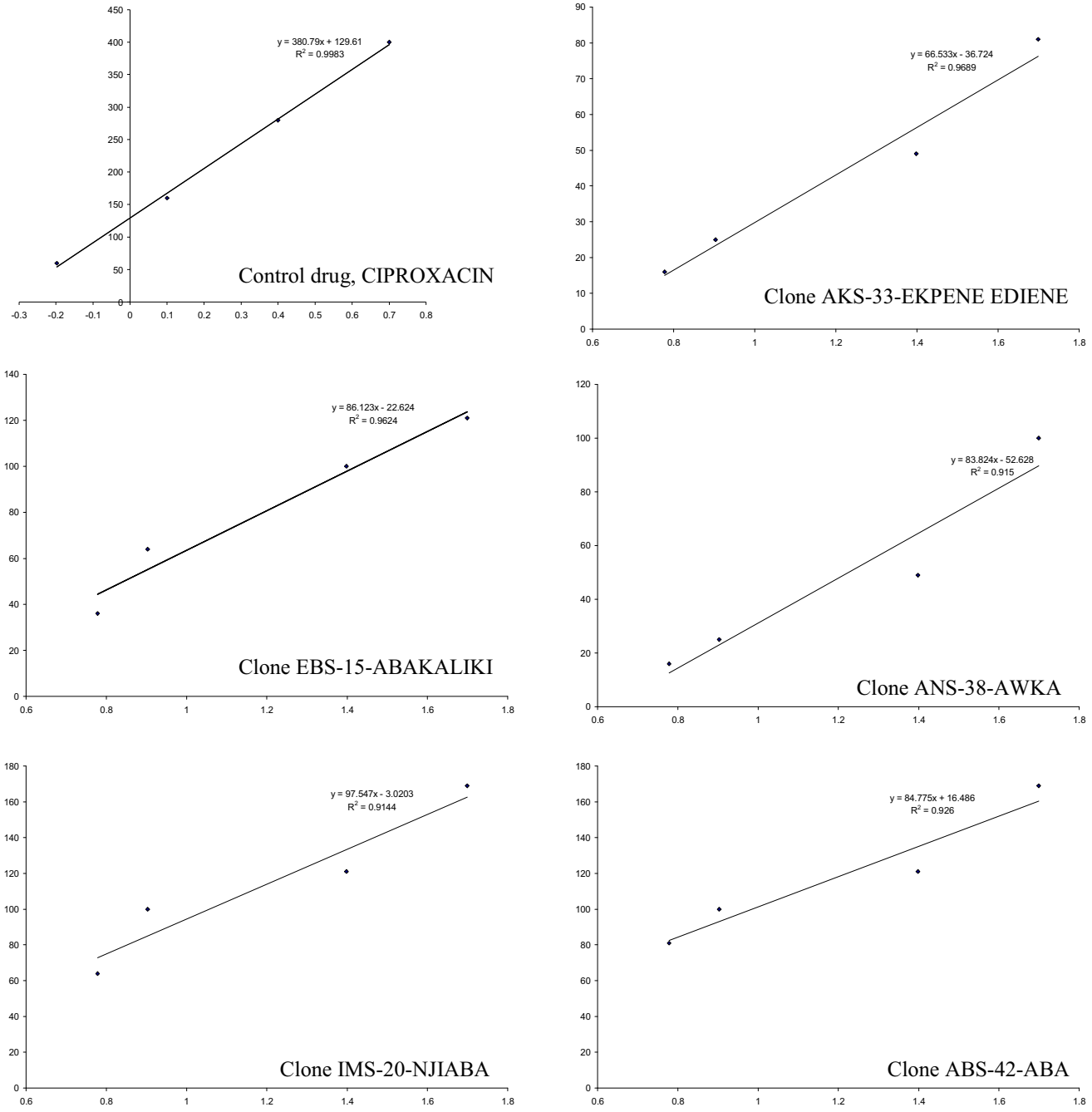

Fig. 1 Graphs depicting inhibition zone diameter squared (IZD^2; Y-axis) against the concentration of the extracts squared ($[Extract]^2$; X-axis) of the five clones and control.

Table 5 Correlation coefficients of the phytochemical contents and the MIC of *G. latifolia* clones.

	Alkaloid (%)	**Phenols (mg/100 g)**	**Tannins (%)**	**MIC (mg/ml)**
Alkaloid (%)	–	-0.381*	-0.149	0.565**
Phenol (mg/100 g)		–	-0.288	-0.677**
Tannin (%)			–	-0.85
MIC (mg/ml)				–

* =significant at 5% probability level
** =significant at 1% probability level

Table 6 Direct (diagonal) and indirect effects of the phytochemicals on MIC recorded on *S. typhi.*

Phytochemical	**Alkaloids (%)**	**Phenols (mg/100 g)**	**Tannins**	**Genotypic correlation**
Alkaloid (%)	[0.353]	0.209	0.003	0.565
Phenol (mg/100 g)	-0.135	[-0.548]	0.006	-0.677
Tannin (%)	-0.053	0.153	[-0.02]	0.085
Residual				0.431

tively correlated with tannins. **Fig. 2** facilitates an understanding of the nature of the cause and effect. It shows that MIC is the result of the phytochemicals and residual (unknown) factors on *S. typhi*. The three phytochemicals are interrelated among themselves and consequently, each influences the MIC by a direct contribution and by acting in combination with other factors with which it is correlated. The direct and indirect effects of the phytochemicals on MIC recorded on *S. typhi* shows that alkaloids had a direct positive effect on the MIC value (**Table 6**). Conversely, phenols and tannins had direct negative effects (–0.548 and –0.02, respectively) on MIC. Furthermore, alkaloids had an indirect positive effect on MIC through phenols and tannins whereas phenols had an indirect negative effect through alkaloids and tannins.

Genetic effects of clones on phytochemicals and MIC

The genetic effects of the clones on phytochemicals and

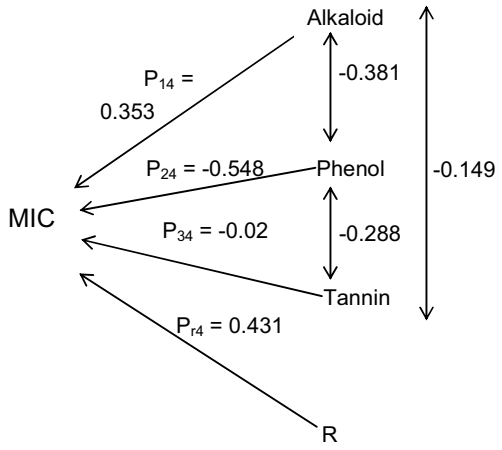

Fig. 2 A path diagram and coefficient of factors influencing minimum inhibitory concentration (MIC) of leaves of *G. latifolia* clones on *S. typhi*.

Table 7 Variance estimates and heritability (broad sense) for phytochemical composition and resultant MIC of the different *G. latifolia* clones.

Traits	2008[a]				2009[b]			
	σ_e^2	σ_g^2	σ_{ph}^2	H_{bs}^2	σ_e^2	σ_g^2	σ_{ph}^2	H_{bs}^2
				%				%
Alkaloids	0.056	21.89	21.95	99.73	0.045	18.56	18.61	99.73
Phenols	0.007	4.71	4.72	99.79	0.005	5.20	5.21	99.80
Tannins	0.008	6.16	6.17	99.84	0.006	7.11	7.12	99.86
MIC	0.002	5.61	5.62	99.82				

[a], MIC was determined in 2008.
[b], MIC was not determined in 2009.
where σ_e^2= error variance; σ_g^2 = genetic variance; σ_{ph}^2 = phenotypic variance and H_{bs}^2 = heritability in broad sense

MIC were closely related to the phenotypic effects of the clones on the determined characters (**Table 7**). Alkaloids had the highest genetic and phenotypic variance while phenols had the least. The broad sense heritability estimate was very high for all the characters ranging from 99.73% for alkaloids to 99.84% for tannins.

DISCUSSION

Phytochemical composition of *G. latifolia* clones and plant parts

Significant differences in the phytochemical components of the clones suggest the genetic contribution of the different clones to their constituents. This gives room for selection of clones that are high in phytochemical components from abundantly available clones in forests. The report of Gamaniel and Akah (1996) on the presence of phytochemicals (alkaloids, saponins, tannins, flavonoids and glycosides) on leaf extracts of unclassified *G. latifolium* species agrees with the results of this research having discovered high levels of the determined phytochemicals. However, the result of this research further indicated genetic differences in the concentration of the phytochemicals in the different clones tested. Agbo *et al.* (2005) had earlier reported clonal variability in the leaf size (big and small), colour of leaves (dark and light green) and bitterness level (very bitter and slightly bitter) of clones within this species. *G. latifolia* genotypes also exhibit significant differences and heterotic expression in certain vitamins and lycopene (Agbo *et al*. 2011). Alkaloids have been shown to confer bitterness to plants (Kupchan 1971) and significant differences in alkaloid levels in the clones in the present study suggest varied levels of bitterness in them. Such results of genetic contributions in the composition of varietal produce have also been reported in other crop species (Ogundipe *et al*. 1989; Sharanabasappa *et al*. 2007; Agbo *et al*. 2009).

The species also showed a specialized distribution of phytochemicals in the different plant parts. Phenols were higher in the leaves while alkaloids and tannins were more abundant in the stems. This result corroborates other findings (Alston and Turner 1963; Sharanabasappa *et al*. 2007) on the specialized availability of different phytochemicals on different parts of the plants. The abundance of tannins in the stems of plants was in agreement with the findings of Harbone (1991), who had earlier reported that tannins occur more in the woody tissue of plants. The concentration of phytochemicals in different parts of a plant provides room for conservation, especially if the desired phytochemical is in the leaves. This implies harvesting of leaves only which helps to conserve the stems to grow new vines and leaves.

Anti-bacterial effects of clones and their parts at varying concentrations

The increased IZD of the clones with an increase in extract concentration showed that the anti-microbial activity of the extracts is dependent on concentration and allows for the appropriate concentration level for use to be selected. The IZD of the leaves of clone ABS-42-ABA (13 mm at 50 mg/ml) was higher than the IZD of ethanolic extracts of *Lasienthera africanum* seeds and *Aframomum melegueta* leaves (7.5 and 10.5 mm at 50 mg/ml, respectively), as reported by Adegoke and Adebayo-tayo (2009) and Doherty *et al*. (2010), respectively. This suggests higher efficacy in the use of *G. latifolia* against *S. typhi* than the two other plants. Meanwhile, a high IZD of the clones supports the report of Stafford and Warren (1963) on the usefulness of the studied phytochemicals for medicinal purposes against some bacteria. A higher IZD for clone ABS-42-ABA corroborates with the clone also having an MIC closest to the control drug, ciprofloxacin. The significant differences in antimicrobial activity among the clones and the plant parts could be due to differences in the levels of phytochemicals brought about by variability in the genetic effects of the clones. The antimicrobial effect of the clones tested in this study agrees with the reports of other researchers (Eleyinmi 2007; Adegoke *et al*. 2009; Kaur and Arora 2009; Rankumar *et al*. 2009; Doherty *et al*. 2010) on the antibacterial activities of *G. latifolium* and other plant species on *S. typhi*. The results of the present study further showed the existence of genetic effects as the different clones tested had significantly varying levels of phytochemicals and antimicrobial activities. Furthermore, higher antimicrobial activity of the leaves than stems suggests that the causal phytochemicals directly responsible for the antimicrobial activity of these plants against *S. typhi* are concentrated more in the leaves.

Correlation and path analysis of *G. latifolia* traits to MIC

The significant negative correlation between phenols and MIC indicates that an increase in phenols will lead to a reduced MIC. On the other hand, the significant positive correlation between alkaloids and MIC showed that increasing levels of alkaloids will cause MIC to increase, too. The correlation observed between phytochemicals and MIC values was explained further by path analysis, which showed that phenols had a higher magnitude of effect on lowering the MIC values of the test plants, which are preferable to higher MIC values. A direct positive effect of alkaloids on MIC indicates its direct influence on the occurrence of higher positive MIC values. The positive indirect effect of alkaloids through phenols further stresses the inhibitory activity of alkaloids on the ability of phenols to lower MIC values. Alkaloids were earlier reported to have analgesic properties and not to have an antimicrobial effect (Saravanakumar *et al*. 2009). Phenols which are hydrolysed tannins on the other hand, have been reported to show antibacterial activities against a broad spectrum of microorganisms

(Scalbert 1991; Saravanakumar *et al*. 2009). The inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bonding with vital proteins such as microbial enzymes (Saravanakumar *et al*. 2009). Path analysis has been shown by other researchers to be very useful in providing additional information to determine the direct and indirect contributions to an effect (Dewey and Lu 1959; Milligan *et al*. 1990; Baiyeri *et al*. 2000; Agbo and Obi 2005). It also provides an effective means of untangling direct and indirect causes of an association and measures the relative importance of each causal factor. The implication of phenols as the causal factor responsible for high antimicrobial activity of the plant species on *S. typhi* agrees with the description of phenols as having broad spectrum antimicrobial activity (Ann and Fullick 1994). Ann and Fullick (1994) also showed that modern antiseptics are often phenol derivatives, effective in destroying bacteria. The higher level of phenols in the leaves showed higher IZD and lower MIC values than the stems, also suggesting that phenols are the causal factor responsible for higher IZD and lower MIC observed in the study.

Genetic effects of clones on phytochemicals and MIC

The high level of broad sense heritability estimates for the characters suggests the low level of environmental effects on the values of the determined traits. This is stressed further by the closeness in the estimated genotypic and phenotypic variances of all the characters. This further shows the high contributions of the genetic components of the clones in the values of phytochemical compositions and resulting MIC value on *S. typhi* as the levels of the phytochemicals are similar in both years suggesting low environmental effects. The traits are thus highly heritable and give room for easier selection of a desired clone as has been reported by other authors for ease of selection of desired plants with high heritability value from a large population (Allard 1960; Agbo and Obi 2005ab). Hence, clone ABS-42-ABA with a higher level of phenols, was identified as having higher antibacterial activity against *S. typhi* and could be selected because its traits showed high heritability estimates. High heritability suggests that similar values will be obtained in succeeding generations of the species, especially as the species can be propagated vegetatively, hence does not require further genetic recombination.

CONCLUSIONS

This study has shown that high levels of phytochemicals are present in different *G. latifolia* clones and significant differences occur in the phytochemical composition of the five clones tested due primarily to variability in genetic effects of the clones. Furthermore, the varying levels of phytochemicals of the clones and their parts resulted in varying levels of IZD from the clones and their parts. The leaves of all clones showed higher IZD than other parts. Similarly, clone ABS-42-ABA had higher antimicrobial activity (2.15 mg/ml) on *S. typhi* than any other clone. The correlation and path analysis of the phytochemicals on the MIC values showed that phenol had a significantly higher negative correlation with MIC and a higher negative direct effect on MIC. This suggests that phenols had a higher magnitude of effect in lowering the MIC value. Antibacterial properties shown by the clones and their parts provide a scientific basis and thus validate the use of the plant in treatment of typhoid fever and other bacterial diseases. Also, the varying efficacy by different genetic resources and plant parts with respect to their phytochemical contents and anti-microbial activity against *S. typhi* could be a basis for further selection and improvement of the species for pharmacological purposes against typhoid fever.

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