European Journal of Biology and Medical Science Research

Vol.8, No.3, pp.43-52, June 2020

Published by ***ECRTD- UK***

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

# PHYTOCHEMICAL SCREENING OF PODS OF *INDIGOFERA TINCTORIA* L. (URI) AND ITS ANTIBACTERIA AND ANTIFUNGI PROPERTIES

**Adjeroh, L.A.,[1] Nwachukwu, M.O.,[1] Nnokwe, J.C., Azorji, J.N[2]., and Osinomumu, I.O. [1]**

*[1]Department of Biology, Federal University of Technology, Owerri, Imo State Nigeria*

*[2]Department of Biological Sciences, Hezekiah University Umudi, Imo State Nigeria.*

*Corresponding author's email: nwachukwumichaelo@yahoo.com*

**ABSTRACT:** *The phytochemical screening of Indigofera tinctoria L. (Uri) pod extracts using hot ethanolic, cold ethanolic and aqueous extracts showed the presence of Alkaloids, Flavonoids, Saponins, Tannins and Cardiac glycosides. Alkaloids were present in all the three different extracts, saponins was absent in cold ethanolic extract but present in the others, flavonoids was present in only cold ethanolic extract, cardiac glycosides were present in cold ethanolic and aqueous extract but absent in hot ethanolic extracts. Antimicrobial activity of Soxhlet ethanolic and aqueous extracts in different concentration were used against the following pathogenic bacteria isolates like Escherichia coli, Salmonella typhi, Staphylococcus auerus and Bacillus cereus using Disk diffusion method. The undiluted Soxhlet ethanolic extract showed that S. typhi has the highest zone of inhibition of 25 mm, followed by B. cereus, S. auerus at 11 mm and E. coli having the least zone of inhibition at 8 mm. In undiluted concentration of aqueous extract, B. cereus has the highest zone of inhibition of 11 mm, followed by E. coli at 8 mm, S. auerus at 7 mm and S. typhi at 6 mm being the least. Also in Soxhlet ethanolic extract at dilution 1:1 concentration, the highest zone of inhibition is B. cereus at 10 mm, followed by S. typhi at 7 mm, while there was no zone of inhibition against E. coli and S. auerus. While in aqueous extract at dilution 1:1, B. cereus and S. typhi has the same zone of inhibition of 7 mm and no zone of inhibition was observed against E. coli and S. auerus. Similarly, in Soxlet ethanolic extract of 1:2 dilution concentration, zone of inhibition was observed only on B. cereus as 7 mm and no zone of inhibition was observed in the remaining isolates. For aqueous extract at dilution 1:2, there was no zone of inhibition against all the isolates. Hence the percentage antibacterial activity of both extract indicated that B. cereus with cumulative zone of inhibition of 47 mm has thre highest percentage zone of inhibition at 37.30%, followed by S. typi at 45 mm against 35.71%, S. auerus at 18 mm against 14.29% and E. coli at 16 mm against 12.70% as the least percentage of antibacterial activity. Conversely, same extracts tested against fungal isolate like Aspergillus tereus, did not exhibit antifungal properties. Thus the results obtained from this study suggested that Indigofera tinctoria pod validate the use of undiluted extracts of this species in ethnomedicine and could provide a lead in the treatments of bacterial infections.*

**KEYWORDS**: Phytochemical, Pods, Indigofera *Tinctoria* L. (Uri), Antibacteria, Antifungi

## INTRODUCTION

*Indigofera* is a pea-family genus found from Asia through South Africa. It contains more than 750 species of shrubs, trees, and herbs in the pea family (Fabaceae). Indigofera plants are pink/white flowered perennials and subshrubs that range from dwarfs to small trees. Because of their long-flowering habit and easy-to-grow nature, indigofera have become garden favorites. Certain species (*Indigofera tinctoria* and *Indigofera suffruticosa*) are used by tropical farmers to produce the dark blue dye called indigo. The cultivation of indigo plants and the extraction of the dyestuff were an important industry in India up to the beginning of the 20th century. Synthetic indigo, developed about that time, gradually replaced natural indigo as a dyestuff. The plants are native to tropical and subtropical regions worldwide. Indigo species are highly variable in appearance but are generally silky or hairy with compound leaves. The rose, purple, or white flowers are borne in showy spikes or clusters, and the fruit is a pod, usually with a thin partition between the seeds (Encyclopedia Britannica 2018). Medicinal plants represent a rich source of antimicrobial and antifungal agents. In last few years, a number of studies have been conducted on medicinal herbs in different countries to prove the efficiency of antimicrobial and antifungal potential. Medicinal plants possess immunomodulatory and antioxidant properties. Plants contain several phytochemicals which possess strong antioxidant activities (Senthilkumar & Venkatesalu, 2009). These antioxidant play an important role in the prevention of chronic ailments such as heart diseases, cancer, diabetes, hypertension, stroke and Alzheimer's disease by protecting the cells from damage caused by free radicals-the highly reactive oxygen compound. Thus, with this background, the present study was undertaken with an aim of evaluating the antibacterial and antifungal properties of ethanolic and aqueous extracts of *Indigofera tinctoria* pod.

**Fig. 1**: Image of the pod of *I. tinctoria* (a) Fresh pod and (b) Dry pod

Multidrug resistance pathogenic microbes have risen so high. This has been attributed to indiscriminate consumption of antibiotics (Shariff, 2001). More so the synthetic chemicals resulting from antimicrobial agents are found to suppress the immune resistance of the body to fight against diseases, thus resulting in the failure to control further incidence of microorganisms. Hence from the light of the above there is need to provide an alternative antimicrobial agents from natural source such as plants, that will be effective in fighting various infectious diseases without compromising human health. This study was conducted to evaluate the antibacteria and antifungi properties of ethanolic and aqueous extracts of *Indigofera tinctoria* pods.

## MATERIAL AND METHODS

The study was carried out at Federal University of Technology, Owerri between the months of July and October, 2018. Owerri is the capital of Imo State found on longitude $5^{0}30^{1}$ north and $70^{0}10^{1}$ East. It lies in the tropical rainforest region of South Eastern Nigeria. There are two distinct seasons in the area; the dry season (November-March) and rainy season (April- October). The dry season is associated with very high humidity of about 80-85%; and very heavy rainfall. Temperature varies according to season between $25^{0}$ c to $32^{0}$c in sunny days. The chief occupation of the local people is farming, but due to over-farming and high population density, the soil has greatly degraded. The cash crops include oil palm, raffia palm, rice, groundnut, melon, cotton, cocoa, rubber, and maize. Consumable crops such as yam, cassava, cocoyam and maize are also produced in large quantities. However, there are few traders, private business owners, artisans, civil servants and professionals like Doctors, Engineers and Lawyers.

### The Study Locations

The study took place in Biological Science Laboratory, Industrial Chemistry Laboratory, both in Federal University of Technology Owerri and finally at Anthony Van-Leeuwenhoek Research Centre Ihiagwa Owerri.

### Sample Collection and Identification

Fresh pod of *Indigofera tinctoria* was collected from Awlaw and Egwu-achi village in Oji-River Local Government Area of Enugu State, Nigeria in the month of July, 2018. And were identified by the chief and taxonomist of the department of Biology, Federal University of Technology Owerri. Dr. C. M. Duru.

### Sample Preparation/Processing

The freshly collected pods of *Indigofera tinctoria* were washed gently in a tape running water, then shade dry for $\mathbf{1^{1/2}}$ month, and finally oven dry at $40^{0}$c in Biology laboratory. The dried pod was weighed with an electronic weighing balance, so as to obtain a constant weight before pulverizing. Then the pod was pulverized to a very fine powder using mortar and pestle in Biology Laboratory. The homogenized powder was stored in an airtight Bijou bottle for further use.

**Preliminary Qualitative Phytochemical Screening/Analysis**

Preliminary qualitative phytochemical screening was done with meathod of Harbone (1998), Parekh and Chanda (2007). The pod of I. tinctoria extracts (i.e. soxhlet extract using ethanol, cold ethanolic, and aqueous extract) were all analyzed for the presence/ absence of saponins, flavonoids, alkaloids, tannins, and cardiac glycosides.

**Test for Saponins:** The presence of saponins was determined by Frothing test.2ml of the pod extract was vigorously shaken with 2ml distilled water and was allowed to stand for 10 minutes and classified for saponin content as follows: No froth indicate absence of saponins and stable froth more than 1.5 cm indicated the presence of saponins (Kapoor et al., 1969).

**Test for Flavonoids:** 2ml of the pod extract was dissolved in dilute NaOH. A yellow solution that turns colourless on addition of con. HCl indicates the presence of flavonoids.

**Test for Alkaloids**: 2ml of the pod extract was acidified with 1% HCl (i.e. 1ml of con. HCl in 99ml of distilled water) and was treated with few drops of Wagner reagents in a test tube. A reddish brown precipitates indicates the presence of alkaloids (Salehi-Surmaghi et al., 1992).

**Test for Tannins:** 2ml of the extract was treated with about 10mls of distilled water and then few drops of 1% ferric chloride (FeCl3) solution were added. The occurrence of blue-black, green or blue greenish precipitate indicates the presence of tannins (Segelman et al., 1969).

**Test for Cardiac glycosides:** Keller-kiliani test was performed to assess the presence of cardiac glycosides. 2ml of the extract was treated 2ml of glacial acetic acid containing 1 drop of ferric chloride solution. To this solution a few drops of concentrated sulphuric acid ($H_2SO_4$) was added. A brown ring formation at the interphase indicates the presence of deoxy sugar characteristics of Cardiac glycosides (Ajaiyeobu, 2002).

**Test Organisms (Bacterial and Fungi)**

Pure culture of 24 hours *staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi*, and *Escherichia coli* of bacteria origin and an already cultivated culture of *Aspergillus terreus* were all collected from Anthony Van-Leeuwenhoek Research Centre Ihiagwa Owerri, Imo State in Nigeria.

**Screening for Antimicrobial Assay**

Antimicrobial activity was screened by agar well diffusion method (Perez *et al*., 1990). The plant extracts were tested for antimicrobial activity against bacterial pathogens such as *staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella typhi*. And *Aspergillus terreus* as fungal pathogen.

**Screening for Bacteria Pathogen**

12 different plate were prepared, containing the solidified MHA, then the test bacteria were inoculated into each of the plate by streak plate method, using sterile inoculating loop, with each test organisms inoculated into three different plate and labeled appropriately with marker, the plates were separated into three different sets such as A, B, and C. Each set, contain the test bacterium. They were evenly spread over the medium by streaking. Then two (2) wells of 6mm were made in each of the plate with a sterile cork borer. The plant extracts were also labeled using code such as 1 and 2, where 1= Soxhlet Ethanolic extract and 2 = Aqueous extract of pod

of *I. tinctoria*. In the first set of plate labeled 'A', the extracts were pipetted without any dilution and filled the hole, while in the second set label 'B', the extracts was diluted with sterile water in a ratio of 1:1 and then pipetted to fill the hole, conversely in the last three set, the extracts were diluted with sterile water in a ratio of 1:2, before pipetting into the hole. Finally the plates where incubated in an upright position for 24 hours at $37^0$C, after incubation the plates where observed for the formation of clear inhibition zone around the well indicating the susceptibility of the extracts. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well.

**Screening for Fungal Pathogen**

Two (2) conical flask with aluminum foil cover were sterilized alongside with already prepared medium of Potato Dextrose Agar (PDA) with autoclave. The medium were poured into the conical flask after autoclaving, and the undiluted ethanolic and aqueous extracts of *I. tinctoria* pod, were added to the medium and shaked thoroughly for proper mixing. The mixture was poured into two different sterile petri dish labeled according to the type of extracts used and allowed to solidify. The spore of *Aspergillus tereus*. Were inoculated into the medium using sterile inoculating loop and the plate was incubated in an upright position for more than 24 hours, after which the plate was observed for the degree of susceptibility.

## RESULTS

The results of the study showed that *I. tinctoria* pod exhibited both antibacterial and antifungal activities. The degree of activities depend on the type of pathogenic microbe used. The potency of the pod extracts was due to the presence of the following bioactive compound found in all the extracts; alkaloids, flavonoids, tannins, saponins and cardiac glycosides. The occurrence of these bioactive compounds and the results of antibacterial and antifungal are represented in the table below.

Table 1. Qualitative Phytochemical Analysis of *I.* tinctoria (Uri) Pod Extracts

| **Phytochemical screened** | **Type of Extract/Indication** | | |
|---|---|---|---|
| | **Hot Ethanolic (Soxhlet)** | **Cold Ethanolic** | **Aqueous** |
| Alkaloids | + | + | + |
| Flavonoids | − | + | − |
| Saponins | + | − | + |
| Tannins | + | − | + |
| Cardiac glycosides | − | + | + |

Where−=Absence of parameter checked and +=Presence of parameter

From the table above, Alkaloids is present in all the extracts of *I. tinctoria* pod, Flavonoids is present in cold ethanolic extract but absent in both hot ethanolic and aqueous extracts, Saponins and Tannins are absent in cold ethanolic extracts but present in hot ethanolic and aqueous extracts, while Cardiac glycosides is absent in hot ethanolic extract but present in both cold ethanolic and aqueous extracts.

**Table 2:** Antibacterial activity of *I. tinctoria* (uri) Pod at Different Concentration

| **Type of Extract** | **Concentration Per Well** | **Diameter of Inhibition Zone (millimeter)** | | | |
|---|---|---|---|---|---|
| | | **TEST ORGANISM** | | | |
| | | **E** | **B** | **Sa** | **S** |
| 1a | Undiluted | 8 | 12 | 11 | 25 |
| 2a | Undiluted | 8 | 11 | 7 | 6 |
| 1b | 1:1 | - | 10 | - | 7 |
| 2b | 1:1 | - | 7 | - | 7 |
| 1c | 1:2 | - | 7 | - | - |
| 2c | 1:2 | - | - | - | - |
| **Total** | | **16** | **47** | **18** | **45** |

**1** = soxhlet ethanolic extract of *I. tictoria* pod, **2** = Aqueous extract of *I. tictoria* pod, **a** = undiluted concentration of the extract, **b** = dilution in 1:1 (i.e. 1 ml of sample + 1 ml of sterile water), **c** = dilution in 1:2 (1 ml of sample + 2 ml of sterile water), **E** = *Escherichia coli*, **B** = *Bacillus cerus*, **Sa** = *Staphylococcus aureus*, **S** = *Salmonella typhi*

From table 2 above, Soxhlet ethanolic and aqueous extracts were used in various concentration such as diluted and undiluted concentration, the dilution was done in a concentration of 1:1, 1:2 and they were all used against *Escherichia coli*, *Salmonella typhi*, *Bacillus cereus*, and *Staphylococcus auerus*. The highest zone of inhibition measured in millimeter was 25 mm against *S. typhi*, followed by *B. cereus* at 12 mm, *S. auerus* at 11 mm and *E. coli* having the least zone of inhibition at 8 mm, for undiluted concentration of soxhlet extract. In aqueous undiluted concentration, *B. cereus* has the highest zone of inhibition at 11 mm, followed by *E. coli* at 8 mm, *S. auerus* at 7 mm and *S. typhi* having the least zone of inhibition at 6 mm, for soxhlst extract at dilution 1:1 concentration, the highest zone of inhibition was *B. cereus* at 10 mm, followed by *S. typhi* at 7 mm, while there was no zone of inhibition in *E. coli* and *S. auerus* respectively. While in aqueous extract at dilution 1:1 concentration *B.cereus* and *S.typhi* has the

same zone of inhibition at 7 mm, while there was no zone of inhibition in *E,.coli* and *S. auerus*. In the soxhlet ethanolic extract at dilution 1:2 concentration, zone of inhibition was observed only in *B. cereus* at 7 mm, while there was no zone of inhibition in the rest of the test isolates. In the aqueous extract at dilution 1:2 concentration, there was no zone of inhibition against all the isolate.

**Table 3:** Cumulative Percentage Antibacterial Activity of Soxhlet ethanolic and Aqueous Extracts of *I. tinctoria* (uri) Pod

| **Test bacteria** | **Total zone of inhibition (mm)** | **Percentage (%)** |
|---|---|---|
| *E. coli* | 16 | 12.70 |
| *B. cereus* | 47 | 37.30 |
| *S. aureus* | 18 | 14.29 |
| *S. typhi* | 45 | 35.71 |
| **Total** | **126** | **100.00** |

From the table 3 above, Bacillus cereus has the highest susceptibility to the pod extract of *I. tinctoria*, with the total inhibition zone of 47 at 37.30%, followed by *Salmonella typhi* with total inhibition zone of 45 at 35.71%, *Staphylococcus aureus* with total inhibition zone of 18 at 14.29% and *Escherichia coli* has the least susceptibility, with the total inhibition zone of 16 at 12.70%.

**Anti-fungal Properties of *Indigofera tinctoria* pod (Uri) Extracts**


**Plate 2:** Image of antifungal result for aqueous extracts of pod


**Plate 3:** Image of antifungal result for ethanolic extracts of pod

From the above, there was growth on both extracts, but more pronounced on the plate with aqueous extracts of the pod than that of ethanolic pod extracts.

## DISCUSSION

The finding of this present study on the phytochemical screening of pods of *Indigofera tinctoria*, using the following extracts; Hot ethanolic extract (Soxhlet), Cold ethanolic extract and Aqueous extract revealed the presence of these bioactive compounds; Alkaloids, Saponins, tannins, Flavonoids and Cardiac glycosides. This result is not far from the results of the phytochemical components of some plants as observed by the works of Mainasara *et al*., 2011; Ukpabi *et al*., 2012; and Zafilaza *et al*., 2018, who worked on the phytochemical screening of *Calotropis procera, Costus afer* and *Euphorbia thymoflia* respectively. The result showed that Alkaloids are present in all the extracts of *I. tinctoria* pod, Flavonoids is present in cold ethanolic extract but absent in both hot ethanolic and aqueous extracts, Saponins and Tannins are absent in cold ethanolic extracts but present hot ethanolic and aqueous extracts, while Cardiac glycosides is absent in hot ethanolic extract but present in both cold ethanolic and aqueous extracts. The reason why aqueous extract have the highest phytochemicals may be due to the type of solvent used in the extraction process which happen to be water.

Antimicrobial activities of both hot ethanolic and aqueous extracts, showed that all the extracts exhibited antibacterial properties which is comparably with the works done by Chuah *et al*., 2014 on Antimicrobial Activities of Plant Extracts against Methicillin-Susceptible and Methicillin-Resistant *Staphylococcus aureus*; Iroha *et al*., 2014 on Medicinal Efficacy of Methanol and Ethanol Crude Extracts of *Mangifera indica* Leaf; Abeer *et al*., 2014 on Antimicrobial Activities and Phytochemical Analysis of the Essential Oil of *Ocimum basilicum*, Collected from Jeddah Region, Saudi Arabia; Addai-Mensah *et al*., 2014 on Evaluation of Antibacterial Potentiation of Crude Extracts of *Phyllanthus amarus, Tamarindus indica* and *Cleome viscosa* and Their Formulation. Soxhlet ethanolic and aqueous extracts were used in various concentration such as diluted and undiluted concentration, the dilution was done in a concentration of 1:1, 1:2 and they were all used against *Escherichia coli, Salmonella typhi, Bacillus cereus*, and *Staphylococcus auerus*. The highest zone of inhibition measured in millimeter was 25 mm against *S. typhi*, followed by *B. cereus* at 12 mm, *S. auerus* at 11 mm and *E. coli* having the least zone of inhibition at 8 mm, for undiluted concentration of soxhlet extract. In aqueous undiluted concentration, *B. cereus* has the highest zone of inhibition at 11 mm, followed by *E. coli* at 8 mm, *S. auerus* at 7 mm and *S. typhi* having the least zone of inhibition at 6 mm, for soxhlst extract at dilution 1:1 concentration, the highest zone of inhibition was *B. cereus* at 10 mm, followed by *S. typhi* at 7 mm, while there was no zone of inhibition in *E. coli* and *S. auerus* respectively. While in aqueous extract at dilution 1:1 concentration *B.cereus* and *S.typhi* has the same zone of inhibition at 7 mm, while there was no zone of inhibition in *E,.coli* and *S. auerus*. In the soxhlet ethanolic extract at dilution 1:2 concentration, zone of inhibition was observed

only in *B. cereus* at 7 mm, while there was no zone of inhibition in the rest of the test isolates. In the aqueous extract at dilution 1:2 concentration, there was no zone of inhibition against all the isolate. This result shows that zones of inhibition were higher when the extracts were undiluted than when they were diluted. This invariably means that as the concentration goes down, the inhibitory activirty equally reduces. The cumulative antibacterial activity showed that *B. cereus* (47 mm) (37.30%) was highest, followed by S. typhi (45 mm) (35.71%), *S. auerus* (18 mm) (14.29%) and *E. coli* (16 mm) (12.7%). The result shows the cumulative effect of the extracts was highly noticed in *B. cereus* and *S. typhi*, meaning that the extracts can be effective in treating diseases caused by these organisms.

It was also observed that all soxhlet ethanolic and aqueous extract did not exhibit antifungal properties, which is completely contrary to the work of Racowski *et al*., (2016) who worked on Antifungal Activity of Infusions from Fresh Oregano, Laurel and Rosemary Leaves and Their Commercial Essential Oils against *Acremonium* sp. The difference may be due to the methods of extraction, purification of extract, and inability to reconstitute the extracts with other solvents.

## CONCLUSION

In conclusion, the results of present findings indicates the presence of bioactive compounds in the extracts of *Indigofera tintoria* (L.) pod, which greatly contributes to the antibacterial activities as observed in some of the extracts. Zone of inhibition is more pronounced on that of undiluted concentration, thus indicating its potential as a good therapeutic agents.

## RECOMMENDATION

- The use of the extracts are effective in treating bacterial infections
- Better methods of purification should be developed so as to evaluates its antifungal properties
- The plant should be cultivated in Botanical garden so as to ensure its availability for combating various pathogenic diseases
- Also other antimicrobial potential like antiviral should also be evaluated
- Ethno-pharmacology and trado-medical practices should be encouraged
- Research is needed to discover other traditional uses of *Indigofera tintoria* pod in health and nutrition.

## References

Abeer, H. A., Ba-Hamdan, Magda, M. A., & Sameera, O. B. (2014). Antimicrobial activities and Phytochemical Analysis of the Essential Oil of *Ocimum basilicum*, Collected from Jeddah Region, Saudi Arabia. *Journal of Microbiology Research*, **4**(6A): 1-9

Ajaiyeobu, E. O. (2002). Phytochemical and antibacterial activity of *Parkia biglobosa* and *Parkia bicolor* leaf extracts. *African Journal Biomedical Resource*, **5**: 125-129.

Chuah, E. L., Zakaria, Z. A. Suhaili, A. Abu Bakar, S. & Desa, M. N. M. (2014). Antimicrobial Activities of Plant Extracts against Methicillin-Susceptible and ethicillin-Resistant *Staphylococcus aureus.Journal of Microbiology Research*, **4**(1): 6-13.

Encyclopedia Britannica (2018). Indigofera. Retrieved from https://www. britannica. com/plant/indigo- plant-genus August 10, 2018.

Harborne, J. B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Chapman and Hall, London, ISBN-13: 9780412572708, pp. 302.

Iroha, I., Ejikeugwu, C., Nwakaeze, E., Oji, A., Afiukwa, N., Nwuzo, A. 2014.Medicinal Efficacy of Methanol and Ethanol Crude Extracts of *Mangifera indica* Leaf.*Journal of Microbiology Research*, **4**(5): 180-182

Kapoor, L. D., Singh, A., Kapoor, S. L., & Srivastava, S. N. (1969). Survey of Indian medicinal plants for saponins, alkaloids and flavonoids. Lloydia, **32**: 297-302.

Mainasara, M. M., Aliero, B. L., Aliero, A. A., & Dahiru, S. S (2011). Phytochemical and Antibacterial Properties of Calotropis Procera (Ait) R. Br. (Sodom Apple) Fruit and Bark Extracts. *International Journal of Modern Botany*, 1(1): 8-11.

Senthilkumar, A., & Venkatesalu, V. (2009). Phytochemical analysis and antibacterial activity of the essential oil of Clausena anisata (Willd.) *International Journal of Integrative Biology*, 5(2): 116-120.

Shariff, Z. U. (2001). Modern herbal therapy for common ailments. Nature Pharmacy Series (Volume 1). Spectrum Books Limited, Ibadan, Nigeria. In Association with Safari Books (Export) Limited, United Kingdom. pp. 9-84.

Parekh, J., & Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal Biomedical Research*, **10**: 175-181.

Segelman, A. B., Farnsworth, N. R., & Quimby, M. D. (1969). False negative saponins test results induced by the presence of tannins. Lloydia, **32**: 52-58.

Ukpabi, C. F., Agbafor, K. N., Ndukwe, O. K., Agwu, A., Nwachukwu, S. N. (2012). Phytochemical Composition of *Costus Afer* Extract and Its Alleviation of Carbon Tetrachloride – induced Hepatic Oxidative Stress and Toxicity. International Journal of Modern Botany, 2(5): 120-126.

Zafilaza, A., Andriantsimahavandy, A., Randrianarivo, R. H., Ramamonjisoa, D. J., Andrianainarivelo, M. (2018). Ethnobotanical, Antimicrobial and Phytochemical Screening of *Euphorbia Thymoflia L.* (*Kinononono madiniky*) to cure the Menstrual Hemorrhage of a Woman Sakalava Bemazava in the Northern Region of Madagascar. International Journal of Plant Research, **8**(1): 1-7.