Environment and Ecology 43 (3A) : 812—818, July—September 2025
Article DOI: https://doi.org/10.60151/envec/VPYM9004
ISSN: 0970-0420 (Print), ISSN: 3049-3625 (Online)

Antifungal Properties of Selected Medicinal Plants Used by Bodo Tribes of Kokrajhar, BTR, Assam, India

Sanjib Brahma, Phami Brahma

Received 5 June 2025, Accepted 24 July 2025, Published on 26 August 2025

ABSTRACT

The Kokrajhar district of BTR, Assam is the home of many ethnic forest dwellers. The Bodos are the major tribe of this region who live in close proximity to the forest. Since time immemorial Bodos used different medicinal plant to cure different ailments. Though numbers of plant species are used in traditional way but scientific study of many of them is not done yet in this region. Viewing this limitation an attempt has made to find out the antimicrobial properties of selected three numbers of commonly used plant species viz. *Andrographis paniculata, Moringa oleifera* and *Psidium guajava.* Antifungal activity of three selected plant species against *Aspergillus* sp. of onion was studied. Extract of compounds from studied plant species was done by using three different solvents viz. distilled water, ethanol and acetone. The antifungal activity of various plant extracts was screened against the test organism using Agar Well Diffusion Method. During study it was found that all the three numbers of investigated plant species have more or less antimicrobial efficacy. The ZOI (Zone of Inhibition) in aqueous extract found in studied plant species were 12 mm in *A. paniculata,* 10 mm in *M. oleifera* and *P. guajava* have 13mm . In ethanol extract *A. paniculata* 12 mm, *M. oleifera* 10 mm and *P. guajava* 14 mm was found. In acetone extract the ZOI was *A. paniculata* 8 mm, *M. oleifera* 9 mm and *P. guajava* 10mm. Out of three studied plant species the ethanol extract of *Psidium guajava* shows the highest ZOI of 14 mm against the test microorganism.

Keywords Antifungal, Medicinal plant, *Andrographis paniculata, Moringa oleifera, Psidium guajava,* Bodo tribe, Kokrajhar.

INTRODUCTION

Nature is abundant and full of life. With the development of human civilization, both the plants and their products have been used as food and medicine. Since ancient times, nature has played a significant role in Indian culture. The ancient Vedas and other scriptures mention medicinal use mostly dependent on plants (Pandey *et al.* 2013). Products made from various plant parts are thought to be safe for ingestion by humans and are a source of several medicinal

Dr Sanjib Brahma[1]*, Phami Brahma[2]

[1]Associate Professor & HOD, [2]Assistant Professor

Department of Botany Science College, Kokrajhar, Assam, India

Email: sbsanjibbrahma@gmail.com
*Corresponding author

agents and bioactive chemicals (Stohs and Hartman 2015). The plant possesses antimicrobial qualities which could be due to their abundance in essential bioactive compounds.

Antimicrobial refers to the substance that inhibits the growth or kills microorganisms like bacteria, fungi and viruses. They play a crucial role in combating infections and preventing the spread of diseases. Antimicrobial can be classified into different categories based on their target microorganisms. Antibiotics can be defined as the naturally produced microbial products or their derivatives that kill or inhibit the growth of other microorganisms. Antibiotics specifically target bacteria, while antiviral are designed to combat viral infections and antifungal are used to treat fungal infections

In recent years, there has been a growing interest in developing new antimicrobial agents and alternative treatment options. Researchers are exploring natural compounds such as plant extracts and essential oils, which have shown promising antimicrobial properties. This could potentially lead to the development of new drugs that are less prone to resistance.

Prior to the development of pharmaceutical medicinal products, humans relied heavily on herbal remedies. Historically, medicinal plants have been utilized to both prevent and treat illnesses. Currently, medicinal plants are used to manufacture about 25% of synthetic drugs (Pan *et al.* 2013). In comparison to contemporary synthetic antibiotics, they are thought to have less adverse effects and are well-known for their antibacterial qualities (Kumar *et al.* 2012). It is therefore preferable to create medicines with their efficacy, constituents, and long-term adverse effects all taken into account. Traditional healers use nearly every component of certain plants as medicine.

North East India including Kokrajhar district has the advantage of topography and climatic conditions which influence the vegetation. The local community people are very close to the nature thus, the direct access into the nature has led to the development of traditional medicine. According to Redo *et al.* (1989) over the past 20 years, there has been increased interest in investigation of new antimicrobials from natural sources. Different plant extracts from medicinal plants have been tested to identify the sources of the therapeutic effects. As a result, some natural products have been approved as new antimicrobial drugs but still there is an urgent need to identify novel substances that are active towards the pathogens with high resistance. A huge number of plant-derived medicines were used for centuries to fight against microbial infections and to minimize inflammation. They are believed to have low toxicity hence safe for therapeutic used (Sadlon and Lamson 2010). Plant derived metabolites have been valuable sources of different antimicrobial and antibacterial properties are reasonably harmless to man. These natural products can eliminate the expensive and insufficient supply of synthetic antimicrobial compounds (Rahman *et al.* 2014).

The pharmacological qualities of various plants can be extracted using solvents, such as ethanol, methanol, acetone, hexane, chloroform, and should be investigated for biological active compounds. These properties include antifungal, antibacterial, antiviral and antiparasitic effects which can help to mitigate pathogens in plants as well as in humans causing various diseases. Therefore, modern scientific research must concentrate on these challenges in order to validate the traditional claims made on various plants.

MATERIALS AND METHODS

Study area

The main objective of this study was to evaluate the antifungal activity of the studied plant samples against *Aspergillus* sp. isolated from onion. For this study the plant samples were collected from different sites of Kokrajhar district viz. Baganshali, Daloabari, Ganganagar, Aflagaon. The Kokrajhar district is located between the longitude 90.16'0" E and latitude 26.24'0" N in the North-eastern region of India in the state of Assam. The favorable climatic conditions in this part of India provide various endemic plants to attain sustainability making it rich in biodiversity. The Kokrajhar district lies extreme north of the river Brahmaputra. Several medicinal plants are traditionally used as medicine but very few scientific works have been carried out. The Kokrajhar district is pre-dominantly inhabited by Bodo tribe and covers an area of 3169.22 km² geographical area. It has a humid sub-tropical climate with dry winter. The temperature ranges around 8.8°C in winter to 35.5°C in summer with 2,400 mm to 3,000 mm annual rainfall. Highest rainfall occurs during the month of July and August. Relative humidity is very high with average of 86% in summer.

Experimental design

Sample collection and processing

Three numbers of locally used plant species were selected for study (Table 1). The selected plants were collected from several locations across Kokrajhar district. The morphological characteristics of the plants were used to determine and identify them. Various standard literature and e-herbarium were used for identification and authentication of plant species.

Disease free and good health plant leaves were picked for study. The chosen plant leaves were properly cleaned by washing to get rid of any undesired material, dirt, and other foreign particles like grasses or other plant parts were eliminated. Following washing, the plant materials were spread out and allowed to air dry at room temperature in the shade. To speed up drying, the samples were flipped up and down at least twice a day.

After 5 to 7 days, the dried samples were grinded with grinder to obtain fine powder of the sample. During grinding the over-heating of the machine was avoided and grinded with intervals of time and stored in a tight container.

Maceration of plant sample

The finely powdered leaves of shade-dried plant leaves were extracted using three different solvents i.e., distilled water, ethanol, and acetone. A weighing balance was used to weigh twenty grams (20 g) of finely powdered plant material, which was then transferred into a conical flask along with 200 mL of distilled water for aqueous extraction. 20 g of powdered leaves were weighed and put into conical flask along with 200 mL of ethanol and acetone, respectively, for extraction (Okeyo *et al.* 2022). After covering each of the three plant samples with a cotton plug, they were all vigorously shaken for five minutes. The samples were then wrapped with aluminium foil to avoid contamination and evaporation and then left undisturbed for 48 hours.

Table 1. Studied medicinal plant species.

Sl. No.	Species name	Local name	Family
1	*Andrographis paniculata* (Burm.f.) Nees	Kalmeg	Acanthaceae
2	*Moringa oleifera* Lam.	Sojina	Moringaceae
3	*Psidium guajava* L.	Sumpram	Myrtaceae

After 48 hours, all the plant extract solution was filtered using sterile filter paper (Whatman No.1). The extracts (filtrate) were then stored for further use. Following the filtration process, the plant extract solution was transferred to a round bottom flask and concentrated at a Rotatory Evaporator with a lower pressure. The flask was continuously heated in a rotating manner to 40°C for aqueous extraction, 60°C for ethanol, and 50°C for acetone (Okeyo *et al.* 2022). Subsequently, the semi-solid extract was labelled, placed in a sterile petri dish, and preserved in refrigerator for later use.

Isolation and identification of test organism

The test organisms were isolated from infected onion. Onion showing symptoms of black mold disease was collected from Kokrajhar local market. The organism was cultured in prepared Sabouraud Dextrose Agar media (SDA). For the preparation of media, 6.5 g of SDA media was prepared in 100 mL of distilled water. To stop bacteria from growing, Amoxicillin (0.1g), an antibiotic drug, was used. After that, sterile inoculating loops were used to inoculate petri plates with fungal spores using the streaking method. The plates were then incubated for 7 days at 37°C (Saravanan *et al*. 2020).

For pure culture and identification, fungal colonies cultured on SDA media were sub-cultured on fresh SDA media and incubated for further growth of fungal colony. After observing the morphology and colony of the fungal isolates, the fungal colonies were

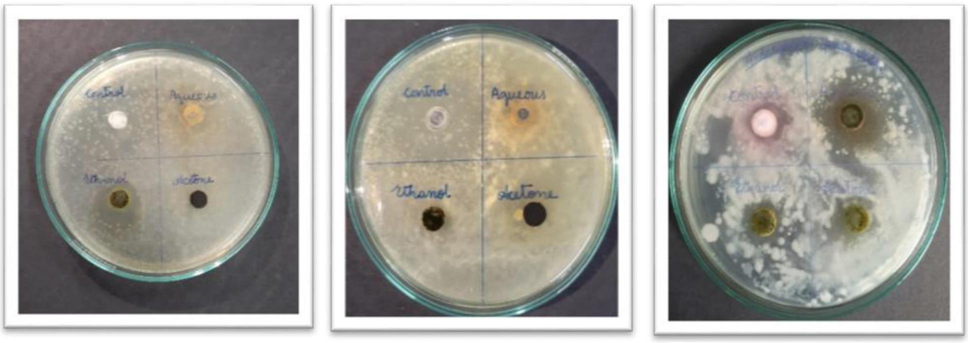

Fig. 1. Triplicates of various extracts of *A. paniculata* showing zone of inhibition.

studied under a microscope (Mailafia *et al.* 2017).

A clean and sterile glass slide was taken and a drop of lactophenol cotton blue was placed. A small segment of fungal mycelium was obtained from the colony and then placed in the slide. A cover slip was gently placed and carefully pressed to remove air bubbles and observed under microscope with ×10 objective lens. The isolated fungal spores were identified based on morphological cultural and microscopical characteristics (Tambuwal *et al.* 2018).

Qualitative screening and antifungal activity assessment

The antifungal activity of various plant extracts was screened against the test organism using Agar Well Diffusion Method. The sterile petri plates were dried at a temperature under hot-air oven to remove the excess water and moisture. The petri plates were then divided into four sections and labelled (positive control, aqueous, ethanol and acetone) for various plant extracts with the help of clean scale.

After dissolving 65 g of Sabouraud Dextrose Agar (SDA) powder in 1000 mL of distilled water, the mixture was autoclaved for 15 minutes at 121°C. It was then allowed to cool to a temperature of 45–50°C and supplemented with amoxicillin to inhibit the growth of bacteria (Suraka *et al.* 2021). After that, the media was poured into sterile, approximately 4mm thick petri dishes. After inoculating the media with fungus strains, it was allowed to dry. To create a well in the middle of each division on the inoculated plates, a sterile cork borer with a diameter of 7 mm was utilized. Thus, making four wells in every plate and each well was then filled with 10 µl of aqueous, ethanol, and acetone plant extract (100 mg/mL), in that respectively.

For positive control, an antifungal agent, Fluconazole (20 mg/mL) was added using micropipette

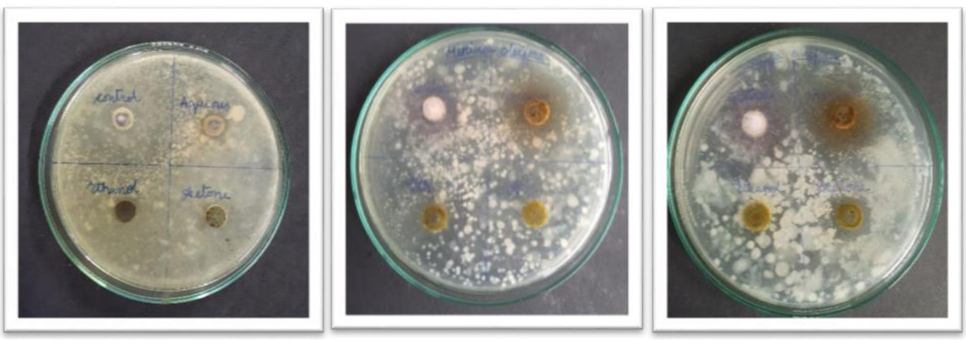

Fig. 2. Triplicates of various extracts of *M. oleifera* showing zone of inhibition.

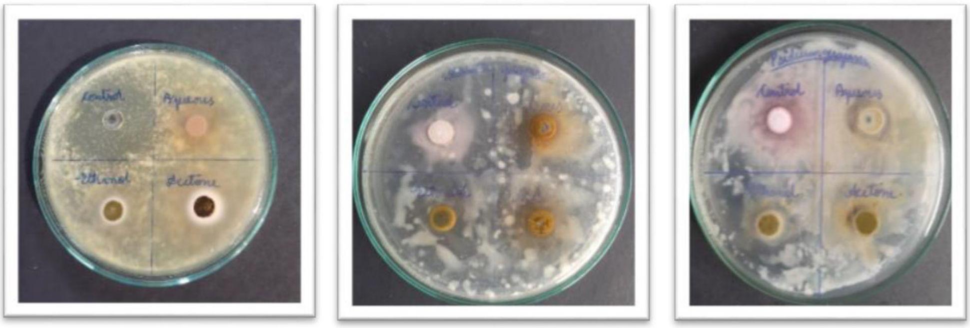

Fig. 3. Triplicates of various extracts of *P. guajava* showing zone of inhibition.

Mathur *et al.* (2011). The plates were then kept with lid closed for half an hour so that the plant extracts diffuse into the media. The plates were then incubated for 18 hours at 37°C (Djadjiti *et al.* 2022).

After proper incubation period, the plates were examined for zones of growth inhibition every 12 hours. A clear ruler was used to measure and record the zones of inhibition in millimeters. The lack of a zone indicates that there was no antifungal action.

RESULTS AND DISCUSSION

By using the agar well diffusion method as reported by Dingle *et al.* (1953) the potential antifungal activity of the aqueous, ethanolic, and acetone extracted fraction of plants was evaluated (Figs. 1-3). In this instance, the inhibition zone was measured by comparing the three readings, and the average was recorded (Table 2).

The average size of the zone of inhibition in the plant sample's acetone, ethanol, and aqueous extracts that displayed inhibition zones against the fungi during the qualitative screening phase was recorded (Table 3).

Table 2. The antifungal activity of the ethanol, acetone, and aqueous extracts when tested against fungus.

Sl. No.	Plant material	Aqueous extract	Ethanol extract	Acetone extract
1	*A. paniculata*	+	+	+
2	*M. oleifera*	+	+	+
3	*P. guajava*	+	+	+

Comments: A positive (+) symbol means that the plant sample extracts have antifungal activity since they prevented the growth of fungus. An inhibition zone is absent when the sign is negative (-).

For positive test, a common antifungal (Fluconazole) was used for standard to compare the antifungal activity with the extracts used in this study. For the negative test, distilled water was used to check if there was any potential contamination. The plant extracts were introduced in the well to diffuse into the surrounding agar using the Agar Well Diffusion Method, forming a concentric circle that inhibits or kills susceptible microorganisms. This inhibited region is known as zone of inhibition (ZOI) and it is measured in mm.

In this study, the screening of anti-fungal activity of *P. guajava* leaf extracts against *A. niger* revealed the highest zone of inhibition. Largest zone of inhibition was observed with ethanol extract with 14 mm ZOI and aqueous extract with 13 mm ZOI and acetone

Table 3. Antifungal activity of various extract, positive and negative control showing inhibition zone against fungi.

Sl. No.	Plant material	Aqueous extract (inhibition zone mm)	Ethanol extract (inhibition zone mm)	Acetone extract (inhibition zone mm)
1	*A. paniculata*	12	12	08
2	*M. oleifera*	10	08	09
3	*P. guajava*	13	14	10
4	Positive-control (Fluconazole)	12	12	12
5	Negative-control (Distilled water) .	0.00	0.00	0.00

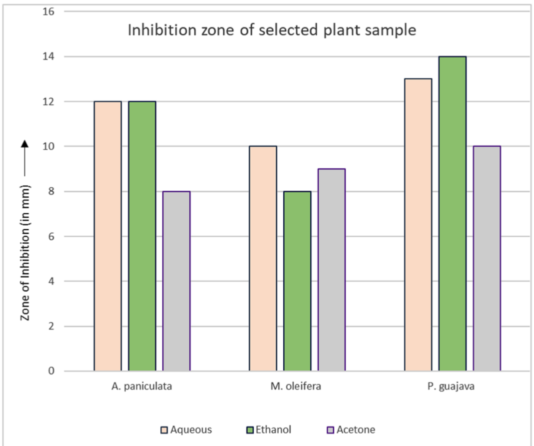

Fig. 4. Zone of inhibition exhibited by different plant extracts against *Aspergillus niger.*

extract with 10 mm ZOI (Fig. 4) *A. paniculata* plant extracts showed the second most antifungal activity against *A. niger.* The highest ZOI was recorded in the ethanol extract with 12 mm ZOI and aqueous extract with 12 mm ZOI followed by acetone extract showing 8 mm ZOI. In case of *M. oleifera* leaf extract, the aqueous leaf extract showed highest ZOI with 10 mm ZOI and the acetone extract showed 9 mm ZOI and the lowest ZOI was recorded in ethanol extract with 8 mm ZOI.

Among all the three plant extracts, *P. guajava* showed highest antifungal activity using all the three solvent extracts against *A. niger.* These results demonstrated that the ethanolic extract had a stronger inhibitory impact on the tested organism than the aqueous and acetone extracts, suggesting that the ethanolic extract is more potent against the organism under test. The findings of this study suggest that *P. guajava* leaves can be extracted using ethanol as a solvent to treat Aspergillosis, which is often caused by *A. niger* and affects immunocompromised persons.

The results showed that the investigated plant extract's antifungal efficacy against *A. niger* varied from one another. The highest antifungal activity was seen in the ethanol extracts of all plants except in *M. oleifera* when compared to the aqueous and acetone extracts. Of all the ethanolic plant extracts tested against *A. niger, P. guajava's* ethanol extract demonstrated the strongest antifungal activity, with a zone of inhibition (ZOI) of approximately 14 mm in diameter.

P. guajava also showed highest ZOI among other plant extracts using aqueous solvent and acetone extract with diameter of 13 mm and 10 mm ZOI respectively. The possible reason for difference in potency maybe due to higher extracting ability of the ethanol than acetone and distilled water. The bio-active properties of the sample plants may also lead to such outcome. However, the result of this study shows that all the tested plants i.e., *A. paniculata, M. oleifera* and *P. guajava* showed antifungal properties. The antifungal activity of the plants may vary depending on the type of solvent used for extraction and the test organism and their resistance capacity.

The antifungal activity of all the plants studied in this experiment revealed varied antifungal potency against *A. niger*. The ethanol extracts of all the tested plants were found to have maximum antifungal activity compared to both aqueous and acetone extracts. Ethanol extract of *P. guajava* possessed highest potent antifungal activity amongst the other ethanolic plant extracts against *A. niger* showing highest zone of inhibition (ZOI) of about 14 mm diameter. *P. guajava* also showed highest ZOI among other plant extracts using aqueous solvent and acetone extract with diameter of 13 mm and 10 mm ZOI respectively. Thus, can be recommended as a promising candidate for biological control of fungal pathogen. The possible reason for difference in potency among various tested plants maybe due to higher extracting ability of the ethanol than acetone and distilled water. The bio-active properties of the sample plants may also lead to such outcome. However, the result of this study shows that all the tested plants i.e., *A. paniculata, M. oleifera* and *P. guajava* showed antifungal properties. The antifungal activity of the plants may vary depending on the type of solvent used for extraction and the test organism. This suggests that these plant extracts may possess therapeutic potential for treating diseases caused by *A. niger,* such as Aspergillosis, Allergies and various respiratory tract infections. Additionally, these plants could prove beneficial in controlling fungal infestation in fruits and vegetables, thereby mitigating the post-harvest diseases particularly in onions.

REFERENCES

Djadjiti, N., Aziz, M. A., Esther, O. K., & Dane, S. (2022). Antimicrobial Activities of *Moringa oleifera* and *Psidium guajava* against Bacterial and Fungal Strains. *Fungal Genomics & Biology*, 12(1),1-4.

Dingle, J., Reid, W. W., & Solomons, G. L. (1953). The enzymatic degradation of pectin and other polysaccharides, II-applications of the cup-plate assay method to the estimation of enzyme. *Journal of the Science Food and Agriculture,* 4(3),149-155. https://doi.org/10.1002/jsfa.2740040305

Kumar, A., Dora, J., Singh, A., & Tripathi, R. (2012). A review on king of bitter (Kalmegh). *International Journal of Research in Pharmacy and Chemistry,* 2(1),116-124.

Mathur, A., Singh, R., Yosuf, S., Bharadwaj, A., Verma, S. K., Babu, P., Gupta,V., Prasad, G. B. K. S., & Dua, V. K. (2011). Antifungal activity of some plant extracts against clinical pathogens. *Pelagia Research Library, USA, Advances in Applied Science Research,* 2 (2), 260-264.

Mailafia, S., Okoh, G. R., Olabode, H. O. K., & Osanupin, R. (2017). Isolation and identification of fungi associated with spoiled fruits vended in Gwagwalada market, Abuja, Nigeria. *Veterinary World,* 10(4), 393-397. https://doi.org/10.14202/vetworld.2017.393-397

Okeyo, G. O., Charimbu, K. M., Nyaanga, J., & Mendes,T. (2022). Antibacterial activity of guava, moringa, camphorbush and pelargonium extracts against bacterial wilt (*Ralstonia pseudosolanacearum* sp. Nov.) of potato. *Saudi Journal of Biological Sciences*, 29(12), 103438

Pandey, M. M., Rastogi, S., & Rawat, A. K. S. (2013). Indian Traditional Ayurvedic System of Medicine and Nutritional Supplementation. *Hindawi, Evidence-based Complementary and Alternative Medicine,* Volume 2013(1), 1-12. https://doi.org/10.1155/2013/376327

Pan, S. Y., Zhou, S. F., Gao, S. H., Yu, Z. L., Zhang, S. F., Tang, M. K., Sun, J. N., Ma, D. L., Han, Y. F., Fong, W .F., & Ko, K. M. (2013). New Perspectives on How to Discover Drugs from Herbal Medicines: CAM′s Outstanding Contribution to Modern Therapeutics. *Wiley, Evidence-Based Complementary and Alternative Medicine,* Volume 2013 (1),1-25. https://doi.org/10.1155/2013/627375

Rahman, M. M., Ahmad, S. H., Mohamed, M. T. M., & Rahman, M. Z. A. (2014). Antimicrobial Compounds from Leaf Extracts of *Jatropha curcus, Psidium guajava,* and *Andrographis paniculata. Hindawi, The Scientific World Journal,* Malaysia,Volume 2014(1),1-8. https://doi.org/10.1155/2014/635240

Redo, M. C., Rios, J. L., & Villar, A. (1989). A review of some antimicrobial compounds isolated from medicinal plants reported in the literature 1978–1988. *Phytotherapy Research,* 3(4), 117-125. https://doi.org/10.1002/PTR.2650030402

Sadlon, A. E., & Lamson, D. W. (2010). Immune-modifying and anti-microbial effects of eucalyptus oil and sample inhalation devices. *Alternative Medicine Review,*15(1), 33-47.

Saravanan, A., Jayasree, R., Kumar, P. S., Varjani, S., Hemavathy, R. V., Jeevanantham, S., & Yaashikaa, P. R. (2020). Production of pigment using *Aspergillus tamari*: New potential for synthesizing natural metabolites. *Environmental Technology & Innovation,* 19 https://doi.org/10.1016/j.eti.2020.100967

Stohs, S. J., & Hartman, M. J. (2015). Review of the Safety and Efficacy of *Moringa oleifera. Phytotherapy Research,* 29(6), In press.

Suraka, B., Ibrahmin, D., Suleman K., Usman, U., & Usman, S. I. (2021). Antifungal activity of *Moringa oliefera l*eaves crude-extract against *Aspergillus flavus* and *Rhizopus stolonifer. Dutse Journal of Pure and Applied Sciences, Nigeria*, 7(4b), 9-17.

Tambuwal A. D., Muhammad, I. B., Alhaji, S., Muhammad, S., & Ogbiko, C. (2018). Morphological and biochemical characterization of isolated *Aspergillus niger, Saccharomyces cerevisiae* and *Zygomonas mobilis* from local indegineous sources. *GSC Biological and Pharmaceutical Sciences,* 5(3), 086-094. https://doi.org/10.30574/gscbps.2018.5.3.0147