GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Preliminary phytochemical screening for various secondary metabolites, quantitative and qualitative analysis of Yemeni *Aloe vera* and *Aloe vacillans* flower extracts

Asma`a Mohsen Al-Wajih [1], Amina Mohammed El-Shaibany [1,*], Salwa Mohammed Raweh [1] and Mahmoud Mohamed El –Aasser [2]

[1] *Department of Pharmacognosy, Pharmacy College, Sana`a University, Sanaa, Yemen.*
[2] *The Regional Center for Mycology and Biotechnology, Al-Azhar University, 11787, Nasr City, Cairo, Egypt.*

GSC Biological and Pharmaceutical Sciences, 2022, 21(02), 202–210

Publication history: Received on 08 October 2022; revised on 21 November 2022; accepted on 23 November 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.21.2.0437

Abstract

The name *Aloe* is from the Greek *Aloe* and refers to the bitter juice from the leaves of these plants. It is probably derived from the earlier Arabic word "Allah" meaning "shining bitter substance," The bitterness results from the presence of aloin and *Aloe*-emodin, *Aloe* is referred to as the 'Miracle Plant' and 'Healing Plant'. The Egyptians called *Aloe* "the plant of immortality.

A comprehensive screening study will be carried out for the *A. vera* and *A. vacillans* flower collection from the Ibb government of Yemen. This study's main objectives are to identify, collected, shade dry, and Fraction the *Aloe vera* and *Aloe vacillans* flowers.

Preliminary phytochemical screening to investigate the chemical composition of both extracts revealed the presence of flavonoids, phenolic compounds, carbohydrates, protein, and sterols in the methanolic extract of the *Aloe Vacillans* flower. Saponin, carbohydrate, flavonoid, steroids, protein, and phenolic compounds were found in the methanolic extract of the *Aloe vera* flower.

Keywords: *Aloe vera; Aloe Vacillans*; Phytochemical screening; Extraction; Fractionation

1. Introduction

Aloe, historically used as a laxative in a large dose or a stomachic in a small dose in the form of juice (or whole leaf) extract and as a healing agent of burns skin ailments, and wounds in the form of fresh juice for more than 20 centuries, fell into disuse in the West as the seat of civilization moved to the temperate zones where the tropical plant could not survive the freezing winters. (Park & Lee, 2006; Mukherjee et al., 2013).

From the ecological nature of the *Aloe* plant, *Aloe* originated in Africa and the history of its use dates back almost 6000 years. In 4000 B. C., the *Aloe* plant was engraved in intaglio on an Egyptian temple fresco. A Sumerian clay tablet from 2200 B. C. was the first document to include *Aloe* among the plants of great healing power. (Park & Lee, 2006; Mukherjee et al., 2013).

1.1. Identification and Collection of Plants

The *Aloe vera* and *Aloe Vacillans* flowers are collected from Badaan and Mytam zone in the morning and afternoon from Ibb city which is located in Yemen during 2018/2019. The taxonomic identification of *Aloe Vacillans* flowers was done

* Corresponding author: Amina Mohammed El-Shaibany
Department of Pharmacognosy, Pharmacy College, Sana`a University, Sanaa, Yemen.

by Dr. Hassan Ibrahim, Professor of Plant Taxonomy, and Botany Section-Department of Biology, Faculty of Science, and Sana'a University, Yemen. *Aloe vera* flowers were identified and authenticated by Dr. Ali Ahmed Abbas Al-Agmi, Department of Biology, Faculty of Education, Dhamar University, Yemen.

1.2. Preparation of Sample

Flowers were plucked from the Inflorescence, cleaned carefully of dirt by a dry piece of cloth, and divided into small portions. Then place the small divided parts separately on clean dry paper away from sunlight and moisture to dry with daily checking of the flower portions. Flowers were powdered finely, weighed after powdering, and then stored in airtight containers at room temperature.

1.3. Extraction

Dried powders of *Aloe vera* and *Aloe Vacillans* flowers were extracted by macerate in a bottle with methanol, each bottle was shacked well and filtered using WHATMAN® filter paper. This procedure was repeated se*veral* times until the total compounds were extracted from the powder. Then the combined filtrates evaporated using a rotary evaporator under reduced pressure at 45 °C. The residual solvent of the extracts was dried and tested the activity (Solaiman, et al., 2015).

2. Qualitative screen

Various phytochemical tests which were performed under the heading of phytochemical screening are mentioned below:

2.1. Phytochemical Tests

2.1.1. Molisch's test for carbohydrates

Two drops of molisch‟s reagents were added to about 5 mg of the extract in a 5 ml aqueous solution in a test tube. 1 ml of conc. H_2SO_4 was allowed to flow down the side of the inclined test tube so that the acid formed a layer beneath the aqueous solution without mixing within. a red ring was formed at the common surface of the two liquids which indicated the presence of carbohydrates. On standing or shaking a dark-purple solution was formed. Then the mixture was shaken and diluted with 5 ml of water. The dull violet precipitate was formed immediately (Parimelazhagan, 2015; Trisha, 2017; Banu, & Cathrine, 2015).

2.1.2. Barfoed's test for reducing sugar

An equal volume of filtrate and Barfoed's reagent are mixed and heated in a water bath. A red precipitate confirms the presence of sugar. (De Silva, et al., 2017)

2.1.3. Benedict's test

A mixture of plant extract and the Benedict reagent is heated in a water bath for 2 minutes and a characteristic-colored precipitate indicates the presence of sugar. (De Silva, et al., 2017; Banu & Cathrine, 2015).

2.1.4. Borntragers's test for anthraquinone glycosides

1 ml of sample solution was shaken with 5 ml of chloroform in a test tube for at least 5 minutes then again shaken with an equal volume of 10% ammonia solution. A bright pink, red, or violet color was developed in the aqueous (upper) layer in the presence of free anthraquinones. (Bandiola, 2018).

2.1.5. Tests for alkaloids

A small volume of each extract was neutralized by adding 1 or 2 drops of dilute H_2SO_4. This neutralized solution was treated with a very small amount of the following reagents and the respective color and precipitate formation (Mir, et al., 2013) was observed:

- Mayer's reagent: Formation of white and cream color precipitate indicated the presence of alkaloids.
- Hager's reagent: The formation of a yellow crystalline precipitate indicated the presence of alkaloids.
- Wagner's reagent: Formation of brownish-black ppt indicated the presence of alkaloids.
- Dragendroff's reagent: Formation of orange or orange-red precipitate indicated the presence of alkaloids.

2.1.6. Test for saponins

- Frothing test: about 0.5 ml of extract was shaken vigorously with water in a test tube. If a thing was produced and it was stable for 1-2 minutes and persisted in warming, it was taken as preliminary evidence for the presence of saponins. (Trisha, 2017).

2.1.7. Test for flavonoids

- Shinoda's test: One mL of the extract is added with 0.5 mL of hydrochloric acid and magnesium metal. The presence of flavonoids is confirmed by reddish coloration. (Bandiola, 2018).

Lead acetate test

Extracts were treated with a few drops of lead acetate solution. The formation of a yellow color precipitate indicates the presence of flavonoids. (Santhi, Sengottuvel, 2016).

H2SO4 test

Extracts were treated with a few drops of H2SO4. The formation of orange color indicates the presence of flavonoids. (Santhi, & Sengottuvel, 2016).

2.1.8. Test for steroids

Salkowski's test

The extract is treated with chloroform and the filtrate of that is treated with a few drops of acetic anhydride. Then the solution is boiled and cooled and the formation of a brown ring at the junction indicates the presence of phytosterols

2.1.9. Test for Protein

Dissolve 100 mg extract in 10 mL of distilled water and filter it through Whatman No. 1 filter paper.

Biuret test

To 2 mL of filtrate, add one drop of 2 % copper sulfate solution. To this, add 1 mL of ethanol (95 %) followed by an excess of potassium hydroxide pellets. The pink color in the ethanol layer indicates the presence of proteins. (Parimelazhagan, 2015).

Xanthoproteic test

The plant extract is treated with a few drops of conc. Nitric acid. The formation of yellow color indicates the presence of proteins (De Silva, et al., 2017).

2.1.10. Test for Fixed Oils and Fats

Saponification test

Add a few drops of 0.5 N alcoholic potassium hydroxide solutions to a small quantity of extract along with a drop of phenolphthalein. Then, heat the mixture in a water bath for 2 h. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats. (Banu, & Cathrine, 2015).

2.1.11. Detection of Phenolic Compounds

Lead acetate test

- Dissolve 50 mg extract in distilled water, and to this, add 3 mL of 10 % lead acetate solution. A bulky white precipitate indicates the presence of phenolic compounds. (Banu, & Cathrine, 2015; Bandiola, 2018).

Ferric Chloride test

The extract (50 mg) is dissolved in 5 ml of distilled water. Ferric chloride solution is added to these few drops of neutral 5%. The dark green color indicates the presence of a phenolic compound (Banu, & Cathrine, 2015)

2.1.12. Test for Gums and Mucilages

Dissolve 100 mg extract in 10 mL of distilled water, and to this, add 25 mL of absolute alcohol with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilages. (Parimelazhagan, 2015).

3. Qualitative Analysis by Thin Layer Chromatography (TLC)

TLC Silica gel 60 F 254, Merck. Germany was used throughout the study to identify the compound in the extract. The organic solvents used include chloroform (CHCl3), ethyl acetate (EtOAc), and general-purpose grade and as a result, they were freshly distilled before use.

The extracts were assayed by thin-layer chromatography (TLC). The different solvent systems used which was prepared on Sigma glass tanks and allowed to dissolve for a few seconds so that a good separation can occur. The plate was placed in a previously saturated TLC chamber with mobile phase the Solvent system I; chloroform (20ml), Solvent system II; chloroform: methanol (18:2), Solvent system III; chloroform: ethyl acetate (18:2). *Aloe vera* and *Aloe Vacillans* extracts and their fraction were spotted on a separated TLC plate. The first plate represents *Aloe vera* extracts and their fraction and the second plate represents *Aloe Vacillans* extracts and their fractions. The dissolved extracts and fractions were spotted at different points on a plate, maintaining the same distance from one edge to another. The marked spots were labeled T, Hx, E.A, Ch, But, and Aq representing total extract, hexane, ethyl acetate, chloroform, n-butanol, and aqueous fraction respectively. A total of 6 extracts and fractions were spotted. The solvent system was allowed to travel a predetermined distance of 15 cm from the origin. Upon separation of the compounds, the TLC plates were air-dried in a fume cupboard. The developed chromatograms were first inspected under UV light (Vilber Lourmat, France) at 254 nm and 365 nm wavelengths. Each chromatogram was then analyzed for the presence of bioactive constituents by spraying with appropriate reagents; methanolic FeCL3 for phenolic compound, and Alcl3 for flavonoids (Wamutu,2016; Adams,2013).

3.1. Quantitative screen

Plants are a rich source of secondary metabolites that have medicinal and aromatic properties. Secondary metabolites such as alkaloids and phenolics generally produced by plants for their defense mechanisms have been implicated in the therapeutic properties of most medicinal plants. Hence, quantification of these metabolites will aid to discover new and effective drugs from plant sources and also to scientifically validate the existing traditional practices. Quantification of a large group of phytochemicals such as phenolics and flavonoids is quantified in this context (Devanaboyina, et al., 2013; Trisha, 2017).

3.1.1. Total Phenolic Content Assay

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method, TPC was assayed by using the Folin-Ciocalteau method, with a volume of 3mL of Folin-Ciocalteau (10%) mixed with 5 µL (0.05mL) plant extract and 0.8mL sodium bicarbonate (7.5%). The reaction solution was incubated at room temperature for 30min. The absorbance of the mixture was measured at 765nm using Milton Roy (Spectronic 1201) spectrophotometer. The TPC was expressed as mg gallic acid equivalents (GAE)/g extract. (Singleton, & Rossi, 1965; Singleton, 1999; Chang, 2002)

3.1.2. Total Flavonoid Content Assay

Total Flavonoid Content (TFC) was quantified by using the Chang et al. (2002) method. Briefly, 0.1mL extract was mixed with 3.90mL distilled water and with 0.3mL sodium nitrite (5%) solution, allowed to react for 5min. Then 0.3mL aluminum chloride (10%) solutions were added. The mixture was allowed to react further for 6min. After this time, the mixture solution was treated with 2mL of 1mM-1 sodium hydroxide. Finally, 2.4 mL of distilled water was added to all samples. The absorbance was read at 510nm against a sample blank without reaction using Milton Roy (Spectronic 1201) spectrophotometer. The TFC of the extracts is expressed as mg quercetin equivalents (QE) /g extract. (Singleton, & Rossi, 1965; Singleton, 1999; Chang, 2002)

3.2. Liquid-liquid fractionation

All separation processes involve the division of a mixture into se*veral* discrete fractions. These fractions may be obvious, physically discrete divisions, such as the two phases of liquid-liquid extraction. The type of fractionation depends on the individual sample and the aims of the separation. (Cannell, 1998). Solvent partitioning of extracts allows a finer separation of the plant constituents into fractions of different polarities.

Based on the sequential fractionation of an extract between two phases of different polarity, The partition well is done according to The modified Kupchan partition method (Kupchan, 1970)

4. Methodology

4.1. Crude extract and fractions yields

Dry powdered flowers of *Aloe vera* and *Aloe Vacillans* which weighed (731 g) and (2457.2 gm) respectively was subjected to sequential extraction by soaking the extract in methanol. after that the total extract was subjected to fractionation with different solvent according to polarity index, starting with hexane, chloroform, ethyl acetate, and finally n-butanol. The number of crude extracts and their fraction obtained and percentage yields were recorded and tabulated in table 1

Table 1 Yield of *Aloe vera* and *Aloe Vacillans* extract

plant	*Aloe vera*		*Aloe Vacillans*	
	Mass in grams	**% Yield**	**Mass in grams**	**% Yield**
Total extract	5 g	0.6%	100g	4.06 %
Hexane fraction	0.717	0.19%	5.8 g	0.23%
Chloroform fraction	0.833	0.11%	3.88 g	0.15%
ethyl acetate fraction	1.956	0.26%	0.53 g	0.02%
n-butanol fraction	0.981	0.13%	5.87 g	0.23%
Aqueous fraction	1.239	0.16%	4.24 g	0.17%

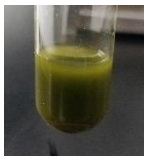
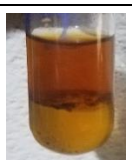
4.2. Qualitative screen

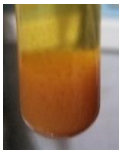
4.2.1. Phytochemical test

In a preliminary phytochemical study of *Aloe vera* and *Aloe Vacillans* flower extracts, different qualitative tests were performed for some major groups of phytochemical constituents. In this analysis, *Aloe Vacillans* flower showed the presence of various groups of phytochemical constituents like flavonoids, phenolic compounds, carbohydrates, protein, and sterols. While *Aloe vera* flower showed the presence of carbohydrates, saponin, flavonoids, Steroids, protein, and phenolic compounds The results are shown in Table 2

Table 2 Phytochemical test of *Aloe vera* and *Aloe Vacillans* flower extract

Phytochemical Constituents	**The test is done for identification**	**Result**	***Aloe Vacillans***	**Result**	***Aloe vera***
Alkaloids	Mayer's test	-		-	
	Hager's test	-		-	

	Wagner's test	-		-	
	Dragendroff's Test	-		-	
Carbohydrates	Molash's test	+		+	
	Barfoed's test	+		+	
	Benedict's test	+		-	
Glycoside	Bontrager's Test	-		-	
Saponin	Frothing test	-		+	
Flavonoids	Shinoda's test	-		-	
	Lead acetate Test	+		+	
	H2SO4 test	-		-	
Steroids	Salkowski's test	+		+	

Phenolic compound	Lead acetate	-		+	
	FeCl3	+		+	
Protein	Biuret test	-		-	
	Xanthoproteic Test	+		+	
Fixed Oils and Fats	Saponification Test	-		-	

(-) = Negative Result (+) = Positive Result

4.2.2. Qualitative Analysis by Thin Layer Chromatography TLC

The TLC analysis was done for the methanol extract of *Aloe Vacillans* flower and *Aloe vera* and fractions to investigate the presence of some secondary metabolites using different solvent systems and different spray reagents. The number of detected spots with their Rf values is mentioned in the table. Also, some TLC plate pictures after spraying are shown in the figure.

4.2.3. Quantitative screen

The total phenolic content of methanolic extracts of both *Aloe vera* and *Aloe Vacillans* flowers was 150.44 mg, and 98.07 mg gallic acid equivalent per gram of extract respectively, while the total phenolic content of n-Hexane fraction of *Aloe vera* flower was 46.35 mg of gallic acid equivalent per gram of fraction see table, the flavonoid content of methanolic extracts of both *Aloe vera* and *Aloe Vacillans* flowers was 70.38 mg, 94.71 mg of quercetin equivalent per gram of extract powder, while flavonoid content of n-Hexane fraction of *Aloe vera* flower was 28.39mg of quercetin equivalent per gram of fraction see table

The result of the quantitative analysis of the phytochemical constituents is presented in the table. The result showed high content of phenolic compound in *Aloe vera* (150.44 mg), followed by *Aloe Vacillans* (98.07mg), and low content of phenolic compound in an n-Hexane fraction of *Aloe vera* flower (46.35 mg). The result of the flavonoid content showed high in *Aloe Vacillans* (94.71 mg) followed by *Aloe vera* (70.38mg), and low content of flavonoid in an n-Hexane fraction of *Aloe vera* flower (28.39 mg).

5. Discussion

The methanol extract of both *Aloe vera* and *Aloe Vacillans* flower was evaluated for their phytochemical constituents. *Aloe Vacillans* flower extract contained flavonoids, phenolic compounds, carbohydrates, protein, and sterols. However, carbohydrate, saponin, flavonoid, steroid, protein, and phenolic compounds were found in *Aloe vera* flower extract these phytochemical results agreed with Martnez-Sánchez, et al., 2020; Debnath, et al., 2018 for the *Aloe vera* flower.

The total phenol content in both *Aloe vera* and *Aloe Vacillans* flower extracts was evaluated in the present study. The highest amount of phenolic content was present in *Aloe vera* (150.44mg of GAE/g of crude extract) and the lowest was in *Aloe Vacillans* extract (98.07mg of GAE/g). While the highest total flavonoid content was present in *Aloe Vacillans* flower extract (94.71 mgQE/g), while the lowest was in *Aloe vera* flower extract (70.38 mgQE/g). These results are generally higher than the results obtained by a previous study which was done on the *Aloe vera* flower. The phenolic content was 17.52 ±1.34 mg GAE per 100 g of dry mass for the extracts, and the total flavonoid content was found to be 13.20±0.09 mg catechin equivalent (CE)/100 g dry extract (Debnath et al., 2018).

6. Conclusion

Phytochemical screening revealed the presence of flavonoids, phenolic compounds, carbohydrates, protein, and sterols in the methanolic extract of the *Aloe Vacillans* flowers. Saponin, carbohydrate, flavonoid, steroids, protein, and phenolic compounds were found in the methanolic extract of the *Aloe vera* flowers.

It's important to mention that it is the first phytochemical screening for the Yemeni *Aloe vera* and *Aloe Vacillans* flowers.

Compliance with ethical standards

Acknowledgments

The authors are grateful to The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Disclosure of conflict of interest

The authors declare that they have no competing interests.

References

[1] Park, Y. I., & Lee, S. K. (2006). New perspectives on *Aloe* (Vol. 8). New York: Springer. pp.7~26

[2] Gasa, Z. P. (2012). Effect of a crude *Aloe vera* leaf extract on diet-induced metabolic dysfunction in growing rats (Doctoral dissertation, Faculty of Health Sciences, University of Witwatersrand),9

[3] Mukherjee, P. K., Nema, N. K., Maity, N., Mukherjee, K., & Harwansh, R. K. (2013). Phytochemical and therapeutic profile of *Aloe vera*. *Journal of Natural Remedies*, 14(1), 1-26.

[4] Solaiman, A. H. M., Nishizawa, T., Sultana, N., Sarker, B., Rahman, R., Shahjahan, M., ... & Hasanuzzaman, M. (2015). Antimicrobial and antioxidant activity analysis of some medicinal plants of Bangladesh. *Adv Plants Agric Res*, 2, 00057.

[5] Parimelazhagan, T. (2015). Pharmacological assays of plant-based natural products (Vol. 71). *Springer*.81~83

[6] Martínez-Sánchez, A., López-Cañavate, M. E., Guirao-Martínez, J., Roca, M. J., & Aguayo, E. (2020). *Aloe vera* flowers, are a byproduct with great potential and wide application, depending on the maturity stage. *Foods*, 9(11), 1542.

[7] Bandiola, T. M. B. (2018). Extraction and qualitative phytochemical screening of medicinal plants: a summary. *International journal of pharmacy*, 8(1), 137-143.

[8] Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International Journal of Advanced Research in Chemical Science*, 2(4), 25-32.

[9] Cannell, R. J. (1998). How to approach the isolation of a natural product. *In Natural products isolation* (pp. 1-51). Humana Press.

[10] Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*, 10(3).

[11] De Silva, G. O., Abeysundara, A. T., & Aponso, M. M. W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *Am J Essent Oil,* 5(2), 29-32.

[12] Debnath, T., Ghosh, M., Lee, Y. M., Nath, N. C. D., Lee, K. G., & Lim, B. O. (2018). Identification of phenolic constituents and antioxidant activity of *Aloe* barbadensis flower extracts. *Food and Agricultural Immunology*, 29(1), 27-38.

[13] Devanaboyina, N., Ramalakshmi, N., Sudeepthi, B. S., Hemachakradhar, K., & Raju, N. P. (2013). Preliminary phytochemical screening, quantitative estimation, and evaluation of the antimicrobial activity of Alstonia macrophylla stem bark. *International Journal of Science Inventions Today*, 2(1), 31-39.

[14] Kupchan, S. M. (1970). Recent advances in the chemistry of terpenoid tumor inhibitors. *Pure and Applied Chemistry*, 21(2), 227-246.

[15] Mir, A. H., Sexena, M., & Malla, M. Y. (2013). An acute oral toxicity study of methanolic extract from Tridex procumbens in Sprague Dawley's rats as per OECD guidelines 423. *Asian Journal of Plant Science and Research*, 3(1), 16-20.

[16] Mir, M. A., Sawhney, S. S., & Jassal, M. M. S. (2013). Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. *Woodpecker Journal of Pharmacy and Pharmacology*, 2(1), 1-5.

[17] Parimelazhagan, T. (2015). Pharmacological assays of plant-based natural products (Vol. 71). *Springer*.81~83

[18] Santhi, K., & Sengottuvel, R. (2016). Qualitative and quantitative phytochemical analysis of Moringa concanensis Nimmo. *Int. J. Curr. Microbiol. App. Sci*, 5(1), 633-640.

[19] Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants using a folding-ciocalteu reagent. *In Methods in enzymology* (Vol. 299, pp. 152-178). Academic Press.

[20] Cannell, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture,* 16(3), 144-158.

[21] Solaiman, A. H. M., Nishizawa, T., Sultana, N., Sarker, B., Rahman, R., Shahjahan, M., ... & Hasanuzzaman, M. (2015). Antimicrobial and antioxidant activity analysis of some medicinal plants of Bangladesh. *Adv Plants Agric Res*, 2, 00057.

[22] Trisha, F. A. (2017). *Phytochemical Screening, Antioxidant & Antimicrobial Investigations of Methanolic Extract of Bark of Garcinia Cowa* (Doctoral dissertation, East West University).

[23] Wamutu, K. R. (2016). Antibacterial antifungal and phytochemical screening Of the plant species Lannea schweinfurthii. (*ENGL.*), chapter3, p35.