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# Phytochemical and Anti-Fungal Screening of the *Averrhoa bilimbi* Fruit Extract against *Aspergillus Niger*

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## Abstract

Aspergillosis is a fungal infection and is a continuing problem in the world today. The respiratory infection increases the risk to those who are immunocompromised. Evidence shows that the *Averrhoa bilimbi* fruit contains the following pharmacological properties such as antihypertensive, antidiabetic, antithrombotic, cytotoxic, hypolipidemic, hepatoprotective, antimicrobial, anthelmintic, and antioxidant properties. However, the anti-fungal property of this medicinal plant has not been established. The objective of this study is to determine the bioactive constituents and investigate the antifungal ability of *Averrhoa bilimbi* fruit extract against *Aspergillus niger* organism. The study utilized a complete randomized design to determine the phytochemical properties and anti-fungal efficacy of *Averrhoa bilimbi* fruit extract against *Aspergillus niger*. The extraction of *Averrhoa bilimbi* fruit was tested according to the procedure of the Standards and Testing Division of the Department of Science and Technology (DOST). Qualitative analysis was also used to determine the phytochemical constituents of the fruit extract. An anti-fungal Assay was done to test the susceptibility of the extract to the *Aspergillus niger.* A positive control (clotrimazole) was used as a comparison. The result of anti-fungal screening of the extract reveals a complete inhibitory activity with mild reactivity against the test organism *Aspergillus niger,* which is comparable with clotrimazole. It is therefore recommended that similar studies be conducted to establish the anti-fungal property of the plant.

**Keywords**: anti-fungal property, *Aspergillus niger*, *Averrhoa bilimbi*, anti-fungal assay

## INTRODUCTION

Plants have been used for medicinal purposes for ages, and such use has not changed even until today. *Averrhoa bilimbi* (*A. bilimbi*) is a medicinal plant in the *Oxalidaceae* family and has been used for different purposes (Ahmed & Alhassan 2016). The plant was originally found in Southeast Asia in countries such as West Malaysia and Indonesia. It has been cultivated in Bangladesh, Indonesia, Malaysia, the Philippines, Singapore, and Thailand. The plant has been an important source of medicine since the old times. It has been used for therapeutic purposes as recorded in Egyptian medicinal papyrus which was written in the fourteenth century. *A. bilimbi* also has pharmacological activities which have been reported as antihypertensive, antidiabetic, antithrombotic, cytotoxic, hypolipidemic, hepatoprotective, antimicrobial, wound healing, antihelminthic, and antioxidant.

*Averrhoa bilimbi* is also known as cucumber tree, *kamias*, and *belimbing asam*. The leaves of this plant have been discovered to have not only antimicrobial but also anti-fungal properties; however, more studies can be made on the fruit of *A. bilimbi* (Aziz, 2016).

According to the Centers for Disease Control and Prevention (CDC), for over 180 species of *Aspergillus*, less than 40 of them have been identified to affect human infections. Species *Aspergillus niger* is an opportunistic filamentous fungus, and it is one of the most familiar to genus *Aspergillus* species. It induces "black mold" disease in specific vegetables and fruits such as grapes, onions, peanuts, and apricots. In addition, black mold is prevalent to cause hazards if food is contaminated. American Thoracic Society stated that *Aspergillus* lives not just in soil, plants, or decaying materials, but it also exists in the dirt in your home, rugs, heating and ventilation ducts, certain foods, including dried fish and dried cannabis plants. *Aspergillus niger* has the least chance to cause diseases to humans compared to other *Aspergillus* species. Rarely, critically underlying lung disease such as aspergillosis can occur and cause severe illness in the person's lungs.

In the study conducted by Juman et al. (2019), it was revealed that *Averrhoa bilimbi* fruit extracts exhibit a wider inhibitory zone than the leaves' extract against pathogenic *Candida* species that causes candidiasis if left untreated.

The incidence of fungal infections in humans has caused a significant rise in the past three decades. Usually, a fungal infection does not affect much to low-risk individuals but is a high risk to those who are in long-term use of antibiotics, immunosuppressive therapies, intensive care unit patients those who are infected with HIV, and invasive surgical procedures such as organ transplant and stem cell transplant (Gou et al., 2019).

A case reported that *Aspergillus niger* has been the cause of fungal pneumonia but is rarely known and did not respond to the drug voriconazole in a patient who has been into a long-term steroid treatment (Person et al., 2010).

However, regardless of the progress in the making of anti-fungal drugs, fungi are still a threat to human health and can spread in healthcare settings, according to the Centers for Disease Control and Prevention (CDC). There is a relation between the making of anti-fungal drugs and the several factors that contribute to the emerging opportunistic pathogens and increasing drug-resistant strains that hinder the effective treatment against opportunistic infections. The continuing spike range in the number of individuals being immunocompromised is not addressed properly, even by powerful and effective drugs; hence, the development of new antifungal agents should be created to fight against drug-resistant microorganisms (Mazu et al., 2016). The study will determine the phytochemical properties of *Averrhoa bilimbi* fruit extract through qualitative analysis and to test the anti-fungal efficacy of the *Averrhoa bilimbi*.

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## RESULTS

*Table 1: Phytochemical Test for Plant Constituent of Averrhoa bilimbi*

| Constituents | Results |
|---|---|
| Sterols | (-) |
| Triterpenes | (+) |
| Flavonoids | (+) |
| Alkaloids | (+) |
| Saponins | (+) |
| Glycosides | (+) |
| Tannins | (-) |

***Note:*** (+) Indicates the presence of constituents, (-) Indicates the absence of constituents

Table 1 displays the *Averrhoa bilimbi* fruit extract's phytochemical analysis results. It showed the positive (+) presence of alkaloids, flavonoids, glycosides, saponins, triterpenes, and the absence (-) of sterols and tannins.

Among the seven phytochemical constituents of *Averrhoa bilimbi* extracts, bioactive fatty acid-containing constituents were triterpenes, alkaloids, saponins, glycosides, while ethanol-containing constituents showed only the presence of the flavonoids. Fatty acids and ethanol are potent in disrupting pathogenic fungal membranes thus, inhibiting their reproduction. As we can see in the test, there is the absence of tannins and sterols, which are ethanol; these are essential in inhibiting the growth of molds.

The proteins and membranes which determine the composition of structure and functions of the cell are the primary target of ethanol to invade because by targeting this, it inhibits glucose and amino acids thereby, reducing its capability to multiply in the process of reproduction (Eleutherio et al., 2019).

It was found that tannins acted to inhibit spore germination and mycelial growth. Tannins target the cell wall causing disruption in the permeability of the membrane leading to spillage of sugar as one of the intracellular contents. *In vivo* test showed a remarkable p-value of $< 0.05$ reduction in an artificially inoculated citrus fruit against *P. digitatum* and decreased disease status of mold green on citrus fruit significantly by 70% in the study of Zhu et al. (2019).

The phytochemical test manifests a lack of some essential bioactive constituents effective in suppressing the growth of fungi; thus, it appears to be less effective compared to clotrimazole.

### Chemical Constituents Interpretation Flavonoids

Flavonoids (or bioflavonoids), also called Vitamin P; and citrin, are usually abundant in fruits, vegetables, seeds, nuts, stems, flowers, tea, wine, propolis, and even in honey. They are secondary types of metabolites coming from plants and are ubiquitous in cell photosynthesizing. Over time, this biochemical constituent has been the choice as the active ingredient utilized to combat human diseases. Based on studies, the broad spectrum of infectious diseases such as parasitic infections,

opportunistic infections, resistant bacterial infections, tuberculosis, fungal infections, viral infections, and in varied cancers, flavonoids can be highly useful. (Bose et al., 2018).

The study of Guerra et al. (2017) showed that Cou-UMB16 in Benzopyrone tested and showed a significant result that suppresses the *Aspergillus* species.

***Triterpenes***

According to Chudzik et al. (2015), they discovered that natural compounds such as triterpenes displayed a broad spectrum of biological effects. In addition, demonstrated to have anti-viral, anti-bacterial, anti-oxidative, anti-fungal, anti-inflammatory properties, chemo-preventive, and anti-fungal properties.

***Alkaloids***

Alkaloids in plants have been long-used therapeutically for the last thousands of years, and it is compound liable for its effectiveness was found not until the 19$^{th}$ century. Alkaloids belong to one of the biggest classes of natural products that have different variety of chemical entities. Some alkaloids are highly dangerous to animals, but these are helpful to be used as sedatives, antiseptics, analgesics, and act as anti-fungal and anti-bacterial agents (Bribi, 2018). In some studies, the gathered information about alkaloids shows a very good effect as an anti-cancer mechanism (Lu et al., 2012). In the review study conducted by Khan et al. (2018), alkaloids obtained in various plants shows anti-fungal effects against diverse fungi. In addition, alkaloids possess little immunity, but these require in-depth studies and clinical trials for validity. Hence, this shows the chances of natural alkaloids being considered as promising anti-fungal agents.

***Saponins***

Saponins belong to a wide group of compounds present in many plants. Saponins are broken down into triterpenoid and steroid glycosides. They are found in most herbs and vegetables. Saponins were found to have an anti-inflammatory mechanism that aids in skin inflammation and edema. Also, it was reported that saponin extracts from ginseng exhibit neovascularization in burn skin that accelerates wound healing (Kim et al., 2011). According to Niziol-Lukaszewska and Bujak (2018), plants are high in saponins which are usefully applied as active agents in cosmetics because of their antioxidant, regenerative mechanism, and promising anti-aging capabilities. In the past years, the use of saponins as a possible content in the development of a drug posed a great challenge. Although saponins have detergent-like properties, few steroidal saponins were accessed in the market as a drug, such as Di-ao-xin-xu-kang for cardiovascular and cerebrovascular diseases in China for the past ten years and Chuan-shan-long injection for rheumatism (Yang, 2006).

***Glycosides***

Naturally, glycosides are plants' secondary metabolites that have magnificent therapeutic effects. There is also a compound glycoside in which glycosidic linkage sugar is attached. For many years, glycosides have been displaying promising therapeutic agents to treat various diseases, to name a few, cancer, myocardial injury, and diabetes. Saponins can activate the biological process of hemolysis as well. Also, they possess anti-thrombolytic, anti-viral,

antioxidant, anti-fungal, and antidepressant mechanisms. For instance, some other studies have shown the use of glycosides against various strains of fungi *Aspergillus fumigatus*. However, the weakness of anti-fungal efficacy of some glycosides credited to the numbers of sugars attached to the aglycon part of steroidal nuclei that fueled the polarity of the compound, causing the direct attack to be less effective (Khan et al., 2017).

*Table 2: Anti-fungal Activity Test of Averrhoa bilimbi*

| Sample/Control | Replicate 1 1 (mm) | Replicate 2 1 (mm) | Replicate 3 1 (mm) | Total Mean Zone of Inhibition | Reactivity | Inhibitory |
|---|---|---|---|---|---|---|
| Fresh Fruit of Kalamyas Extract (10mm) | 10.00 | 10.00 | 10.00 | 10.00 | 2 | +++ |
| Positive Control Clotrimazole (10mm) | 16.83 | - | - | 16.83 | 3 | +++ |
| Negative Control Sample-free disk (10mm) | 0.00 | - | - | 0.00 | 0 | (-) |

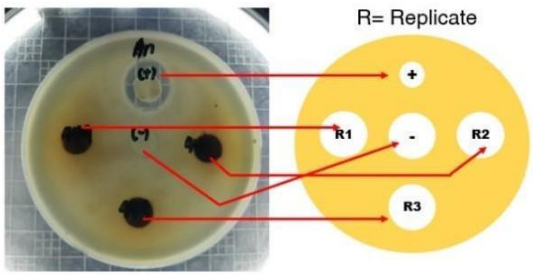
**Note:** Inhibitory Activity Rating: (-) negative; (+) slight; (++) partial; and (+++) complete

Reactivity Rating:

1- None (No detectable zone around or under specimen)
2- Slight (Some malformed or degenerated cells under the specimen)
3- Mild (Zone limited under the specimen)
4- Moderate (Zone extends 5-10 mm beyond specimen)
5- Severe (Zone extends greater than 10 mm beyond specimen)

Table 2 represents the disc diffusion test result of *Averrhoa bilimbi* fruit extract against *Aspergillus niger.*

*Figure 1: Zone Inhibition and Reactivity of Averrhoa bilimbi Fruit Extract*



The *Averrhoa bilimbi* fruit extract underwent Disk Diffusion Method to determine if there is anti-fungal resistance/susceptibility against *Aspergillus niger*.

Table 2 shows that the extracted sample of the fresh fruit of *kalamyas* produces complete inhibitory activity with mild reactivity against the specimen, *Aspergillus niger*. However, the susceptibility of *A. niger* in comparison to clotrimazole, the positive control, clotrimazole propagated moderate reactivity and complete inhibitory activity as compared to *Averrhoa bilimbi* fruit extract.

On the other hand, the sample-free disc that was used as a negative control presented no reactivity or inhibitory activity in opposition towards the specimen.

In Figure 1, all disc size samples and controls measured 10 millimeters (mm). The reactivity of *Averrhoa* extract was tested in three replicates. In replicate 1, the zone of inhibition revealed 10 mm, replicate 2 revealed the zone of inhibition at 10 mm, and replicate 3 had the zone of inhibition revealed as 10 mm against *Aspergillus niger*. To compute, the total mean zone of inhibition of *Averrhoa* replicates is equal to zero, exhibiting mild reactivity (2), meaning the inhibition zone is limited under the filter paper disc. On the other hand, clotrimazole which served as the positive control, exceeded its disc size of 10 mm to 16.83 mm. The total mean zone of inhibition of clotrimazole was 16.83 mm.

Clotrimazole exhibits moderate reactivity (3), which implies that the zone of inhibition extends 5 to 10 mm past the specimen. However, the sample-free disc, used as the negative control, showed zero (0) number of inhibitions, resulting in zero in a total mean zone of inhibition. This implies a lack of detectable zone around or under the specimen. For the inhibition, it was referred to as the "halo" seen in the plate as (+) in Figure 1. For the inhibitory activity rating, this refers to a qualitative description. If there is a colony present or if it is clear in the zones of inhibition. A cartoon representation is provided below in Figure 2.

*Figure 2: Cartoon representation of Inhibitory Activity*

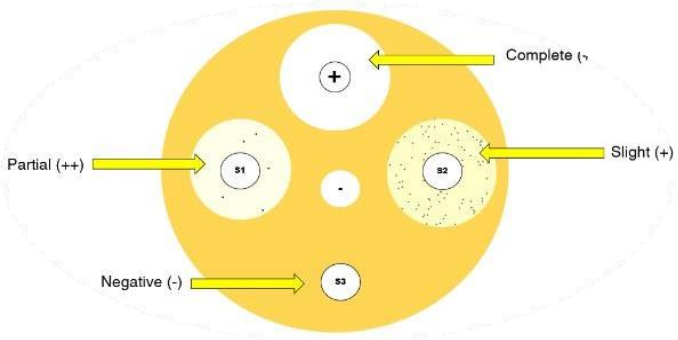



Figure 2 represents the inhibitory activity rating of the *Averrhoa bilimbi* against *Aspergillus niger*. In the photo provided, there are four discs presented. First, the positive control disc, which appeared (+++) complete inhibitory rating which indicates that there is no colony growth in the zone of inhibition observed. Second is sample 1, where the picture showed (++) partial inhibitory

activity, which indicates limited growth of the fungal colony in the zone of inhibition. The third is sample 2, where the picture showed a slight (+) inhibitory activity that indicates a minimal growth of the fungal colony in the zone of inhibition. Lastly, sample 3, where the picture showed (-) inhibitory activity that indicates a complete growth of the fungal colony in the zone of inhibition and that the sample exhibits a failure to inhibit the growth of fungus.

The test result revealed that *Averrhoa bilimbi* extract and clotrimazole produced the same (+++) complete inhibitory rating. This implies that clotrimazole and *A. bilimbi* extract have equivalent rates with regard to their effectiveness in inhibiting the growth of *Aspergillus niger*.

Broad-spectrum antifungal agents such as clotrimazole inhibit fungal activity can be used as a remedy for various infections like vaginal yeast, ringworm, athlete´s foot, and oral thrush. It inhibits the synthesis of ergosterol, which is an important component of the fungal cell membrane, and destroys its permeability status. Clotrimazole is necessary for the biosynthesis of ergosterol since it targets the specific enzyme called lanosterol 14 a-demethylase. Thus, it prohibits the production of ergosterol following cell lysis (National Library of Medicine, 2021)

Clotrimazole excipients consist of polysorbate 60, benzyl alcohol, cetostearyl alcohol, cetyl esters wax, sorbitan monostearate, octyldodecanol, purified water, and sorbitan monostearate in which these inactive ingredients are potent in the killing of fungus that causes infection. ("DailyMed - CLOTRIMAZOLE ANTIFUNGAL- clotrimazole cream," 2021).

Benzyl alcohol, cetostearyl alcohol, and octyldodecanol are ethanol. According to Rogawansamy (2015), fungal spores can be killed with a high concentration of ethanol. Its killing effectiveness is within the maximum of 70% ethanol. The primary goal of ethanol is to attack the proteins and membranes of the fungus, which determine its structure and functions. In the process, it eventually inhibits glucose and amino acids, thus decreasing its ability to grow, much more in cell division of its reproduction (Eleutherio et al., 2019).

On the other hand, sorbitan monostearate and octyldodecanol are fatty acids. According to Bhattachryya et al. (2020), fatty acids have powerful derivatives that break pathogenic fungal membranes such as *Candida* and *Trichophyton*. Fatty acids display antimicrobial effectivity by attacking various cellular functions, including fatty acid metabolism and protein synthesis thus, perturbing the cell membrane.

In the phytochemical test result, among the seven phytochemical constituents of *Averrhoa bilimbi* extracts, bioactive fatty acid-containing constituents were triterpenes, alkaloids, saponins, and glycosides, while ethanol containing constituents showed only the flavonoids. Fatty acids and ethanol are potent in disrupting pathogenic fungal membranes, thus inhibiting their reproduction. In this case, clotrimazole and *Averrhoa bilimbi* fruit extracts have the same complete inhibitory effects against *Aspergillus niger* but not within its reactivity effects. Clotrimazole exhibits moderate reactivity, while *Averrhoa bilimbi* showed mild reactivity. In this test, there is the absence of tannins and sterols, which are essential in inhibiting the growth of molds. In the study of Zhu et al. (2019), they have found that tannins acted on inhibiting spore germination and mycelial growth. Tannins target the cell wall causing disruption in the permeability of the membrane leading to spillage of sugar as one of the intracellular contents. Tannins showed a

remarkable p-value of < 0.05 reduction in an artificially inoculated citrus fruit against *P. digitatum* in vivo test and decreased disease status of mold green on citrus fruit significantly by 70%.

In the study of Rana et al. (2016), they have found that the methanolic extract of A. bilimbi fruit part displayed a positive anti-fungal effect on *C. albicans*, *A. niger*, and *S. cerevacae.*

In addition, the in vitro screening of *Averrhoa bilimbi* leaves done by Carandang et al. (2017) found that its methanolic extract has convincing effectiveness against *Microsporum canis*, a fungus that causes skin infections such as tinea capitis, tinea corporis, and ringworm.

Further, Kumari (2017) stated that the more the sample extract from the *Averrhoa* fruit and leaf is concentrated, the more it gives an acceptable inhibitory response in opposition towards certain selected Gram-negative bacteria and Gram-positive bacteria.

## CONCLUSION

It can be concluded that *Averrhoa bilimbi*, specifically its fruit extract, possesses essential bioactive compounds such as alkaloids, flavonoids, glycosides, saponins, and triterpenes that influence its anti-fungal ability against *Aspergillus niger* organism.

## REFERENCES

Alhassan, A. M., & Ahmed, Q. U. (2016). *Averrhoa bilimbi* Linn.: A review of its ethnomedicinal uses, phytochemistry, and pharmacology. Journal of Pharmacy & Bioallied Sciences, Wolters Kluwer-Med know Publication 8(4), 265-271. https://doi.org/10.4103/0975-7406.199342

Aspergillosis-Types of Fungal Diseases-Fungal Diseases- CDC. (2019) November 4. https://www.cdc.gov/fungal/diseases/aspergillosis/index.html

Aziz, N. A. (2016). A Review of the Antimicrobial Properties of three Selected Underutilized Fruits of Malaysia. International Journal of Pharmaceutical and Clinical Research, 8(9), 1278–1283

Batac, M., & Denning, D. (2017). Serious fungal infections in the Philippines.

European Journal of Clinical Microbiology & Infectious Diseases, 36. https://doi.org/10.1007/s10096-017-2918-7

Bhattacharyya. A., Sinha. M., Singh. H., Patel. R. S., Ghosh. S., Sardana. Kabir., Ghosh. S., Sengupta. S. (2020). Mechanistic Insight Into the Antifungal Effects of a Fatty Acid Derivative Against Drug-Resistant Fungal Infections.

Microbiol., 08 September 2020 https://doi.org/10.3389/fmicb.2020.02116

Bongomin, F., Gago, S., Oladele, R. O., & Denning, D. W. (2017). Global and Multi-National Prevalence of Fungal Diseases- Estimate Precision. Journal of Fungi, 3 (4). https://doi.org/10.3390/jof3040057

Bose, S., Sarkar, D., Bose, A., Subhash, CM. (2018). Natural Flavonoids and its Pharmaceutical Importance. The Pharma Review July- August 2018. PMID: 22876957 DOI: 10.2174/13895713804999775

https://www.researchgate.net/publication/328577501 Natural Flavonoids and Its Pharmaceutical Importance

Bribi, N. 2018. Pharmacological Activity of Alkaloids: A Review. Asian Journal of Botany (2018) Volume 1 doi:10.63019/ajb.vli2.467

Carandang, VC., Hernandez, MR., Alvaira CE. (2017). In vitro Screening of Anti- Fungal Effect of *Averrhoa bilimbi* leaves Methanolic Extract Against Microsporum canis. Lyceum of the Philippines- St. Cabrini College of Allied Medicine Research Vol.2 No. 2 March 2017 https://lpulaguna.edu.ph

Chudzik, M., Karzonek-Szlacheta, I., Krol, IW. (2015). Triterpenes as Potentially Cytotoxic Compounds. Open Access Molecules. Molecules 2015, 20, 1610- 1625; doi: 10.3390/molecules20011610. www.mdpi.com/journal/molecules

Crowley, D. C., Gallager C. (2014). Clotrimazole as a pharmaceutical: past, present and future., Journal of Applied Microbiology/Volume 117. Issue 3/ p. 611-617. https://doi.org/10.1111/jam.12554
https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/jam.12554

DailyMed. US National Library of Medicine. Clotrimazole Antifungal- Clotrimazole cream. https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=e03d9368- e1b4-4e37-87e4-3ee241916aed

Eleutherio, E. (2019). Molecular Mechanisms Involved in Yeast Fitness for Ethanol Production. Advances In Biotechnology Microbiology, 12(5). doi: 10.19080/aibm.2019.12.555847

Gou, T., Ye, Y., Huang, L., Cheng, B. (2019). Recurrent fever of unknown fungal infection in a low-risk patient a case report. August 2019-Volume 98 Issue 33 pe16908. doi:10.1097/MD.0000000000016908

Guerra, F., Araújo, R., Sousa, J. P., Silva, V. A., Pereira, F. O., Mendonça-Junior, F., Barbosa-Filho, J. M., Pereira, J. A., & Lima, E. O. (2018). A new coumarin derivative, 4-acetatecoumarin, with anti-fungal activity and association study against Aspergillus spp. Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology], 49(2), 407–413. https://doi.org/10.1016/j.bjm.2017.06.009

Gunawan, AC., & Paano, A. (2013). Structure Elucidation of Two New Phytol Derivatives, A New Phenolic Compound and Other Metabolites of *Averrhoa bilimbi*. *DLSU Res. Congress*, 1–8. Kumari, S. C. (2017). Studies on Bilimbi. *International Journal of All Research Education and Scientific Methods*, *5*(7) https://www.researchgate.net/publication/324476579_Pharmacological Activity of Alkaloids A Review

Juman, M., Nordin, ML., Woldegiorgis, A., Zulkiple, SA. (2019). In Vitro Study of Antifungal Activity of *Averrhoa bilimbi* Extracts Against Candida Species.

International Journal of Veterinary Science. P-ISSN: 2304-3075, E-ISSIN: 2305-4360. www.ijvets.com

Khan, H., Khan, Z., Amin, S., Mabkhot, Y. N., Mubarak, M. S., Hadda, T. B., Maione, F. (2017). Plant Bioactive Molecules Bearing Glycosides as Lead Compounds for the Treatment of Fungal Infection: A review. Science Direct Elsevier. 2017 Elsevier Massons SAS. http://dxdoi.org/1.1016/j.biopha.2017.06.077

Khan, H., Mubarak, M., Amin, S., (2017). Anti-fungal Potential of Alkaloids as An Emerging Therapeutic Target. National Library of Medicine National Center for Biotechnology Information. https://pubmed.ncbi.nlm.nih.gov/27440186

Kim, YS., Cho, IK., Jeong, MJ., Jeong, SJ., Nah, SY., Cho, YS., Kim, SH., Go, A., Kim, S.E., Seong Soo Kang, SS., Moon, CH., Kim, JC., Kim, SH., Bae, CS. (2011). Therapeutic Effect of Total Ginseng Saponin on Skin Wound Healing. PMC US National Library of Medicine National Institutes of Health. Doi: 10.5142/jgr.2011.25.3.360 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3659532

Kumari, C. (2017). Studies on Bilimbi. International Journal of All Research Education and Scientific Methods (IJARESM) ISSN: 2455-6211, Volume 5, Issue 7, July-2017, Impact Factor: 2.287. https://www.researchgate.net/publication/320892417 Studies on Bilimbi

Lima, V., Mélo, E., & Santos Lima, LD. (2001). Physicochemical Characteristics of Bilimbi (*Averrhoa bilimbi* L.). *Revista Brasileira de Fruticultura*, *23*(2), 421– 423. https://doi.org/10.1590/S0100-29452001000200045

Lu, J., Bao, J., Chen, X., Huang, M., & Wang, Y. (2012). Alkaloids Isolated from Natural Herbs as the Anticancer Agents. *Evidence-Based Complementary And Alternative Medicine*, *2012*, 1-12. doi: 10.1155/2012/485042

Mazu, T. K., Bricker B.A., Flores-Rozas, H., Ablordeppey, S. (2016). The Mechanistic Targets of Anti-fungal Agents: An Overview. National Library of Medicine National Institute of Health. Mini Rev Med Chem. 2016; 16(7). 555-

578. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5215921

Mokhtar, S. I., Aziz, N. (2016). Antimicrobial properties of *Averrhoa bilimbi* Extracts at Different Maturity Stages. Journal of Medical Microbiology & Diagnosis.

Mokhtar and Aziz, J Med Microb Diagn 2016, 5:3 doi:10.4172/2161- 0703.1000233

National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 2812, Clotrimazole. https://pubchem.ncbi.nlm.nih.gov/compound/Clotrimazole.

Niziol-Lukaszewska, Z., Bujak, T. (2018). Saponins as Natural Raw Materials for Increasing the Safety of Bodywash Cosmetic Use. Journal of Surfactants and Detergents/ Volume 21, Issue 6 p.767. https://doi.org/10.1002/jsde.12168

Person, AK., Chudgar, SM., Stout, JE. *Aspergillus niger*: An Unusual Cause in Invasive Pulmonary Aspergillosis. Journal of Medical Microbiology

Rana SMM., Billah, MM., Hossain MS., Saifuddin AKM., Islam SKMA, Banik S., Naim Z., Raju GS. (2016) Susceptibility of Microorganism to Selected Medicinal Plants in Bangladesh. Asian Pacific Journal of Tropical Biomedicine 2014; Volume 4, Issue 11, 911-917

Rogawansamy, S., Gaskin, S., Taylor, S., Pisaniello, D. (2015). An Evaluation of Antifungal Agents for the Treatment of Fungal Contamination in Indoor Air Environments. International Journal of Environmental Research and Public Health. nt J Environ Res Public Health. 2015 Jun; 12(6): 6319–6332.

Yang, C. H., Zhang, Ying., Jacob, M. R., Khan, S. I., Zhang, Y. J., Li, X. C., (2006). Anti-fungal Activity of C-27 Steroidal Saponins. American Society for Microbiology. doi:10.1128/AAC.50.5.1714.2006. https://www.ncbi.nlm.nih.gov/pmc/articles

Zhu, C., Lie, M., Andargie, M., Zeng, J., Li, J., (2019) Antifungal activity and mechanism of action of tannic acid against Penicillium digitatum. Elsevier, Physiology and Molecular Plant Pathology Vol 107, August 2019, Page 1.