FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 5 No. 2, June, 2021, pp 109 – 113
DOI: https://doi.org/10.33003/fjs-2021-0502-601

IN VITRO EVALUATION *OF ANISOPUS MANNII* AND *LEPTADENIA HASTATA* FOR ANTIBACTERIAL POTENTIAL AGAINST BACTERIAL ISOLATES FROM DIABETIC WOUND

[*1]Sani, S. B., [3]Usman, B. M., [2]Hayatu, M., [1]Mardiyya, A. Y., [1]Bilkisu, A., [1]Umma, M., [2]Namadina, M. M., [1]Hassan, M. I., [1]Safiyanu, I., [1]Jibril, A., [1]Ajingi, Y. S. and [1]Sale, A. I.

[1]Department of Biology, Kano University of Science and Technology Wudil, P.M.B. 3244 Kano State, Nigeria.
[2]Department of Plant Biology, Bayero University Kano, P.M.B 3011 Kano State, Nigeria.
[3]Medical Laboratory Scientist, Corporate Head Office, Nigerian Ports Authority, 26/28 Marina, P.M.B. 12588 Lagos State, Nigeria

[*]Corresponding Author's Email: sakinasanibuhari@yahoo.com

ABSTRACT
Leptadenia hastata and *Anisopus mannii* – are perennial plants of family Asclepiadaceae. They are widely distributed in West Africa and are locally used as anti-diabetic agents in Northern Nigeria. This study was conducted to investigate the phytochemical constituent and antibacterial activity of the crude ethanol extract of the *Leptadenia hastata* and *Anisopus mannii* against some bacterial isolates from diabetic wound. The phytochemical screening was carried out using standard protocol and antibacterial activity was determined by agar well diffusion method followed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on the plant extract that showed activity. Result of phytochemical screening reveals the present of tannins, phenols, flavonols, saponins, and alkaloids in all the plants extract except in *Anisopus mannii* where alkaloid is absent. Also, result from antibacterial activity of *leptadenia hastata* demonstrated inhibition zones ranging from 11.5±0.71 to 20.0±0.00 at various concentrations (8mg/ml and 4mg/ml) against the tested organisms (*Citrobacter* specie, *E.coli*, *Proteus vulgaris.*) with MIC and MBC values ranging from 2mg/ml-8mg/ml. Hence, the ethanol extract of *Leptadenia hastata* can be considered as new therapeutic agent for the treatment of diabetic wound infection. Further studies need to be carried out to investigate toxicological effect and diabetic wound healing property of the plant.

Keywords: Antibacterial, Asclepeadeceae, *Anisopus mannii*, *Leptadenia hastate, phytochemical, diabetic wound*

INTRODUCTION

Leptadenia hastate (Pers.) Decne also known as Yadiya in Hausa language is a widely distributed tropical African herb used as vegetable. The leaves of the plant are commonly used in northern Nigeria for the treatment of diabetes mellitus (Shinkafi *et al.,* 2015; Negbenebor *et al.,* 2017; Sani *et al.,* 2019). Other traditional uses include; yellow fever (Ali *et al.,* 2017), general well-being (Kankara *et al.,* 2015), hypertension, catarrh and skin diseases (Dambatta and Aliyu, 2011). Local healers in Burkina Faso also used the plants for trypanosomiasis, skin diseases and wound healing (Haruna *et al.,* 2017). Antidiabetic, anti-inflamatory and in vitro wound healing effect of the plant were also validated (Thomas, 2012).

Anisopus mannii N.E.Br. is a herbal plant nativite to Africa, especially prominent in the central and western tropical regions. The folkloric use of *Anisopus mannii* as antidiabetic plant is common in northern Nigeria (Sani *et al.,* 2019) hence the name 'kashe zaki' meaning 'sweat killer'. Antioxidant (Aliyu *et al.,* 2010), antibacterial (Sani *et al.,* 2009) and hypoglycemic (Osibemhe *et al.,* 2017) potential of the plant was also reported. The use of medicinal plants to cure various human ailments is widely practiced in the world (Kumar, 2016). For centuries, people have turned to natural remedies to cure common ailments such as colds, allergy, upset stomachs and toothaches and the trend is constantly increasing (Kumar, 2016). Herbal medicine has been recognized by WHO as essential components for primary health care and about 11% of the 252 drugs are derived from plants (Taylor, 2000).The beneficial medicinal effects of plant materials typically result from the combination of plants secondary metabolites known as phytochemicals (Kothari *et al.,* 2012) . Phytochemicals are chemical compounds that occur naturally in plants (Varma, 2016). With rich source of phytochemicals, plant kingdom provide relief against several health complications such as heart attack, cancer, diabetes, malaria, jaundice, wound healing, inflammation, bacterial and viral infections, helminthiases throughout the world (Swargiary,2017).

According to the Wound Healing Society, wounds are physical

injuries that result in an opening or break of the skin that cause disturbance in the normal skin anatomy and function (Alam *et al.,* 2011). They result in the loss of continuity of epithelium with or without the loss of underlying connective tissue (Agyare *et al.,* 2016). Most chronic wounds are ulcers that are associated with is chaemia, diabetes mellitus, venous stasis disease, or pressure (Agyare *et al.,* 2016). If the wounds are not well treated, they can be infected. Infected wounds heal more slowly, re-epithelialisation is more prolonged, and there is also the risk of systemic infection (Inngjerdingen *et al.,* 2004). The most common pathogens associated with infected wound in diabetes are aerobic gram-positive bacteria, particularly *Staphylococcus aureus* and beta-hemolytic *streptococci*, aerobic gram-negative, particularly *Escherichia coli*, *Proteus species*, *Klebsiellaspecies* and anaerobic organism, *Bacteroides species*, *Clostridium species*, *Peptococcus* and *Peptostreptococcus specie* (Bader, 2008).

The use of medicinal plants in wound management and care involves disinfection, debridement, and the provision of adequate environment for natural healing process (oguntibeju, 2019). The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains (Ellof, 1998). Many researchers have reported the antibacterial activity of medicinal plants against diabetic foot infection isolates. These include *Psidium guaiava*, *Azadirachta indica*, *Tridax procumbens* (Lakshmi *et al.,* 2016), *Terminalia chebula*, *Cinnamomum zeylancium* Suresh *et al.,* 2012).

Therefore, the aim of this research was to investigate the phytochemical constituents and antibacterial activity of *Leptadenia hastata(Pers.) Decne* and *Anisopus mannii N.E.Br.* against some bacterial isolates from diabetic wound.

MATERIALS AND METHODS

Collection and identification of plant materials for study

Fresh leaves of *leptadenia hastata* and whole plants *Anisopus manniii* were collected by herbalist from Itfan forest at kibiya local government area and identified by botanist in the herbarium of Bayero University Kano. The identified specimens were deposited in the herbarium with vourcher number BUKHAN248 for *leptadenia hastata* and BUKHAN211 for *Anisopus manniii.*

Preparation of the extracts

The collected plant parts were washed under running water and shade dried at room temperature (25ºC) for two weeks. The dried parts were then pulverized using electric grinder and fifty grams of each powdered plant materials was macerated with 500ml of ethanol kept on a rotary shaker at 150rpm with constant agitation for 72hrs. Thereafter, it was filtered through 8 layers muslin cloth and then re filtered through whatman No. 1 filter paper. The filtrate was then concentrated in a rotary evaporator at 40ºC and dried at room temperature

Qualitative Phytochemical screening

Detection of Alkaloids

Fifty milligram of solvent free extract was stirred with few ml of dilute hydrochloric acid and filtered.

Mayer's test **:** 1 or 2 drops of Mayer's reagent (Potassium mercuric iodide)were added by the side of the test tube, to 1ml of the extract. A white or creamy precipitate indicates the presence of alkaloids.

Hager's test: 2 drops of Hager's reagent were added to 1ml of each extract. A reddish brown precipitate observed indicates the presence of alkaloids in each extract (Olusola *et al.,* 2012)

Tannins

One milliliter of freshly prepared 10% KOH was added to 1ml of the extracts. A dirty white precipitate observed in each extract showed the presence of tannins.

2 drops of 5% FeCl3 were added to 1 ml of the extracts. A greenish precipitate indicates the presence of tannins in the three extracts (Olusola *et al.,* 2012)

Saponins

Frothing test: 2ml of the extracts in a test tube was vigorously shaken for two minutes. Frothing observed in the extracts tested indicates the presence of saponins.

Emulsion test: 5 drops of olive oil were added to 3ml of the extracts in a test tube and the mixture was vigorously shaken. A stable emulsion formed indicates the presence of saponins (Olusola *et al.,* 2012)

Detection of Flavonoids

Alkaline reagent test: 1ml of 10% NaOH was added to 3 ml of the extract. A yellow colouration showed the presence of flavonoids in each extract (Olusola *et al.,* 2012)

In 0.5 ml of filtrate of each of the plant parts extracts, 5 ml of dilute ammonia was added, followed by addition of 1 ml of concentrated sulfuric acid to it. The presence of flavonoids was detected by yellow coloration of the solution that disappears on standing (Roy *et al.,* 2015)

To 4mg/ml of the extract a piece of magnesium ribbon was added followed by concentrated hydrochloric acid drop wise. A color change ranging from orange to red indicates flavones; red to crimson indicates flavonoids (Yusah'u, 2011)

Phenols

Ferric chloride test: Small quantities of alcoholic and aqueous extracts were dissolved in 2 ml of distilled water separately, and into it, few drops of 10% aqueous ferric chloride solution were added. Ablue or green color was produced which indicating the presences of phenols (Roy *et al.,* 2015).

Antibacterial screening of the extracts

Preparation of concentrations of the crude extracts

Eight milligram of each crude extracts was dissolved in 1ml Dimethyl- sulfoxide (DMSO) to obtained a concentration of 8mg/ml (stock solution) . This is followed by doubling dilution in DMSO to obtain another concentration of 4mg/ml.

Test organism

The test bacterial isolates(*Citrobacter* specie, *E.coli*, *Proteus vulgaris*) used for this analysis were pure clinical isolates from diabetic wound obtained from microbiology laboratory, Aminu

Kano Teaching Hospital, Kano state Nigeria. The isolates were maintained on nutrient agar slants and kept in a refrigerator (4ºC) prior to use.

Inocula standardization

A loopful of the isolate was picked using sterile wire loop and emulsified in 4ml of sterile physiological saline followed by proper shaking. The turbidity of the suspension was matched with that of 0.5 McFarland Standard (Yusha'u, 2011).

Agar well diffusion method

This was carried out as reported by Mann *et al*. (2008) with some modifications. Standardized inoculum (5×10cfu/ml) of each test bacterium was spread on sterile Muller Hilton agar plates so as to achieve even growth. The plates were allowed to dry and a sterile cork borer of diameter 6.0mm was used to bore four wells in the agar plates. Subsequently, a 100µl volume of the different concentrations (4mg/ml and 8mg/ml) of the extracts were introduced in the wells of the Mueller-Hinton agar culture using pasteure pipette. Ciprofloxacine (30µg) was used as a positive control and DMSO was use as a negative control. The plates were incubated at 37ºC over night. Zones of inhibition produced by the extracts against the test isolates were observed and measured.

Minimum inhibitory concentration (MIC) method

The MIC was carried out on the extracts that showed antibacterial activity against the isolates by the agar well diffusion method. The MIC of the extracts was determined using the tube dilution method as reported by Nuhu and Odinaka (2017) with some modification. Equal volume of the extract and Mueller-Hinton broth i.e 2ml each were dispensed into sterilized test tubes to obtain concentrations of 8mg/ml to 2mg/ml. The organism (0.1ml of standardized inoculum) was inoculated into each tube containing the broth and the extract. Tubes containing broth and plant extracts without inocula served as a negative control while tubes containing broth and inocula served as a positive control. The tubes were incubated at 37ºC for 24hrs. The lowest concentration in the series without visible sign of growth was considered to be the MIC.

Minimum Bactericidal Concentration Method

Minimum Bactericidal concentration (MBC) was carried out as described by Lar *et al.* (2011) with some modifications. The MBC was determined by first selecting the tubes or the least concentrations of extracts that showed no turbidity during the MIC determination. One loopful from each of these tubes was sub-cultured onto the surface of the extract free Mueller-Hinton agar and incubated at 37ºC for 24hrs. The lowest concentration at which no growth was observed on the agar was noted as the MBC.

RESULTS AND DISCUSSION

Result from phytochemical screening as shown in table 1 reveal the presence of saponin, tannins, phenols, flavanoids and alkaloids in all the tested plant extracts except in *A.mannii* were alkaloids is absent. The presence of Tannins, phenols, flavonoids, alkaloids and saponins in the leaves of extract of *L.hastata* is in accordance to the study carried out by Haruna *et al.,* (2017).

Flavonoids are low molecular weight polyphenolic antioxidants naturally present in fruits, vegetables (Nyamai *et al.,* 2015). The therapeutic effect of flavonoids includes antihyperglycemic effect, anticancerous and anti-inflammatory effect (Nyamai *et al,* 2016). Tannins are also polyphenols that are obtained from various parts of different plants such as leaves, bark, fruit, fruit pod and root (Nyamai *et al.,* 2016). They are reported to exhibits antimicrobial (Edeoga et al., 2005), anti-diabetic (Kang *et al.,* 2011)) and anti-cancerous activity (Cassidy *et al.,* 2000).

Alkaloids are phytochemicals that contain nitrogen and are derived from various amino acids. Alkaloids are known to have blood glucose lowering activity, antioxidant activity (Nyamai *et al.,* 2016), anti-hypertensive, antimalarial activity (Dholi *et al.,* 2011) and antimicrobial activity (Edeoga *et al.,* 2005).

Saponins are plant compounds that occur either as steroid alkaloids, glycosides of triterpenoids or steroids. These phytochemicals are known to have hypocholesterolaemic, immunostimulant, hypoglycemic effect and anti-carcinogenic properties (Ros, 2000).

In this study, evaluation of antibacterial activity of ethanolic extract of the studied plants was carried out by agar well diffusion method against both gram positive and gram negative bacterial isolates from diabetic wound. The mean zone of inhibition of the extracts are shown in table 2, it can be observed that the extract of *Leptadenia hastate* exhibited varying degrees of antibacterial activity against all the tested organisms. The mean zone of inhibition of the extract increases with increase in the concentration of the extract. The highest zone of inhibition of 20.0±0.00mm was observed by citrobacter specie at a concentration of 8mg/ml. The antibacterial effect of *Leptadenia hastata* against *E.coli* and *Proteous specie* was in conformity to the study of Ali *et al.* (2019*)*who reported the inhibitory effect of ethanolic leaves extract of leptadenia hastata against some gastro-intestinal isolates. Another study by Umaru *et al.* (2018) also showed antibacterial activity of various extracts of *Leptadenia hastata* against Salmonella typhi. In this presence study the antibacterial effect of *Leptadenia hastata* bacterial isolates from diabetic wound could be attributed to the presence of the above phytochemicals. The ethanolic extract of *Anisopus mannii* did not show any activity against the tested organisms. But other researchers (Aliero and Wara, 2009; Sani *et al*, 2009; Oludare *et al*., 2016) reported the antibacterial activity of the plants against some isolates from other diseases. This could be due to the absent of alkaloid which is present in *Leptadenia hastata.*and alkaloid has been reported by Ali *et al*. (2019) to in inhibit bacterial growth.

The MIC and MBC of ethanolic extract of *Lepdenia hastata* was shown in table 3. The MIC values ranging from 2mg/ml-8mg/m (table 3) and the MBC values ranges from 2mg/ml-4mg/ml. A study carried out by Ali et al. (2019*)* on antibacterial activity of ethanol extract of *Leptadenia hastata* against some gastro-intestinal isolates also reported MIC and MBC values ranging

between 6.25-50mg/ml. According to Agyare *et al.* (2014) extract exhibiting antibacterial activity where MIC values is below 8mg/ml possess very effective antibacterial activity.

Table 1: Phytochemical constituents of *L.hastata* and *A.manniii* ethanol extracts

Plant extract	Saponins	Tannins	Phenols	Flavnoids	Alkaloids
L.hastata	+	+	+	+	+
A.manniii	+	+	+	+	-

Table 2: showed the antibacterial activity of *L.hastata* and *A.manniii* ethanolic extracts against the test organisms

Plant extract	Extract Concentration (mg/ml)	Mean zone of inhibition(mm) of the extract on the test organism		
		Citrobacter specie±SD	*E.coli*±SD	*Proteus vulgaris*±SD
Anisopus mannii	*8*	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
	4	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Leptadenia hastata	*8*	20.0±0.00	16.0±0.00	11.5±0.71
	4	18.0±0.00	11.5±0.71	0.0±0.00
Ciprofloxacin (30µg)		32.5±2.12	23.0±1.41	21.5±0.71
DMSO		0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

Data given are mean of two replicates ± standard deviation.

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic extracts of *L.hastata* on the test organisms

Plant extract	MIC and MBC on the isolates in mg/ml					
	Citrobacter specie		*E.coli*		*P.vulgaris*	
	MIC	MBC	MIC	MBC	MIC	MBC
L.hastata	2	2	2	2	8	4

CONCLUSION

It can be concluded that the crude ethanol extract of *L.hastata* exhibited antibacterial effect against citrobacter specie, E.coli and proteous vulgaris which are isolates from diabetic wound. This could be a baseline by pharmacologist for the development of new antibiotic for the treatment of diabetic wound infection. This also may validate the folkloric use of *Leptadenia hastata* in the treatment of diabetic foot infection. Further in vivo and toxicological studies need to be carried out to validate its folkloric use.

REFERENCES

Agyare C., Duah B.Y., Bekoe,E.P., Hensel, A,, Dapaah,S.O. and Appiah, T. (2016). Review: African medicinal plants with wound healing properties. Journal of Ethnopharmacology, 177: 85–100.

Agyare, C., Kisseih, E., Kyere, I.Y. and Sampane, O.P.P. (2014). Medicinal plants used in wound care: Assessment of wound healing and antimicrobial properties of *Zanthoxylum leprieurii*. *Biological Sciences and Pharceutical Research*, 2(8):081-089.

Alam,G., Singh, M.P. Singh A. (2011). Wound healing potential of some medicinal plants. International Journal of Pharmaceutical Sciences Review and Research 9(1):136-145

Ali, M., Diso, S.U., Minjibir, A.A., Sani, J.A., Nas, .FS., Yahya, A. (2017). Assessment of medicinal plants used in treatment of various ailments in Kano city Nigeria. Asian J. Biores. Technol. 2(4):1-11.

Ali, M., Imam, I.U., Abdallah, M.S. (2019). Antibacterial Activity of Leptadenia Hastata Leaves Extracts against Some Gastro-Intestinal Isolates. Archives in Biomedical Engineering & Biotechnology. 1(3):1-5.

Aliero,A.A. and Wara, S.H. (2009). Validating the medicinal potential of *Leptadenia hastata*. African Journal of Pharmacy and Pharmacology, 3(6): 335-338.

Aliyu, A.B., Ibrahim,H., Musa, A.M., Ibrahim, M.A., Oyewale, A.O. and Amupitan, J.O. (2010). In vitro evaluation of antioxidant activity of African Journal of Biotechnology, 9(16);2437-2441.

Bader, M. (2008). Diabetic foot infection. American Academy of Family Physicians, 78(1):71-79

Cassidy, A., Hanley, B., Lamuela-Raventos, R.M. (2000). Isoflavones, lignans and stilbenes-origins, metabolism and potential importance to human health. Journal of the Science of Food and Agriculture. 80: 1044-1062.

Danbatta, S.H. and Aliyu, B.S. (2011). A survey of major ethno medicinal plants of Kano North, Nigeria, their knowledge and uses by traditional healers. Bayero Journal of Pure and Applied Sciences. 4(2):28-35

Dholi, S.K., Raparla, R., Mankala, S.K., Nagappan, K. (2011). Invivo Antidiabetic evaluation of Neem leaf extract in alloxan induced rats. Journal of Applied Pharmaceutical Science, 1 : 100-105

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4:685 – 688

Eloff, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? J Ethnopharmacol., 60(1):1-8

Haruna, A., Mann, A., Ogbadoyi, E.O. (2017). Phytochemical composition and antitrypanosomal activity of leaf extract of *Leptadenia hastata(Pers) Decne*. Bayero Journal of Pure and Applied Sciences. 10(2):292-299.

Inngjerdingen, K., Inngjerdingen, K., Nergård, C.S., Diallo, D., Mounkoro, P.P. and Paulsen, B.S. (2004). An ethnopharmacological survey of plants used for wound healing in Dogonland, Mali. West Africa. Journal of Ethnopharmacology, 92: 233–244.

Kang, N.J., Shin, S.H., Lee, H.J., Lee, K.W. (2011). Polyphenols as small molecular inhibitors of signaling cascades in carcinogenesis. Pharmacology & Therapeutics. 130: 310-324.

Kankara, S.S., Ibrahim, M.H., Mustafa, M., Go, R. (2015). Ethnobotanical survey of medicinal plants used for traditional maternal healthcare in Katsina state, Nigeria. S. Afr. J. Bot. 97: 165-175.

Kothari, V.,Gupta, A. and Naraniwal, M. (2012). Extraction methods for preparation of bioactive plants extract; a comparative study. LAP LAMBERT academic publication.

Kumar, A.S. (2016). Medicinal plants: Future source of new drugs. International Journal of Herbal Medicine. 4(4): 59-64.

Lakshmi, S.S., Chelladurai, G. and Suresh, B. (2016). In vitro studies on medicinal plants used against bacterial diabetic foot ulcer (BDFU) and urinary tract infected (UTI) causing pathogens. J Parasit Dis. 40(3):667–673.

Lar, P.M., Ojile, E.E., Dashe, E. and Oluama, J.N. (2011). Antibacterial activity of *Moringa olefera* seed extracts on some gram negative bacteria isolates. African Journal of Natural sciences. 14: 57-52.

Mann, A., Yahya Y., Banson A. and Ajayi G.O. (2008). Phytochemical and antibacterial screening of *Anogeissus leiocarpus* against some microorganisms associated with infectious wound. African Journal of Microbiology Research, 2:060-062.

Negbenebor, H.E., Shehu, K., Mukhtar, M.F., Oiza, A.Z., Nura, S. and Fagwalawa, L.D. (2017). Ethno Botanical Survey of Medicinal Plants Used by Hausa People in the Management of Diabetes Mellitus in Kano Metropolis, Northern Nigeria. European Journal of Medicinal Plants. 18(2):1-10.

Nuhu, A.A. And Odinaka, U.F. (2012). Phtochemical creanng and antimcrobal activity of Sacocephalus pobeguini L. Africican Journal of Pharmaceutical research and development, 9(1):86-92

Nyamai, D.W., Arika, W., Ogola, P.E., Njagi, E.N.M, Ngugi, M.P. (2016). Medicinally Important Phytochemicals: An Untapped Research Avenue. Journal of pharmacognosy and phytochemistry 4(1):35-46.

Nyamai, D.W., Mawia, A.M., Wambua, F.K., Njoroge, A. and Matheri, F. (2015). Phytochemical Profile of Prunus africanaStem Bark from Kenya. Journal of Pharmacognosy & Natural Products. 2015; 1: 110.

Oguntibeju, O.O. (2019). Medicinal plants and their effects on diabetic wound healing, Veterinary World, 12(5): 653-663.

Oludare, O.T., Olufunke, A., Desoji,C.A. and Joshua, A. (2016). Antimicrobial activity and phytochemical composition of crude ethyl acetate extracts of *Anisopus mannii* on some selected clinically important microorganisms. International Journal of Pharma Sciences and Research. 7(5):231-139.

Olusola, A., Olutayo O., Michael A. and Adepoju A.J. (2012). Antimicrobial activity and Elemental analysis of *Cassia siberiana* leaves Using Atomic Absorption Spectrometer . *J.* Nat. Prod. Plant Resour.2 (1):9-18.

Osibemhe, M., Lawal, N., Umar, D., Omaji, G.O. and Jibiya, S.A. (2017). Effect of aqueous extract of *Anisopus manni* stem in Alloxan-induced diabetic rats. Science World Journal 12(3):7-8.

Rao, N., and Lipsky, B.A. (2007). Optimising antimicrobial

therapy in diabetic foot infections. Drugs. 67:195-214.

Ros,E. (2000). Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. Atherosclerosis. 151: 357-379.

Roy, P., Abdulsalam, F.I., Pandey, D.K., Bhattacharjee, A. , Reddy, N.E. and Malik, T. (2015). Evaluation of antioxidant, antibacterial, and antidiabetic potential of two traditional medicinal plants of India: *Swertia cordata* and *Swertia chirayita*. Pharmacognosy Research. 7(1):S57-S62.

Sani, D., Sanni, S. and Ngulde, S.I. (2009). Phytochemical and antimicrobial screening of the stem aqueous extract of *Anisopus mannii*. Journal of Medicinal Plants Research. 3(3):112-115.

Sani, S.B., Aliyu, B.S., Haruna, M., Yahya, S.M., Mardiyya, A.Y., Ajingi, Y.S., Wada, L.H. and Abba, H. (2019). Local plants and diabetes management: folkloric practices in metropolitan Kano, Nigeria. Bayero Journal of Pure and Applied Sciences. 12(1):268-273.

Shinkafi, T.S, Bello L, Hassan, S.W, Ali, S. (2015). An ethnobotanical survey of antidiabetic plants used by Hausa-Fulani tribes in Sokoto, Northwest Nigeria. J. Ethnopharmacol. 172(22), 91-99.

Suresh, A., Muthu,G., Suresh, G., Premnath, R., Gopinath, P., Moses, A. and Ramesh, S. (2012). Screening of antibacterial properties of indian medicinal plants against multi drug resistant diabetic foot ulcer isolates. International Journal of Phytopharmacology. 3(2): 139-146.

Swargiary, A. (2017):Recent trends in traditionally used medicinal plants and drug discovery. Asian Journal of Pharmacy and Pharmacology. 3(4):

Taylor, L. N.D. (2000). Plant Based Drugs and Medicines, Rain Tree Nutrition

Thomas, S.D. (2012). *Leptadenia hastata*: A Review of its Traditional uses and its Pharmacological Activity. Medicinal chemistry. 2(7):148-150.

Umaru, I.J., Badruddin,F.A., Assim, Z.B.and Umaru, H.A. (2018). Antimicrobial properties of leptadenia hastata (pers) decne leaves extract. International Journal of Pharmacy and Pharmaceutical Sciences. 10(2):149-152.

Varma N. (2016). Phytoconstituents and Their Mode of Extractions: An Overview. Research Journal of Chemical and Environmental Sciences, 4(2):8-15.

Yusha'u, M. (2011). Phytochemical screening and antibacterial activity of *Hibiscus sabdariffa* extracts against some urinary tract isolates. Best journal. 8(2):83-86.