# Antimicrobial Activity And Phytochemical Screening Of Split Gill Mushroom (Schizophyllum Commune) Ethanolic Extract

**Ricky B. Acanto, Van Helen S. Cuaderes**

**Abstract:** Split gill mushroom (Schizophyllum commune) is one of the most widespread fungal species with various uses among communities, especially in the countryside. With its favorable characteristics, this macrofungi shows potential to be utilized in the field of natural products. The study aims to ascertain the antimicrobial effects and determine the active components present in the ethanolic extracts. The extract's antibacterial activity was determined using Kirby-Bauer Disk Diffusion Assay, antifungal susceptibility through Poisoned Food Method, and minimum inhibitory concentration (MIC) through Microtiter Well Assay. Qualitative phytochemical analysis was carried out using the test tube method. Results revealed that gram-negative bacteria were resistant to the different concentrations of the extracts; however, gram-positive test bacteria were susceptible to any extract concentration. Additionally, MICs showed that a lower concentration of the extract was required to inhibit the growth of gram-positive than the gram-negative bacteria. The increasing concentration of extracts showed more significant fungal growth inhibition. Saponins, tannins, alkaloids, flavonoids, terpenoids, proteins, and carbohydrates were identified through the phytochemical screening. Ethanolic extracts of S. commune showed effectiveness against the growth of gram-positive bacteria and fungi due to different active components present. Further studies may be conducted to explore the potential of this macrofungi.

**Index Terms**: antimicrobial activity, ethanolic extract, macrofungi, phytochemical screening, Schizophyllum commune

## 1 INTRODUCTION

Since human civilization's inception, we have relied on our natural environment for survival for food, shelter, medicine, and other raw materials. Our ancestors have used other living organisms, primarily plants, to source these materials for various purposes. Through time, we have explored nature to improve our state of living. One of the aspects of human existence being explored continuously is discovering compounds from other living organisms with engaging biological activities to produce products for our benefit, specifically in medicine, as most of the active ingredients of medicine have been from natural products as the source [1]. In recent times, we are racing to discover new sources of active compounds to address the increasing need for antimicrobial products and antibiotic resistance.

It was reported that most natural products had been extracted from plants and bacterial sources; however, fungal species have not been fully explored in the past 5-10 years and include the metabolic mechanisms involved for the synthesis of a small number of these secondary metabolites of the fungal phylum Basidiomycota[2]. Mushrooms, specifically split gill mushroom (Schizophyllum commune), are under this phylum and benefit humans for millennia. Their benefits include a range of bioactive compounds as antimicrobial [3], cytotoxic [4], and other compounds which can be a substantial source of pharmaceuticals and agrochemicals product [5], [6]. Split gill mushroom (S. commune) is a widespread fungus characterized as a tiny, light-brown fungus that clings and multiplies on a moist, decaying tree branch, especially after a prolonged rain [7]. It was also documented that Aeta communities in the Philippines had used this fungus as food and medicine [8]. Laboratory analysis of samples revealed that Schizophyllum species produce a natural polysaccharide called schizophyllan, which possesses antimicrobial effects [9]. This study focused on S. commune, commonly known as split gill mushroom, by determining the antimicrobial activity on gram-positive and gram-negative bacteria and its antifungal activity. Additionally, the ethanolic extracts' phytochemical screening was done to determine the active compounds present in the extract. The utilization of macrofungi with known therapeutic claims provides cheaper alternative medicine that humans can utilize. Using natural products to treat infections caused by gram-negative and gram-positive bacteria and parasitic fungal species would lessen the drawback of using the synthetic antimicrobial agent with adverse side effects on humans and the environment. In addition, extracts may be incorporated into other products such as lotions, soaps, creams, and others to help prevent the growth of infectious microorganisms when applied externally and may also help treat external infections. The focus of the study was to evaluate the antimicrobial activity and the phytochemical composition of the ethanolic extract of S. commune.


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## 2 MATERIALS AND METHODS

### 2.1 Collection, identification, and preparation of S. commune

The fresh samples were collected by local folks in the forest of Minapasuk, Calatrava, Negros Occidental. Documented and photographed using the digital camera. The samples were identified using the macroscopic characteristics of the specimen and compared with the published literature and online identification keys like MycoKey Morphing Mushrooms Identifier and musroomsobserver.org. The identified mushroom sample was cleaned from unwanted debris and transported to the CHMSC Science Laboratory for air-drying.

### 2.2 Extraction of the sample

Extraction of the sample was done at the Negros Prawn Producers Cooperative (NPPC) Diagnostic and Laboratory – a

certified ISO/IEC 17025: 2005 in Bacolod City, Negros Occidental, Philippines. The air-dried samples were thinly cut and pulverized using a mortar and pestle. The ground sample was immediately plunged into 95% ethanol to avoid enzymatic activity and macerated for about 48 hours before extraction. The mixture was filtered using the Buchner funnel. The collected filtrate was subjected to the rotary evaporator with controlled temperature and revolution to achieve the desired consistency of the extract. A flame test was done to ensure that no alcohol mixture was present in the extract.

### 2.3 Phytochemical screening

Phytochemical screening of S. commune ethanolic crude extract was carried out using the test tube method described by [10], [11]. The extract was screened for saponins, tannins, alkaloids, flavonoids, terpenoids, glycosides, proteins, amino acids, and carbohydrates

### 2.4 Antibacterial assay

#### 2.4.1 Preparation of McFarland's standard.

A 100 mL of McFarland's standard was prepared by mixing 99.5 mL of 1% sulfuric acid and 0.5 mL of 1.75% $BaCl_2.2H_2O$. The turbidity is equivalent to 1.0 x $10^8$ CFU/mL bacteria. Bacteria were isolated from a pure culture of E. coli, and S. aureus was transferred to sterile buffered phosphate or distilled water solution. The turbidity was adjusted using a spectrophotometer at 600 nm wavelength compared to the absorbance of McFarland's standard, which is 0.089.

#### 2.4.2 S. commune extract dilution and preparation of impregnated disc.

A stock solution of the resulting extract was prepared by dissolving 100 mg of extract per 1 ml of sterile distilled water - which was considered as 100% extract concentration. The concentrations, 75%, 50%, and 25%, were made by diluting the concentrated extract with appropriate volumes of sterile distilled water. A 7 mm diameter filter paper discs were prepared and sterilized. The discs were impregnated with the extracts, dried in an incubator at 37°C for 18 to 24 hours, and immediately used for the sensitivity test.

#### 2.4.3 Sensitivity testing for gram-positive bacteria and gram-negative bacteria.

The antibacterial assay was analyzed using the Kirby-Bauer Disk Diffusion method. The disk diffusion method is the most widely used to evaluate the antimicrobial activity of plant extracts or microbial extracts. In sensitivity testing, Staphylococcus aureus and Escherichia coli were used for gram-positive and gram-negative bacteria, respectively. The agar plate surface was inoculated by spreading the volume of microbial inoculum (based on McFarland's standard) over the entire agar surface. The impregnated disks were inoculated into the agar plates and followed by incubation of the plates for 24 hours at 37 $^0$C. After incubation, the Vernier caliper was used to measure the diameter of the zone of inhibition for each discs used. The measurement obtained from the individual discs was recorded and compared with the standard table to determine the sensitivity zone whether the tested bacterial species are sensitive to S. commune ethanolic crude extract.

### 2.5 Antifungal Susceptibility Testing

The poisoned Food Method was used to determine the antifungal activity of the extracts. The treatments tested were in the following concentrations: 4000 ppm, 2000 ppm, 1000 ppm, 500 ppm, 250 ppm, and no treatment. Malt extract-Salt-Agar (MESA) medium with the respective concentrations of the S. commune extracts were prepared and sterilized at 121°C, 15 psi pressure for 15 minutes. Fifteen (15) ml of the medium was separately poured into Petri plates, allowed to cool and solidify. After solidifying the medium, a 5mm disc of a seven-day-old culture of the Aspergillus flavus was inoculated into MESA at the center of the Petri plates – these plates were incubated at room temperature for five days. The Petri plates containing media without the extract but with the same amount of distilled water served as control. The colony diameter was measured in mm after incubation. Each treatment was repeated three times. The fungi toxicity of the extract in terms of percentage inhibition of mycelial growth was calculated using the formula:

$$\text{Percent Inhibition} = \left(\frac{C - T}{C}\right) \times 100$$

where C = Average increase in mycelial growth in control plate and T = Average increase in mycelial growth in treatment plate

### 2.6 Minimum Inhibitory Concentrations (MIC) Assay

An antimicrobial agent's minimum inhibitory concentration (MIC) is the lowest concentration at which visible growth of microorganisms is inhibited following an incubation period. [12]. To determine the MIC of the ethanolic extracts of S. commune on both gram-negative and gram-positive bacteria, Microtiter Well Assay was employed, and the cultures were incubated at 37 $^o$C for 48 hours.

### 2.7 Statistical Analysis

Mean, Standard Deviation, and Percentage were the descriptive statistical tools employed in the study.

### 2.8 Interpretation of the Mean Zone of Inhibition. Interpreting the mean zone of inhibition of negative control, positive control, and Schizophyllum commune ethanolic crude extract is based on the American Society for Microbiology [13].

| Zone of Inhibition (mm) | Description |
|---|---|
| < 14 mm | Resistant |
| 15-18 mm | Intermediate |
| > 19 mm | Susceptible |

## 3 Results and Discussions

### Antimicrobial Activity of S. commune Ethanolic Extracts

Antibacterial Activity of S. commune. The S. commune ethanolic extracts, as depicted in Table 1, showed varied antibacterial activity on gram-negative and gram-positive bacteria, the E. coli, and S. aureus cultures, respectively. The positive control, Tetracycline, showed the greatest mean zone of inhibition of 24.06 mm against E. coli which is described as susceptible. On the other hand, E. coli was resistant to the different extract concentrations: 100% S. commune Ethanolic Extract (SCEE) (M=7.98, SD=.43). Other concentrations (75% and 50% SCEE) and the negative control (sterile DMSO) showed no inhibition against the test bacteria. Thus, SCEE showed weak or no antimicrobial effect on gram-negative bacteria depending on the concentration. In terms of the

antimicrobial activity of SCEE against gram-positive bacteria, E. coli were susceptible with 100% SCEE (M=32.69, SD=1.26), 75% SCEE (M=26.14, SD=.43), and 50% SCEE (M=25.88, SD=.26). The activity of the gram-positive bacteria was also susceptible (M=45.06, SD=.56) with the positive control. The negative control showed no inhibition. Results imply that the SCEE shows potential against gram-positive bacteria.

***Table 1. The Zone of Inhibition of the Ethanolic Crude Extracts of Schizophyllum commune***

| Treatment | Mean Zone of Inhibition (mm) | SD | Description |
|---|---|---|---|
| Escherichia coli | | | |
| Tetracycline (Positive Control) | 24.06 | .48 | Susceptible |
| S. commune Ethanolic Extract | | | |
| 100% | 7.98 | .43 | Resistant |
| 75% | 0.00 | .00 | Resistant |
| 50% | 0.00 | .00 | Resistant |
| DMSO (Negative Control) | 0.00 | .00 | Resistant |
| Staphylococcus aureus | | | |
| Tetracycline (Positive Control) | 45.06 | .56 | Susceptible |
| S. commune Ethanolic Extract | | | |
| 100% | 32.69 | 1.26 | Susceptible |
| 75% | 26.14 | .43 | Susceptible |
| 50% | 25.88 | .26 | Susceptible |
| DMSO (Negative Control) | 0.00 | .00 | Resistant |

Note: ≤ 14 mm, resistant; 15-18 mm, intermediate; ≥ 19 mm, susceptible

The study results conformed with [14], where the activity of the methanolic extracts of S. commune showed inactive effects on gram-negative bacteria, including E. coli. However, the results of the same author were in contrast with the present results on the antibacterial activity against gram-positive bacteria, S. aureus. Results have shown that methanolic extracts were inactive in inhibiting the bacterium; on the other hand, the present study showed that ethanolic extracts were susceptible to S. aureus. Though both studies used alcohol as a solvent, the difference lies in the type of alcohol used [15] that at 95% concentration, ethanolic extract showed a more significant inhibition zone than methanolic extract. Results further confirmed the antibacterial activity of S. commune ethanolic extracts on S. aureus, with the average diameter of the zone of inhibition was 19 ± 1 mm [16]. However, the same study is contrasted by the mean zone of inhibition was 17 ± 1 mm on E. coli. Antifungal Activity of S. commune. Table 2 shows the antifungal activity of SCEE in terms of percent inhibition measured in terms of radial growth of Aspergillus flavus. The data was based on the radial growth of the fungus after five (5) days of incubation. Results showed the ethanolic extract of S. commune exhibited 86.47% inhibition with 4000 ppm concentration, which showed the most significant inhibition; followed by 2000 ppm with 81.98% inhibition, 1000 ppm with 77.40% inhibition, 500 ppm with 52.91% inhibition, and 250 ppm with 2.96% inhibition, the least of the treatments. The negative control was used as a basis for determining the percent inhibition of the treatments. Results imply that the higher the concentration of the extracts, the greater will be the percent inhibition.

***Table 2. The percent inhibition of the different concentrations of ethanolic crude extracts of Schizophyllum commune***

| Concentration | Mean Radial Growth (mm) Day 5 of Incubation | % inhibition |
|---|---|---|
| 4000 ppm | 10.96 | 86.47 |
| 2000 ppm | 14.60 | 81.98 |
| 1000 ppm | 18.31 | 77.40 |
| 500 ppm | 38.14 | 52.91 |
| 250 ppm | 78.60 | 2.96 |
| No Treatment | 81.00 | 0 |

The antifungal activity of S. commune is supported by [17], where a review of the antifungal activity of mushrooms showed that S. commune has low minimum inhibitory concentrations against fungal species such Lentinus sp., Microporus affinis, and Microporus xanthopu. The review, however, didn't show activities of mushrooms against A. flavus. Minimum Inhibitory Concentration (MIC) S. commune. The Minimum Inhibitory Concentration (MIC) Assay results of the ethanolic crude extracts of S. commune are shown in Table 3. Results showed that the MIC value of the extract was 156.3 µg/mL against the gram-positive bacterium S. aureus. On the other hand, 312.5 µg/mL was required to inhibit the growth of the gram-negative bacterium, E. coli. Thus, less concentration of the extract was needed to inhibit the growth of gram-positive bacteria than the gram-negative bacteria.

***Table 3. The minimum inhibitory concentration assay results of the ethanolic crude extracts of Schizophyllum commune***

| Test Microorganism | SCEE Concentration |
|---|---|
| S. aureus | 156.3 µg/mL |
| E. coli | 312.5 µg/mL |

The minimum inhibitory concentration (MIC) of the present study with values of 156.3 µg/mL and 312.5 µg/mL for S. aureus and E. coli, respectively, were less than the reported value with a range of 3.0–8.0 mg/mL (3000 – 8000 µg/mL) [9], in which oxidized schizophyllan was utilized. The result suggests that the ethanolic extract is needed less than oxidized schizophyllan in inhibiting the growth of bacteria, both gram-positive and gram-negative.

**Phytochemical Screening**

The ethanolic crude extract of S. commune revealed saponin, tannins, alkaloids, flavonoids, terpenoids, proteins, and carbohydrates. On the other hand, the test showed negative results for glycosides and amino acids. These secondary

metabolites, as a result of plant metabolism, exhibit physiological activities in humans. The results of the qualitative phytochemical screening of S. commune are shown in Table 4.

***Table 4. Phytochemical Screening of Ethanolic Crude Extract of Schizophyllum commune***

| Secondary Metabolites | Reagents | Positive Results | Experimental Results |
|---|---|---|---|
| Saponin | Distilled water | Continuous frothing | + |
| Tannins | 1% gelatin solution | Green to a black precipitate | + |
| Alkaloids | Mayer's reagent | Brick-red precipitate | + |
| Flavonoids | Lead acetate solution | Black cloud/black precipitate | + |
| Terpenoids | Sulfuric Acid solution | A dark brown/black precipitate | + |
| Glycosides | Ferric chloride solution | Upper layer: bluish-green color Lower layer: brownish-red color | - |
| Proteins | 4% sodium hydroxide and 1% copper sulfate solution | Violet/pink color formation | + |
| Amino Acids | 5% Ninhydrin solution | Purple color formation | - |
| Carbohydrates | α-naphthalene solution | Brownish-red precipitate | + |

(+) presence (-) absence

It has long been recognized that phytochemical compounds such as alkaloids, saponins, tannins, flavonoids, and terpenoids are biologically active and thus contribute to the extract's antimicrobial activity, which explains their traditional use in medicine [18]. These compounds are present from the ethanolic extract of S. commune. A wild Schizophyllum commune strain obtain from Achanakmar-Amarkantak Biosphere Reserve was examine for its potential to produce bioactive chemicals. Two phenolic compounds were found via GCMS analysis of the samples, wild and in vitro, which suggest that s. commune's wild strain may be a source of the new bioactive chemicals with antibacterial and antioxidant activity [19]. A similar study concluded that the Dacryopinax spathularia (Schwein) and Schizophyllum commune (Fries) have potent mycochemical constituents, including tannins and saponins flavonoids, alkaloids which are responsible for the significant impact on the impact of both macrofungal extracts. The results may open the way to use the above two macrofungi as potent antimicrobial dietary sources or develop a new potent antibacterial drug [20]. Meanwhile, the phytochemical screening of Caloncoba echinata using different solvents revealed carbohydrate, glycoside, alkaloid, protein, amino acid, terpenoids, tannin, and flavonoids with their respective test reagents. Its antibacterial sensitivity testing using Mueller Hilton agar revealed that it had only a minimal inhibitory effect on Escherichia coli and Staphylococcus aureus, the two bacteria known to cause wound and skin infections. This reaffirms Sierra Leone's traditional use of the medicinal herb Caloncoba echinata [21]. Alkaloids are mainly used as drugs. The oldest and best-known prescription, Quinine, reduces fever, malaria treatment, and artificial flavoring in tonic water. Other than quinine, some uses of alkaloid-derived drugs are: control fibrillation, heart block, white-blood-cell cancers, relieves nasal decongestion, and serves as an antidote to nerve gas and insecticide poisoning [22]. Saponins are glucosides with foaming characteristics, have beneficial effects on blood cholesterol levels, cancer, bone health, and immune system stimulation [23]. Tannins are quite caustic or sharp, providing a distinctive astringency taste. Tannins coagulate proteins and mucosal tissues by forming an insulating and protective layer on the skin that soothes irritation and pain. It has astringent, hemostatic, antiseptic, and toning properties (Editors, 2014). Flavonoids are natural compounds with variable phenolic structures: aglycones, glycosides, and methylated derivatives. It possesses many biological properties like antioxidants, hepatoprotective activity, antibacterial activity, anti-inflammatory activity, anticancer activity, and antiviral activity [25]. Terpenoids, also known as terpenes, are the most significant natural compounds used to treat human diseases. Terpenoids are known for their range of biological activities against cancer, inflammation, malaria, and antibacterial and antiviral (infectious diseases) [26].

Whereas protein is a significant component in the food system, and the demand for protein supply has increased substantially in recent decades. Proteins are used in various industries, but it has promising applications in pharmaceutical and cosmetic industries [27]. Many carbohydrates and carbohydrates derivatives are used as therapeutics and diagnostics such as antibiotics and anticoagulants [28], [29].

## 4 Conclusion

Based on the study's findings, it is concluded that the ethanolic crude extract of S. commune effectively inhibits the growth of gram-positive bacteria but not with gram-negative bacteria. A lower concentration of the extract is needed to inhibit the growth of the gram-positive bacteria. Meanwhile, a higher concentration of the extract effectively inhibits the growth of fungi. The active compounds present in the extract are responsible for its antimicrobial activity. Antimicrobial assays may be performed using other solvents to extract the other active components. Antimicrobial assays may be performed using other solvents to extract the other active components.

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## References

[1] A. L. Harvey, "Natural products in drug discovery," Drug Discovery Today, vol. 13, no. 19–20. pp. 894–901, Oct-2008.

[2] C. Schmidt-Dannert, "Biosynthesis of terpenoid natural products in fungi," Adv. Biochem. Eng. Biotechnol., vol. 148, pp. 19–61, 2015.

[3] A. H. S. Mirfat, S. Vikineswary, M. Ahmad, H. Salahuddin, N. Abdullah, and V. Sabaratnam, “Antimicrobial activities of split gill mushroom Schizophyllum commune Fr,” 2014.
[4] M. Kaur et al., “Schizophyllum commune induced genotoxic and cytotoxic effects in Spodoptera litura,” Sci. Rep., vol. 8, no. 1, p. 4693, Dec. 2018.
[5] J. V. Pham et al., “A Review of the Microbial Production of Bioactive Natural Products and Biologics,” Front. Microbiol., vol. 0, no. JUN, p. 1404, 2019.
[6] F. Alberti, G. D. Foster, and A. M. Bailey, “Natural products from filamentous fungi and production by heterologous expression,” Appl. Microbiol. Biotechnol. 2016 1012, vol. 101, no. 2, pp. 493–500, Dec. 2016.
[7] N. Ortega, “Kurakding fungi or Common split-gill | Project Noah,” 2012.
[8] O. Tantengco and E. M. Ragragio, “Ethnomycological survey of macrofungi utilized by Ayta communities in Bataan, Philippines,” Curr. Res. Environ. Appl. Mycol. (Journal Fungal Biol., vol. 8, no. 1, pp. 104–108, 2018.
[9] G. C. Jayakumar, S. V. Kanth, B. Chandrasekaran, J. Raghava Rao, and B. U. Nair, “Preparation and antimicrobial activity of scleraldehyde from Schizophyllum commune,” Carbohydr. Res., vol. 345, no. 15, pp. 2213–2219, Oct. 2010.
[10] A. M. Aguinaldo, E. I. Espeso, B. Q. Guevarra, and M. G. Nonato, “Phytochemistry Section,” in A Guidebook to Plant Screening: Phytochemical and Biological, 5th ed., B. Q. Guevarra, Ed. Manila: Research Center for Natural Sciences and University of Santo Tomas Publishing House, 2004, pp. 23–64.
[11] P. Tiwari, B. Kumar, M. Kaur, G. Kaur, and H. Kaur, “Phytochemical screening and Extraction: A Review,” Internalionale Pharm. Sci., vol. 1, no. 1, pp. 98–106, 2001.
[12] J. M. Andrews, “Determination of minimum inhibitory concentrations,” J. Antimicrob. Chemother., vol. 48, no. SUPPL. 1, pp. 5–16, 2001.
[13] J. Hudzicki, “Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information,” Am. Soc. Microbiol., no. December 2009, pp. 1–13, 2012.
[14] A. Mirfat, A. Noorlidah, and S. Vikineswary, “Scavenging activity of Schizophyllum commune extracts and its correlation to total phenolic content,” J. Trop. Agric. Fd. Sc., vol. 38, no. 2, pp. 231–238, 2015.
[15] K. Bacon, R. Boyer, C. Denbow, S. O’Keefe, A. Neilson, and R. Williams, “Evaluation of different solvents to extract antibacterial compounds from jalapeño peppers,” Food Sci. Nutr., vol. 5, no. 3, pp. 497–503, May 2017.
[16] T. Khardziani, E. Metreveli, K. Didebulidze, and V. Elisashvili, “Screening of Georgian medicinal mushrooms for their antibacterial activity and optimization of cultivation conditions for the split gill medicinal mushroom, Schizophyllum commune BCC64 (Agaricomycetes),” Int. J. Med. Mushrooms, vol. 22, no. 7, pp. 659–669, 2020.
[17] M. Alves, I. Ferreira, J. Dias, V. Teixeira, A. Martins, and M. Pintado, “A Review on Antifungal Activity of Mushroom (Basidiomycetes) Extracts and Isolated Compounds,” Curr. Top. Med. Chem., vol. 13, no. 21, pp. 2648–2659, Dec. 2013.
[18] O. Wintola and A. Afolayan, “The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of Hydnora africanaThunb. used as antidysenteric in Eastern Cape Province, South Africa,” BMC Complement. Altern. Med., vol. 15, no. 1, Sep. 2015.
[19] A. M. Tripathi and B. N. Tiwary, “Biochemical constituents of a wild strain of Schizophyllum commune isolated from Achanakmar-Amarkantak Biosphere Reserve (ABR), India,” World J. Microbiol. Biotechnol. 2013 298, vol. 29, no. 8, pp. 1431–1442, Mar. 2013.
[20] A. Kumar, M. Kumar, S. Ali, and M. P. Sinha, “Anti-pathogenic efficacy of Indian edible macrofungi Dacryopinax spathularia (Schwein) and Schizophyllum commune (Fries) against some human pathogenic bacteriae,” J. Emerg. Technol. Innov. Res., vol. 6, no. 1, pp. 695–704, 2019.
[21] L. Koroma, L. M. Kamara, T. B. R. Yormah, and G. M. T. Robert, “Phytochemical screening and antimicrobial sensitivity of extracts of the traditional medicinal plant Caloncoba echinata in Sierra Leone,” Int. J. Sci. Technol. Res., vol. 7, no. 8, pp. 1–6, 2018.
[22] M. Nikolic and S. Djordjevic, “Alkaloids in the pharmaceutical industry: Structure, isolation, and application,” Hem. Ind., vol. 57, no. 10, pp. 471–478, 2003.
[23] S. Shibata, “Saponins with Biological and Pharmacological Activity,” Springer, Berlin, Heidelberg, 1977, pp. 177–196.
[24] A. Editors, “Uses of tannins and tannosomes,” Access Sci., 2014.
[25] S. Kumar and A. K. Pandey, “Chemistry and biological activities of flavonoids: an overview.,” ScientificWorldJournal., vol. 2013, p. 162750, Dec. 2013.
[26] W. Bidigare, Robert R; Wang, Guangyi; Tang, “Terpenoids As Therapeutic Drugs and Pharmaceutical,” Nat. Prod. Drug Discov. Ther. Med., p. (197-227), 2005.
[27] Y. Luo and T. Wang, “Pharmaceutical and Cosmetic Applications of Protein By-Products,” in Protein Byproducts, Elsevier, 2016, pp. 147–160.
[28] J. Lonngren, “Carbohydrates and the pharmaceutical industry,” Pure &App/. Chem, vol. 61, no. 7, pp. 1313–1314, 1989.
[29] A. B. A. Ahmed, M. Adel, P. Karimi, and M. Peidayesh, “Pharmaceutical, Cosmeceutical, and Traditional Applications of Marine Carbohydrates,” Adv. Food Nutr. Res., vol. 73, pp. 197–220, Jan. 2014.