Available: DOI: https://doi.org/10.54117/gjpas.v2i1.95 *Research article*

Gadau Journal of Pure and Allied Sciences
Gadau J Pure Alli Sci, 2(1): 50-57 (2023)
ISSN: 2955-1722
DOI: https://doi.org/10.54117/gjpas.v2i1.95

Antibacterial effects of the leaves and twigs of *Turraea vogelii* on some enteric pathogens

Haishat Y. Olufadi-Ahmed [1], Philip A. Idowu [2], and Haruna J. Audu [3]

[1]Department of Pharmaceutical Microbiology & Biotechnology, University of Ilorin, Ilorin, Nigeria.
[2]Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria.
[3]Department of Pharmaceutical Microbiology & Biotechnology, Kaduna State University, Kaduna, Nigeria.

**Correspondence: ah.olufadi@unilorin.edu.ng*

Abstract

Qualitative and quantitative phytochemical analysis as well as antibacterial analysis of extracts of *Turraea vogelii* (Hook F.) leaves and twigs on some enteric pathogens were reported in the current study. The qualitative phytochemical screening of the leaf extracts showed the presence of saponins, flavonoids, cardiac glycosides, anthraquinones, terpenoids, steroids and alkaloids. In addition, the quantitative phytochemical screening showed that the greatest percentage yield was from the methanol extract with alkaloids (8.8%) and terpenoids (8.7%) showing the highest concentrations. The antibacterial effects of hexane, ethyl acetate and methanol extracts of *Turraea vogelii* at concentrations 80 mg/mL, 40 mg/mL and 20 mg/mL each were studied by the pour plate method. Clinical isolates of *Escherichia coli, Salmonella typhi* and *Proteus mirabilis* (five each) obtained from University College of Health (UCH), Ibadan, Nigeria were employed as test organisms. Gentamicin was used as control at concentration of 10 µg/mL. Preliminary antimicrobial assay using only methanol showed antimicrobial activity in both leaves and twigs. Results showed that *Escherichia coli* was most susceptible to all extracts of the leaves while *Proteus mirabilis* was the least susceptible. For the twigs, *Salmonella typhi* was most susceptible to the hexane extract, *E. coli* was most susceptible to ethyl acetate extract and *Proteus mirabilis* was most susceptible to the methanol extract. Minimum Inhibitory Concentration (MIC) of leaves showed that its hexane extract is bacteriostatic at 40 mg/mL but at 20 mg/mL, it showed bacteriostatic activity against *Proteus mirabilis*. The methanol extract of the twigs had an MIC of 20 mg/mL for most of the isolates collected except the *Salmonella typhi* that had an MIC of 40 mg/mL. Minimum Bactericidal Concentration (MBC) of the ethyl acetate extract of leaves and methanol extract of twigs was 40 mg/mL. This study has demonstrated the antibacterial effect of leaves and twigs extracts of *T. vogelii* on some enteric pathogens.

Article History

Received: 04/03/2023
Accepted: 27/03/2023
Published: 24/04/2023

Keywords
Turraea vogelii;
Leaves;
Twigs extract;
Antibacterial;
Enteric pathogens;
Clinical isolates

How to cite this paper: Olufadi-Ahmed, H.Y., Idowu, P.A., and Audu H.J. (2023). Antibacterial effects of the leaves and twigs of *Turraea vogelii* on some enteric pathogens. *Gadau J Pure Alli Sci, 2(1): 50-57*. https://doi.org/10.54117/gjpas.v2i1.95.

1.0 Introduction

Herbal medicine has been widely formulated and used as an integral component of primary health care in Nigeria, Republic of China, Ethiopia and Argentina. Assortments of herbal preparations are being used to treat various kinds of microbial diseases (Akinyemi *et al*., 2005). Various studies have shown us that medicinal plants contain bioactive constituents known as secondary metabolites that are responsible for their beneficial properties (Alfalluous *et al*., 2017).

Turraea vogelii are trees or shrubs scattered mainly in Asia, Australia, Tropical Africa and Madagascar belonging to the genus *Turraea* and the family Meliaceae (Walkty *et al*., 2014).

This plant is a woody climber that can grow up to 5m high. It can also grow as an understory scandent shrub.

In Nigeria, it is commonly known as *Ovioza* in Edo and *aha omode* in Yoruba (Burkhill, 1985). Traditionally, the plant is used to treat stomach pains, eczema and blennorrhoea. Its leaves, root and bark are claimed to be used in the treatment of cough, stomach ache, whooping cough, impotence, filariasis and as an aphrodisiac (Odugbemi, 2008). The plant has also been proven to possess anti-inflammatory activity (Olumoh-Abdul *et al.*, 2019), cytotoxic and antiplasomodial activity (Ogbole *et al.*, 2016) as well and potent antioxidant properties (Olufadi-Ahmed & Idowu, 2019).

Data on *T. vogelii* antibacterial activities in Nigeria is limited. This, therefore, formed the basis for this study. The study was designed to investigate the qualitative and quantitative constituents as well as the antibacterial effect of the leaves and twig extracts of *T. vogelii* on three enteric pathogens. Both root and bark of the plant were used in this study for investigative purposes since ethno-botanically, both parts are used for several ailments.

2.0 Materials and Methods

2.1 Collection of Organisms

Fifteen clinical isolates (enteric pathogens) were used in this research. All the clinical strains were collected from the University College Hospital, Ibadan and characterized and identified at the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria. These include five strains of *Salmonella typhi*, five strains of *Proteus mirabilis* and five strains of *Escherichia coli.*

2.2 Plant Material Collection and Identification

The plant sample of was collected at Onigambari forest reserve, Oyo state. Identification and authentication was carried out at Forestry Research Institute of Nigeria (FRIN) Ibadan, Oyo State and assigned the voucher specimen number FHI 110392.

2.2.1 Qualitative and Quantitative Phytochemical Analysis

Phytochemical screening was carried out on the methanol, ethyl acetate and hexane fractions of *T. vogelii* leaves to test for the presence of alkaloids, flavonoids, saponins, tannins, anthraquinones, tannins, steroid, cardiac glycosides and phenolic compounds according to protocol by Trease and Evans, 1989.

2.2.2 Preparation of the Leaves and Twigs Extract

The leaves and twigs of the medicinal plant were chopped into smaller pieces and air-dried for three weeks and subsequently milled. 400g of each part of the pulverized plant material was successively extracted by soxhlet extraction using hexane, ethyl acetate and methanol in order of increasing polarity using the method described by Trease and Evans, 1989. The obtained extracts were evaporated to dryness and the concentrate used in the antimicrobial screening at dilutions of 20 mg/mL, 40 mg/mL and 80 mg/mL using their extraction solvents.

2.3 Antimicrobial Agents

The chemotherapeutic agent used in the antimicrobial assay as positive control against the test organisms was gentamicin at 10µg/mL while the negative controls include 40% methanol, ethyl acetate and hexane.

2.4 Preparation of Microbial Culture

Overnight bacterial cultures were obtained by sub-culturing from stored slopes. A small inoculum size was fetched from the slopes using a flamed inoculating loop into 5mL nutrient broth in a test-tube. These were then properly labelled and incubated for 18 hours at 37^{o}C.

2.5 Susceptibility Testing

All the test microorganisms were tested for their susceptibility to the plant extracts by means of agar diffusion technique (CLSI, 2014). The pour plate method was used for this assay.

2.6 Preparation of the Pour Plates

0.1mL of each organism was taken and put into 9.9mL of sterile distilled water to obtain 10^{-2} inoculum concentration of the organism.

From the diluted organism (10^{-2}), 0.2mLwas taken into the prepared sterile nutrient agar cooled to about 40-45^{o}C, then poured into sterile petri dishes and allowed to solidify for about 45-60 minutes. Using a sterile cork-borer of 8mm diameter, the wells were made according to the number of test-tubes for the experiment. For this work, 8 wells were made. The graded concentrations of extracts were put into the wells accordingly including the controls.

The plates were left on the bench for about 1 hour to allow the extract diffuse properly into the nutrient agar. The plates were the incubated for 18-24 hours at 37^{o}C.

2.7 MIC and MBC

2.7.1 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of each extract was determined by agar dilution method. The plant extracts were prepared in graduated decreasing concentrations and seeded into nutrient broth until it produced turbidity equal to the 0.5 McFarland standard No. 1 from which the organisms were then streaked on each plate. All plates were incubated at 37^{o}C for 24 hours. The MIC was taken as the minimum inhibitory concentration of plant extracts that inhibited discernible bacterial growth on the plates.

2.7.2 Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined for all the isolates by sub-culturing from all the test mixtures that failed to show growth in the tube of MIC on Nutrient agar plates using a sterile wire loop and incubated at 37^{o}C for 24 hours. The MBC was recorded as the lowest concentration of plant extract inhibiting growth.

2.8 Chromatography

2.8.1 Column Chromatography of Plant Extracts

Ethyl acetate extract of *T. vogelii* was first analysed on a pre-coated TLC plate using a solvent system containing ethyl acetate-hexane (3:7) and ethyl acetate-hexane (7:3) as a mobile phase. This was viewed under the UV lamp at 536nm and sprayed with vanillin sulphate. 5g of ethyl acetate extract of *Turraea vogelii* leaves was weighed and dissolved in acetone. Silica gel was poured into the dissolved extract and set aside to dry. Two hundred grams of silica gel was also weighed and placed in a beaker where hexane was poured into it to make slurry and stirred to remove air bubbles. The column was clamped onto a retort stand firmly and packed with the silica gel slurry. Hexane was poured into the slurry to achieve complete packing of the silica gel before the dry extract was introduced and sealed off with cotton wool to prevent disturbance of the extract bed. A little amount of hexane was left above the bed to prevent caking of the silica gel. 400ml of hexane/ethyl acetate mixture in a ratio 9:1 was initially introduced into the column and tap was opened to collect eluate in aliquots of about 30ml each. Several hexane/ethyl acetate mixtures in other ratios were introduced sequentially viz (80:20, 70:30, 60:40: 50:50, 40:60, 30:70 and 0:10). In other to elute other compounds of that may not be polar enough to be eluted by hexane/ethyl acetate mixture, 200ml of ethyl acetate/methanol mixture was used in ratio 9.5:0.5, 9:1, 8.5:1.5, 8:2, 7:3 and finally washed with 0:10. They were successively poured and eluates collected in well labelled bottles. These fractions are then subjected to thin layer chromatography (TLC).

2.8.2 Thin Layer Chromatography

147 eluates were collected and spotted on TLC plate before being developed in a solvent tank. The developed plates were allowed to dry and then viewed under UV lamp at 365nm to see the bands. The plate was then sprayed using vanillin sulphate. Those fractions with like bands under the UV lamp are then pooled together and concentrated to be used in other bioassays.

2.9 Antimicrobial Assay of Pooled Fractions

All 147 fractions of approximately 30ml each was pooled to only 6 fractions based on the TLC readings. An inoculum size of 0.1ml of each organism was taken and put into 9.9ml of sterile distilled water to obtain 10^{-2} inoculum concentration of the organism. From the diluted organism (10^{-2}), 0.2ml was taken into the prepared sterile nutrient agar cooled to about 40-45^{o}C, then poured into sterile Petri dishes and allowed to solidify for about 45-60 minutes. Using a sterile cork-borer of 8mm diameter, the wells were made according to the number of test-tubes for the experiment. For this work, 6 wells were made. The different extracts were put into the wells accordingly including the controls. The plates were left on the bench for about 1 hour to allow the extract diffuse properly into the nutrient agar. The plates were the incubated for 18-24 hours at 37^{o}C.

3.0 Results and Discussion

The quest for safer and more effective antimicrobial therapies is necessitated by the resistance that pathogens soon develop against current drugs. Plants being reservoirs of various types of bioactive molecules are target of extensive research world-wide (Walkty *et al*., 2014; Sahreen *et al*., 2010; Elgayyar *et al*., 2001).

In this study, the phytochemical components of different solvent extracts of *T. vogelii* leaves were quantitatively analysed. The different extracts exhibited diverse phytochemical profiles depending on polarity of solvents used (Table 1). out of the three extracts used (methanol, ethyl acetate and hexane), the methanol extract exhibited the most occurrence of phyto-constituents such as saponins, flavonoids, cardiac glycosides, anthraquinones, terpenoids, steroids and alkaloids followed closely by the ethyl acetate fraction which showed the presence of tannins, flavonoids, anthraquinones, terpenoids, phenols and alkaloids. The presence and absence of any phytochemical constituent depends on the polarity of solvent medium used for its extraction as well as the physiological properties of individual taxa. (Pradeepa *et al*., 2016).

Table 2 shows the result of quantitative analysis of the phytochemical constituent of *T. vogelii* leaves. Here, phyto-constituents such as alkaloids, flavonoids, saponins, tannins, terpenoids and total phenol were quantitatively analysed. The greatest percentage yield observed came from the methanol extracts with alkaloids (8.8%) and terpenoids (8.7%) showing the highest concentration. This is followed by the ethanol extract also with alkaloid (7.7%) as the highest concentration observed.

The Multiple Antibiotics Resistance Index (MARI) of the clinical isolates used in this study is represented in the figure above. It largely shows that many of these enteric pathogens display multiple resistance to antibiotics and may pose a threat in the treatment of infections they may cause.

Alkaloids are compounds that are diverse in structure and have been reported to possess antimicrobial activity by inhibiting activity of enzymes (Othman *et al*., 2019) or by intercalating into cell wall and DNA of bacteria (Padey and Kumar, 2013).

Tannins which is consumed as a food product in plants and vegetables is known to hamper bacterial reproduction by stopping the activity of certain enzymes that are important in microbial metabolism (Geidan *et al*., 2007; Sharma *et al*., 2013).

Table 1: Qualitative phytochemical screening of extracts of *T. vogelii* leaves

Test	Hexane fraction	Ethyl acetate fraction	Methanol fraction
Saponins	-ve	-ve	++ve
Tannins	-ve	+ve	-ve
Flavonoids	+ve	+ve	++ve
Cardiac glycosides	-ve	-ve	+ve
Anthraquinones	-ve	+ve	+ve
Terpenoids	+ve	+ve	+ve
Steroids	+ve	-ve	+ve
Phenol	-ve	+ve	-ve
Alkaloids	-ve	+ve	+ve

Interpretations
+ve: present
++ve: abundantly present
-ve: absent

Table 2: Quantitative phytochemical analysis of extracts of *T. vogelii* leaves

Sample	% Alkaloids content	% Flavonoid content	% Saponin content	% Tannin content	% Terpenoid content	% Total phenol
Hexane fraction	-	0.05	-	-	4.30	-
Ethyl acetate fraction	7.70	2.23	-	0.23	0.90	0.19
Methanol fraction	8.80	2.17	2.90	-	8.70	-

- Absence of phytochemical constituent

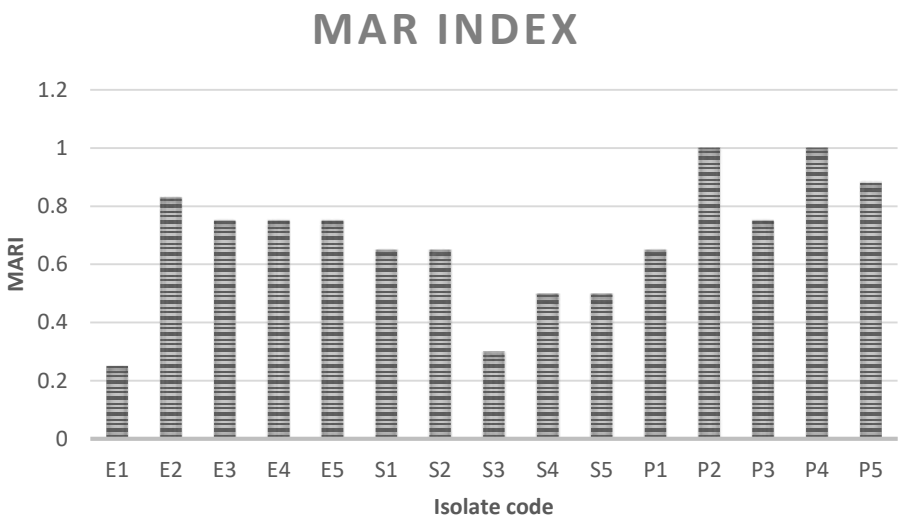

Figure 1: Multiple Antibiotics Resistance Index of isolates to selected antibiotics

Table 3: Antimicrobial susceptibility of enteric pathogens to extracts of *Turraea vogelii* leaves and twigs.

		DIAMETER OF ZONES OF INHIBITION (mm)														
		Salmonella typhii					***Escherichia coli***					***Proteus mirabilis***				
Extract	**Conc.mg /ml**	**S1**	**S2**	**S3**	**S4**	**S5**	**E1**	**E2**	**E3**	**E4**	**E5**	**P1**	**P2**	**P3**	**P4**	**P5**
Hexane extract of *T. vogelii* twigs	80	14	17	16	16	14	12	12	17	14	24	12	13	14	14	12
	40	12	22	14	18	22	12	12	13	12	15	11	12	12	12	12
	20	20	28	20	12	14	15	16	11	10	12	11	10	10	10	11
Ethyl acetate extract of *T. vogelii* twigs	80	12	0	14	12	14	12	16	14	14	18	14	15	16	0	22
	40	10	0	14	14	16	10	22	13	14	14	14	14	14	0	20
	20	10	0	0	10	14	10	16	12	18	13	18	12	12	18	14
Methanol extract of *T. vogelii* twigs	80	10	0	0	10	14	0	0	16	12	0	16	14	15	18	12
	40	0	0	12	10	12	0	0	14	0	12	12	12	12	15	0
	20	0	0	0	12	12	0	0	10	0	11	0	11	10	0	10
Hexane extract of *T. vogelii* leaves	80	22	20	20	16	16	16	20	10	18	18	0	0	12	14	20
	40	10	14	18	14	14	25	18	0	16	18	0	12	12	12	10
	20	18	14	16	14	20	12	16	12	14	22	20	13	14	12	16
Ethyl acetate extract of *T. vogelii* leaves	80	14	14	16	24	18	18	16	16	25	25	20	14	16	20	25
	40	16	16	14	16	12	12	14	14	20	20	25	12	14	12	12
	20	14	12	14	14	18	22	10	11	20	16	24	0	12	20	16
Methanol extract of *T. vogelii* leaves	80	0	0	12	12	10	0	10	12	9	11	13	12	12	12	11
	40	0	0	10	10	12	0	0	0	10	9	11	0	10	0	0
	20	14	0	0	14	12	0	0	0	10	10	0	0	0	0	0
Gentamicin	10 µg/mL	16	18	20	24	26	18	11	20	19	17	24	12	15	0	13

Table 4: Minimum inhibitory concentration (MIC) of leaves and twigs extracts of *Turraea vogelii*

	Minimum Inhibitory Concentrations in mg/ml														
	Salmonella typhii					*Escherichia coli*					*Proteus mirabilis*				
Extract	S1	S2	S3	S4	S5	E1	E2	E3	E4	E5	P1	P2	P3	P4	P5
Hexane extract of *T. vogelii* twigs	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Ethyl acetate extract of *T. vogelii* twigs	80	80	80	80	80	80	80	80	80	80	40	80	80	80	40
Methanol extract of *T. vogelii* twigs	20	20	20	20	20	40	40	40	40	40	20	20	20	20	20
Hexane extract of *T. vogelii* leaves	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Ethyl acetate extract of *T. vogelii* leaves	40	40	40	40	40	40	40	40	40	40	20	40	40	40	20
Methanol extract of *T. vogelii* leaves	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	10	>80	>80	40	10

Table 5: Minimum bactericidal concentration (MBC) of leaf and twig extracts of *Turraea vogelii*

	Minimum Bactericidal Concentrations in mg/ml														
	Salmonella typhii					*Escherichia coli*					*Proteus mirabilis*				
Extract	S1	S2	S3	S4	S5	E1	E2	E3	E4	E5	P1	P2	P3	P4	P5
Hexane extract of *T. vogelii* twigs	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl acetate extract of *T. vogelii* twigs	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80
Methanol extract of *T. vogelii* twigs	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Hexane extract of *T. vogelii* leaves	ND	ND	ND	ND	>80	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl acetate extract of *T. vogelii* leaves	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Methanol extract of *T. vogelii* leaves	80	80	80	80	>80	80	80	80	80	80	80	80	80	80	80

ND= Not determined

Table 6: Antimicrobial assay of ethyl acetate fractions of the leaves of *T. vogelii* obtained from Column fractionation

CONC	100µg/ml						1mg/ml						10 mg/mL					
ORG	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Sal4	-	11	-	-	-	-	11	9	-	22	-	ND	ND	12	-	ND	22	ND
Sal5	-	-	-	-	-	-	-	-	-	22	-	ND	ND	-	-	ND	18	ND
Ecoli3	13	-	-	-	-	-	9	-	-	-	12	ND	ND	12	-	ND	18	ND
Ecoli5	-	-	-	10	-	-	-	9	-	12	-	ND	ND	-	-	ND	-	ND
Prot1	-	-	-	11	-	-	10	10	-	-	-	ND	ND	10	-	ND	-	ND
Prot5	-	-	10	18	12	-	-	-	-	18	-	ND	ND	-	-	ND	28	ND

- = no zone of inhibition ND = Not determined

The tannins successfully extracted by the solvent, ethyl acetate may be responsible for the slightly better than standard antimicrobial effect observed. The phenol content, although low may also be a contributing factor to the antimicrobial potency of the ethyl acetate fraction. Phenols isolated form medicinal plants are well documented to possess useful pharmacological and biological properties such as antiviral, antimicrobial, anti-inflammatory and cytotoxic activity which validates their use of the plant in ethnomedicine (Lee *et al*., 2013). Considering that the percentage concentration of alkaloids quantified in this research is the highest, we might be right to say that the bacterial killing ability of the extract is justified by the presence of alkaloids.

Multiple Antibiotics Resistance Index (MARI) is a parameter that indexes phenotypes of clinical isolates that exhibit resistance to three or more antibiotics (Mustapha *et al.*, 2020). MARI of the clinical isolates collected is represented in Figure 1. There is a high MARI observed in all isolates in this study ranging from 0.25 to 1. A MARI value of >0.2 suggests multiple antibiotics resistance and is an indication of the presence of highly resistant bacteria (Joseph *et al.*, 2017).

It was discovered in this research that the ethyl acetate fraction of the leaves of *Turraea vogelii* was most potent extract of the leaves while the methanol fraction of the twigs of *Turraea vogelii* was most active extract against the enteric pathogens followed closely by the ethyl acetate fraction. This is probably due to the polarity of the constituents in the extract. Ethyl acetate, being polar aprotic will extract compounds of like polarity and so will methanol which will remove polar compounds. Preliminary antimicrobial screening of the methanol extract of plant leaves and twigs revealed promising antimicrobial effect of the plant after it displayed wide zones of inhibition against gentamicin used as control.

Table 3 shows the antimicrobial action of different fractions of the extract against the enteric pathogens. The ethyl acetate fraction of the leaves showed the best activity of all the leaf fractions while the methanol fraction of twigs showed the best activity of the entire twigs fraction followed closely by its ethyl acetate fraction.

The Minimum Inhibitory Concentration is the lowest concentration of an antimicrobial that will inhibit the growth of a micro-organism after overnight incubation (Peng and Mabberly, 2008). It is generally regarded as the most basic laboratory measurement of activity of an antimicrobial agent against an organism (**Turnidge *et al*., 2003)**. Table 4 shows the MIC of leaves and twigs of *Turraea vogelii* extracts. Inhibition of activity of the pathogens was most prominent at 40 mg/mL of the ethyl acetate fraction of the leaves with the exception of two strains of *Proteus mirabilis* (P1 and P5) that were inhibited at 20 mg/mL. The hexane and methanol fractions of the leaves showed that the MIC of those fractions may be at a concentration greater than 80 mg/mL. As for the twigs, its methanol fraction exhibited an MIC ranging from 20-40 mg/mL while its ethyl acetate **fraction showed MIC ranging from 40-80 mg/mL.** According to Fabry *et al.* (1998), active crude extracts are those having MIC values <8000µg/mL. With this, it is safe to conclude that the ethyl acetate fraction of leaves and methanol fraction of twigs of *T. vogelii* are active and contain compounds that could be developed to elicit better antimicrobial activity that will compare favourably with the drug positive control which killed the organism within the same contact time though at a lower concentration. The Minimum Bactericidal Concentration for the methanol extract of twigs and ethyl acetate extract of leaves of *T. vogelii* was 40 mg/mL for all isolates while the rest showed an MBC of ≥80 mg/mL. MBC of the hexane fractions were not determined because their MIC was greater than 80 mg/mL as represented on Table 5. Yet again, the most potent fractions are the ethanol extract of twigs and ethyl acetate fraction of leaves.

Table 6 shows the results of the antibacterial action of the ethyl acetate fractions of the plant leaves obtained by column fractionation. 147 fractions of approximately 30ml each that were obtained from the column fractionation and pooled to only 6 which were used for assay at 100 µg/mL. The active antimicrobial principle may not have been in all the fractions due to the lack of zones of inhibition observed in some fractions. Fractions A, C, D, E showed slight antimicrobial activity at 100 µg/ml. Fraction D however showed the best antimicrobial activity at a higher concentration of 1mg/ml but could not be determined at 10 mg/mL due to insufficiency of extract. Fraction E was very active against the selected organisms at 10 mg/mL.

The present report of antibacterial activity of crude extracts of *T. vogelii* against enteric pathogens was based on in-vitro study. It is therefore necessary to test the activity in an in vivo study. Also, to have better application of these crude extracts in enteric pathogen infections, other bacterial strains of these pathogens should be tested in the future.

4.0 Conclusion

The *in vitro* antimicrobial activities of the crude extracts of hexane, methanol and ethyl acetate fractions of leaves and twigs of *Turraea vogelii* were investigated and it was observed that the extracts of the plant possesses antimicrobial activity comparable to Gentamicin against the selected pathogens. In conclusion, it can be said that the plant has good potential for development of new antibacterial drugs. Further studies still needs to be carried out in order to identify, isolate and elucidate active constituent of bioactive extracts.

Declarations

Acknowledgement

Heartfelt appreciation goes to Mr T.K. Odewo of the Federal Research Institute of Nigeria for collection and identification of the plant and Mr Sunday of Pharmaceutical Microbiology Laboratory, University of Ibadan, Nigeria for all his technical assistance in this research.

Ethics approval and consent to participate

Not Applicable

Consent for publication

All authors have read and consented to the submission of the manuscript.

Availability of data and material

Not Applicable.

Competing interests

All authors declare no competing interests.

Funding

There was no funding for the current report.

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