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CYTOTOXICITY, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF THE LEAVES AND STEM BARK EXTRACTS OF *VITELLARIA PARADOXA*

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ABSTRACT

The effective use of *Vitellariaparadoxa* in traditional medicine for treatment of various ailments including inflammation, fever, skin irritation, dermatitis, sunburn, rheumatism, diarrhea, stomach ache and ulcers are well established. This study focused on the cytotoxicity of the plant parts using Brine Shrimp Lethality Assay (BSLA), antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Kleibsiella pneumonia* and *Escherichia coli* using disc diffusion and minimum inhibitory concentration methods. Result of cytotoxicity showed that the leaves crude extract (VPL 01) is more cytotoxic with LC_{50} 15.17µg/mL followed by the methanol fraction of both the leaves (VPL 04) and the stem bark (VPS 04) with LC_{50} values 23.21 and 19.35µg/mL respectively. The result of the antioxidant assay obeys the Beer-Lambert law over the useful range with both the leaves and stem back of *Vitellariaparadoxa* showing great antioxidant potency in all fractions tested. Themethanolic fraction standout above other factions with IC_{50} of 9.64µg/mL and 6.50µg/mL for the leaves and stem bark fractions respectively followed by the moderately polar ethyl acetate fraction which recorded IC_{50}16.96µg/mLand16.38µg/mL for leaves and stem back in comparison with standard ascorbic acid and BHT which recorded 4.77µg/mL and 9.18µg/mL respectively. The antimicrobial activity showed that the ethanol leaves extract had the maximum zone of inhibition against the entire test organisms, while the methanol fraction showed maximum inhibition against *B. cereus* (18±0.73 mm), *S. aureus* (19±1.41 mm) and *K. pneumonia* (19±0.03 mm) with MIC values of 62.5µg/mL for all the organisms respectively. These studies indicated that bioactive molecules present in *Vitellariaparadoxa*may be used as a prototype for development of new drugs as pharmaceutical raw materials.

Keywords: *Vitellariaparadoxa*, cytotoxicity, antioxidant, antimicrobial

INTRODUCTION

Medicinal importance of plants has been attributed to the presence of their natural products that are believed to activate, catalyse or initiate some curative reactions in humans, some of these natural products includes; terpenoids, phenolic acids, lignans, tannins, flavonoids, quinones, coumarins and alkaloids (Richard,*et al.*, 2014; Sanders, 2019).Traditional medicine is used to maintain health, prevent, diagnose and treat physical and mental illnesses based on theories, beliefs and experiences (Sanders, 2019). About 80% of the world populations are dependent on traditional medicines for their primary health care needs (Sofowora, *et al.*, 2013), thus making it necessary to investigate the safety of consumption of these materials as well as the medicinal potencies.

Vitellariaparadoxa (Shea tree)has long been used in traditional medicine for treatment of various ailments including inflammation, fever, skin irritation, dermatitis, sunburn, rheumatism, diarrhea stomach ache and ulcers (Bauerand Moll, 1942; Moore, 2008).Moreover according to an Ethno-botanical survey, the bark of the trunk and stem bark of *Vitellariaparadoxa*has been used historically for the treatment of cancer (Jiofack *et al.*, 2010), which is one of the most life-threatening diseases that occur as a result of deregulating proliferation of abnormal cells which invades and disrupts surrounding tissues (Al-mehdar*et al.*, 2012).

This research investigates the safe use of this plant *via* cytotoxicity test using brine shrimp lethality assay (BSLA), antioxidant activity studies using DPPH radical scavenging assay and antimicrobial activity of*Vitellariaparadoxa*.

MATERIALS AND METHODS

Collection, Identificationof Plant Materials

Fresh leaves and stem bark of *Vitellariaparadoxa* were collected from the premises of Yako (Kiru local Government area of Kano State) and were identified by Prof. B. S. Aliyu on 9th January, 2019 at the Department of Plant Science, Bayero University Kano, Nigeria.

The identified plant materials were air dried in the laboratory at room temperature (26°C) for 2 weeks, after which it was pulverized into uniform fine powder. The powdered plant materials were weighed using an electric weighing machine.

Extraction of Plant Materials

The powdered plant material was extracted using soxhlet method of extraction with ethanol as the extraction solvent (Soxhlet, 1897). 60g of the powdered leave and stem bark of the plant sample was separately packed in a muslin cloth (thimble)

and inserted into the Soxhlet extractor. 200 ml of the solvent was added and heated for 4hrs at relatively low temperature below the boiling point of the solvent to avoid denaturing of metabolites. At the end of the extraction period, the solvent was allowed to evaporate to obtain the crude extracts. The method was repeated in order to obtain enough extracts for further analyses.

The crude extract was macerated into three sub-fractions in order of increasing polarity i.e. *n*-hexane, ethylacetate and methanol. The fractions were evaporated at reduced temperature and were appropriately labeled as VPS and VPL for the stem bark and leaves respectively. 01, 02, 03, and 04 for crude (ethanol), *n*-hexane, ethyl acetate, and methanol fractions respectively.

Cytotoxicity Assay

Hatching Shrimp

Brine shrimp eggs, *Artemiasalina* were hatched in seawater. After 48hrs incubation at room temperature, the larvae were attracted to one side of the vessel with a light source and collected by pipette. Larvae were separated from eggs by aliquoting them three times in small beakers containing seawater (Ibrahim and Abdullahi, 2015).

Brine Shrimp Lethality Assay

Toxicity of the extract was monitored by the brine shrimp lethality test according to the method described by Lilybeth and his co-workers (2013), with slight modification. Each of the extract/fraction (1 mg/mL) was dissolved in methanol, from which 5000, 500 and 50 µL of each solution was transferred into vials corresponding to 1000, 100 and 10 µg/mL respectively. This was allowed to evaporate to dryness in about 24 h at room temperature. Each dosage was tested in triplicate (9 per test sample). Sea water (4 mL) and 10 larvae were introduced into each vial. The final volume of solution in each vial was adjusted to 5 mL with sea water immediately after adding the shrimps, and DMSO as a negative control was prepared as a drug-free. Survivors were counted after 24 h, and LC_{50} was determined by probit analysis using SPSS version 20 to establish the therapeutic index.

Antioxidant Activity

DPPH Assay

The free radical scavenging activity of the plant extracts against 2,2-diphe-nyl-1-picrylhydrazyl (DPPH) radical was determined according to the standard method with slight modification (de-Oliveira*et al.*,2012). Each sample of stock solution (1.0 mg/L) was diluted to final concentration of 1000, 500, 250, 125,61.5, 31.3, 15.63 and 7.82 µg/mL. Total of 50 µM DPPHmethanolic solution (160 µL) was added to sample solution (40 µL) and allowed to react at room temperature for 30 min in dark. The absorbance of the mixturewas measured at 517 nm. Ascorbic acid (AA) and butylatedhydroxyl toluene (BHT) were used as positive controls. Lower absorbance of the reaction mixture indicates higher radical scavenging activity, and vice versa. Inhibitions of DPPH radical in percent (% I) were calculated using the formula:

$$\% I = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where; A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance values of the test compounds. The sample concentration that provides 50% inhibition (IC_{50}) was determined by probit analysis using SPSS 20.

Antimicrobial Activity

Test Microorganism

Two Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Kleibsiella pneumonia* and *Escherichia coli*) were obtained from the Microbiology Laboratory of Bayero University, Kano and were grown on nutrient agar (NA).

Disc diffusion Method

Antimicrobial activity of the extracts was determined using standard procedure with slight modification (Bhuiyan *et al.*, 2011*;* Saini, *et al.,* 2005). The suspension (400 µL) of the test bacteria was spread on the nutrient agar (NA) and the disc (6 mm diameter) impregnated with 10 µL of the target compounds and DMSO (negative control) was placed on the inoculated agar, which was incubated for 24 h at 37°C. Streptomycin sulfate (10µg/mL) was used as the positive controls for test bacteria. Clear inhibition zones around the discs indicated the presence of antimicrobial activity and the activity was determined by measuring the zone of inhibition that appeared after the incubation period. DMSO was used as a negative control and all tests and analyses were carried out in triplicate.

Minimum inhibitory concentration (MIC)

The MIC was determined by the broth micro dilution method using 96-well micro-plates as describe in the literature with modification (Teh, *et al.,* 2017).The inoculate of the microbial strains was prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Each sample (1.0 mg) was dissolved in DMSO (1 mL) to obtain 1000 µg/mL stock solution. A number of wells were reserved in each plate for positive and negative controls. Sterile broth (100 µL) was added to the well from row B to H. The stock solutions of samples (100 µL) were added to the wells in rows A and B. Then, the mixture of samples and sterile broth (100 µL) in row B was transferred to each well in order to obtain a twofold serial dilution of the stock samples (concentration of 1000, 500, 250, 125, 62.5, 31.3, 15.6 and 7.8 µg/mL). The inoculums (100 µL) were added to each well and a final volume 200 µL was obtained in each well. Streptomycin sulfate was used as positive controls and plates were incubated at 37°C for 24 h. Microbial growth was indicated by the presence of turbidity and a pellet at the bottom of the well.

RESULTS AND DISCUSSION

The degree of lethality was observed to be proportional to the concentration of the extracts/fractions with highest observed mortality at the highest concentrations (1000µg/mL) in all the samples. In general, analysis of the result showed that the leaves of the plant displayed significant activity than the stem bark **(Table 1)**. However, the leaves crude extract (VPL 01) is more cytotoxic with LC_{50} 15.17µg/mL followed by the methanol fraction of both the leaves (VPL 04) and the stem bark (VPS 04) with LC_{50} values 23.21 and 19.35µg/mL respectively. No mortality was observed in the negative control (DMSO). This preliminary assessment is the most extensive study estimated to represent over 90% of the studies in which *Artemia* is used as an experimental test organism. Several reports demonstrated the correlation between the result of the LC_{50} and that of oral toxicity in mice justifying the bioactive potentials of the plant

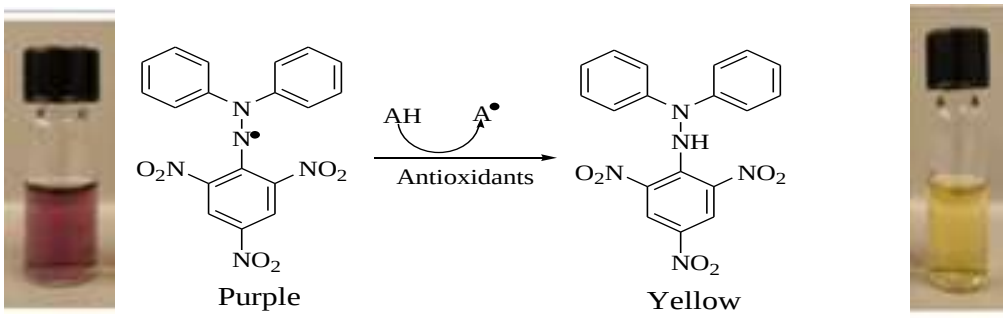
materials due to different classes of secondary metabolites (Moore, 2008). Additionally, result from this assay can serve as a lead to isolation of novel chemical compounds with medicinal properties.

Table 1: Brine Shrimp Lethality Assay of *Vitellariaparadoxa*

Extracts/ Fractions	Conc. (µg/mL)	Number of Surviving Nuplii After 24 h			Total Number of Death	% Mortality	LC_{50} (µg/mL)
		T1	T2	T3			
VPS 01	10	5	8	8	03	10.00	
	100	3	1	5	21	70.00	42.79
	100	3	3	2	22	73.33	
VPS 02	10	9	9	10	2	6.67	
	100	7	6	9	8	26.67	1184.42
	1000	6	6	4	14	46.67	
VPS 03	10	9	5	7	9	30.00	
	100	4	1	3	22	73.33	31.89
	1000	3	0	1	26	86.67	
VPS 04	10	6	6	5	13	43.33	
	100	3	5	2	20	66.67	19.35
	1000	1	1	1	27	90.00	
VPL 01	10	4	6	7	13	43.33	
	100	2	1	4	23	76.67	15.17
	1000	2	0	0	28	93.33	
VPL 02	10	10	8	9	3	10.00	
	100	5	5	8	12	40.00	357.12
	1000	4	4	4	18	60.00	
VPL 03	10	7	6	7	10	33.33	
	100	2	5	5	18	60.00	37.75
	1000	1	2	0	27	90.00	
VPL 04	10	6	7	5	12	40.00	23.21
	100	3	2	5	20	66.67	
	1000	1	1	1	27	90.00	
DMSO		10	10	10			

Key: VPS: *Vitellariaparadoxa* Stem Bark ; VPL: *Vitellariaparadoxa* Leaves

Natural antioxidants, including polyphenols, flavonoids, vitamins, and volatile chemicals, have been identified particularly from different plant parts (Ibrahim and Abdullahi, 2015).The effect of antioxidants on DPPH radical scavenging isthought to be due to their hydrogen-donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Brighente, *et al.*, 2007).



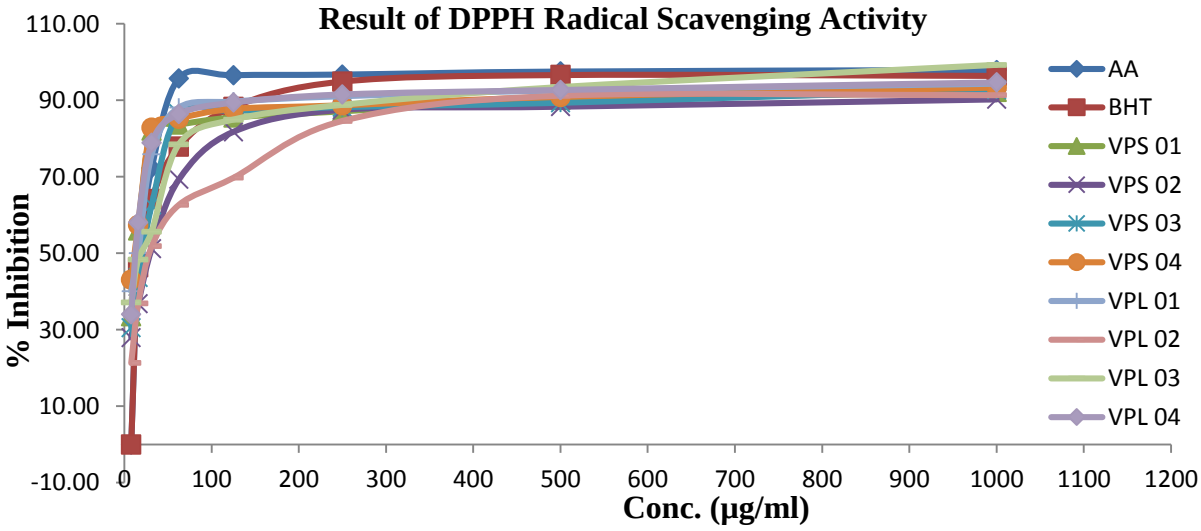
Both the leaves and stem bark of *V. paradoxa* displayed effective radical scavenging property against DPPH. The assay is based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is usually reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine. DPPH can accept an electron or hydrogen radical to become a stable, diamagnetic molecule and can be oxidized only with difficulty irreversibly. DPPH shows a strong absorption band at 517 nm due to its odd electron and solution appears a deep violet colour, the absorption vanishes as the electrons pairup. The resulting decolorization is dependent on the number of electrons donated by the active constituent. The result of the spectrophotometric absorption and concentration of the samples/standard obeys the Beer-Lambert law over the useful range.
Both leaves and stem back of *Vitellariaparadoxa* show great antioxidant potency in all extracts tested, with methanolic extract as the standout fraction above other extracts with IC_{50} of 9.64µg/mL and 6.50µg/mL for the leaves and stem bark fractions respectively. However, moderately polar ethyl acetate extract recorded a good but lower antioxidant activity with 16.96µg/mLand16.38µg/mL for leaves and stem back in comparison with standard ascorbic acid and BHT which recorded 4.77µg/mL and 9.18µg/mL respectively **(Table 2 and Scheme 1)**.

Table 2: Result of the Antioxidant of the leaves extracts of *Vitellariaparadoxa*

Conc. (µg/ml)	1000	500	250	125	62.5	31.3	15.8	7.8	IC_{50} (µg/ml)
AA	97.94	97.53	96.74	96.57	95.74	82.28	74.11	52.23	4.77
BHT	96.93	96.16	94.90	92.27	87.88	72.16	51.03	49.33	9.18
VPS 01	92.20	90.14	87.14	85.55	83.67	82.08	56.01	33.58	9.13
VPS 02	90.24	88.32	87.54	81.77	69.32	51.20	36.78	27.97	26.44
VPS 03	92.83	89.35	88.23	87.25	86.52	62.65	43.64	30.49	16.38
VPS 04	93.31	90.87	88.67	87.73	85.22	82.84	57.38	43.06	6.50
VPL 01	94.36	92.59	91.09	89.71	88.12	75.91	50.03	40.05	9.89
VPL 02	91.35	91.22	84.62	69.76	62.67	51.85	36.88	21.32	33.94
VPL 03	99.24	93.44	88.79	84.94	78.50	55.65	48.39	37.16	16.96
VPL 04	94.70	92.72	91.55	89.42	86.46	78.92	58.06	34.07	9.64

AA: Ascorbic Acid; BHT: Butylatedhydroxy Toluene; VPS: *Vitellariaparadoxa* Stem Bark

VPL: *Vitellariaparadoxa* Leaves; Result are means of three replicates



Scheme 1: Result of the DPPH Radical Scavenging Activityfor the Extracts/Fractions of *V. paradoxa*

The antimicrobial properties of the extracts/fractions of both the leaves and stem bark of *V. paradoxa* at various concentrations against Gram-positive (*B. cereus*and*S. aureus*) and Gram-negative (*K. pneumonia* and *E. coli*) were assessed in this study. The

results revealed that the ethanol and methanol extracts of the leaves efficiently suppressed the growth of the tested microorganisms with variable potency. As shown in Table 3, the ethanol leaves extract had the maximum zone of inhibition against the entire test organisms. The methanol fraction showed maximum inhibition against *B. cereus* (18±0.73 mm), *S. aureus* (19±1.41 mm) and *K. pneumonia* (19±0.03 mm) with MIC values of 62.5µg/mL for all the test organisms. The ethanol extract of the stem bark was also active against the Gram-positive bacteria with zone of inhibition of 16±0.12 mm against *B. cereus* and 19±0.01 mm against *S. aureus*; and the extract was active at minimum inhibitory concentration of 125µg/mL and 150µg/mL against the Gram-positive bacteria respectively.

Table 3a: Zone of inhibition (mm) of the extracts of *V. paradoxa*

Extracts	Test Microorganims (mm)			
	B. cereus	*S. aureus*	*K. pneumonia*	*E. coli*
VPS 01	16±0.12	19±0.01	15±0.43	12±0.11
VPS 02	6±0.10	9±0.51	5±0.03	--
VPS 03	11±0.32	13±0.00	11±0.17	11±0.06
VPS 04	13±0.11	12±0.62	12±0.43	14±0.21
VPL 01	19±0.54	21±0.07	17±0.02	20±0.18
VPL 02	11±0.31	11±0.09	13±0.00	9±0.40
VPL 03	14±0.22	13±0.07	14±0.18	14±0.21
VPL 04	18±0.73	19±0.01	19±0.03	15±0.19
Streptomycin Sulfate	20±0.30	21±0.24	20±0.08	24±0.21
DMSO	--	--	--	--

--: No activity; Result are means of three replicates.

Table 3b: Minimum inhibitory concentration (MIC) (µg/ml) of the extracts *V. paradoxa*

Extracts	Test Microorganisms (µg/ml)			
	B. cereus	*S. aureus*	*K. pneumonia*	*E. coli*
VPS 01	125	150	200	200
VPS 02	500	500	300	--
VPS 03	250	250	250	300
VPS 04	125	125	150	125
VPL 01	62.5	125	125	62.5
VPL 02	200	250	200	300
VPL 03	200	200	200	200
VPL 04	62.5	62.5	62.5	125
Streptomycin Sulfate	7.8	7.8	7.8	7.8
DMSO	--	--	--	--

NA--: No activity; Result are means of three replicates. Diameter of the disc (6mm)

CONCLUSION

The results of all the assays conducted justify the importance of the *V. paradoxa* in traditional medicine of tropical region of Africa. The medicinal applications of the plant parts are due to the presence of natural metabolites of different molecular compositions. It was observed in this study that the polar extracts/of both the leaves and stem bark of *Vitellariaparadoxa* demonstrated significant cytotoxic, antioxidant and antimicrobial activity. The antioxidant activity displayed by the plant may be due to the presence of phenolic compounds present in the different parts of the plant which has already been reported (Steven, *et al.,* 2003). These studies indicated that bioactive molecules present in *Vitellariaparadoxa*can be used as a prototype for development of new drugs as a source of antioxidant, antidiabetic, antitumor and antimicrobial pharmaceutical raw material. For further studies, effective eco-friendly isolation and characterization techniques should be employed to determine the bioactive components through activity-guided assays.

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REFERENCES

Al-mehdar, H., Abdallah, H.M., Osman, A.M.M. and Abdel-Sattar, E.A. (2012). *In-vitro* cytotoxic screening of selected Saudi medicinal plants. *J. Nat. Med.*, 66(2):406-412.

Bauer, K.H. and Moll, H. (1942). The contents of the shea butter nut, the seed of *Butryospermumparkii*. *Arch. Pharm.,* 280: 37-45.

Bhuiyan, M.M.H., Hossain,M.I., Mahmud, M.M. and Al-Amin, M.M. (2011). Microwave-assisted Efficient Synthesis of Chalcones as Probes for Antimicrobial Activities. *Chem. J.,* 1(1): 21–28.

Brighente, I.M.C., Dias, M., Verdi, L.G. and Pizzolatti, M.G. (2007). Antioxidant Activity and Total Phenolic Content of Some Brazilian Species.*Pharm. Bio.,* 45(2): 156-161.

de-Oliveira,A.M.F., Pinheiro, L.S., Pereira,C.K.S., Matias,W.N., Gomes, R.A., Chaves, O.S., de-Souza, M.F.V., de-Almeida, R.N. and de-Assis, T.S. (2012). Total Phenolic Content and Antioxidant Activity of Some Malvaceae Family Species. *Antioxid*.,1: 33-43.

Hossain, M.A., Akhtar,S.M. and Sadri, S.A. (2017). Two new flavonoids from *Adeniumobesum* grown in Oman. *J. King Saud Univ. Sci*.,29: 62–69.

Ibrahim, N. S. and Abdullahi, M. (2015). Cytotoxicity, Total Phenolic Contents and Antioxidant Activity of the Leaves Extract of *Annonamuricata. ChemSearch J*., 6(1): 46–51.

Jiofack, T., Fokunang, C., Guedje, N., Kemeuze, V., Fongnzossie, E., Nkongmeneck, B.A., Mapongmetsem, P.M. andTsabang, N. (2010).Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. *Int. J. Med.Sci.,* 2:60–79.

Moore, S. (2008). The role of *Vitellariaparadoxa* in poverty reduction and food security in the Upper East region of Ghana. *EarthEnv.,* 3:209–245.

Richard, S.T., Bruno, P.T., Jean,N.N.,Didiane, M.Y., Sylvain,N.N., Stéphanie, M.C.G., Landry, L.L., Armel, H.N.K., Paul,F.M. andAhsana, D.R. (2014). Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants.*Asian Pac. J. Trop. Med.,* 14: 272-278.

Saini, R.K., Choudhary, A.S., Joshi, Y.C. and Joshi, P. (2005). Solvent Free Synthesis of Chalcones and their Antibacterial Activities. *E-J. Chem.,* 2(4):224–227.

Sanders, D.L. (2019). Standing in the Shadows of Plants. *Plts. Pple.Plts*., 1(3): 130–138.

Sofowora, A., Ogunbodede, E., Onayade, A. (2013). The role and place of medicinal plants in thestrategies for disease prevention. *Afr. J. Trad.Compl.Altern. Med.*,10(5):210-229.

Steven, M., Zeev, W. andNissim, G. (2003). Phenolic constituents of shea(*Vitelleriaparadoxa*) kernel. *J. Agric. Food Chem*., 51: 6268-6273.

Teh, C.H., Nazni, W.A., Nurulhusna, A.H., Norazah, A. and Lee, H.L. (2017). Determination of antibacterial activity and minimum inhibitory concentration of larval extract of fly *via*resazurin-based turbidometricassay.*BMCMicrobiol.,* 17: 36–39.