South Asian Research Journal of Natural Products

Volume 7, Issue 1, Page 1-8, 2024; Article no.SARJNP.110724

Phytochemical, Anti-microbial and Chromatographic Studies of Root Bark Extracts of *Acacia ataxacantha*

Duguryil, Z. P. [a], Gambo, N. N. [a*], Chukwu, A. C. [a], Ogori, B. O. [a] and Denji, K. B. [a]

[a] Chemistry Department, Federal College of Education, P.M.B. 27, Pankshin, Plateau State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/110724

Original Research Article

Received: 23/10/2023
Accepted: 27/12/2023
Published: 02/01/2024

ABSTRACT

The paper is a report of an experimental work designed to carry out the phytochemical, antimicrobial and chromatographic studies on the polar extracts of the root bark of *Acacia ataxacantha*. The plant materials were extracted with n-hexane and thereafter successively extracted with ethyl acetate, methanol and water in that order. Phytochemical screening of the extracts revealed the presence of saponins, tannins, flavonoids steroids and anthraquinones, thin layer chromatography of the crude extracts gave two distinct spots for each of the extracts. Rf values obtained for methanol extract stood at Mf_1(0.340) and Mf_2(0.520) and those got for water extract remained as Wf_1 (0.300) and Wf_2 (0.625). Column chromatographic separation of the crude extracts resulted in 12 fractions for methanol extracts while 13 fractions were eluted from the water extracts. These were pooled into two fractions for methanol extract as ME_1 and ME_2 and water extract fractions as WE_1 and WE_2. Crude extracts as well as the purified fractions were tested on eight bacteria namely, *Pseudomonas aeruginosa*, *Escherichia coli*, S*taphylococcus aureus, Bacillus spp, Klebsiella pneumonia, Proteus mirabilis* and *Salmonella typhi,* and three fungi which included

**Corresponding author: Email: gambonanbol@yahoo.com;*

Candida albicans, Mucur species and *Aspergillus fumigatus*. Both methanol (ME_1 and ME_2) and water fractions (WE_1 and WE_2) exhibited both antimicrobial and antifungal potencies against all the microorganisms.

Keywords: Acacia ataxacantha; anthraquinones; antibacterial and antifungal.

1. INTRODUCTION

Over 800 species of the genus *Acacia* have been reported and they are found all over the world [1]. *Acacia* is a monophyletic genus of flowering plant in the legume family, fabaceace, commonly known as thorne three or shittah tree. *Acacia ataxacantha* is one of these species [2]. "*A. ataxacantha* commonly known as flame thorn is an African tree species" [3]. A thorny multi- stem, woody plant that can be scrambling shrub growing 5 to 8m tall. Sometimes the plant may adopt a more tree-like development, thus growing up to 10m tall with a stem diameter of 20 to 30cm.

"Acacia ataxacantha is wide – spread in Sub-Saharan Africa, spreading from Senegal in the West to Sudan in the North – East. Its spread extends to South – West into Namibia and South Africa. The plant may also be found in kwazulu Natal" [4]. In Nigeria, *Acacia ataxacantha* is found in the middle belt and northern part where it is sometimes planted to form dense Impenetrable hedges [5].

"The leaf of *A. ataxacantha* possesses alternate venation, pinnate with spine that carried 5 – 12 pairs of pinnae. On twig, spines are short, clearly pointing down. The flowers have cream to white colour with a long transition auxiliary, 4 to 5 cm long and arranged on stem, 10 to 15mm are sometimes isolated in pairs. The persistent prickles are profused on young twigs, but can also be found on older wood. Unlike most *acacias*, the prickles are not in pairs, but scattered along young twigs hence, the name ataxacantha" meaning "orderless prickles." The fruit pods are flattened, brownish – red when dry" [6].

"Preparations from different parts of *A. ataxacantha* have been used in African folk medicine for the treatment of wide spectrum of human and livestock diseases" [4]. "Therefore, the dough sheet of the plant is used topically in the treatment of abscesses. The leaf decoction *A. ataxacantha* is claimed to be used orally in fabrile convulsions. Its bark has been effectively used against tooth decay and by inhalation in the case of bronchitis and cough" [7,1]. "In Nigeria, pods and seeds of *Acacia ataxacantha* are used to treat dysentery" [8]. "The roots of the plant are used in Kenya for the treatment of joint and back pain" [9]. "In Namibia, the stem bark is dissolved in boiling water and the filtrate drunk for the treatment of pneumonia [10,4] *Acacia ataxacantha* has been used in the treatment of viral diseases. Reports have also shown that the root part of the plant has been used in the treatment of constipation and abdominal pains" [11,12].

The effectiveness of the plant against some pathogens has been confirmed by modern scientific studies. For example, ethylacetate extract of the stem bark of the plant showed activity against *Staph. aureus, Staphylococcus epidermidis, and Enterococcus feacalis*. The stem bark extracts in n-hexane has also shown some antifungal activity against *Aspergillus strains*. The phytochemical examination of the plant's extract indicated the presence of tannins, alkaloids, flavonoids, steroids, Coumarins, saponins and naphthaquinones [6].

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

Fresh plant materials of *Acacia ataxacantha* were harvested in Mararaba Pushit village of Mangu Local Government Area of Plateau State, Nigeria. They were collected from their wild plants on the field.

Identification and authentication of the plant were confirmed immediately after harvest by experts in the Department of Botany, University of Jos, Plateau State, Nigeria. The materials were then air-dried at ambient temperature for two weeks. They were ground into powder, sieved with a 1.5mm by 2mm plastic sieve. The sieved powder was then stored in sterile polythene bags for further use.

2.2 Method of Extraction

"Two hundred (200g) grams of the powdered root bark of the plant was wrapped in a filter paper

and carefully placed into the porous thimble. The thimble was then inserted into the inner tube of the Soxhlet extractor. The loaded thimble was then fitted to a 500cm^3 round-bottomed flask containing hexane to boil over a heating mantle until the vapour passed up through the tube into the condenser. The condensed solvent extracted the plant material in the thimble into the flask. The process was allowed to run for 4 hours. At the end of 4 hours the process was stopped when it was obvious that extraction was no longer taking place by the solvent. The wrapped plant materials were removed from the thimble and dried before returning it into the thimble. This process was repeated using ethyl acetate at 30-40 ^{0}C for 12 hours. Methanol, at 50 – 60 ^{0}C for 8hours and water was allowed at 60-70 ^{0}C for 12hours in that order. Each extract was concentrated in *vacuo* with the aid of a rotary evaporator, and the respective extract concentrates were kept in desiccators to dry for at least 3 days before further test were carried out on them" [3].

2.3 Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out on the crude extracts according to standard methods [13-15]. The plant extracts were screened for the presence of alkaloids, cardiac glycosides, flavonoids, steroids, anthraquinones, saponins tannins, glycosides, terpenoids, and others. Results of phytochemical screening are shown in Table 1.

2.3.1 Test for cardiac glycosides

2.3.1.1 Keller killiani test

"A small quantity (0.5 g) of the extract was dissolved in a little quantity of glacial acetic acid containing one drop of iron (iii) chloride solution. This was then under- laid with concentrated sulphuric acid. No brown ring was observed at the interface between the glacial acetic acid and the sulphuric (VI) acid. This indicated the absence of a deoxy sugar characteristics of cardenolides" [15].

2.3.2 Test for saponins (Frothing test)

"Small portions of each of the plant extracts were shaken vigorously with 10.0 cm^3 of distilled water in a test tube. The test tubes were placed in a water bath. Frothing which persisted on warming was taken as evidence for the presence of saponins" [3].

2.3.3 Test for anthraquinones: Borntrager's test

":0.5 g each of the plant extract was shaken with 2.0 cm^3 of benzene and filtered. 4.0 cm^3 of 10 % ammonia solution was added to the filtrate. The resultant mixture was shaken and the presence of a red colour in the ammonia solution (lower layer) phase was taken for the presence of free anthraquinones" [3].

2.3.4 Test for steroids and Terpenes

"A solution of each of the extracts was made by dissolving 0.5 g of the extract in 2.0 cm^3 of chloroform. The solution was subjected to Liebermann-Burchard test where a bluish – green or blue colour was taken for the presence of steroids while pink colour was taken for the presence of terpenes" [3].

2.3.4.1 Liebermann-burchard test

"To the solution of the plant extract in chloroform, 3.0 cm^3 of acetic anhydride was added, mixed gently and allowed to cool in ice. This was followed by the careful addition of a few drops of concentrated H_2SO_4. A pink colour was observed" [3].

2.3.5 Test for flavonoids

i. **Lead acetate test:** 0.5 g of the extract was dissolved in 5cm^3 of distilled water and 1cm^3 of 10 % lead acetate was added to it. The appearance of a yellow precipitate was considered a positive test for flavonoid.

ii. **Iron (ii) chloride test:** Two drops of iron (ii) chloride were added to a solution of the plant extract prepared by dissolving 0.5 g of the crude extract in water. A dark colouration was taken for the presence of phenolic compounds.

2.3.6 Test for Tannins

"About 0.5 g of each of the plant extract was boiled with 10cm^3 of distilled water for 5 minutes. The solution was filtered and filtrate obtained. To 2.0 cm^3 of the cooled filtrate, a few drops of ferric chloride was added. The absence of a blue-black, green or blue-green precipitate was taken for the absence of tannins" [3].

2.3.7 Test for alkaloids

"0.5 g of each extract was stirred in 5 cm^3 of 2M aqueous hydrochloric acid and the resultant solution was Mayer's reagent. A second 1cm^3 was treated with Dragendorf's reagent. The absence of a creamy white precipitate with Mayer's reagent and orange-red colour or turbidity with Dragendorf's reagent indicated preliminary evidence for the absence of alkaloids" [3].

3. ANTIMICROBIAL STUDIES (Bioassay)

3.1 Preparation of Media

Various culture media used were prepared according to the manufacturers' instructions.

3.1.1 Nutrient agar

To prepare nutrient agar, 28 g of nutrient agar powder was weighed and suspended in 500cm^3 of distilled water in a conical flask. This was then brought to boil to dissolve the medium completely. The resultant solution was made up to 1dm^3 (1000 cm^3) mark of conical flask with distilled water. The mixture was placed in a water bath and sterilized in an autoclave for 15 minutes at a pressure of 15 atmospheres and a temperature of 121 ^{0}C. The medium was allowed to cool to a temperature of 40 ^{o}C when 20 cm^3 of this stock solution was then dispensed into each petri dish and allowed to gel [16,17].

3.1.2 Chocolate Agar (CA)

Chocolate agar powder was weighed as instructed by the manufacturer. This was dissolved in 1 dm^3 (1000 cm^3) of sterile distilled water. The resultant mixture was autoclaved for 15minutes at 121 ^{o}C. After autoclaving, this was allowed to cool to 45 ^{o}C. 10cm^3 of sterile defibrinated blood was added to the resultant mixtures. It was then well mixed after which it was poured into petri dishes and allowed to set [18].

3.1.3 Preparation of McFarland's standard

To carry out this, 1 cm^3 of concentrated sulphuric acid was added to 99 cm^3 of distilled water to make 1 % v/v of solution of the acid.

Similarly, 0.5 g of anhydrous barium chloride was dissolved in 50 cm^3 of distilled water to make 1 % v/w solution of barium chloride.

A 0.5 cm^3 of the barium chloride solution was added to 99.5 cm^3 of sulphuric acid solution and thoroughly mixed. The test isolate was inoculated into peptone water and their densities adjusted to 0.5 McFarland's Standard [18].

3.1.4 Preparation and standardization of inoculums

The growth method was used to prepare the inoculum for the bacteria and fungi that were used for bioassay.

The eight colonies of bacteria were picked from overnight growth on agar and inoculated into broth and incubated for about 18 to 24 hours at 37 ^{o}C. Chocolate agar broth was used for the spores of fungi and also incubated but at 25^{0}C for 48 hours. The turbidity produced by the organisms was adjusted to match 0.5 McFarland.

3.1.5 Inoculation of the plates and application of the extracts

"To inoculate the plate, one drop of the adjusted sub-cultured nutrient broth was applied to the surface of the nutrient agar (NA) and chocolate agar (CA) and evened to cover the surface of the agars with microbes. One microbe was inoculated to one plate making a total of 15 plates for 15 microorganisms. After 30 minutes, four wells were punched on the plate using a sterile cork borer of 5mm diameter, 2 for the water and methanol extracts, 1 for negative control and 1 for positive control. A 0.1 cm^3 of the extract from each solvent (equivalent to 20 mg of the extract) was dropped into each appropriately labeled well. Into the remaining two wells, sterile distilled water and ciprofloxacin (a positive control for bacteria used) of the same concentration as the extracts were introduced to serve as negative and positive controls respectively for the bacteria. For the remaining seven wells in fungi plates, distilled water and ketoconazole (a positive control used for fungi) of the same concentration as the extracts were introduced to serve as negative and positive controls respectively. The inoculated plates were left on the table for 1 hour to allow for proper diffusion. Agar plates were incubated aerobically at 37 ^{0}C while the chocolate agar, containing the seven fungi was incubated for 48 hours at 25 ^{0}C. Zones of inhibition produced after incubation

were measured by linear measurement of diameter" [3].

4. THIN LAYER CHROMATOGRAPHY

4.1 Spotting the Plates

"Commercially prepared Tlc plates of adsorbent made of silica gel G was used. The solid test sample of 10mg was placed in a watch glass and dissolved with a few drops of ethylene chloride. A small capillary pipette was filled by dropping the pulled end into the solution to be examined. The pipette was emptied to the thin layer plate at a point about 1 cm from the bottom. The spotting was repeated. It was, however, ensured that the solvent was allowed to evaporate between applications" [3].

4.2 Developing Tlc Plates

"A solvent system of methanol: Chloroform (7:3v/v) was best for the separation of water extract while the same methanol: Chloroform (4:1v/v) was best for the separation of methanol extract. The same solvent systems that gave neat separation with qualitative thin layer chromatography were used for the column chromatography" [3].

4.3 Column Chromatographic Seperation of Crude Extracts

To separate the fractions (active components) of *A. ataxacantha* from the crude extracts, column chromatographic method as described in literatures [19] was adopted.

5. RESULTS AND DISCUSSION

5.1 Results

The results of the preliminary phytochemical screening of the crude polar (water and methanol) extracts of the root back of *A. ataxacantha* is as presented in Table 1. The results for the susceptibility studies of both crude extracts and chromatographic fractions of the plant are shown in Tables 2 and 5. Tlc results and those of the pooled column chromatography fractions are presented in Tables 3 and 4.

5.2 Discussion

Qualitative phytochemical examination conducted on both methanol and water extracts of the plant part under study using conventional methods revealed the presence of saponins and flavonoids in both plant extracts while only methanol extracts exhibited the presence of tannins, steroids and anthraquinones. These classes of compounds have been reported to have curative activity against a number of pathogens and therefore could suggest their use traditionally for the treatment of various illnesses [20].

The *in vitro* antimicrobial test shown in table 2 revealed that methanol extract was active against all bacteria involved in the study except *Bacillus spp.* on which activity was not pronounced (9mm was taken as bench mark for sensitivity). Water extract on the other hand only inhibited the growth of *Staphylococcus aureus* and *Klebsiella pneumonia*. Methanol extract was also found to show some activity against two (2) of the fungi used for the study. These included *Candida albican* and *Mucor spp*. Water crude extract showed some sensitivity against *Aspergillus fumigatus*. Both methanol and water crude extracts were not active against the rest of the fungi: *Penecillium spp, Aspergillus flavus, Trichphyton megninii* and *Trichophyton rubrum* (Any extract with diameter zone of inhibition below 5mm against fungi was regarded inactive).

For the fact that these crude extracts greatly inhibited the growth of *P. aeruginosa, E. coli, K. pneumonia and S. pneumonia* (Bacteria reputed for causing various kinds of bacterial infectious diseases) as well as *Candida albican* and *Mucor species* (fungi responsible for causing fungal infections like candidiasis and mucormycosis respectively) gives further credence to the traditional use of the plant in the treatment of microbial infections.

Qualitative thin layer chromatography of both methanol and water crude extracts gave two spots each: Mf_1, Mf_2, Wf_1 and Wf_2 respectively. Their R_f values are presented in Table 3.

Also, for column chromatography, a total of 12 fractions were collected for methanol extract while 13 were eluted from water extract. These were pooled into two fractions for each extract according to their polarity. The result is shown in Table 4 as ME_1, ME_2, WE_1 and WE_2.

Table 5 summarizes the results of microbial tests carried out on the yellow and oily methanol and water fractions. The results showed that the methanol fraction, ME_1 greatly inhibited the growth of all the bacteria used for the study

except *Proteus mirabilis*. The fraction ME_1 exhibited a greater inhibition of growth against *Staphylococcus aureus*. *Klebsiella pneumonia, Streptococcus pneumonia* and *Salmonella typhi* when compared with that of the standard drug ciprofloxacin.

Water fraction WE_1 also demonstrated a pronounced inhibition of growth against all the pathogens. These bacteria have been implicated in causing various infectious diseases in human [21].

Table 1. Phytochemical constituents of the polar extracts of the root back of *Acacia ataxacantha*

Metabolites	1	2	3	4	5	6
Extractions						
EMeOH	++	+++	++	++	+	+
EH_2O	+++	-	++	+++	-	-

Key:EMeOH = Methanol extract; (+++)High concentration; EH_2O = Water extract; (++) Moderate
1 = Saponins 2 = Tannins (+) Trace.
3 = flavonoids;4 = carbohydrates;5 = steroids;6 = Anthraquinones

Table 2. Antimicrobial activity test of crude extracts from the root back of *Acacia ataxacantha*

S/N Test Organism	Diameter Zone of Inhibition (mm)		
(Bacteria)	**MeOH**	**Water**	**+ve Control**
1. *Pseudomonas aeruginosa*	19	00	19
2. *Escherichia coli*	09	00	13
3. *Staphylococcus aureus*	10	15	20
4. *Bacillus spp*	08	00	20
5. *Klebsiella pneumonia*	10	11	30
6. *Streptococcus pneumonia*	10	00	10
7. *Proteus mirabilis*	10	00	13
8. *Salmonella typhi*	10	00	15
(Fungi)			
1. *Candida albican*	06	00	0.5
2. *Penecillium spp*	00	00	08
3. *Aspergellus flavus*	00	03	10
4. *Aspergillus fumigatus*	00	05	12
5. *Trichophyton megninii*	00	00	0.3
6. *Trichophyton rubrum*	00	00	00
7. *Mucur specie*	11	00	12

Key: i. +ve control for Bacteria ≡ Ciprofloxacin; ii. -ve control for bacteria ≡ Sterile distilled water; iii. Diameter of zone of inhibition ≡ Water measure in mm; iv. +ve control for fungi = Ketoconazole (Antifungal drug)

Table 3. R_f values of component of methanol and water extracts

Fractions	Mf_1	Mf_2	Wf_1	Wf_2
R_f values	0.340	0.520	0.300	0.625

Table 4. Pooled column chromatography fractions

Fractions	Solvent System
ME_1 (2 – 6)	Methanol: Chloroform (20:80)
ME_2 (7 – 12)	Methanol: Chloroform (50:50)
WE_1 (2 – 5)	Methanol: Chloroform (30:70)
WE_2 (5 – 13)	Methanol: Chloroform (60:40)

Table 5. Antimicrobial test with various fractions of methanol and water extracts of the root back of *A. ataxacantha*

Microoganisms	Diameter of zones of inhibition (mm)				Standard control	
(**Bacteria)**	**ME_1**	**ME_2**	**WE_1**	**WE_2**	**CIPRO**	**KETO**
Pseudomonas aeruginosa	25	19	20	15	30	–
Escherishia coli	30	15	30	20	35	–
Staphylococcus aureus	27	15	20	15	25	–
Bacillus spp	15	10	20	00	25	–
Klebsiella pneumonia	20	00	20	20	10	–
Streptococcus pneumonia	25	00	25	00	20	–
Proteus mirabilis	00	00	15	15	25	–
Salmonella typhi	15	15	19	00	10	–
(**Fungi**)						
Candida albican	15	11	16	00	–	28
Mucor spp	30	17	22	00	–	19
Aspergellus fumigatus	20	20	10	15	–	20

Key: CIPRO = ciprofloxacin; KETO = ketoconazole; (–) standard control not used

The methanol fractions ME_1 and ME_2 and the water fractions WE_1 and WE_2 were all found to also demonstrate antifungal activity by greatly inhibiting the growth of *Mucor species* and *Aspergillus fumigatus*. These fungi have been linked to be responsible for various fungal infections [20].

6. CONCLUSION

In conclusion, the results of this investigation, has demonstrated that the root part of the plant may contain potent active constituents that are beneficial in the treatment and prevention of microbial diseases.

The results also substantiate the findings in literature [20] that revealed that extracts of the root bark of *Acacia ataxacantha* have both antibacterial and antifungal properties.

Further purification and characterization of the methanol fractions ME_1 and ME_2 and water fractions WE_1 and WE_2 are ongoing.

ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge the Tertiary Education Trust Fund (TETFund) of Federal College of Education, Pankshin Plateau State-Nigeria for providing the funds for this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Batiha GE, Akhtar N, Alsayegh AA, Abusudah WF, Almohmadi NH, Shaheen HM, Singh TG, De Waard M. Bioactive compounds, pharmacological actions, and pharmacokinetics of genus *Acacia*. Molecules (Basel, Switzer-land). 2022;27(21):7340.
2. Britannica T. Editors of Encyclopaedia. (*acacia. Encyclopedia Britannica;* 2023. Available:https://www.britannica.com/plant/acacia
3. Gambo NN, Okooboh MA, Ibejekwe SJ, Ogori BO, Chindo IY, Agada GO. Phytochemical and chromatographic studies of the polar extracts of the leaves of Cassia alata Linn.
4. Hedimbi M, Chinsembu KC. Ethnomedicinal study of plants used to manage HIV/AIDS related disease conditions in the Ohangwena Region, Namibia International Journal of Medicinal plant Research. 2012;(1):4-11.
5. Dukku U. *Acacia ataxacantha*: A nectar plant for honey bees between two dearth periods in the Sudan Savanna of northern Nigeria. Bee World. 2003;84:32-34.
6. Amoussa AMO, Lagnika L, Sanni A. *Acacia ataxacantha* (Bark): Chemical composition and antibacteria activity of extracts. International Journal of Pharmacy and Phamaceutical Sciences. 2014; 6(11):15-18.
7. Amoussa AM, Lagnika L, Sanni A. Acacia ataxacantha (bark): Chemical composition and antibacterial activity of the extracts.

International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6:138-141.
8. MacDonald I, Joseph OE, Harriet ME. Documentation of Medicinal plants sold in the markets in Abeokuta, Nigeria. Tropical Journal of Pharmaceutical Research. 2010;9(2):12-15.
9. Kereru PG, Gachanja AN, Keriko JM, Mungai G. Traditional Medicines among EMBU and Mbeere People of Kenya. African Journal of Complementary Alternative medicine (Afr. J. CAM) 2007;1 (3):29-31.
10. Kereji AE. Evaluation of Herbal Drugs used to treat Fungal and Bacterial Diseases in Mbeere, Eastern Kenya. International Journal of Herbal Medicine. 2013;1(4):85-87.
11. Olasunmoye KK, Kehinde FY. Ethnobotanical survey of medicinal plants used in treating viral infections among yoruba tribe of South West Nigeria. African Journal of Microbiology research. 2011;5 (19):11-13.
12. Pekzar MJ, Chan ECS, Kieg NR. Microbiology: Concept and Application. Mac Graw Hill Inc. New York. 1993;P967.
13. Zaman K, Pathak K. Pharmacognostical and phytochemical studies on the leaf and stem bark of Annona reticulata L. Journal of Pharmacognosy and Phytochemistry. 2013;1:1-8.
14. Sofowora A. Medicinal plants and traditional medicine in Africa. John Wiley and Sons Ltd. London. 1993;209.
15. Trease GE, Evans WC. Pharmacognosy (15thed) W.C. Evans; Edinburgh W.B. Saunders. 2002;585.
16. Oboh PA, Abulu EO. The anti-microbial activities of extracts of sidium guajava and citounaurantifolia. Nigerian Journal of Biotechnology. 1997;8:25-28.
17. Monica C. District laboratory practice in tropical countries (part 2).U.K. Cambridge University Press. 2000;32-34.
18. Bhutia MO, Thapa N, Tamang JP. Molecular characterization of bacteria, detection of enterotoxin genes, and screening of antibiotic susceptibility patterns in traditionally processed meat products of Sikkim, India. Frontiers in microbiology. 2021;11:599606.
19. Vogel AI. A Textbook of practical organic chemistry (ed) B.S. Furniss, London: Lognman. 1989;156-157)^{a} (164-168)^{b}.
20. Aba OY, Ezuruike IT, Ayo RG, Habila JD, Ndukwe GI. Isolation, antibacterial and antifungal Evaluation of α – amyrenol from the root extract of A. *ataxacantha* D.C. Scholars Academic Journal of Pharmacy. 2015;4(2):124–131.
21. Marius H, Kazhila CC. Ethanomedicinal study of plants used to manage HIV/AIDs related diseases conditions in the ohangwena region, Namibia International Journal of Medicinal plant Research. 2012; 1(1):4-11.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/110724