Evaluation of Antibacterial Activity of Stem Bark Extracts of *Diospyros mespiliformis* and *Boswellia serrata* against Some Clinical Bacterial Isolates Obtained from Haemorrhoid Lesions

Mohammed, B.[1] and Danazumi, U.I.[2]
[1]Department of Microbiology, Bayero University Kano, BUK, P.M.B 3011, Nigeria,
E-mail: bshchemiron@yahoo.com; Tel: 07012789177

Abstract: Bacterial resistance to antibiotics is one of the major problems encountered in the field of chemotherapy worldwide. Some pathogenic bacteria are becoming resistant to most potent antibiotics used in hospitals and clinics; this necessitate for the search of new and promising antibacterial agents. In view of that, the antibacterial activity of stem bark extracts of *D. mespiliformis* and *B. serrata* locally used for the treatment of haemorrhoids against bacterial isolates sourced from haemorrhoid lesions in patients attending Aminu Kano Teaching and Murtala Muhammad Specialist Hospitals, Kano was investigated. Aqueous and ethanolic extracts of the stem bark were obtained and screened for phytochemical constituents using cold maceration technique. Swabs were aseptically collected using sterile swabsticks and screened for bacterial pathogens. Isolates were identified using cultural, biochemical and molecular techniques. Antibacterial activity of low and high concentrations of the extracts was determined using agar well diffusion technique. The results of the phytochemical screening of the extracts revealed the presence of phenols, cardiac glycosides, steroids, flavonoids, tannins and anthraquinones in the extracts. The bacterial species identified were Enteropathogenic *Escherichia coli, Proteus vulgaris, Proteus mirabilis, Enterobacter agglomerans, Enterobacter aerogenes, Salmonella arizonae, Salmonella enterica, Salmonella Typhi, Shigellla dysentriae and Citrobacter freundii*. The results of the antibacterial activity of the extracts revealed low activity of both the low and high concentrations of the aqueous and ethanolic extracts of the two plants against the test organisms; zones diameter of inhibition ranging from 6.00-12.45mm were recorded; the well diameter was 6mm. In conclusion, the crude stem bark extracts of the two plants possessed weak antibacterial activity. It is recommended that other plants' parts should be tested for antibacterial activity.
Keywords: Phytochemical screening, Antibacterial activity, Clinical isolates, Agar well diffusion technique

INTRODUCTION

Several plants are locally used in the treatment of some medical conditions such as haemorrhoids in Hausa Communities of Northern Nigeria. Among the plants widely used to locally treat haemorrhoids include the *Diospyros mespiliformis* and *Boswellia serrata*; the stem barks are usually boiled in water and the infusions are taken orally or the powdered form of the stem bark are mixed with water to form a paste which is applied externally to the affected area or mixed with liquid food such as gruel or pap. Burkill (1995) reported that the chemical constituents of *D. mespiliformis* possess some therapeutic importance. The bark preparations of the plant are administered to treat cough, bronchial diseases, tuberculosis, syphilis, leprosy and are applied externally to wounds, ulcers, bruises, and furuncles (Ahmed and Mahmud, 2017).

Boswellia serrata is a moderate to large sized branching tree of the family Burseraceae; it grows in dry mountainous regions of India, Africa and Middle East (Siddiqui, 2011). The plant has been used for centuries to treat various chronic inflammatory diseases (Mansur *et al*., 2014). Resins from the plant have been used to treat haemorrhoids locally; this was probably attributed to its anti-inflammatory property (Sara and Ikram, 2018). In view of high morbidity and mortality of sepsis 25-30% (Lee *et al.,* 2016) and wide usage of the two plants in ethno-medicine more especially treatment of haemorrhoids, the study was conducted to evaluate the antibacterial activity of the stem bark extracts of *D. mespiliformis* and *B. serrata* against bacteria isolated from haemorrhoid lesions from patients attending Murtala Muhammad Specialist and Aminu Kno Teaching Hospitals, Kano.

MATERIALS AND METHODS

Two hundred swabs from hemorrhoids lesions were collected from the outpatients department of Aminu Kano Teaching Hospital and Murtala Muhammad Specialist Hospital Kano and transported to the Microbiology Laboratory at the Department of Microbiology, Bayero University Kano aseptically using standard protocols for analyses.

Identification of the Bacterial Isolates

Swab samples from haemorrhoid lesions were ascetically collected and cultured for potentials pathogens. Bacterial isolates were identified based on cultural, biochemical and molecular techniques as follows:

Biochemical Tests

The following biochemical tests were carried out as recommended for the biochemical screening of Gram negative bacilli; triple sugar iron test (TSI), Urease, Indole, Citrate Utilization, Voges Proskauer, and Methyl Red (Cheesbrough, 2006; Adeoye, 2007).

Identification of Bacteria Isolates using Molecular Technique

The commercial identification kit (microgen) were prepared according to the manufacturer's instructions and were used for the identification of the enterobacteriacae and non fastidious bacteria , the kits contains 12 micro wells in which each sample was placed, the species of the bacteria were identified based on colour change within 18 to 24 hours depending on the samples. The set up were connected to software that generates eight digit codes specific to each organism (CLSI, 2012).

Collection of Plant Materials

The two plants were identified and authenticated at the Department of Plant Biology, Bayero University, Kano. The stem bark *of Boswellia serrata* locally called "*Ararrabi* in Hausa and *Diospyros mespiliformis* locally called *Kanya* were carefully washed using clean tap water, dried and placed under shade in an aerated place and then ground into fine powder using mortar and pestle that were sterilized with 70% ethanol as described by Sofowora (1993).

Extraction of the plants materials

Fifty gram of stem bark powder of *Boswellia serrata* and *Diospyros mespiliformis* were soaked in 500ml of absolute ethanol and distilled water and extracted using cold maceration method as described by Sofowora (1993). Fifty gram of each stem back powder was percolated in 500ml of 99% ethanol and distilled water in 1 litre conical flask and stored for 3 days with constant shaking. The mixture was then filtered using Whatman filter paper. The filtration obtained were evaporated using rotary evaporator for ethanol and water bath set at 40^{o}C for the aqueous. Extracts recovered were stored at 4^{o}C in a refrigerator.

Phytochemical Screening of *D. mespiliformis* and *B. serrata* Extracts

Phytochemical screening for the detection of some plant secondary metabolites (saponins, phenols, cardiac glycosides, steroids, tarpenoides, tannins, alkaloids and anthraquinones) was carried out as described by Sofowora (1993).

Antibacterial Activity Testing

Antibacterial activity of low concentrations (500, 250, 125 and 62.5µg/ml) and high concentrations (400, 200, 100 and 50mg/ml) of the plant extracts were tested against the bacterial isolates using agar-well diffusion method (CLSI, 2012). Standardized inoculum (1.5×10^{8} CFU/ml) of each isolate was emulsified on the surface of Mueller Hinton Agar using sterile swab sticks. Then, wells of 6mm diameter were dug on the agar and 100µl of each concentration was added in to each well using 1ml BD syringe and labeled appropriately. The extracts were allowed to diffuse in to the medium for 15 minutes and then the plates were incubated at 37˚C for 24hrs in inverted position. Zone diameters of inhibition were measured using centimeter ruler and recorded in millimeter (CLSI, 2012).

RESULTS

Ten bacterial species were identified from the swabbed with Enteropathogenic *E. coli* having the highest percentage occurrence (24%), *Shigella dysentriae* (20.20%) and *Enterobacter aerogenes* (15.38%) were found to be the second and third in terms of percentage of occurrence, while the least in terms of percentage of occurrence were *Citrobacter fruendii* (3.80%) and *Salmonella arizonae* (1.29%) (Table 1).

The phytochemical constituents detected were phenols, saponins, tannins, cardiac glycosides, tarpenoides, anthraquinones, while alkaloids were only detected in *B. serrata* aqueous extracts (Table 2).

Both low and high concentrations of aqueous extracts of *B. serrata* exhibited low performance on the test organisms (zone diameters of inhibition ranged from 6.00-9.61mm), however, the high concentrations performed slightly better than the low concentrations (Table 3).

High concentrations of ethanolic extracts of *B. serrata* recorded better activity on the isolates (zone diameters of inhibition ranged from 6.00-12.1mm); however, the two extracts had low activity (Table 4).

The activities of both low and high concentrations of aqueous and ethanolic extracts of *D, mespiliformis* against the clinical isolates were low (Table 5 and 6).

Table 1: Bacterial Species and their Percentage of Occurrences from Haemorrhoid Lesions Collected from Murtala Muhammad Specialist and Aminu Kano Teaching Hospital, Kano

S/N	Code	Organisms identified	No. of Occurrence	%OC
1.	00064075	Enteropat. *E. coli*	20	24.0
2.	15611111	*P. vulgaricus*	9.0	10.24
3.	15433757	*P. mirabilis*	10	11.52
4.	17538401	*E. agglomerans*	7.0	7.97
5.	10113230	*E. aerogenes*	13	15.38
6.	67301140	*S. arizonae*	1.0	1.29
7.	36401160	*S. enteric*a	4.0	5.12
8.	44108660	*S. Typhi*	6.0	7.68
9.	35673513	*S. dysentriae*	17	20.20
10.	00221122	*C. freundii*	3.0	3.80
		Total	**90**	**100**

Table 2: Phytochemical Constituents of Stem Bark Extracts of *D. mespilformis* and *B. serrata*

Phytochem. constituents	***D. mespiliformis* Ethanolic**	***D. mespiliformis* aqueous**	***B. serrata* ethanolic**	***B. serrata* aqueous**
Phenols	+	+	+	+
Cardiac glycosides	+	+	+	+
Steroids	+	+	+	-
Tarpenoids	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	-	-	-	+
Tannins	+	+	+	+
Anthraquinones	+	+	+	+
Saponins	-	+	+	+

Key: + = present, - = absent

Table 3: The mean diameter zone of inhibition of *Boswellia serrata* aqueous extracts at low at high concentrations

Isolates	**Concentration (mg/ml)**					**Concentrations (µg/ml)**			
	400	**200**	**100**	**50**	**Cont.**	**500**	**250**	**125**	**62.5**
Enteropthogenic *E. coli*	9.12	8.11	7.60	6.88	24.5	6.00	6.00	6.00	6.00
Proteus vulgaricus	6.98	6.14	6.00	6.11	20.8	6.00	6.00	6.00	6.00
Proteus mirabilis	9.22	7.87	7.00	7.20	23.5	6.00	6.11	6.00	6.00
Enterobacter agglomerans	7.22	8.10	6.23	7.80	23.1	6.00	6.44	6.00	6.00
Enterobacter aerogenes	9.61	8.20	7.86	6.50	24.0	6.00	6.00	6.00	6.00
Salmonella arizonae	8.45	7.22	7.00	6.43	21.2	6.20	6.00	6.00	6.00
Salmonella enteric	9.44	7.00	6.00	6.45	23.4	6.00	6.00	6.00	6.00
Salmonella Typhi	11.0	8.96	7.89	6.80	23.0	6.11	6.00	6.00	6.00
Shigella dysentriae	10.8	7.96	7.50	6.90	23.9	6.00	6.00	6.00	6.00
Citrobacter freundii	12.5	8.76	8.36	6.00	22.5	6.00	6.00	6.00	6.00
p-value-0.00818									
p-value-0.00000									

Key: Cont.-Control (Augmentin), 6mm = diameter of Cork Borer

Table 4: The mean diameter zone of inhibition of *Boswellia serrata* ethanolic extracts against the bacterial isolates

Isolates	Concentration (mg/ml)					Concentrations (µg/ml)			
	400	200	100	50	Cont.	500	250	125	62.5
Enteropthogenic *E. coli*	10.0	8.11	7.60	7.40	24.5	6.99	6.77	6.00	6.00
Proteus vulgaricus	9.89	9.40	8.00	7.98	20.8	7.00	6.89	6.12	6.00
Proteus mirabilis	9.14	7.98	6.00	7.87	23.5	7.20	6.11	6.00	6.00
Enterobacter agglomerans	12.1	9.23	8.23	7.90	23.1	7.80	6.44	6.09	6.00
Enterobacter aerogenes	10.5	8.90	7.86	7.00	24.0	6.98	6.50	6.00	6.00
Salmonella arizonae	9.10	8.34	8.00	7.22	21.2	6.93	6.60	6.20	6.00
Salmonella enteric	9.46	8.40	6.76	6.45	23.4	6.00	6.00	6.00	6.00
Salmonella Typhi	9.00	9.96	7.89	6.77	23.0	6.50	6.20	6.00	6.00
Shigella dysentriae	8.18	8.08	7.96	7.86	23.9	6.50	6.20	6.00	6.00
Citrobacter freundii	12.7	8.76	8.30	8.00	22.5	7.00	6.80	6.00	6.00
p-value-0.00810									
p-value-0.00000									

Key: Cont.-Control (Augmentin 30µg/disk)

Table 5: The mean diameterzone of inhibition of *Diospyros mespiliformis* ethanolic extracts against the bacteria isolates

Isolates	Concentration (mg/ml)					Concentrations (µg/ml)			
	400	200	100	50	Cont.	500	250	125	62.5
Enteropthogenic *E. coli*	8.99	8.20	7.90	7.40	24.5	6.98	6.87	6.50	6.00
Proteus vulgaricus	7.98	7.50	7.00	6.98	20.8	6.70	6.00	6.00	6.00
Proteus mirabilis	8.00	7.28	7.00	6.87	23.5	6.65	6.40	6.00	6.00
Enterobacter agglomerans	9.11	8.65	8.00	7.91	23.1	7.80	6.45	6.04	6.00
Enterobacter aerogenes	8.50	8.20	7.22	7.00	24.0	6.65	6.40	6.30	6.00
Salmonella arizonae	8.12	8.00	8.00	7.54	21.2	6.94	6.60	6.40	6.00
Salmonella enteric	9.90	8..40	7.00	6.76	23.4	6.45	6.00	6.00	6.00
Salmonella Typhi	9.00	9.96	7.89	6.77	23.0	6.50	6.20	6.00	6.00
Shigella dysentriae	8.18	8.08	7.96	6.86	23.9	6.50	6.20	6.00	6.00
Citrobacter freundii	9.50	8.55	8.55	8.01	22.5	6.60	6.90	6.00	6.10
p-value-0.00780									
p-value-0.00000									

Key: Cont.-Control (Augmentin)

Table 6: The mean zone diameter of inhibition of *Diospyros mespiliformis* aqueous extracts against bacterial isolates

Isolates	Concentration (mg/ml)					Concentrations (µg/ml)			
	400	200	100	50	Cont.	500	250	125	62.5
Enteropthogenic *E. coli*	9.80	8.90	8.98	8.00	24.5	7.96	7.80	6.50	6.40
Proteus vulgaricus	8.87	8.50	8.00	7.98	20.8	7.60	7.30	7.00	6.00
Proteus mirabilis	8.00	7.65	7.20	7.00	23.5	6.66	6.41	6.00	7.00
Enterobacter agglomerans	8.43	8.60	7.98	7.61	23.1	7.00	7.00	6.07	7.00
Enterobacter aerogenes	8.30	8.30	8.22	7.75	24.0	7.56	7.40	7.30	7.00
Salmonella arizonae	8.12	8.23	7.50	7.94	21.2	7.24	7.60	7.40	6.00
Salmonella enteric	10.46	9.40	9.00	8.74	23.4	8.45	8.00	7.91	7.00
Salmonella Typhi	10.70	9.46	8.54	8.00	23.0	7.50	7.20	6.98	6.50
Shigella dysentriae	10.00	9.08	9.96	8.86	23.9	7.55	7.22	7.00	7.20
Citrobacter freundii	9.80	9.65	9.65	9.00	22.5	8.98	8.60	8.00	7.50
p-value-0.00800									
p-value-0.00000									

Key: Cont.-Control (Augmentin 30µg/disk)

DISCUSSION

Ten bacterial species were isolated in the study and all are members of the family enterobacteriaceae; this could be attributed to the fact that the sources of the swab samples were haemorrhoid lesions located at the rectal area along the gastro-intestinal tracts. Members of the family enterobacteriaceae reside in the gastro-intestinal tracts of human and other animals.

The aqueous and ethanolic extracts of the two plants were found to be rich in phytochemical constituents, but the aqueous and ethanolic extracts of *D. mespiliformis* and the ethanolic extract of *B. serrata* were found to lack alkaloids. This finding was not in conformity with the findings of Abdullahi *et al.* (2019); Abdulrahman *et al.* (2014), who detected alkaloids in methanolic stem bark extracts of *D. mespiliformis*.

The findings of the antibacterial activity of the plants extracts revealed that both low and high concentrations had weak activity on the bacterial isolates. This finding was similar to the findings of Shagal *et al.* (2011), Dangoggo *et al.* (2016), Ahmed and Mahmud (2017), Yadima *et al.* (2017) who recorded weak activities of *D. mespiliformis* extracts on clinical isolates of bacteria. Similarly, Mansur *et al.* (2014) recorded low activity of high and low concentrations of *B. serrata* on *E. coli, Enterobacter aerogenes, Proteus vulgaris* and *Klebsiella pneumoniae*.

There was significant difference (p-value=0.00000) between the activity of the control antibiotic (augmentin) and that of high and low concentrations of aqueous extracts of *B. serrata*. The control drug was found to perform effectively on the bacterial isolates with zones diameter of inhibition within the acceptable limit set by CLSI (2012).

Highest concentration (400mg/ml) of the extracts was found to exhibit higher activity compared to other lower concentrations tested. The susceptibility patterns of the ten species of bacteria were found to be not significantly different from each other, except *P. vulgaris* that showed significant variation (p-value=0.00810) in its susceptibility pattern.

Similarly, there was no significant difference (p-value 0.02113) between the activities of aqueous and ethanolic extracts of *B. serrata,* this could probably be attributed similarity in the phytochemical constituents of the extracts. This implies that the stem bark preparations of the two plants used locally in the treatment of haemorrhoids have insignificant activity against bacterial pathogens colonizing haemorrhoid lesions.

The ethno-medicinal application of *B. serrata* in the treatment of haemorrhoids could be attributed to anti-inflammatory effect of the plant rather than its antibacterial property. Resins extracted from *B. serrata* have been reported to possess anti-inflammatory activity (Siddiqui, 2011; Sara and Ikram, 2018).

The high and low concentrations of the aqueous and ethanolic extracts of *D. mespiliformis* were also found to have weak activity against the bacterial isolates, however the aqueous extracts had slightly higher activity than the ethanolic extracts but no statistical significant difference (p-value 0.0868). This variation might be linked to the presence of alkaloids in the aqueous extracts which were not detected in the ethanolic extracts.

CONCLUSION AND RECOMMENDATIONS

From the findings of this study, it can be concluded that both low and high concentrations of aqueous and ethanolic extracts of *B. serrata* and *D. mespiliformis* have weak activities (7.98 – 12.7mm zone diameter of inhibition at 400mg/ml concentration) on the clinical bacterial isolates for the study. The bacterial isolates were sensitive to augmentin ($\geq$ 20mm zone diameter of inhibition). It can be recommended that synergistic potential of the two plants' extracts should be investigated; likewise, toxicity potentials of the two plants should be assayed.

REFERENCES

Abdullahi, A. E., Dahiru, S., Mohammed, M. S., Abubakar, A. and Adamu, Z. H. (2019). Phytochemical and Antimicrobial Screening of the Methanolic Extracts of *D. mespiliformis* Hochst. *Pharmacognosy Journal,* 11 (2):362-368.

Abdulrahman, B., Mammam, M., Suleiman, M. M., Ahmed, A. and Bello, A. (2014). Preliminary Phytochemical Screening of *D. mespiliformis*. *The Journal of Agriculture and Natural Resources Sciences,* 1 (4):12-8.

Adeoye, A. (2007). *A Textbook for Medical Laboratory Practice*. First Edition, Distributed by Edoson Publishers a Division of Angus Eddson (Nig.) Ltd. Pp 117-156.

Ahmed, A. H. and Mahmud, A. F. (2017). Pharmacological Activities of *D. mespiliformis*. A Review. *Int. Journal of Pharmacy and Biological Sciences*, 7(4):93-96.

Burkill, H. M. (1995). The Useful Plants of West Tropical Africa. Volume 1. Pp 200 – 209.

Cheesbrough, M. (2006). *District laboratory practice in tropical countries.* Part 1, second edition, Pp. 12 – 17.

Clinical and Laboratory Standards Institute (CLSI) (2012). *Performance Standards for Antimicrobial Susceptibility Testing; Approved standard*- Eleventh edition. M02-A11. Vol 32(1): 1 – 200.

Dangoggo, S. M., Hassan, L. G., Sadiq, T. T. and Manga, S. B. (2016). Phytochemical Analyses and Antibacterial Screening of Leaf of *D. mespiliformis* a*nd Ziziphus spin-Christi*. *International Journal of Biochemistry Research and Review,* 12 (4):1-9.

Lee, C. R., Lee, J. H., Park, K. S., Kim, Y. B., Jeong, B. C and Lee, S. H. (2016). Global Dissemination of Carbapenemase Producing *K. pneumoniae* Epidemiology, Genetic Context Treatment Option and Detection Methods. *Microbial,*7:895-900.

Mansur, I. S., Sudheer, A., Sambasivarao, K., and Matcha, B. (2014). Antimicrobial Activity of Frankincense of *B. serrata. International Journal of Current Microbiology and Applied Sciences,* 3 (19): 1095 -1101.

Sara, N. J.W. and Ikram, M. E. (2018). A Comparative Study of Anti-inflammatoryand Chemical Analysis of Ziziphus spina-Christi Leaves and *B. serrata* Gum Dry Distillates. 6th Edition of Int. Conference on Pharmacognosy and Medicinal Plants Amsterdam, Netherlands.

Shagal, M. H., Kubmarawa, D. and Alim, H. (2011). Preliminary Phytochemical Investigation and Antimicrobial Evaluation of Roots, Stem bark and Leaves Extracts of *D. mespiliformis*. *International Research Journal of Biochemistry and Bioinformatics*, 2 (1):011-015.

Siddiqui, M. Z. (2011). Antiinflammatory Activity Resins from *B. serrata*. *Indian Journal of Pharm. Sciences,* 73 (3):255-261.

Sofowara, A. (1993): Medicinal plants and Traditional medicine in Africa, Spectrum Book LTD, Ibadan. Nigeria: 289.

Yadima, S. G., Murtala, M. and Bello, A. U, (2017). Phytochemical Screening and Antibacterial Activities of Aqueous Extracts of *D. mespiliformis, Mitragyna reticulatum* and *Tamarindus indica* in Northern Nigeria. *Arid Journal of Engineering, Technology and Environment,* 13 (3): 420-427.