Original Article

ANTIFUNGAL ACTIVITY OF LALLEY (*Lawsonia Inermis*) LEAVES ON SELECTED CLINICAL ISOLATES

Enwa Felix Oghenemaro[1*], Oyubu L. Obaro[2], Jewo Augustina Oghenevwaerhe[3], Efurhievwe Omizuomerhan Ashley[4]

[1*,4]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Delta State University, Abraka.
[2]Department of Science Laboratory Technology, Faculty of Science, Delta State University, Abraka.
[3]Department of Microbiology, Faculty of Science, Delta State University, Abraka
*Corresponding Author: Enwa Felix Oghenemaro

DOI: 10.47750/pnr.2022.13.S09.1234

Abstract

Nature has been the source of the basic needs like food, clothing, shelter and medicines. Natural products derived from plants have been used to help mankind sustain healthy living. In recent years, herbal remedies have been considered as an alternative medicine. These plant-based drugs are reported to have been successfully used to cure hypertension, jaundice, skin disease and other infectious diseases. Some of the skin diseases are those arising from fungi infections such as ringworm, athletes' foot, dandruff, etc. This study is focused on demonstrating the antifungal activity of Lawsonia inermis on dandruff isolates. The pulverized Lalley leaves were gotten from a local outlet in Kaduna and was extracted with 70% methanol and with the aid of cold maceration and partitioned to get chloroform and aqueous extract. 30 dandruff samples were collected from students in Delta state University and was labelled F1-F30 for identification. The samples were cultured and isolated in Saboraud dextrose agar (SDA), SDA mixed with Tween 80, and SDA mixed with Power oil vegetable oil to simulate normal hair conditions. Identification using biochemical tests like catalase, fermentation and microscopy tests were done. Phytochemical screening was carried out, Tannin, steroids, saponins, alkaloids, terpenoids were present, while cardiac glycosides and flavonoids were absent. Ten (10) samples were catalase negative, and 20 were positive. All the samples fermented the sugars used (lactose, sucrose and glucose), and all the samples were gram positive. Samples F16 and F27 are Trichophyton tonsurans and they showed resistance to the extracts but were susceptible to the positive control, Nystatin. The other samples were Malassezia furfur and Malassezia pachydermatis respectively. The highest antifungal activity of the extracts was at 100mg/ml and the least at 3.125mg/ml. From this study, it shows that Nystatin is active against T. tonsurans but mot against the Malassezia species. In conclusion, the findings in this study supports the use of these plant extracts in the ethnomedical treatment of dandruff caused by Malassezia species.

Keywords: Lawsnia inermis; Antifungal, Dandruff.

1. INTRODUCTION

Throughout ages, man has relied on nature for their basic needs such as food, clothing, shelter, and medicines. Plants have formed the basis considered by many to offer an alternative treatment and prevention of various diseases. It is also believed by traditional medical practitioners that the phytocomponents present in herbal medicine have better agreeable effects with the human system (Chit et al, 2012).

Herbal remedies are based on the fact that plants contain natural active ingredients that can be used in the treatment of illnesses and betterment of health. It is the use of plants and plant extracts to treat various diseases. These

remedies are therapeutic regimens that comprises of several compounds that interact with multiple targets rather than consisting of a single compound or component that only interacts with a single target (Wang et al., 2012)

Natural products derived from plants have been used to help mankind sustain healthy living. These natural products have been used for thousands of years and it is estimated that 80% of the world's population rely on herbal medicine for their health care.

Since those early times, herbal therapy based on traditional knowledge has been used to treat human ailments. (Gonzalez et al, 2016). Due to its presumed effectiveness, accessibility and widespread acceptability, the use of herbal medicine has risen globally (Juan, 2021).

In recent years, herbal remedies have been considered as an alternative/complementary medicine. Plants based drugs are reported to have been successfully used to cure hypertension, cancer, tuberculosis, jaundice, diabetes, skin diseases and many other infectious diseases (Mohd et al., 2019).

Some of the skin diseases plant remedies treat include those arising from fungi infections such as ringworm, dandruff, athletes' foot, etc.

Herbal medicine is always described as a treatment regimen that consists of a concerted pharmacological intervention of various substances that interact with multiple targets, rather than a single drug that interacts with a single target (Wang et al., 2012)

Much research has been concentrated on medicinal plants in the last decade, since they are thought to be a reservoir of diverse types of bioactive compounds with distinct therapeutic and pharmacological effects (Ahmed et al, 2017). The understanding of how medicinal plants are used as medications in many traditional medical systems has been crucial in the development of novel drugs for orthodox medicine (Ahmed et al, 2017). Even the modern pharmaceutical industry relies heavily on the diversity of secondary metabolites in plants, of which at least 12,000 have been isolated, accounting for less than 10% of all secondary metabolites (Akharaiyi et al, 2012)

Fungi infection, also called mycosis is caused by a fungus that invades the tissues and can cause a disease that confines to the skin, spreads into bones, tissues, organs or affects the whole body (Debra, 2022)

In addition to other places, fungi can be found in the air, soil, water, and plants. Some fungus can be discovered naturally in the human body.

Just like there are good and bad bacteria, there are good and terrible fungi. Hazardous fungi that invade the body can be challenging to get rid of since they can survive in the environment and reinfect the person who is trying to get better. Depending on the type, a fungal infection can cause a variety of symptoms, however some common ones are as follows: Itching, as well as skin changes such as redness, cracking, or peeling. Some of the most common fungal infections include jock itch, ringworm, yeast infections, athletes' foot, and dandruff.

Dandruff is a disorder that causes skin flakes on the scalp. Itching can also be experienced. Most people get dandruff at some point in their lives, but it is more frequent between the ages of adolescence and middle age. Seborrheic dermatitis, allergic responses, psoriasis, and eczema are some of the possible reasons. Seborrheic dermatitis is caused by an overreaction to Malassezia, a yeast that lives on the scalp. The age of a person, the weather, stress levels, medical conditions, and the hair products they use all contribute to the development of dandruff.

Poor hygiene is not a concern, although if a person does not wash or brush their hair frequently, the flakes may be more evident. (Yvette, 2020)

2. MATERIALS AND METHODS

2.1. Materials:

Sterile swab stick, normal saline, syringe, test tubes, test tube racks, culture media (Nutrient agar, Sabouraud Dextrose agar, mannitol salt agar, MacConkey agar, Cetrimide agar, peptone water, nutrient broth, miu agar, Urease broth base), sterile water, microscope, incubator, autoclave, refrigerator, beam balance, measuring cylinder, beaker, wire loop, glass holder, Bunsen burner, EDTA bottle. The various reagents and equipment used for this work were obtained from the laboratories of Pharmaceutical Microbiology Department of the Faculty of Pharmacy Delta State University, Abraka (DELSU).

2.2. Methods

2.2.1. Collection and Extraction of Plant Material

The already pulverized form of lawsonia inermis was gotten from a local outlet in Kaduna state.

The pulverized leaves of lawsonia inemis was extracted with 70% methanol with the aid of a Cold maceration technique for a period of 72 hours. It was thereafter filtered with a sieve. The resulting extract was weighed (101.2g) and stored in a refrigerator.

Fraction A/ Crude Extract

The pulverized leaves of lawsonia inemis was extracted with 70% methanol with the aid of a Cold maceration technique for a period of 72 hours. It was thereafter filtered with a sieve. The resulting extract was weighed (101.2g) and stored in a refrigerator.

Fraction B / Chloroform Extract

45.7g of the extract was weighed out for the partitioning and was dissolved in equal amounts of methanol and distilled water (100ml each) and was transferred into a separating funnel. 200ml of chloroform was added to the separating funnel which was then covered and shaken properly. The funnel was left to stand for a while before another 100ml of chloroform was added and shaken. The chloroform extract was thereafter removed and weighed (1.55g) and stored in a refrigerator.

Fraction C/ Aqueous Extract

200ml of water was added to the remaining extract in the separating funnel and was shaken again before leaving to stand for a while. The resulting aqueous extract was removed, weighed (29.69g) and stored in a refrigerator.

2.2.2. Phytochemical Analysis (Screening)

Preliminary phytochemical analysis of the extract of the root bark of Lawsonia inermis was carried out by the methods described by Enwa et al (2016) and Okafor et al (2021) with little modification.

2.2.3. Collection of Clinical Specimens (Dandruff)

Specimen collection was carried out from May 2022 to June 2022; a total of 30 Dandruff samples of students of Delta State University, Abraka was collected.

A sterile flexible swab stick was rubbed in the student's hair to obtain a good sample the sterile swab sticks were labelled and transferred to microbiological investigation in the pharmaceutical microbiology laboratory, Faculty of Pharmacy, Delta State University, Abraka for further laboratory investigations.

2.2.4. Sterilization of Materials

Glassware's such a test tubes, beakers, measuring cylinder were wrapped in foil paper and sterilized in an autoclave at 121 C for 15 minutes. Cork borers were sterilized by cleaning with cotton wool soaked with 99% methanol and flames over a Bunsen burner. The work area was also sterilized by cleaning it with cotton wool soaked in disinfectant before each work is carried out. Media used in the research was sterilized by autoclaving at 121 C for 15 minutes and inoculating wire loops were sterilized by heating to redness using the Bunsen burner before each use.

2.2.5. Preparation of Media

The various media for incubation of the microbial were prepared according to the manufacturer's instruction and were sterilised by autoclaving at 121 C for 15 minutes

2.2.6. Culturing Of Dandruff Isolates

The work area was swabbed with cotton wool and a disinfectant solution. Saboraud dextrose broth was prepared in test tubes and the sterile dandruff samples was inoculated into them respectively by dipping the bud of the thirty-dandruff containing swab stick into the individual broth medium and was left for 24 hours to permit fungi growth. The test tubes were labelled F1 TO F30 respectively.

2.2.7. Isolation of the Fungi

The work area was swabbed with cotton wool and a disinfectant solution. 65g of saboraud dextrose agar (SDA) is to be prepared in 1000ml of water. 45.5g of SDA was weighed and dissolved with 700ml of water to prepare 30 agar plates to isolate the 30 dandruff samples. The mixture was swirled gently and transferred into a conical flask. The flask was plugged with wads of cotton wool wrapped in aluminium foil to prevent evaporation. The mixture was sterilized in an autoclave at 121°c for 15 minutes. After sterilization, the media was allowed to cool to 45°c and 0.005ml of ciprofloxacin was introduced into the mixture to inhibit the growth of bacteria. The sterile medium was poured into sterile petri dishes and left to solidify. The plates were labelled F1 to F30 respectively.

The procedure was repeated to prepare SDA needed to make 60 more agar plates; 30 for the addition of Tween 80, and the other 30 for the addition of Power oil vegetable oil. The plates were also labelled F1 to F30 respectively. Vegetable oil was added to reference the growth of dandruff despite the presence of hair creams that are greasy and other hair products. The cultured plates were incubated at 37°C for 24 hours.

Bijou bottles were sterilized and used to prepare slants by pouring prepared Saboraud agar halfway into the bottles and kept at a 45° angle until the agar solidified. This was done to prevent contamination of the samples. Fungus from the cultured plates with growth were inoculated using a flamed wire loop into the slants for isolation. The slants were labelled F1 to F30 and was incubated at 37°C for 24 hours.

2.2.8. Confirmation of Identity of Test Microorganism

All confirmatory biochemical test were carried out as described by Anie et al (2015) and Enwa (2014)

2.2.8 Determination of Minimum Inhibitory Concentrations (Mic) Of Plant Fractions

The plant fractions minimum inhibitory concentration was determined to know its antifungal activity strength against the isolated organisms of the dandruff isolates. The test done is called the Agar Diffusion Test.

Fraction A (crude extract), fraction B (chloroform extract), fraction C (aqueous extract), were prepared to obtain a known concentration of 3.125mg/ml to 100mg/ml. Thereafter, 20mls of saboraud dextrose agar was added to 1ml of each faction concentrations prepared in a 2-fold serial dilution and properly mixed together by swirling gently in a radial and longitudinal fashion and was allowed to solidify at room temperature. The fungus suspensions of each inoculum were prepared and streaked on the surface of each media. The MIC is then determined by zone of inhibitions.

2.2.9 Statical Analysis

The data obtained were evaluated using Statistical Package for Social Sciences, Version 22 (SPSS 22) and then, data was summarized using graphs, frequency tables, means and standard deviations.

2.2.10. Ethics approval

Ethical approval was obtained from the Research and Ethical Committee, Delta State University Teaching Hospital (DELSUTH), Oghara

3. RESULTS

3.1 Phytochemical analysis

Investigations on the phytochemical screening of the methanolic extract of the pulverized leaves of Lawsonia inermis (table 1), indicated the presence of saponins, tannins, alkaloids, steroid and terpenoids in the crude extract. Cardiac glycosides and flavonoids were absent.

The phytochemical analysis of the methanolic extracts of the pulverized leaves of lawsonia inermis are shown in Table 1:

Table 1: Results of Phytochemical Test

TEST	OBSERVATION	INFERENCE
Tannins	Black colouration observed	Present
Steroids	Brown ring at the interface	Present
Cardiac glycosides	Brown ring at the interface	Absent
Saponins	Persistent foam formation/ frothing	Present
Alkaloids	Brown colouration with dragendoffs	Present
Flavonoids	Transparent solution observed	Absent
Terpenoids	Brown ring at the interface	Present

Table 2: Biochemical test on Microbial isolates

S/N	Shape	G/S	Catalase	Sucrose	Glucose	Lactose
1	Cocci	-	+	+	+	+
2	Cocci	-	+	+	+	+
3.	Oval	+	+	+	+	-

4.	Cocci	-	+	-	-	-
5.	Oval	+	+	-	-	-
6.	Cocci	-	+	-	-	-
7.	Cocci	-	+	-	-	-
8.	Cocci	-	+	-	-	-
9.	Oval	+	+	-	-	-
10.	Oval	+	-	+	+	-
11.	Cocci	-	+	+	+	+
12.	Cocci	-	-	-	-	-
13.	Cocci	-	+	-	-	-
14.	Cylindr	+	+	-	-	-
15.	ic	-	+	-	-	-
16.	Cocci	-	-	-	-	-
17.	Cocci	-	+	-	-	-
18.	Cocci	+	+	-	-	-
19.	Oval	-	-	-	-	-
20.	Cocci	-	+	-	-	-
21.	Cocci	-	-	-	-	-
22.	Cocci	-	+	-	-	-
23.	Cocci	+	-	-	-	-
24.	Oval	+	-	-	-	-
25.	Oval	-	-	-	-	-
26.	Cocci	-	+	-	-	-
27.	Cocci	-	+	-	-	-
29.	Cocci	-	-	-	-	-
30.	Cocci	-	+	+	+	+
	Cocci					

KEYS: ISO: Isolates +: Positive -: Negative

G/S: Gram staining

S/C: Simon Citrate MSA: Mannitol Salt Agar

3.2 Results of Zone of Inhibition

Table 3: Results of Zone of Inhibition for Fraction A (Crude)

Sample	100mg/ ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	Positive control
F10	14.00	10.66	9.00	9.00	7.00	0.00
F12	18.33	17.33	16.00	13.00	12.66	0.00
F16	0.00	0.00	0.00	0.00	0.00	11.00
F19	15.66	14.00	13.66	11.00	10.33	0.00
F27	0.00	0.00	0.00	0.00	0.00	17.00
F2	17.66	17.00	17.33	14.66	12.33	0.00
F3	20.00	19.00	15.66	14.00	11.00	0.00
F4	22.33	19.33	17.00	15.66	11.66	0.00
F5	15.66	14.33	14.00	12.66	10.00	0.00
F30	18.00	17.00	15.33	15.66	10.00	0.00

Table 4: Results of Zone of Inhibition for Fraction B (Chloroform Extract)

Sample	100mg/ ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	Positive control
F10	20.00	18.33	17.00	15.66	14.00	0.00
F12	18.00	17.33	16.66	13.33	12.66	0.00
F16	0.00	0.00	0.00	0.00	0.00	12.00
F19	16.66	15.00	14.66	12.00	11.33	0.00
F27	0.00	0.00	0.00	0.00	0.00	17.00
F2	15.66	14.00	10.66	0.00	0.00	0.00
F3	21.00	17.66	16.00	16.00	13.66	0.00
F4	17.00	15.66	13.00	13.00	11.66	0.00
F5	16.00	12.00	10.66	9.00	7.66	0.00
F30	20.00	18.00	17.00	17.00	15.66	0.00

Table 5: Results of Zone of Inhibition for Fraction C (Aqueous Extract)

Sample	100mg/ ml	50mg/ml	25mg/m	12.5mg/ml	6.25mg/ml	Positive control
F10	12.00	11.66	9.00	6.66	6.00	0.00
F12	17.66	14.00	14.00	11.67	10.00	0.00
F16	0.00	0.00	0.00	0.00	0.00	10.00

F19	16.00	13.00	12.66	0.00	0.00	0.00
F27	0.00	0.00	0.00	0.00	0.00	16.00
F2	9.00	8.66	8.66	7.66	5.66	0.00
F3	19.00	17.66	16.00	13.66	11.00	0.00
F4	19.00	15.66	13.66	11.00	10.00	0.00
F5	11.00	10.00	9.00	0.00	0.00	0.00
F30	16.00	15.66	13.66	11.00	9.00	0.00

Table 6: Results of Minimum Inhibitory Concentration of All Fractions (Mg/Ml)

SAMPLE	CRUDE	CHLOROFORM	AQUEOUS
F10	6.25	3.125	12.5
F12	12.5	3.125	12.5
F16	--	--	--
F19	50	25	12.5
F27	--	--	--
F2	6.25	3.125	6.25
F3	6.25	3.125	12.5
F4	6.25	6.25	12.5
F5	6.25	12.5	12.5
F30	100	50	100

4. DISCUSSION

The result from the phytochemical screening of the methanolic extracts of lawsonia inermis revealed the presence of tannins, saponins, alkaloids, terpenoids and steroids. This result agrees with the findings of Musab et al., (2016) and Dharmesh et al., (2020) who reported the presence of tannins and terpenoids in the methanolic leaf extract of lawsonia inermis. The presence of these secondary plant metabolites in the plant extracts is an indication that the plant is of pharmacological importance (Adebayo and Ishola, 2009). These secondary metabolites have been found to be present in plants at various parts and levels of growth (Adeshina et al., 2007).

Methanol has been constantly used for the extraction of bioactive constituents in plants and yield indicating that it is a good solvent for the extraction of bioactive compounds in the plant sample (Adebayo-Tayo, et al., 2010). In this study, the antifungal activity of methanolic leaf extracts of lawsonia inermis were tested against Malassezia and Trichophyton tonsurans.

Samples F16 and F27 are T. tonsurans and showed resistance to the leaf extract but were susceptible to the positive control; Mycony's Nystatin oral suspension 100,000IU/ml. The other samples were Malassezia species, F14 being Malassezia furfur and the others Malassezia pachydermatis.

The results in this study indicates that the increase in concentrations of the methanolic leaf extracts gave corresponding increase in mean zones of inhibition as shown in tables 3, 4 and 5. The highest antifungal activity was observed at 100mg/ml for the crude extract with a mean score of 22.33mm against Malassezia pachydermatis. From the investigation, it was observed that Trichophyton tonsurans was resistant to the methanolic extract of lawsonia inermis. This can be seen in their mean zones of inhibition as presented in table 3, 4 and 5.

Nystatin (control) at 100,000IUmg/ml exhibited mean zone of inhibition of 17.00mm against Trichophyton tonsurans. From this study, it shows that Nystatin is active against Trichophyton tonsurans but not against Malassezia species. The MIC was determined by agar well diffusion method using different dilutions. The result showed that the least concentration of extract that had activity on the isolates was 3.125mg/ml. Thus, the antifungal activity of the methanolic extract of L. inermis can be ascribed to the presence of bioactive compounds present in it as the presence of these compounds in plant extracts have been revealed to exhibit medicinal and physiological activities.

5. CONCLUSION

The result of this study has justified the ethnomedicinal uses of Lawsonia inermis for the development of antifungal agents for the effective treatment of fungi infection, this activity is as a result of the chemical constituents possessed by the plant. The leaf extract was active against M. furfur and M. pachydermatis.

Therefore, the ability of the extracts to inhibit the growth of the tested fungal species is an indication of the broad-spectrum antimicrobial potential of Lawsonia inermis which makes it a potent source of antifungal drugs. In conclusion, the finding in this study supports the use of these plant extracts in ethnomedicinal treatment of dandruff caused by Malassezia species. Lawsonia inermis could be a promising potential of new antifungal drugs.

REFERENCES

1. Adebayo, E.A. and Ishola, O.R. (2009). Phytochemical and antimicrobial screening of crude extracts from the root, stem, bark and leaves of Bridelia ferruginea. African Journal of Biotechnology, 8(4): 650-653.
2. Adeshina, GO., Onaolapo, J.A., Ehimidu, J.O, Odama, L.E. and Kunle, O.F (2007). Phytochemical and toxicology activity of the leaf extracts of Alchornea cordifolia (Schum and Thonn). Nigerian Journal of Pharmaceutical Research, 6(1): 19-24.
3. Anie CO, Nwabuokei IG, Oghenejobo M, Enwa FO. (2015). The antibacterial effect of the leaf extract of
4. Buchholzia coriacae (Capparidaceae) on gramnegative nasal isolates. Scholars Academic Journal of Pharmacy; 4(4): 226-231
5. Benitez, L.L. and Carver, P.L. (2019). "Adverse Effects Associated with Long-Term Administration of Azole Antifungal Agents". Drugs. 79 (8): 833–853.
6. Chit, S.J., Jonathan, S.H., Hani, E.N., (2016). Herbal Medicine: Toxicity and Recent Trends in Assessing Their Potential Toxic Effects. Advances in Botanical Research 79(8): 365-384.
7. Chukwuebuka, E.O, A.P., Mishra, M.R. Goyal (2020). The Development of Nutraceuticals and Traditional Medicine 97(8): 539-558.
8. C.I. Ogbonna, R.O. Robinson and J.M. Abubakar (1985). The distribution of ringworm infections among primary school children in Jos, Plateau State of Nigeria. Mycopathologia Research article 89(7): 101-106.
9. Debra, J. (2022). Fungal infections of the skin. https://www.webmd.com/skin-problems-and-treatments/guide/fungal-infections-skin
10. Dharmash, G., Darpini, S.P., Kishor, K.D., Santosh, K.V., and Timir, B.P. (2020). Phytochemical and Antioxidant Activities of Methanolic extract of Lawsonia inermis L. Bark. Current issues in Pharmacy and Medical Sciences, 33(3): 132-138.
11. Denise, M.A. (2021). Overview of Fungal skin infections. MD, Darthmouth Geisel School of Medicine. 125.
12. Elham, A.S. and Elbasheir, A.M (2014). In Vitro Activity of Lawsonia inermis on some Pathogenic Fungi. Veterinary Research Institute. Journal of mycology.
13. Enwa O Felix, Oghenejobo Michael, Clement O. Anie, Rita A. Ayeh, Antibacterial Screening of the Ethanol and Aqueous Extract of the Fruit Peel of Persea Americana Mill against Selected Enteric Bacteria. Academia Journal of Microbiology Research 2016; 4(3):040-046.
14. Enwa FO (2014). A mini review on the microbiochemical properties of sauerkraut. Afr J Sci Res 3:15–16
15. Gozubuyuk, G.S, E. Aktas and N. Yigit., (2014). An ancient plant Lawsonia inermis: Determination of in vitro antifungal activity against dermatophytes species. Science direct, 24(4). 313-318
16. Jinhui, C., Biao, R., Yaojun, T., (2015). Synergistic Combinations of Antifungals and Anti-virulence Agents to Fight Against Candida albicans. National Library of Medicine. 6(4): 362-371.
17. Juan, J.M (2021). Medicinal Plants and Their Traditional uses in Different Locations. Phytomedicine: A Treasure of Pharmacologically Active Products from Plants. 207-223.
18. Lie-Fen, S.R., and S.Y., Allan, (2012). Recent Trends in Medicinal Plants Research. Advancement in Botanical Research, 62(1): 2-430

19. Liliana, S., Ana, C.A., Caroline, M.M., Wanessa, C.M., (2017). Antifungal Therapy: New Advances in the Understanding and Treatment of Mycosis. National Library of Medicine, 32: 143-147
20. Mehnaz, K., and Talha, J., (2011). Pharmacological Activities of Lawsonia inermis L. International Journal of Biomedical Research 1(2): 62-68.
21. Michael, R., John., R.B., (2014). Development of Drugs from Plants: Regulation and Evaluation. International Journal of Biomedical Research 91(4):385-408.
22. Moraes, D.C., Ferreira, A.P., (2019). Insights on the anticandidal activity of non-antifungal drugs. European Journal of Pharmaceutical and Medical Research. 3(9): 253-259
23. Mohd, S.A., and Iqbal, A., (2019). Herbal Medicine. New Look to Phytomedicine. 3-13.
24. Musab, A.W., Imad, M.T., and Hozeifa, M.H., (2015). Effects of Lawsonia inermis L. leaves' methanolic extract on carbon tetrachloride induced hepatotoxicity in rats. Journal of Intercultural Ethnopharmacology. 5(1): 22-26.
25. N. Chattopadhyay, R. Maurya. (2015). Herbal Medicine. Reference Module in Biomedical sciences. 1-3.
26. Ofonime, O. (2016). Dandruff Aetiology and the Effects of Edible Lipids on the Growth of Isolates. European Journal of Pharmaceutical and Medical Research. 3(9): 71-76.
27. Olaide, O.O., Olaniyi, O., Olayinka, A.O., Akinlolu, G.O.,and Olumayowa, A.O., (2014) The Prevalence and Pattern of Superficial Fungal Infections among School Children in Ile-Ife, South-Western Nigeria. National Library of Medicine.
28. Okafo SE, Enwa FO, Amusile O. Formulation and Evaluation of Antimicrobial Properties of Psidium guajava Ethanol Leaf Extract Creams. Tropical Journal of Natural Product Research, 2021; 5(12):2144-2148
29. Olufunke, F., (2021). Nigeria's Medicinal Plants: Lawsonia inermis (Laali). Punch Newspapers.
30. Shuyuan, L., Hou, Y., Xu, Chen, (2014). Combination of Fluconazole with non-antifungal Agents: A Promising Approach to Cope with Resistant Candida albicans Infections and Insight into New Antifungal Agent Discovery. National Library of Medicine. 43(5): 395-402.
31. Sushim, K.G., Kuo, C.L., Chang, H.C., (2019). In Vitro Propagation and Approaches for Metabolites Production in Medicinal Plants. European Journal of Pharmaceutical and Medical Research. 35(61): 35-55.
32. Yvette, B.O., Reema, P.M., (2020). How to treat dandruff
33. Wang, X., Fangfang, W., Aihua, Z., and Wenfeng, W., (2017). Identification of the Absorbed Constituents of Schisandra Lignans by Serum Pharmacochemistry of TCM. 337-350.