

# Phytochemical and antimicrobial screening of *Globimetula oreophila* (Oliv) van Tiegh and *Phragmanthera capitata* (Spreng) Balle

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**Background:** Medicinal plants will continue to be relevant in the production of lead compounds that can be utilized for its antibacterial activity directly or through chemical modification (s). **Aims:** The present study is conducted to evaluate the antibacterial activity vis-à-vis phytochemical constituents of *Globimetula oreophila* and *Phragmanthera capitata*. **Materials and Methods:** Cup plate agar diffusion assay was used to determine the minimum inhibitory concentrations (MIC) of the extracts of both plants against *Escherichia coli*, *Klebsiella* spp., *Shigella* spp., *Salmonella typhi*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by plotting the diameters of zones of inhibition against the logarithm of concentrations and the straight line graph so generated extrapolated to the value equivalent to the diameter of the cork-borer. The antilog of the corresponding value of concentration was taken as the MIC value. **Statistical Analysis:** All the values of the results of cup plate diffusion assay were expressed as means of two replicates  $\pm$  standard deviation. **Results:** Preliminary phytochemical screening reveals the presence of saponin, tannins, alkaloids, anthraquinones and flavonoid in both plants. Antibacterial screening of both plants showed that both of them were active against all clinical bacterial isolates tested with *P. capitata* having a higher activity than *G. oreophila* on most of the isolates. The ability of both plants to inhibit the growth of *S. typhi* is a major breakthrough in the control of incidence of typhoid fever notwithstanding the reports of resistance to existing antibiotics. **Conclusion:** It therefore follows that the importance of both plants in the management and control of infections with which all the bacterial isolates used for this study were associated cannot be underestimated.

**Key words:** Cup plate agar diffusion, *Globimetula oreophila*, minimum inhibitory concentrations, *Phragmanthera capitata*, *Salmonella typhi*

## INTRODUCTION

Medicinal plants will continue to serve the useful purpose towards curtailing the incidence of resistance to antibacterial agents through either the provision of 'lead' compounds that may assist in the development of new antibacterial agents that will be more potent, and efficacious with little or no toxicity to humans through the process of phytochemical screening or the use of crude extracts from the medicinal plants directly.

While some medicinal plants have been screened for the presence or otherwise of antibacterial effect, many are yet to be screened with the hope that more will be screened along this line of activity. Examples

of plants yet to be screened for their antibacterial activity are *Globimetula oreophila* (Oliv) van Tiegh and *Phragmanthera capitata* (Spreng) Balle, which belong to Loranthaceae family of parasitic mistletoes that are plants with green leaves or stems and suckers that penetrate host tissue and are therefore both photosynthetic and parasitic (hemiparasitic). Loranthaceae (mistletoes) is a large family of about 75 genera and over 900 species.<sup>[1]</sup> The family has three terrestrial, root parasitic genera and 72 genera of aerial branch parasites.<sup>[2]</sup>

Six major genera are found in Nigeria namely: Tapinanthus (Blume) Reichb., Agelanthus Tieghem, Loranthus L., *Globimetula* Tieghem, *Phragmanthera* Tieghem and Englerina Tieghem. Tapinanthus is far more widespread in the Nigerian Savanna.<sup>[3,4]</sup>

*G. oreophila* (Oliv) van Tiegh and *P. capitata* (Spreng) Balle are usually associated with kolanut trees thereby reducing their growth and can result in the death of the trees with heavy infestation. *G. oreophila* is called afomo in Yoruba speaking area of Nigeria, Kauci in Hausa and Children's matches in Eastern Cameroon presumably due to the match-like shape of the flower.

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Mistletoes have been reportedly used for the treatment of cancer, hypertension, diabetes, arthritis, heart problems, insomnia, infertility and pneumonia.<sup>[5-11]</sup>

The use of the plants in the treatment of pneumonia, a bacterial infection, necessitated this study.

This work therefore aimed at evaluating the antibacterial activity of *G. oreophila* (Oliv) van Tiegh and *P. capitata* (Spreng) Balle against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Shigella* spp. as well as *Salmonella typhi* vis-à-vis their phytochemical screening.

## MATERIALS AND METHODS

### Collection and Authentication of Plant Material

Both *G. oreophila* (Oliv) van Tiegh and *P. capitata* (Spreng) Balle samples were collected in Sagamu, Ogun State and authenticated at the Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria where herbarium samples were prepared and deposited with Forestry Herbarium Identification numbers: 108814 and 108815 respectively.

### Plants Extraction

Total 1000 g each of powdered leaves of *P. capitata* and *G. oreophila* was extracted by maceration (cold extraction) at room temperature in methanol for 3 days and the methanol extracts obtained were evaporated in rotary evaporator at 40°C after filtration to constant weights at low temperature. The percentage yield of the extract was thus calculated.

### Phytochemical Screening

Tests for the presence of Alkaloids, Saponins, Tannins, Anthraquinones, Cardiac glycosides and Flavonoids were performed on the crude extracts of *P. capitata* and *G. oreophila*.<sup>[12]</sup>

### Microbiological Test

Clinical isolates of *S. aureus*, *E. coli*, *P. aeruginosa*, *Proteus mirabilis*, *K. pneumoniae*, *S. typhi* and *Shigella* spp. were collected on agar slant from the Pathology Department of Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria and authenticated by conventional biochemical tests such as Gram stain, catalase test, oxidase test, indole test, hydrogen sulphide production as well as sugar fermentation.

A speck of each isolate was suspended in sterile nutrient broth and incubated at 37°C for 24 h.

0.5 McFarland equivalent (0.1 ml) of overnight grown culture of each isolate was seeded in sterile nutrient agar medium, poured and allowed to set in sterile Petri dish. With the aid of a sterile cork-borer of known

diameter (5 mm), wells were bored in the nutrient agar medium to accommodate different concentrations of the crude extracts of the plants. The concentrations of the crude extracts used were 100 mg/ml, 60 mg/ml, 50 mg/ml, 40 mg/ml, 30 mg/ml, 20 mg/ml and 10 mg/ml. Ciprofloxacin injection (5 mg/ml) was used as a positive control while methanol was used as a negative control. The plates were left at room temperature for 1 h to allow diffusion after which the plates were incubated at 37°C for 24 h and finally examined for the presence of inhibition zones that were measured using a transparent ruler and recorded.

### Determination of Minimum Inhibitory Concentration

Diameters of zones of inhibition were plotted against the logarithm of concentrations and the straight line graph so generated was extrapolated to the value equivalent to the diameter of the cork-borer (5 mm). The antilog of the corresponding value of concentration was taken as the MIC value.<sup>[13]</sup>

## RESULTS

The percentage yield of the extracts for both *G. oreophila* and *P. capitata* are 7.4% and 6.95% respectively as shown in Table 1. A preliminary phytochemical study of the plants revealed the presence of saponin, tannins, alkaloids, flavonoids as well as combined anthraquinones as shown in Table 2. The organisms showed varying susceptibilities to the tested concentrations of both plants as exemplified in Table 3.

With *G. oreophila*, no organism showed any zone of inhibition between 10 mg/ml and 40 mg/ml concentrations

**Table 1: Percentage yield of the crude extracts of *Globimetula oreophila* and *Phragmanthera capitata***

Plant's name	Weight of the crude extract	Percentage yield
<i>Globimetula oreophila</i>	74 g	7.4
<i>Phragmanthera capitata</i>	69.5 g	6.95

**Table 2: Phytochemical constituents of *Globimetula oreophila* and *Phragmanthera capitata***

Secondary metabolites tested for	<i>Globimetula oreophila</i>	<i>Phragmanthera capitata</i>
Free anthraquinones	-	-
Combined anthraquinones	+	+
Cyanogenic glycosides	-	-
Cardiac glycosides	-	-
Flavonoids	+	+
Saponin	+	+
Alkaloids	+	+
Tannins	+	+

+ - Presence; - - Absence

**Table 3: Mean zones of inhibition of various concentrations of *Globimetula oreophila* and *Phragmanthera capitata* against selected bacterial isolates**

Organisms used	Mean zones of inhibition of extract of <i>Globimetula oreophila</i> (mm) at different concentrations in mg/ml							Mean zones of inhibition of extract <i>Phragmanthera capitata</i> (mm) at different concentrations in mg/ml							S
	10	20	30	40	50	60	100	10	20	30	40	50	60	100	5
<i>Escherichia coli</i>	0	0	0	0	11.70	11.90	14.30	0	0	0	0	11.60	12.30	14.60	20.00
					SD	SD	SD					SD	SD	SD	
					0.2	0.3	0.4					0.2	0.4	0.4	
<i>Klebsiella pneumoniae</i>	0	0	0	0	13.50	15.10	16.20	0	0	0	0	14.70	15.30	17.40	23.90
					SD	SD	SD					SD	SD	SD	
					0.4	0.1	0.2					0.3	0.4	0.4	
<i>Proteus mirabilis</i>	0	0	0	0	11.20	12.10	14.00	0	0	0	0	11.40	12.20	14.50	26.50
					SD	SD	SD					SD	SD	SD	
					0.3	0.1	1.0					0.5	0.3	0.6	
<i>Pseudomonas aeruginosa</i>	0	0	0	12.80	13.50	14.30	15.60	0	0	0	0	13.30	15.50	17.10	21.00
				SD	SD	SD	SD					SD	SD	SD	
				0.2	0.4	0.4	0.4					0.2	0.7	0.1	
<i>Shigella spp.</i>	0	0	0	0	13.50	13.70	15.10	0	0	0	0	13.10	13.50	14.40	23.00
					SD	SD	SD					SD	SD	SD	
					0.4	0.3	0.1					0.2	0.4	0.4	
<i>Salmonella typhi</i>	0	0	0	0	11.60	12.30	14.40	0	0	0	0	11.90	13.50	15.50	27.30
					SD	SD	SD					SD	SD	SD	
					0.5	0.4	0.5					0.3	0.4	0.5	
<i>Staphylococcus aureus</i>	0	0	0	0	14.70	16.40	18.50	0	0	0	0	14.20	16.40	18.50	27.00
					SD	SD	SD					SD	SD	SD	
					0.4	0.4	0.2					0.2	0.3	0.6	

S – Standard drug (ciprofloxacin); SD – Standard deviation

except the *P. aeruginosa* with 12.8 mm zone of inhibition at 40 mg/ml. However, at higher concentrations of 50, 60 and 100 mg/ml, zones of inhibition as a measure of susceptibility varied among all the tested bacteria with *S. aureus* the most susceptible to all these concentrations. While *P. mirabilis* was the least susceptible organism at 50 and 100 mg/ml concentrations, *E. coli* was the least at 60 mg/ml. This observation may be attributed to the difference in the nature of cell wall of *S. aureus*, a gram-positive bacterium, and *P. mirabilis* and *E. coli*, both gram-negative bacteria. MIC are 19.9 mg/ml, 3.5 mg/ml, 12.1 mg/ml, 14.1 mg/ml, 14.1 mg/ml, 10.0 mg/ml and 8.9 mg/ml for *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Shigella spp.*, *S. typhi*, *P. aeruginosa* and *S. aureus* respectively.

With *P. capitata*, no organism was susceptible to 10, 20, 30 and 40 mg/ml concentrations while higher concentrations of 50, 60 and 100 mg/ml produced varying zones of inhibition among the bacterial isolates used for the study. While *S. aureus* was the most susceptible to all concentrations that produced zones of inhibition, *P. mirabilis* was the least susceptible at 50 and 60 mg/ml concentrations and *Shigella* at 100 mg/ml. MIC are 8.9 mg/ml, 10.0 mg/ml, 7.1 mg/ml, 12.1 mg/ml, 1.5 mg/ml, 14.1 mg/ml and 3.2 mg/ml for *S. aureus*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Shigella spp.*, *S. typhi* and *P. aeruginosa* respectively.

### Statistical Analysis

All the values of the results of cup plate diffusion assay were expressed as means of two replicates  $\pm$  standard deviation.

### DISCUSSION

While *P. capitata* was more active on all bacterial isolates with the exception of *Shigella* on which *G. oreophila* was more active, the same level of activity was displayed on *S. aureus* by both plants. The corollary to this observation is that although both plants were active against all the bacterial isolates used for the study, but while *P. capitata* will be a better choice against infections caused by *E. coli*, *S. typhi*, *P. mirabilis*, *P. aeruginosa*, *K. pneumoniae*, *G. oreophila* will be better against infections caused by *Shigella spp.* such as dysentery while either plants could be used against infections to which *S. aureus* has been associated.

The ability of the extracts of both plants to inhibit *S. typhi* isolate used in the study showed both plants to be promising in controlling the incidence of typhoid fever especially now that there are reports of *S. typhi* acquiring resistance to the existing antibiotics used in its control.<sup>[14]</sup>

Nonetheless, all the bacterial isolates used showed susceptibility to the standard drug used as control in this

study with *S. typhi* having the highest level of susceptibility. This corroborates the choice and use of 4-Quinolones to which ciprofloxacin belongs in the treatment of typhoid fever. 4-Quinolones act by inhibiting DNA gyrase, an enzyme responsible for unwinding and coiling of DNA strand, thereby preventing the process of replication.

However, activity-guided bioassay study on the plants is recommended with a view to identifying which of the phytochemical constituents is responsible for the observed antibacterial activity moreover that there had been reports attributing antibacterial activity to each of saponins, tannins, alkaloids, flavonoids as well as anthraquinones.<sup>[15-19]</sup> This will pave the way for whether the plants can be combined in the treatment of infections to which all the tested bacterial isolates have been associated.

## CONCLUSION

It is evident that the importance of *G. oreophila* and *P. capitata* in the control of bacterial infections cannot be underscored as the methanol extracts of both plants displayed a significant activity against both the gram-positive and gram-negative bacterial isolates used in this study.

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