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Study of the cutaneous toxicity and antifungal activity of Senna podocarpa, a plant used to treat cutaneous affection

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ABSTRACT: The study aims to assess the cutaneous toxicity and antifungal activity of the hydroethanol extract of *Senna podocarpa,* a plant used in traditional medicine. This research is essential to determine both the safety of using the extract on the skin and its efficacy against various fungal infections.
Following OECD guideline 404 (2015), twelve Hyplus rabbits were treated with 200 mg/kg and 500 mg/kg doses of the extract to observe skin reactions, such as erythema and oedema, over 14 days. Antifungal activity was assessed using the double dilution slant tube method, followed by inoculation with *Candida albicans, Aspergillus fumigatus,* and *Trichophyton mentagrophytes*. Antifungal parameters such as MIC, MFC, and IC50 were determined.
Senna podocarpa extract showed no dermal toxicity, with a mean irritation index (MII) of 0, indicating that it is neither irritant nor corrosive to rabbit skin. No skin lesions were observed, and the rabbits' coats grew back 24 hours after application. In addition, the extract did not affect the weight of the rabbits, with those given 500 mg/kg even showing greater weight gain than those given 200 mg/kg. In terms of antifungal activity, the extract inhibited the growth of the fungi tested in a dose-dependent manner. The MIC and MFC were 6.25 mg/mL and 12.5 mg/mL for *A. fumigatus,* 25 mg/mL and 100 mg/mL for *C. albicans,* and 100 mg/mL for *T. mentagrophytes*.

KEYWORDS: *Senna podocarpa,* Antifungal, Cutaneous toxicity.

1 INTRODUCTION

Fungal infections represent a growing threat to global public health, affecting various tissues and organs, including the skin, nails, mucous membranes, and sometimes internal organs. Cutaneous mycoses, in particular, affect around 20-25% of the world's population, with prevalence rates varying considerably between regions, reaching as high as 66% in Côte d'Ivoire [1]. These infections include not only onychomycosis, but also dermatomycoses such as ringworm, athlete's foot, and cutaneous candidiasis. They are often characterised by symptoms such as skin thickening, pruritic lesions, and alterations in nail colour and texture, leading to physical discomfort and a significant psychological impact [2], [3]. Conventional antifungal treatments, although effective in many cases, are increasingly limited by pathogen resistance and adverse side effects [4]. This has led to a growing interest in alternative therapies, including the use of medicinal plants. *Senna podocarpa,* a plant widely used in traditional African medicine, is recognised for its varied pharmacological properties, including its laxative, antimicrobial and antimycotic effects. Its leaves and bark contain bioactive compounds such as flavonoids, anthraquinones and tannins, which are responsible for its antifungal activity. These compounds act by inhibiting the growth of fungi and disrupting their cell structure [5], [6].

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However, the efficacy of a medicinal plant is not enough to guarantee its safety. It is essential to assess the cutaneous toxicity of *Senna podocarpa* before its therapeutic use to prevent possible adverse effects such as allergic reactions or skin irritation. The aim of this study is therefore to assess both the antifungal activity and cutaneous toxicity of this plant on Hyplus rabbits, with a view to determining its potential as a safe and effective alternative treatment for cutaneous mycoses.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The plant used for the various tests was *Senna podocarpa*. It was collected in a village called Gbena, located 7 km from Séguéla in the Worodougou region. The authentication of this plant was carried out at the National Floristic Center (CNF) of the Félix Houphouët-Boigny University (UFHB), where the *Senna podocarpa* specimen is cataloged under the number UCJ009177.

2.2 ANIMAL MATERIAL

The animal material consisted of six (6) Hyplus rabbits, aged between 3 and 4 months and weighing between 1.15 and 2.12 kg. They were acclimatised for 2 weeks in the CNF shadehouse in a 6-compartment hutch with a waste evacuation system that allows the discharge of urine and faeces in order to maintain good hygiene, a feed trough and a bowl for water. The rabbits were marked according to the extract used and the concentration of the extracts.

2.3 PREPARATION OF THE HYDROETHANOL EXTRACT OF S. PODOCARPA

The leaves of S. podocarpa were cut into small pieces, then dried in the open air away from the sun. They were then ground to a fine powder using an electric grinder. One hundred grams (100 g) of each fine powder obtained was added to an Erlenmeyer flask containing 1 L of 70% hydroethanol solution. Maceration lasted 30 minutes. The macerate obtained was then ground and filtered once (1) using a white cloth, three (3) times through cotton wool, and finally once (1) using Whatman No. 1 filter paper. The various filtrates obtained were dried in an oven at 45°C for 48 hours.

2.4 METHOD FOR STUDYING THE CUTANEOUS TOXICITY OF THE HYDROETHANOL EXTRACT OF S. PODOCARPA

The study was conducted in accordance with OECD guideline 404 [7].

2.4.1 PRINCIPLE OF THE IN VIVO TEST

The skin of the animals selected for the experiment was treated with a single dose of Senna podocarpa extract, with untreated areas serving as controls. The degree of irritation or corrosion was observed and recorded according to a scale of values at specified intervals, with a detailed description provided by the experimenter for a complete assessment of the effects. The duration of the study was adapted to assess the reversibility of the effects observed. Animals showing persistent signs of distress and/or pain were euthanised and these signs were taken into account in the evaluation of the results.

2.4.2 PREPARATION OF IN VIVO TESTS

2.4.2.1 ANIMAL SELECTION

The study was conducted on six (6) Hyplus rabbits, aged 3 to 4 months, nulliparous and non-pregnant. They were divided into two groups for each dose of *Senna podocarpa* extract. The Hyplus breed was chosen due to the lack of suitable albino rabbits.

2.4.2.2 PREPARATION OF THE ANIMALS

The rabbits were first weighed and then the dorsal region of their trunk was shorn flush 24 hours before each test, taking care not to leave any scratches on their skin. The animals were marked according to the doses administered.

2.4.2.3 DOSE OF EXTRACT

The hydroethanol extract of *Senna podocarpa* was administered at doses of 200 and 500 mg/kg body weight to the skin of rabbits.

2.4.2.4 EVALUATION OF THE IRRITANT AND CORROSIVE EFFECT

The extract was applied to a 6 cm^2 area of the dorsal region of the trunk of the animals, using a vehicle consisting of alcohol diluted to 10%, considered non-aggressive to the skin. A volume of 0.5 mL of the solution was applied to the test areas.

2.4.2.5 INITIAL TEST

This test required one female rabbit for each dose of extract, for a total of two (02) rabbits. Each rabbit received three successive test patches in different shorn areas. One rabbit received 200 mg/kg body weight and the other 500 mg/kg. The extract was first applied evenly to compresses and then placed on the skin of each rabbit. The compresses were held in place with a non-irritating plaster. The first patch was removed after three (3) minutes, the second after one hour (1 h), and the last after four hours (4 h). At each removal, the presence or absence of skin reactions was noted, with the untreated areas serving as controls. The rabbits were then observed for 14 days. Skin reactions were recorded 24 h, 48 h and 72 h after removal of the last patch. At the end of this period, the animals were weighed.

2.4.2.6 CONFIRMATORY TRIAL

For this test, four (04) rabbits were used, i.e. two (02) rabbits for each dose of 200 and 500 mg/kg body weight. A single patch was applied to the skin of the rabbits for 4 hours. Observation and scoring of skin reactions were performed one hour after patch removal, as well as 24 h, 48 h, and 72 h during the observation period. Animal weights were measured at the end of the study.

2.4.2.7 EVALUATION AND CALCULATION OF SKIN REACTIONS

Reactions were assessed using an arbitrary skin reaction rating scale. Erythema and oedema scores were recorded for each rabbit. The irritant power of each extract or average skin irritation index (ASI) was calculated from the two averages of the parameters (erythema and oedema) and the products studied were classified according to the ASI classification (following the modified Draize classification) [8].

$$ME = \frac{Sum\ of\ all\ erythema\ scores}{Total\ number\ of\ erythema\ scores}$$

$$MO = \frac{Sum\ of\ all\ oedema\ scores}{Total\ number\ of\ oedema\ scores}$$

$$IIM = \frac{ME+MO}{2}$$

ME: Mean erythema; MO: Mean oedema; IIM: irritation index Mean.

The scores obtained during the observation period for the initial trials were determined from the mean of the erythema or oedema scores obtained on the three (03) patches received (3 min, 1h and 4h).

2.5 EVALUATION OF THE ANTIFUNGAL ACTIVITY OF S. PODOCARPA

The agar was prepared according to the manufacturer's instructions and distributed in various test tubes (3cm x 12cm).

2.5.1 INCORPORATION OF EXTRACTS INTO AGAR

The tests were carried out separately for each extract and each fungal species in order to determine the values of the antifungal parameters. Comparison of these parameters will enable the most active plant extract to be selected. The double dilution method in inclined tubes was used to incorporate the extracts into the agar. The pre-cooked agar was poured into 10 test tubes numbered 1 to 10, with 20 mL in tube 1 and 10 mL in the other tubes (2 to 10) in each series.

Of these 10 tubes, 8 contained plant extracts and 2 were control tubes without plant extracts: one serving as a control for the growth of germs (GC) and the other without germs serving as a control for the sterility of the culture medium (SC). In general, and depending on the series of tests, concentrations varied from 1000 µg/mL to 0.38 µg/mL. For the 8 tubes in each series, concentrations varied geometrically by a factor of ½, from tube 1 to tube 8. After incorporation of the extract, the 10 tubes from each series were sterilised in an autoclave at 121°C for 15 minutes, then tilted with a small base at room temperature to allow the agar to cool and solidify.

2.5.2 INOCULUM PREPARATION

For the antifungal assays, samples were prepared individually from 48-hour-old cultures of the three types of fungi on slant agar media. At least one or two isolated colonies were picked from each type of fungus using a 2 mm diameter loop, then mixed in 10 mL of sterilised distilled water. This suspension resulted in a parent suspension noted $10^{0,}$ with a concentration of 10^6 cells/mL. Starting from suspension 10°, suspension 10^{-1} was created by diluting 1 mL of suspension 10^0 in 9 mL of sterilised distilled water to obtain a total of 10 mL, with 10^5 cells per mL [9].

2.5.3 INOCULATION OF CULTURE MEDIA

The fungal species *Candida albicans, Aspergillus fumigatus* and *Trichophyton mentagrophytes* were cultured in all tubes in the series, with the exception of sterility control tube 10. Inoculation was carried out in streaks until exhaustion with 10 µL of suspension 10^{-1} (at a concentration of 10^5 cells/mL), equivalent to 1000 cells inoculated. The cultures were incubated at 30°C in a MEMMERT incubator for 2 to 7 days, depending on the variety of pathogenic fungi examined [9], [10].

2.5.4 GERM COUNT

After two days for *Candida albicans* and *Aspergillus fumigatus* and 5 to 7 days for *Trichophyton mentagrophytes,* the different species of fungi were counted by direct colony counting. The growth of the fungi was assessed in the experimental tubes of each series on the basis of the survival rate, which was calculated in comparison with the growth control tube with 100% survival using the formula below:

S= (n/N) ×100; With: S = Germ survival (expressed as a percentage); N = Number of colonies in the control tube; n = Number of colonies in the experimental tube.

Analysis of the experimental data led not only to the plotting of activity curves, but also to the identification of the following antifungal parameters:

The Minimum Fungicidal Concentration (MFC) corresponds to the lowest concentration of extract that eliminates 99.99% of fungal species compared with the growth control, leaving a survival rate of 0.01%;

The MIC is the lowest concentration of extract above which no growth visible to the naked eye is observed.

The dose required to obtain 50% inhibition (IC_{50}). This corresponds to the quantity of extract that caused 50% inhibition of the growth of the fungal species. This value is obtained visually by analysing the sensitivity curve.

3 RESULTS

3.1 SKIN TOXICITY

The irritant and corrosive effects of the crude extract of *S. podocarpa* were determined by calculating erythema and edema scores and observing the rabbits after the tests over 14 days.

The MII (Mean Irritation Index) of the crude extract of *S. podocarpa* at doses of 200 mg/kg BW and 500 mg/kg BW was 0. Therefore, it is considered non-irritant and non-corrosive to the skin based on the modified Draize classification. Similarly, the results of the confirmatory tests of the crude extract of *S. podocarpa* on the rabbits' skin revealed an MII of 0, further confirming the initial test results.

The rabbits showed slight agitation, without further effects, following the application of the adhesive bandage for a few minutes but eventually calmed down after becoming accustomed to the presence of this foreign body. Additionally, no other skin lesions were observed during the testing and observation periods, and the fur began to regrow within 24 hours.

Effect of *Senna podocarpa* Extract on the Weight Gain of Rabbits

The *Senna podocarpa* extract had no adverse effect on the weight of the rabbits, which gained weight during the observation period. Additionally, the rabbits that received the 500 mg/kg BW dose experienced slightly higher weight gain compared to those that received the 200 mg/kg BW dose.

- Average weight gain of rabbits that received the 200 mg/kg BW dose: 0.63 g
- Average weight gain of rabbits that received the 500 mg/kg BW dose: 0.90 g.

3.2 RESULTS OF ANTIFUNGAL ACTIVITY TEST

The analysis of the impact of hydroethanolic extracts of *Senna podocarpa* on the in vitro growth of *Candida albicans, Aspergillus fumigatus,* and *Trichophyton mentagrophytes* showed that after incubation, the number of colonies in the experimental tubes decreased progressively compared to the control as the concentration of the extract increased.

This demonstrated that the various extracts studied inhibited the in vitro growth of the three fungal species in a dose-dependent manner, allowing the determination of the minimum fungicidal concentrations (MFC) and minimum inhibitory concentrations (MIC).

- In the presence of *Senna podocarpa*
  - MIC = 6.25 mg/mL and MFC = 12.5 mg/mL for *Aspergillus fumigatus*
  - MIC = 25 mg/mL and MFC = 100 mg/mL for *Candida albicans*
  - MIC = 100 mg/mL and MFC = 100 mg/mL for *Trichophyton mentagrophytes*

The antifungal tests were based on counting the colonies in 10 experimental tubes where the growth of the microorganisms was evaluated as a percentage of survival compared to the control tube with 100% survival. These antifungal tests allowed for the graphical determination of the inhibitory concentrations necessary to ensure the survival of 50% of the microorganisms (IC50).

- In the presence of *Senna podocarpa*
  - IC50 = 6.36 mg/mL for *Candida albicans*
  - IC50 = 2.7 mg/mL for *Aspergillus fumigatus*
  - IC50 = 26.75 mg/mL for Trichophyton mentagrophytes

4 DISCUSSION

These results indicate that the crude extract of *Senna podocarpa,* tested at doses of 200 mg/kg and 500 mg/kg in rabbits, did not exhibit any significant irritant or corrosive effects based on the criteria for erythema and edema evaluation. This is confirmed by a Mean Irritation Index (MII) of 0, in accordance with the modified Draize classification for skin irritation. Previous research on extracts of *Senna alata,* a plant from the same family as *Senna podocarpa,* also demonstrated a low potential for skin irritation in laboratory animals. In a study conducted by Yaméogo et al. [11], the topical application of an ethanol extract of *Senna alata* did not induce significant skin reactions, corroborating the non-irritation findings observed with *Senna podocarpa* in the present study. Additionally, a study on *Aloe vera* by Saghir et al. [12] demonstrated that plant extracts often possess soothing properties on the skin, which could explain the absence of skin reactions. This type of skin tolerance may be linked to the presence of bioactive compounds common in plant extracts from the Fabaceae family, to which *Senna podocarpa* belongs. Another study by Ali et al. [13] on *Azadirachta indica* (Neem) showed that even at higher doses, certain plant extracts can not only be non-irritating but also have beneficial effects on the skin, such as reducing skin inflammation. The results of this study align with the non-irritation and good tolerance observations made with *Senna podocarpa.*

Furthermore, the extract did not adversely affect the weight gain of the rabbits during the observation period. The rabbits treated with the higher dose (500 mg/kg) even showed slightly more weight gain than those treated with the lower dose (200 mg/kg). These observations suggest a general tolerance of the extract on the overall health and body weight of the test animals. A study by Oliveira et al. [14] on the effects of *Moringa oleifera* extracts in rats showed that even at high doses, these extracts did not have a detrimental effect on the animals' body weight. On the contrary, as observed in our study with *Senna podocarpa,* some groups showed slightly higher weight gain, suggesting good tolerance and the absence of systemic toxicity.

Additionally, a study by Uche et al. [15] on the effects of *Garcinia kola* revealed that administering extracts at moderate doses did not have adverse effects on the weight gain of the tested rodents and was even associated with a slight increase in

body mass. This result is similar to the observations made in our study, where rabbits exposed to the higher dose of *Senna podocarpa* (500 mg/kg) showed greater weight gain. Regarding the slight initial agitation observed in the rabbits after applying the adhesive tape, it is important to note that in a study by Sethi et al. [16] on rabbits, a similar response was reported during the administration of external substances. According to these authors, this transient agitation is generally attributed to temporary discomfort due to the novelty of the situation rather than a specific reaction to the tested extract. The observations of rapid recovery and normal hair growth in the rabbits align with the findings of a study conducted by Akah et al. [17], where the animals also showed normal hair regrowth after stopping the application of topical extracts, confirming the absence of prolonged side effects.

The antifungal activity tests of hydroethanolic extracts of *S. podocarpa* reveal varying levels of effectiveness depending on the fungal species tested. Among them, *Aspergillus fumigatus* showed the greatest sensitivity to the extract, with a Minimum Inhibitory Concentration (MIC) of 6.25 mg/mL and a Minimum Fungicidal Concentration (MFC) of 12.5 mg/mL. This indicates that relatively low concentrations of *Senna podocarpa* are sufficient to inhibit and kill this fungus. In contrast, *Candida albicans* and *Trichophyton mentagrophytes* required much higher concentrations to achieve similar effects, with respective MICs of 25 mg/mL and 100 mg/mL, and MFCs of 100 mg/mL for both species. These results suggest a relative resistance of *Candida albicans* and *Trichophyton mentagrophytes* compared to *Aspergillus fumigatus.*

The relative effectiveness of the *Senna podocarpa* extract is also highlighted by the IC_{50} (50% Inhibitory Concentration) values. *A. fumigatus* displays the lowest IC50 (2.7 mg/mL), confirming its high sensitivity to the extract. Conversely, *C. albicans* and *T. mentagrophytes* exhibit higher IC50 values, 6.36 mg/mL and 26.75 mg/mL respectively, reflecting their greater resistance to fungal growth inhibition.

These results are consistent with observations from other studies on plant extracts, which also show significant but variable antifungal activity depending on the fungal species. Studies on *Azadirachta indica* (Neem) and *Curcuma longa* (Turmeric) have demonstrated that some fungi are more sensitive than others, as reported by Ali et al. [13] and Rathod et al. [18], respectively.

Compared to other works, the *Senna podocarpa* extract shows superior efficacy. In a study conducted by Bagré [19], *Candida albicans* was inhibited with a total aqueous extract of *Morinda morindoides* at a concentration of 300 mg/mL after 48 hours of incubation, a much higher concentration than that required for *Senna podocarpa*. Additionally, extracts of *Mitracarpus villosus* (MV1) and *Spermacoce verticillata* (SV1) evaluated by Zihiri et al. [20] showed less effective antifungal activity against *Aspergillus fumigatus,* with MFC values of 100 mg/mL for MV1 and 50 mg/mL for SV1. By comparison, *Senna podocarpa* presents an MFC of 12.5 mg/mL, making it four and two times more active than *Mitracarpus villosus* and *Spermacoce verticillata,* respectively.

Senna podocarpa appears to be a promising candidate for the development of new natural antifungal agents, particularly for the treatment of infections caused by *Aspergillus fumigatus*. However, further research is needed to optimize its efficacy against other fungal pathogens such as *Candida albicans* and *Trichophyton mentagrophytes.*

5 CONCLUSION

This study shows that the crude extract of Senna podocarpa is non-irritating and non-corrosive to the skin at the tested doses of 200 mg/kg and 500 mg/kg, with excellent tolerance observed in rabbits. The absence of adverse effects on weight gain further supports the overall safety of the extract. Additionally, the antifungal activity of the hydroethanolic extract of Senna podocarpa demonstrates significant efficacy against Aspergillus fumigatus, although the effectiveness is lower against Candida albicans and Trichophyton mentagrophytes. These findings highlight the potential of Senna podocarpa as a natural antifungal agent, particularly for treating infections caused by Aspergillus fumigatus. However, further studies are needed to optimize its effectiveness against other resistant fungal species and to explore its potential for broader therapeutic applications.

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REFERENCES

[1] A. Konaté, W. Yavo, K.F. Kassi, V. Djohan, K.E. Angora, H. Bossoa-Vanga, P. Barro-Kiki and E.H.I. Menan, «Profile of Onychomycoses in Abidjan (Côte d'Ivoire),» *Journal of Medical Mycology,* vol. 24, pp. 205-210, 2014.

[2] A.K. Gupta, N. Stec, R.C. Summerbell, N.H. Shear, V. Piguet, A. Tosti, B.M. Piraccini, «Onychomycosis: a review. *Journal of the European Academy of Dermatology and Venereology,* » 34, o. 9, pp. 1972-1990, 2020.

[3] S.R. Lipner, R.K. Scher, «Onychomycosis: Treatment and Prevention Strategies,» *Journal of the American Academy of Dermatology,* vol. 80, no. 4, pp. 853-867, 2019.

[4] L.E. Cowen, D. Sanglard, S.J. Howard, P.D. Rogers, D.S. Perlin, «Mechanisms of Antifungal Drug Resistance,» *Cold Spring Harbor Perspectives in Medicine,* vol. 5, pp.19-27, 2015.

[5] O.A. Adebesin, J. Okpuzor, O.O. Iroanya, S.O. Adenekan, C. Aniekwena, «Antioxidant and Cytotoxic Properties of *Senna alata* and *Senna podocarpa* Leaf Extracts,» *Planta Medica,* vol. 79, p. 27, 2013.

[6] M. Adebayo, O. Lawal, A. Sikiru, I. Ogunwande, O. Avoseh, «Chemical Constituents and Antimicrobial Activity of Essential Oil of *Senna podocarpa* (Guill. et Perr.) Lock,» *American Journal of Plant Sciences,* vol. 5, pp. 2448-2453, 2014.

[7] Organisation for Economic Co-operation and Development (OECD), «Guideline 404 for the Testing of Chemicals: Skin Corrosion/Irritation,» OECD Publishing, Paris, 2015.

[8] M. Farmaca, «Guide d'évaluation de l'irritation cutanée selon la classification de Draize modifiée,» Laboratoire di Analisi e Ricerca, Protocol No. 23004660, Bosco Marengo, 2023.

[9] K.E. Kporou, A.K.M. Kra, S. Ouattara, F. Guede-Guina, A.J. Djaman, «Improvement by Chromatographic Fractionation of the Anticandidal Activity of a Hexane Extract of *Miltracarpus scaber* Zucc. on In Vitro Growth of *Candida albicans* and *Candida tropicalis*,» *Phytotherapy,* vol. 8, no. 5, pp. 290-294, 2010.

[10] F. Guédé-Guina, G. Zihiri, C. Bahi, D. Traoré, «Analysis of the Antifungal Properties of Medicinal Plant Extracts,» *Journal of African Ethnopharmacology,* vol. 5, no. 1, pp. 45-53, 1997.

[11] B.G.J. Yaméogo, S. Ouédraogo, B.C. Sombié, M. Zambelongo, F. Wango, H. Zimé-Diawara, R. Semdé, «Formulation of Dermatological Creams Based on Shea Butter and Hydro-Ethanolic Extracts of *Mitracarpus scaber* Zucc. (Rubiaceae) and *Senna alata* (L.) Roxb. (Fabaceae) for the Treatment of Skin Infections,» *African Journal of Pharmaceutical Technology and Biopharmacy (JATPB),* vol. 2, no. 3, 2023.

[12] S.A. Saghir, K. Saeed, M. Athar, «Skin Soothing Effects of Aloe Vera: A Preliminary Study,» *Journal of Dermatological Treatment,* vol. 12, no. 2, pp. 127-130, 2001.

[13] M.I. Ali, T. Ahmed, S.H. Ansari, «Evaluation of the Topical Anti-Inflammatory Activity of *Azadirachta indica* (Neem) in Animal Models,» *Pharmacognosy Research,* vol. 7, no. 1, pp. 37-42, 2015.

[14] J.T.A. Oliveira, S.B. Silveira, K.M. Vasconcelos, B.S. Cavada, R.A. Moreira, «Assessment of Toxicological Effects of *Moringa oleifera* Lam. Leaves in Mice,» *Journal of Ethnopharmacology,* vol. 125, no. 2, pp. 239-242, 2012.

[15] F.I. Uche, P.N. Okafor, «The Effects of *Garcinia kola* Seed Extract on Liver Enzymes, Serum Total Protein and Uric Acid Levels of Adult Rats,» *Journal of Ethnopharmacology,* vol. 159, pp. 43-49, 2015.

[16] N. Sethi, R.K. Srivastava, R.K. Singh, «Assessment of Irritation Potential of Different Surfactant-Based Creams Using In Vivo Methods,» *Indian Journal of Dermatology,* vol. 56, no. 6, pp. 718-722, 2011.

[17] P.A. Akah, J.A. Alemji, O.A. Salawu, T.C. Okoye, V.N. Offiah, «Effects of Administration of Crude Aqueous and Methanol Extracts of *Alchornea cordifolia* Leaves on the Liver Function of Albino Rats,» *Journal of Medicinal Plants Research,* vol. 1, no. 1, pp. 30-33, 2007.

[18] S. Rathod, M. Patel, «Antifungal Activity of *Curcuma longa* Extracts against *Aspergillus niger*,» *Journal of Medicinal Plants Research,* vol. 5, no. 9, pp. 1512-1515, 2011.

[19] I. Bagré, «Evaluation of the Antifungal Activity of Aqueous Extracts of *Morinda morindoides* against *Candida albicans*,» *Journal of Ethnopharmacology,* vol. 90, no. 1, pp. 81-84, 2004.

[20] G. Zihiri, D. Traore, F. Guédé-Guina, «Antifungal Activity of *Mitracarpus villosus* and *Spermacoce verticillata* on Dermatophytes and *Candida albicans,» Journal of Ethnopharmacology,* vol. 112, no. 2, pp. 346-352, 2007.