AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Study of the Cutaneous Toxicity and Antifungal Activity of Senna Podocarpa, A Plant Used to Treat Cutaneous Affection

Agré Don Josette[1*], Assemian Inès Christelle Chadon Alphonsine[2], KONAN Gbê Kouakou N'Dri Ange [1], Bolou Gbouhoury Eric-Kevin[3]

1.Laboratory of Biology and Health, UFR Biosciences, Félix Houphouët-Boigny University, Côte d'Ivoire

2.Laboratory of Biotechnology, Agriculture and Valuation of Biological Resources, UFR Biosciences, Félix Houphouët-Boigny University, Côte d'Ivoire

3.National Center of Floristics, Félix Houphouët-Boigny University, Côte d'Ivoire

ABSTRACT

The study aims to evaluate 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 for 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 IC_{50} were determined. *Senna podocarpa* extract showed no dermal toxicity, with a mean irritation index (MII) of 0, indicating that it is neither irritating nor corrosive to rabbit skin. No skin lesions were observed and the fur of the rabbits regrown 24 hours after application. In addition, the extract did not affect the weight of the rabbits, with those given 500 mg/kg actually 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.

*Corresponding Author Email: agrejosette@yahoo.fr

Received 22 August 2024, Accepted 25 October 2024

Please cite this article as: Josette AD *et al*., Study of the Cutaneous Toxicity And Antifungal Activity of Senna Podocarpa, A Plant Used to Treat Cutaneous Affection. American Journal of PharmTech Research 2024.

INTRODUCTION

Fungal infections are a growing global public health problem affecting various tissues and organs, including the skin, nails, mucous membranes and occasionally internal organs. Cutaneous mycoses affect approximately 20-25% of the global population, with prevalence rates varying widely between regions, reaching up to 66% in Côte d'Ivoire (Konaté et al., 2014). These infections include not only onychomycosis but also conditions such as ringworm, athlete's foot and cutaneous candidiasis. They are often characterized by symptoms such as skin thickening, pruritic lesions, and changes in nail colour and texture, leading to physical discomfort and significant psychological impact (Gupta et al., 2020; Lipner et al., 2019). Although conventional antifungal treatments are generally effective, their efficacy is increasingly limited by pathogen resistance and unwanted side effects (Cowen et al., 2015). This has led to a growing interest in alternative therapeutic approaches, including the use of medicinal plants. *Senna podocarpa*, a plant widely used in traditional African medicine, is known for its diverse pharmacological properties, including laxative, antimicrobial and antifungal effects. The leaves and bark of this plant contain bioactive compounds such as flavonoids, anthraquinones and tannins, which are responsible for its antifungal activity by inhibiting fungal growth and disrupting their cell structure (Adebesin et al., 2013; Adebayo et al., 2014). However, the efficacy of a medicinal plant is not sufficient to guarantee its safety. It is essential to assess the cutaneous toxicity of *Senna podocarpa* prior to its therapeutic use in order to prevent possible adverse effects such as allergic reactions or skin irritation. The aim of this study is therefore to evaluate both the antifungal activity and the cutaneous toxicity of this plant on *Hyplus* rabbits, in order to determine its potential as a safe and effective alternative treatment for cutaneous mycoses.

MATERIALS AND METHOD

Plant material

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

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 shade house in a 6-compartment hutch with a waste evacuation system that allows the removal of urine and

faeces to maintain good hygiene, a food trough and a bowl for water. The rabbits were marked according to the extract used and the concentration of the extracts.

Preparation of the hydroethanol extract of *S. podocarpa*

The leaves of *S. podocarpa* were cut into small pieces and dried outdoors, away from the sun. They were then ground into 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 litre of 70% hydroethanol solution. The maceration lasted 30 minutes. The macerate obtained was then ground and filtered once (1) through a white cloth, three (3) times through cotton wool and finally once (1) through Whatman No. 1 filter paper. The different filtrates obtained were dried in an oven at 45°C for 48 hours.

Method for studying the cutaneous toxicity of the hydroethanol extract of *S. podocarpa*

The study was conducted in accordance with OECD guideline 404 (OECD 404, 2015).

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 fixed intervals, with a detailed description provided by the experimenter for a complete assessment of the effects. The duration of the study was adjusted to assess the reversibility of the effects observed. Animals showing persistent signs of distress and/or pain were humanely killed and these signs were taken into account in the evaluation of the results.

Preparation of in vivo tests

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.

Preparation of the animals

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

Dose of extract

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

Evaluation of the irritant and corrosive effect

The extract was applied to a 6 cm² area of the dorsal trunk of the animals using a vehicle consisting of alcohol diluted to 10%, which is considered to be non-irritant to the skin. A volume of 0.5 ml of the solution was applied to the test areas.

Initial test

This study required one female rabbit per dose of extract for a total of two (2) rabbits. Each rabbit received three consecutive test patches in different shaved 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 dressings were held in place with non-irritating plasters. 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 followed for 14 days. Skin reactions were recorded at 24 h, 48 h and 72 h after removal of the last patch. At the end of this period the animals were weighed.

Confirmatory trial

Four (04) rabbits were used in this study, i.e. two (02) rabbits at each dose of 200 and 500 mg/kg body weight. A single patch was applied to the skin of the rabbits for 4 hours. Skin reactions were observed and graded one hour after patch removal and at 24, 48 and 72 hours during the observation period. The animals were weighed at the end of the study.

Evaluation and calculation of skin reactions

Reactions were graded using an arbitrary skin reaction rating scale. Erythema and oedema scores were recorded for each rabbit. The irritant potential of each extract, or the average skin irritation index (ASI), was calculated from the two mean values of the parameters (erythema and oedema) and the products studied were classified according to the ASI classification (following the modified Draize classification) (Farmaca, 2023).

$$ME = \frac{Sum\ of\ all\ erythema\ scores}{Total\ number\ of\ erythema\ scores}\ ;\ MO = \frac{Sum\ of\ all\ oedema\ scores}{Total\ number\ of\ oedema\ scores}\ ;\ IIM = \frac{ME+MO}{2},$$

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).

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).

Incorporation of Extracts into Agar

The tests were carried out separately for each extract and each fungal species to determine the values of the antifungal parameters. The comparison of these parameters allows the selection of the most active plant extract. 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 was used as a control for the growth of germs (GC), and the other without germs was used as a control for the sterility of the culture medium (SC). In general, and depending on the test series, the concentrations varied from 1000 µg/ml to 0.38 µg/ml. For the 8 tubes in each series, the 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 and then tilted on a small base at room temperature to allow the agar to cool and solidify.

Inoculum preparation

For the antifungal tests, samples were individually prepared from 48-hour-old cultures of the three fungal species on slant agar media. At least one or two isolated colonies of each fungal species were picked with a 2 mm diameter loop and then mixed in 10 mL of sterilised distilled water. This resulted in a parent suspension marked 100, with a concentration of 106 cells/ml. Suspension 10-1 was prepared from suspension 10° by diluting 1 mL of suspension 100 in 9 mL of sterilised distilled water to give a total of 10 mL with 105 cells per mL (Kporou et al., 2010).

Inoculation of culture media

The fungal species *Candida albicans, Aspergillus fumigatus* and *Trichophyton mentagrophytes* were grown in all tubes in the series except sterility control tube 10. Inoculation was carried out in strips to exhaustion with 10 µL of 10-1 suspension (at a concentration of 105 cells/mL), corresponding to 1000 inoculated cells. Cultures were incubated at 30 $^{\circ}$ C in a MEMMERT incubator for 2 to 7 days, depending on the variety of pathogenic fungi studied (Guédé-Guina et al., 1999; Kporou et al., 2010).

Germ count

After 2 days for *Candida albicans* and *Aspergillus fumigatus* and 5 to 7 days for Trichophyton mentagrophytes, the different fungal species were counted by direct colony counting. The growth of the fungi in the experimental tubes of each series was evaluated on the basis of the survival rate, which was calculated in comparison with the growth control tube with 100% survival, using the following formula:

$S=(n/N) \times 100$; where: 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) is the lowest concentration of extract that eliminates 99.99% of the fungal species compared to 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 achieve 50% inhibition (IC_{50}). This is the amount of extract that caused 50% inhibition of growth of the fungal species. This value is obtained visually by analysing the sensitivity curve.

RESULTS AND DISCUSSION

Skin Toxicity

The irritant and corrosive effects of the crude extract of *S. podocarpa* were assessed by measuring erythema and oedema scores and observing the rabbits for 14 days after the tests. The Mean Irritation Index (MII) of the crude extract administered at doses of 200 mg/kg and 500 mg/kg body weight was 0, indicating that the extract is neither irritating nor corrosive to the skin according to the modified Draize classification. Confirmatory test results also showed an MII of 0, supporting the initial findings. The rabbits were slightly agitated for a few minutes after application of the patch, with no further effects, but eventually calmed down as they became accustomed to the presence of this foreign body. In addition, no other skin lesions were observed during the test and observation periods, and the fur began to regrow within 24 hours.

Effect of *Senna podocarpa* extract on weight gain in rabbits

Senna podocarpa extract had no adverse effect on the weight of the rabbits, which gained weight during the observation period. In addition, rabbits receiving the 500 mg/kg bw dose gained slightly more weight than those receiving the 200 mg/kg bw dose. The average weight gain of rabbits at the 200 mg/kg b.w. dose was 0.63 g. The average weight gain of rabbits at the 500 mg/kg BW dose was 0.90 g.

Antifungal Activity Test

Analysis of the effect 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 progressively decreased compared to the control as the concentration of extract increased. This showed that the different extracts studied inhibited the in vitro growth of the three fungal species in a dose-dependent manner, allowing the determination of 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 colony counts in 10 test tubes where the growth of microorganisms was evaluated as a percentage of survival compared to the control tube with 100% survival. These antifungal tests allowed the graphical determination of the inhibitory concentrations required to ensure the survival of 50% of the micro-organisms (IC_{50}).

In the presence of *Senna podocarpa:*

- IC_{50} = 6.36 mg/mL for *Candida albicans*
- IC_{50} = 2.7 mg/mL for *Aspergillus fumigatus*
- IC_{50} = 26.75 mg/mL for *Trichophyton mentagrophytes*

DISCUSSION

These results indicate that the crude extract of *Senna podocarpa*, tested in rabbits at doses of 200 mg/kg and 500 mg/kg, showed no significant irritant or corrosive effects according to the criteria for assessing erythema and oedema. A Mean Irritation Index (MII) of 0, in accordance with the modified Draize classification for skin irritation, confirmed this lack of irritation. Previous studies on *Senna alata*, a species related to *Senna podocarpa*, have also shown a low potential for skin irritation in laboratory animals. In a study by Yaméogo et al. (2023), topical application of an ethanolic extract of *Senna alata* did not induce significant skin reactions, supporting the findings observed with Senna podocarpa in this study. In addition, research on Aloe vera by Saghir and co-workers (2001) showed that plant extracts often have soothing properties on the skin, which may explain the absence of skin reactions here. This skin tolerance may be related to the presence of bioactive compounds commonly found in plant extracts from the Fabaceae family, to which *S. podocarpa* belongs. Another study by Ali et al. (2015) on Azadirachta indica (Neem) showed that certain plant extracts, even at higher doses, may not only be non-irritating but also have beneficial effects on the skin, such as reducing inflammation. These findings are consistent with the observed non-irritation and good skin tolerability of *S. podocarpa.*

In addition, the extract did not adversely affect the weight gain of the rabbits during the observation period. In fact, the rabbits treated with the higher dose (500 mg/kg) showed slightly more weight gain than those treated with the lower dose (200 mg/kg). These observations suggest a general tolerability of the extract on the overall health and body weight of the test animals. A study by Oliveira and co-workers (2012) 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 tolerability and the absence of systemic toxicity.

Furthermore, a study by Uche and collaborators (2015) on the effects of *Garcinia kola* showed that the administration of extracts at moderate doses had no adverse effects on the weight gain of the rodents tested and was even associated with a slight increase in body mass. This result is similar to that observed in our study, where rabbits exposed to the higher dose of *Senna podocarpa* (500 mg/kg) showed greater weight gain. Regarding the slight initial restlessness observed in the rabbits after tape application, it is important to note that a study by Sethi (2011) on rabbits reported a similar response 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 response to the extract being tested. The observations of rapid recovery and normal hair growth in the rabbits are consistent with the findings of a study by Akah and co-workers (2007), where the animals also showed normal hair regrowth after cessation of topical extracts, confirming the absence of prolonged side effects. The antifungal activity tests of hydroethanolic extracts of *S. podocarpa* show different levels of efficacy 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 MICs of 25 mg/mL and 100 mg/mL, respectively, 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 efficacy of *Senna podocarpa* extract is also highlighted by the IC_{50} (50% inhibitory concentration) values. *A. fumigatus* shows the lowest IC_{50} (2.7 mg/ml), confirming its high sensitivity to the extract. Conversely, *C. albicans* and *T. mentagrophytes* exhibit higher IC_{50} values of 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 shown that some fungi are more

sensitive than others, as reported by Ali and collaborators (2015) and Rathod and collaborators (2011), respectively.

The *Senna podocarpa* extract shows superior efficacy compared to other works. In a study conducted by Bagré (2004), *Candida albicans* was inhibited by 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*. In addition, extracts of *Mitracarpus villosus* (MV1) and *Spermacoce verticillata* (SV1) evaluated by Zihiri and coworkers (2007) showed less potent antifungal activity against *Aspergillus fumigatus*, with MFC values of 100 mg/mL for MV1 and 50 mg/mL for SV1. In comparison, *Senna podocarpa* has 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 optimise its efficacy against other fungal pathogens such as *Candida albicans* and *Trichophyton mentagrophytes*.

CONCLUSION

This study shows that *Senna podocarpa* crude extract 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 body-weight gain further supports the overall safety of the extract. In addition, the antifungal activity of the hydroethanolic extract of *Senna podocarpa* shows significant efficacy against *Aspergillus fumigatus*, although the efficacy against *Candida albicans* and *Trichophyton mentagrophytes* is lower. These results highlight the potential of *Senna podocarpa* as a natural antifungal agent, particularly for the treatment of infections caused by *Aspergillus fumigatus*. However, further studies are needed to optimise its efficacy against other resistant fungal species and to explore its potential for broader therapeutic applications.

REFERENCES

1. Konaté A, Yavo W, Kassi KF, Djohan V, Angora KE, Bossoa-Vanga H, Barro-Kiki P and Menan EH. Profile of Onychomycoses in Abidjan (Côte d'Ivoire). *J Med Mycol 2014;* 24, 205-210.
2. Gupta AK, Stec N, Summerbell RC, Shear NH, Piguet V, Tosti A, Piraccini BM. Onychomycosis: a review. J Eur Acad of Dermatol Venereol 2020; 34, 1972-1990.
3. Lipner SR, Scher RK. Onychomycosis: Treatment and Prevention Strategies. *J Am Acad Dermatol 2019*; 80(4), 853-867.

4. Cowen LE, Sanglard D, Howard SJ, Rogers PD, Perlin DS. Mechanisms of Antifungal Drug Resistance. *Cold Spring Harb Perspect Med 2015*; 5, 19-27.
5. Adebesin OA, Okpuzor J, Iroanya OO, Adenekan SO, Aniekwena C. Antioxidant and Cytotoxic Properties of *Senna alata* and *Senna podocarpa* Leaf Extracts. *Planta Med 2013;* 79-27.
6. Adebayo M, Lawal O, Sikiru A, Ogunwande I, Avoseh O. Chemical Constituents and Antimicrobial Activity of Essential Oil of *Senna podocarpa* (Guill. et Perr.) Lock. *Am J Plant Sci 2014*; 5, 2448-2453.
7. Organisation for Economic Co-operation and Development (OECD), "Guideline 404 for the Testing of Chemicals: Skin Corrosion/Irritation," OECD Publishing, Paris, 2015.
8. Farmaca M. Guide d'évaluation de l'irritation cutanée selon la classification de Draize modifiée, Laboratoire d'analyse et de recherche, Protocol No. 23004660, Bosco Marengo 2023 ;140p.
9. Kporou KE, Kra AKM, Ouattara S, Guede-Guina F, Djaman AJ. 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*. *Phytother* 2010; 8(5), 290-294.
10. Guédé-Guina F, Zihiri G, Bahi C, Traoré D. Analysis of the Antifungal Properties of Medicinal Plant Extracts. *J Afri Ethnopharmacol 1997*; 5, 45-53.
11. Yaméogo BGJ, Ouédraogo S, Sombié BC, Zambelongo M, Wango F, Zimé-Diawara H, Semdé R. 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. *Afri J Pharmaceutic Tech Biopharm 2023; 2-3.*
12. Saghir SA, Saeed K, Athar M. Skin Soothing Effects of Aloe Vera: A Preliminary Study. *J Dermatolog Treat 2001;* 12, 127-130.
13. Ali MI, Ahmed T, Ansari SH. Evaluation of the Topical Anti-Inflammatory Activity of *Azadirachta indica* (Neem) in Animal Models. *Pharmacogn Res 2015; 7, 37-42.*
14. Oliveira JTA, Silveira SB, Vasconcelos KM, Cavada BS, Moreira RA. Assessment of Toxicological Effects of *Moringa oleifera* Lam. Leaves in Mice, *J Ethnopharmacol 2012; 125, 239-242.*
15. Uche FI, Okafor PN. The Effects of *Garcinia kola* Seed Extract on Liver Enzymes, Serum Total Protein and Uric Acid Levels of Adult Rats. *J Ethnopharmacol 2015; 159, 43-49.*

16. Sethi N, Srivastava RK, Singh RK. Assessment of Irritation Potential of Different Surfactant-Based Creams Using In Vivo *Methods, Indian J Dermatol 2011;* 56, 718-722.
17. Akah PA, Alemji JA, Salawu OA, Okoye TC, Offiah VN. Effects of Administration of Crude Aqueous and Methanol Extracts of *Alchornea cordifolia* Leaves on the Liver Function of Albino Rats. *J Med Plant Res* 2007; 1, 30-33.
18. *Rathod S, Patel M. Antifungal Activity of Curcuma longa Extracts against Aspergillus niger. J Med Plant Res 2011;* 9, 1512-1515.
19. Bagré I. Evaluation of the Antifungal Activity of Aqueous Extracts of *Morinda morindoides* against *Candida albicans*. *J Ethnopharmacol 2004*; 90(1), 81-84.
20. Zihiri G, Traore D, Guédé-Guina F. Antifungal Activity of *Mitracarpus villosus* and *Spermacoce verticillata* on Dermatophytes and *Candida albicans*. *J Ethnopharmacol 2007; 112 (2), 346-352.*