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Analysis of Phytochemical Components in Fractionated Ethanol Extracts of *Chrysophyllum albidum* Leaves

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Abstract

Phytochemical characterization of medicinal plants is essential for guiding the isolation of bioactive constituents and supporting drug discovery efforts. This study comprehensively assessed the phytochemical composition of the ethanol leaf extract of *Chrysophyllum albidum* (*C. albidum*) using a complete gradient elution column chromatography methodology. The crude ethanol extract was fractionated into 12 fractions (F1–F12), with F1 being non-polar (100% hexane) and F12 being highly polar (100% water). All fractions were subjected to qualitative phytochemical screening to determine the presence of major phytochemical classes, including alkaloids, flavonoids, tannins, saponins, and terpenoids. The findings indicated a heterogeneous distribution of phytochemicals across the fractions, with elevated levels of polar constituents, particularly flavonoids and tannins, detected in the more polar fractions (F5–F11), whereas the less polar fractions (F1–F4) predominantly contained non-polar compounds such as terpenoids. This comprehensive phytochemical profile provides a valuable foundation for future bioactivity-guided isolation and characterization of specific bioactive compounds from *C. albidum* leaves, thereby supporting the development of phytopharmaceuticals derived from this species.

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Keywords: *Chrysophyllum albidum*; African Star Apple; Phytochemicals; Column Chromatography; Ethanol Extract.

INTRODUCTION

Medicinal plants are an important part of traditional medicine and are still a major source of new medicines. The African star apple, or *Chrysophyllum albidum* (G. Don 1837), is a tropical plant that belongs to the Sapotaceae family. It is frequently utilized in African ethnomedicine. People use several parts of the plant, like the leaves, bark, roots, and fruit, for their health benefits. People utilize the leaf extract to cure a number of health problems, including stomachaches, diarrhea, and skin infections (Adebayo *et al*., 2011).

Prior phytochemical investigations of *C. albidum* have detected secondary metabolites, including flavonoids, tannins, saponins, and alkaloids, which are thought to contribute to its recognized antioxidant, antibacterial, and anti-inflammatory properties. But crude extracts are made up of hundreds of different molecules, which make it hard to say which ones are responsible for certain bioactivities (Adekanmi and Olowofoyeku, 2020).

Fractionation is a very important step in separating and cleaning bioactive chemicals from complicated plant extracts. Researchers can make simpler fractions that are easier to analyze and test for biological activity by carefully separating the crude extract based on the polarity of its parts. Column chromatography with a gradient elution system is a good way to get this kind of separation.

This study intends to perform a comprehensive phytochemical analysis of the ethanol leaf extract of *C. albidum* subsequent to methodical separation via column chromatography. The precise goals are to separate the crude ethanol leaf extract into 12 parts (F1-F12) using a gradient solvent method, to conduct qualitative phytochemical screening on each fraction to ascertain the presence of principal groups of phytochemicals. To measure the total amount of phenolic and flavonoid compounds in the fractions in order to find the best sources of these beneficial chemicals.

Phytochemical and Pharmacological Characteristics of *Chrysophyllum albidum* (*C. albidum*) is useful for medicine since it has a lot of different phytochemicals. Numerous research have examined the phytochemical content of various plant parts, yielding diverse

results contingent upon the extraction solvent and specific plant part utilized. Flavonoids, phenolics, tannins, and glycosides have been found in methanolic and aqueous leaf extracts, people generally think that *C. albidum* extracts are good because they have a lot of phenolic and flavonoid compounds, which can help protect the body from diseases caused by oxidative stress (Balogun and Ashafa, 2018). Moreover, the cotyledons from the seeds are used in ointments to address vaginal and dermatological infections (Balogun and Ashafa, 2018). Additionally, proteins, carbohydrates, and resins have also been identified in various parts of the plant (Balogun and Ashafa, 2018).

Adebayo *et al*., (2011) isolated specific compounds from *Chrysophyllum albidum*, including eleagnine, tetrahydro-2-methylharman, and skatole. Eleagnine, in particular, has been recognized as a significant compound responsible for the plant's antimicrobial activity (Anna and Oladapo, 2013; Adebayo *et al*., 2011). The seed cotyledon has been documented for its anti-hyperglycemic and hypolipidemic effects, contributing to its potential medicinal value in managing certain health conditions (Baloglu *et al*., 2019; Balogun and Ashafa, 2018). Studies have delved into identifying and characterizing the active compounds present in the plant, aiming to understand their therapeutic properties and potential applications in healthcare (Ibrahim *et al*., 2021; George *et al*., 2018). This exploration of traditional uses and phytochemical composition underscores the rich traditional knowledge and scientific interest in *Chrysophyllum albidum*, providing a foundation for further investigations into its potential medicinal applications and the development of therapeutic agents (Chacko *et al*., 2012; Donga and Chanda, 2020).

Fractionation is very important for modern study on natural products. It helps to separate complex mixtures so that individual compounds may be isolated. This is important for figuring out their structures and making new medications (Akinmoladun *et al*., 2022). The solvent used for fractionation is very important since it decides which compounds are recovered in each fraction. This work proposes a gradient elution approach that provides a more thorough separation by incrementally enhancing the polarity of the mobile phase. While prior research has assessed the phytochemicals in various extracts and some fractions of *C. albidum,* a comprehensive study employing the suggested precise gradient elution methodology has not been thoroughly recorded for the leaf extract. Research on the root bark of *C. albidum* and other *Chrysophyllum* species has shown that column chromatography is a good way to separate bioactive chemicals. For example, a study on Carissa edulis used column chromatography with several solvent systems to separate

components, which were subsequently identified using GC-MS. This establishes a robust precedence for the methods utilized in the present investigation (Baloglu *et al*., 2019).

MATERIALS AND METHODS

Plant collection and identification

Location of *Chrysophyllum albidum* Trees

Chrysophyllum albidum (*C. albidum*), also known as African star apple, healthy leaves were collected from Anyikang forests in Bekwarra Local Government Area of Cross River State. Several leaves from different parts of the tree were taken to ensure representativeness. Both mature and young leaves were collected for comprehensive identification.

Identification and Confirmation

Healthy leaves of *C. albidum* (African star apple) were collected from Anyikang forests in Bekwarra Local Government Area of Cross River State, authenticated at the herbarium unit department of Agriculture, University of Calabar, Nigeria with voucher specimen number "UNICAL/AGRI/C.A2024/041".

Chemical and Reagents

Chemicals and reagents used were of analytical grades.

Study Area

The study was carried out in the Central Research Laboratory, Federal University Wukari, Taraba State, Nigeria between May 2024 to July 2025.

Extract Preparation

The rinsed leaves were allowed to air dried and Pulverized. 770g of pulverized leaves substance was extracted in 2 L of ethanol for 72 hours and filtered. It was further concentrated under vacuum at 45 ^{oC} using rotary evaporator and water bath, then stored in sealed sterile containers and refrigerated at 2-4 ^{OC} until used (Yakubu *et al*., 2022).

Fractionation

Packing of the column and elution was done according to the method of Yakubu *et al.* (2022). A glass chromatographic column having a dimension of 60 x 5 cm was packed with slurry silica gel (60–120 mesh) and 100% hexane with a layer of cotton wool on top of

the silica gel bed and the mobile phase made of several solvent mixtures with increasing polarity. The eluted fractions were collected in aliquots of 100ml in beakers. To make sure the packing is equal, the column washed with more hexane; using a dry loading procedure, a tiny amount of silica gel absorbed the crude ethanol extract around 20 g. This mixture was carefully put on top of the packed column. Elution was done with a stepwise gradient of solvents that are more polar for each stage, about 200 ml of each solvent system was utilized, and the fractions put in beakers. Solvent combination for the fractionation include F1a and b: 100% Hexane F2a and b: 50% Hexane and 50% Chloroform F3a and b: 100% Chloroform; F4a and b: Chloroform: Ethyl acetate (50:50) F5a and b: Ethyl acetate in full F6a and b: Ethyl acetate and ethanol at a 50:50 ratio F7a and b: 100% ethanol F8a and b: 50% ethanol and 50% methanol F9a and b: 100% Methanol F10a and b: Methanol and Water (50:50) F11a and b: All Water F12a and b: Water and hexane (50:50). After that, the solvents were removed from the pooled fractions using water bath, and dried extracts weighed and kept at 4°C. Using standard methods, the dried fractions (F1-F12) were checked for the presence of main phytochemical classes. Tests for alkaloids: Mayer's and Dragendorff's. Flavonoids: Shinoda test (Mg and HCl) and test with an alkaline reagent. Saponins: Test for froth. Tannins: the ferric chloride test and the gelatin test. Salkowski's test for terpenoids. We utilized spectrophotometric methods to figure out how much total phenolic and total flavonoid is in the fractions that test positive for these chemicals. The Folin-Ciocalteu method was used to find the Total Phenolic Content (TPC), and the results were given as Gallic Acid Equivalents (GAE) per gram of fraction. The aluminum chloride colorimetric method was used to find the Total Flavonoid Content (TFC), and the results were given as Quercetin Equivalents (QE) per gram of fraction. A descriptive tables and chats below show the qualitative results showing whether or not the tested phytochemicals were present.

RESULTS AND DISCUSSION

Phytochemical Profile According to Fraction Polarity

The methodical separation of the ethanol leaf extract of *Chrysophyllum albidum* showed a strong link between the polarity of the eluting solvent and the amount of certain types of phytochemicals. The extract was divided into 12 different fractions (F1-F12), from

non-polar (100% hexane) to highly polar (100% water). This made it possible to separate flavonoids, phenols, saponins, and tannins (Table 1).

Amount of Flavonoids

The examination of flavonoid content revealed an unexpected trend. The largest levels of flavonoids were not in the ethyl acetate and methanol fractions, which are usually high in flavonoids. Instead, they were in the less polar fractions that were eluted with hexane and hexane/chloroform (F1a, F1b, F2a, and F2b). F2b had the highest value, at 472.66 mg/g. On the other hand, the amount of flavonoids went down a lot in the intermediate polarity fractions (F5 and F6) and stayed low in the highly polar fractions (F9-F11). This indicates that the flavonoids in the *C. albidum* leaf extract are mainly non-polar or methylated types, which dissolve better in less polar organic solvents. This observation challenges the prevailing idea that flavonoids are concentrated in medium-polarity fractions, necessitating more exploration into the precise structures of these non-polar flavonoids.

Phenolic Content

The distribution of phenolic compounds among the fractions exhibited a more diverse pattern, indicating the existence of phenols with a broad spectrum of polarity. The moderate to low polarity fractions (F2b, F3a, and F4a) had the most of the substance, but the amount went down continuously as the solvent polarity went up. The fractions with the highest polar properties (F9-F11) have the least amount of phenolic compounds. This means that the more polar, water-soluble phenolic chemicals, including gallic acid derivatives, are either not present in high enough amounts or were successfully separated into other fractions. F2b had the most phenolic content, at 203.48 mg/g.

Saponin Amount

The distribution of saponins followed the expected pattern, with their concentration going up a lot as the polarity of the eluting solvent went up. This is in line with the fact that these chemicals are glycosidic and very polar. The most polar fractions had the most saponins: F11b (281.80 mg/g), F11a (276.96 mg/g), and F7a (274.97 mg/g). These fractions were eluted with 100% water and 100% ethanol, respectively. The first three non-polar fractions (F1-F3) have the least amount of saponin.

The Amount of Tannin

The tannin level was relatively low across all fractions, which is common for leaf extracts compared to barks or roots. The distribution was not a straight line; there were various peaks and valleys. There were significant peaks in F2b (18.53 mg/g), F5b (16.75 mg/g), and F10b (15.99 mg/g). This means that the extract contains several types of tannins with different polarity. The highly polar water fractions (F11a and F11b) have the least amount of tannins.

F1a and b = hexane-100; F2a and b = hexane : chloroform- 50:50; F3a and b = chloroform- 100 F4 aand b = chloroform : ethylacetate- 50:50; F5a and b = ethylacetate - 100; F6 aand b = ethylacetate/ethanol = 50:50; F7a and b = ethanol- 100; F8a and b = ethanol : methonol- 50:50; F9 aand b = methanol- 100; F10a and b = methanol : water- 50:50; F11a and b = water- 100; F12a and b = water : hexane- 50:50.

Table 1. The Phytochemicals Contents of Fractions of *Chrysophyllum albidum* Leaf Extract

FRACTION	FLAVONOID mg/g	PHENOL mg/g	SAPONIN mg/g	TANIN mg/g
1a	399.73	99.82	193.31	8.63
1b	443.52	145.62	194.87	8.08
2a	443.92	162.29	197.76	8.22
2b	472.66	203.48	194.78	18.53
3a	308.77	199.59	190.77	8.52
3b	428.72	192.82	241.10	9.41
4a	426.15	201.71	261.93	10.73
4b	400.09	188.18	247.62	10.99
5a	167.06	184.79	236.15	14.75
5b	129.38	196.21	257.58	16.75
6a	109.13	141.79	267.95	14.78

FRACTION	FLAVONOID mg/g	PHENOL mg/g	SAPONIN mg/g	TANIN mg/g
6b	97.96	105.32	272.77	14.58
7a	97.11	85.93	274.97	12.88
7b	94.53	87.23	273.83	14.68
8a	116.60	85.23	257.47	15.21
8b	121.20	76.51	266.65	6.68
9a	102.16	65.12	265.32	9.48
9b	89.73	55.07	271.95	13.53
10a	92.26	52.96	267.39	6.16
10b	97.26	65.21	266.17	15.99
11a	104.13	61.79	276.96	4.48
11b	161.45	54.98	281.80	4.02
12a	254.23	69.09	266.46	4.98
12b	214.28	61.43	266.31	4.18

Total Flavonoid Contents of Fractions of *Chrysophyllum albidum* Leaf Extract

Figure 4.1 displays a bar chart shows the Total Flavonoid Content (mg/g) in different parts of a crude ethanol extract from the leaves of *Chrysophyllum albidum*. We used solvents with different polarity to separate the fractions. The "a" and "b" samples are copies of each solvent system. Fractions 10a and 10b, which are made up of methanol and water (50:50), had the most total flavonoids, with levels close to 600 mg/g. This means that a mixture of solvents with medium polarity works best for getting flavonoids out of the leaves of this plant. Fractions 1a, 1b (100% hexane), 2a, 2b (hexane:chloroform), and 4a, 4b (chloroform:ethyl acetate) include moderate amounts of flavonoids, usually between 400 and 450 mg/g.

Fractions 5a, 5b (100% ethyl acetate) and 6a, 6b (ethyl acetate:ethanol) likewise show moderate quantities. This means that these solvent systems may extract a lot of flavonoids, but not as much as the methanol:water mix. Fractions 7a, 7b (100% ethanol), 8a, 8b (ethanol:methanol), and 9a, 9b (100% methanol) all have low flavonoid concentration, about 100 mg/g. This means that these very polar solvent solutions might not be the best for getting these specific flavonoids out. Fraction 5a has the least amount with just about 160 mg/g. The findings demonstrate that the polarity of the solvent system markedly influences the extraction yield of flavonoids. Using very polar pure solvents like ethanol (7a, 7b) and methanol (9a, 9b) to extract flavonoids gives you a low amount of them.

Using pure nonpolar solvents like hexane (1a, 1b) to extract gives you a significant amount. A medium-polarity combination of methanol and water (10a, 10b) works best for extraction. The "a" and "b" sample pairings usually have the same amount of flavonoids, but the error bars demonstrate that there are some slight differences. This indicates that the experimental approach was reliable, consistent and could be repeated.

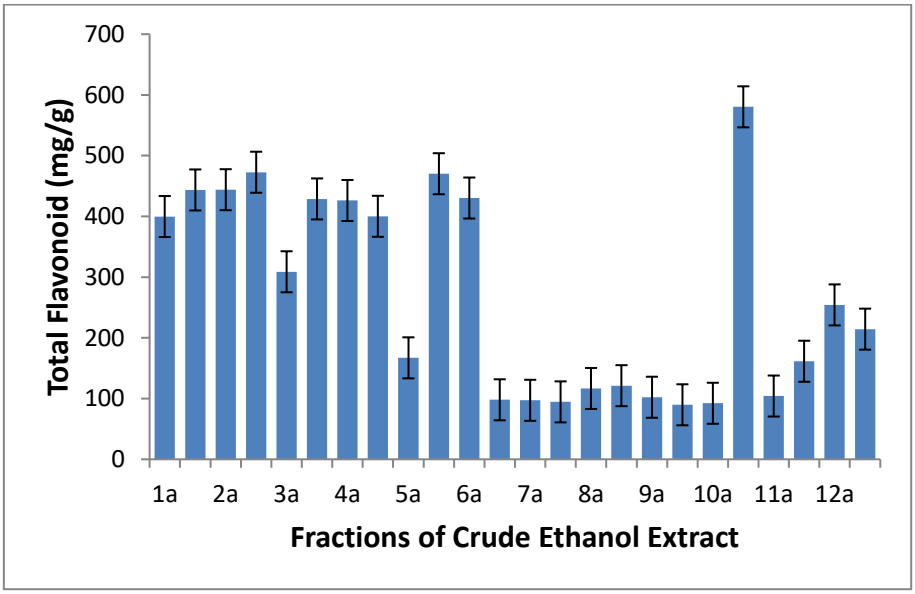


Figure 1: Total Flavonoid Contents of Fractions of *Chrysophyllum albidum* Leaf Extract

F1a and b = hexane-100; F2a and b = hexane : chloroform- 50:50; F3a and b = chloroform- 100 F4 aand b = chloroform : ethylacetate- 50:50; F5a and b = ethylacetate - 100; F6 aand b = ethylacetate/ethanol = 50:50; F7a and b = ethanol- 100; F8a and b = ethanol : methonol- 50:50; F9 aand b = methanol- 100; F10a and b = methanol : water- 50:50; F11a and b = water- 100; F12a and b = water : hexane- 50:50.

Phenol Contents of Fractions of *Chrysophyllum albidum* Leaf Extracts

The amount of phenolic chemicals in the fractions changes a lot depending on the solvent system employed for extraction, as shown by the heights of the bars and the error bars.

The less polar fractions, F2 (hexane:chloroform 50:50) and F3 (100% chloroform), had the highest phenolic concentration. F2b, F3a, and F3b have the highest levels of phenol, which is about 200 mg/g. Fraction F5 (100% ethylacetate) and the first extraction fractions of F4 (50% chloroform:ethylacetate) also have a lot of phenolic content, with an average of 180 to 200 mg/g. The fractions with the lowest phenolic concentration are those that have more polar solvents, including pure methanol (F9), pure water (F11), and methanol:water (F10). The F9a and F9b fractions have the lowest values, which mean that 100% methanol removed less phenolic chemicals. An unexpected result is that fraction F10a (methanol:water 50:50) is a clear outlier because it has a much higher concentration (around 200 mg/g) than the other polar fractions (F9 and F11). This indicates that the 50:50 methanol:water mixture was particularly efficient in extracting a specific category of phenols or that the partitioning method for this sample was distinct. The overall trend shows that phenolic compounds were easier to extract with solvents that were not too polar, such chloroform and ethylacetate, than with solvents that were more polar, like methanol and water. This implies that the phenolic chemicals in *Chrysophyllum albidum* leaves are predominantly of the less polar variety.The phenolic contents for the "a" and "b" replicates are similar for most fractions (F1, F2, F4, F5), with error bars that overlap. This shows that the experimental procedure worked the same way every time. The major difference is between F10a and F10b, which suggests that there is more variability.

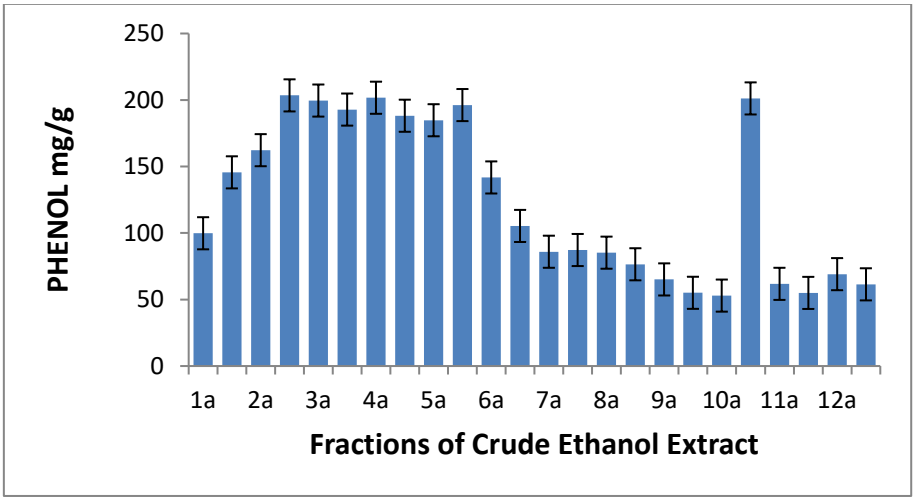


Figure 2: Phenol Contents of Fractions of *Chrysophyllum albidum* Leaf Extracts

F1a and b = hexane-100; F2a and b = hexane : chloroform- 50:50; F3a and b = chloroform- 100 F4 aand b = chloroform : ethylacetate- 50:50; F5a and b = ethylacetate - 100; F6 aand b = ethylacetate/ethanol = 50:50; F7a and b = ethanol- 100; F8a and b = ethanol : methonol- 50:50; F9 aand b = methanol- 100; F10a and b = methanol : water- 50:50; F11a and b = water- 100; F12a and b = water : hexane- 50:50.

Saponin Contents of Fractions of *Chrysophyllum albidum* Leaf Extract

This shows that the saponins in *Chrysophyllum albidum* are chemically diverse since different types of saponins have distinct polarity. This is a common outcome for plant extracts. The fact that the saponins were found in more than one fraction shows that the chromatographic procedure worked to separate them based on their polarity, which proves that the fractionation process was correct. Saponins have a wide variety of polarities, which means that distinct types of saponins were able to be separated along the polarity gradient. The fractions with more polar solvents (such F8, F9, F10, F11, and F12) probably have the more polar saponins. Saponins are glycosides that dissolve in water; therefore they often come out nicely in very polar solvents like methanol and water. The first few fractions (F1 to F3a) use less polar solvents including hexane, chloroform, and ethyl acetate; hence they may have less polar saponins. It is also possible to extract certain saponins into organic solvents.

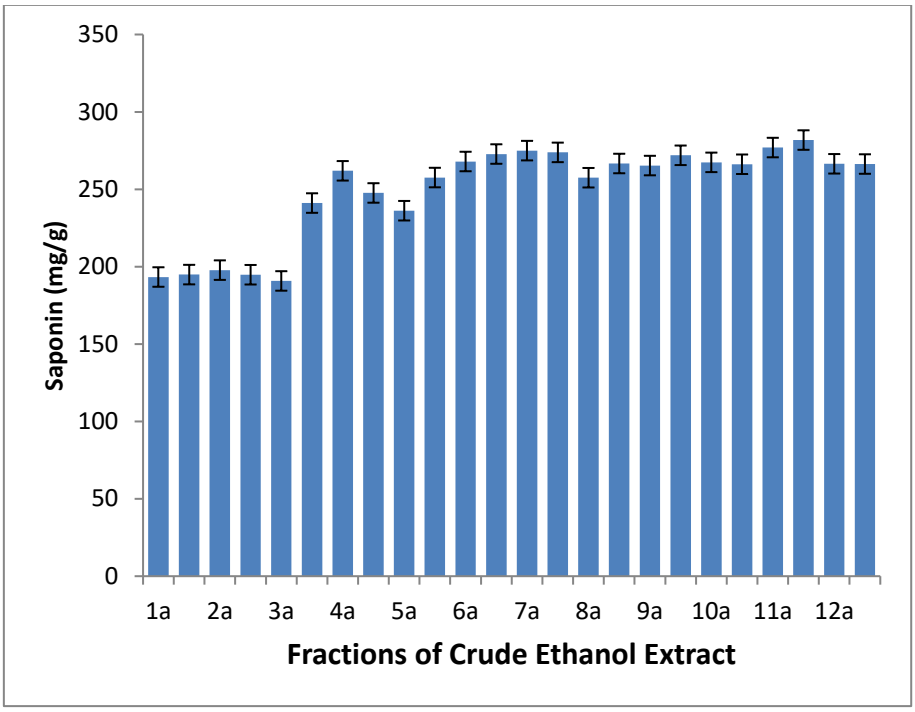


Figure 3: Saponin Contents of Fractions of *Chrysophyllum albidum* Leaf Extract

F1a and b = hexane-100; F2a and b = hexane : chloroform- 50:50; F3a and b = chloroform- 100 F4 aand b = chloroform : ethylacetate- 50:50; F5a and b = ethylacetate - 100; F6 aand b = ethylacetate/ethanol = 50:50; F7a and b = ethanol- 100; F8a and b = ethanol : methonol- 50:50; F9 aand b = methanol- 100; F10a and b = methanol : water- 50:50; F11a and b = water- 100; F12a and b = water : hexane- 50:50.

Tannin Contents of Fractions of *Chrysophyllum albidum* Leaf Extract

It looks like tannins are not evenly spread out in the leaf extract because some fractions (2b, 5b, 10b, 6a, 6b, 7b, 8a, and 9b) have more tannins than others. Instead, they are grouped together in certain percentages based on their chemical features, specifically how polar they are. The presence of distinct types of tannins (such as hydrolyzable and condensed) with varying polarity in the *Chrysophyllum albidum* leaf extract is shown by the fact that high tannin concentration is spread over different fractions. If all tannins were the same, they would probably be in only one or two fractions. The solvent systems that worked best for getting tannins out of the leaf powder were the ones that worked best for the fractions with high tannin content, like 2b, 5b, 6a, 6b, 7b, 8a, and 9b. Solvents including methanol, ethanol, and acetone are typically used to get tannins out of plant materials.

These fractions probably have most of the tannins, therefore they should show the strongest biological activities associated to tannins. Tannins are recognized to be good for health since they fight free radicals, kill bacteria, and make proteins clump together. The fact that tannins were only identified mostly in these specific fractions and not spread out uniformly shows that the chromatographic separation procedure worked. It did a good job of separating distinct substances based on their chemical properties. Other investigations on *Chrysophyllum albidum* have also found that its leaves and stem bark include a lot of tannins. The results from Figure 4.5 support these findings, but they give more particular information on which solvent systems work best to get the active tannin compounds out.

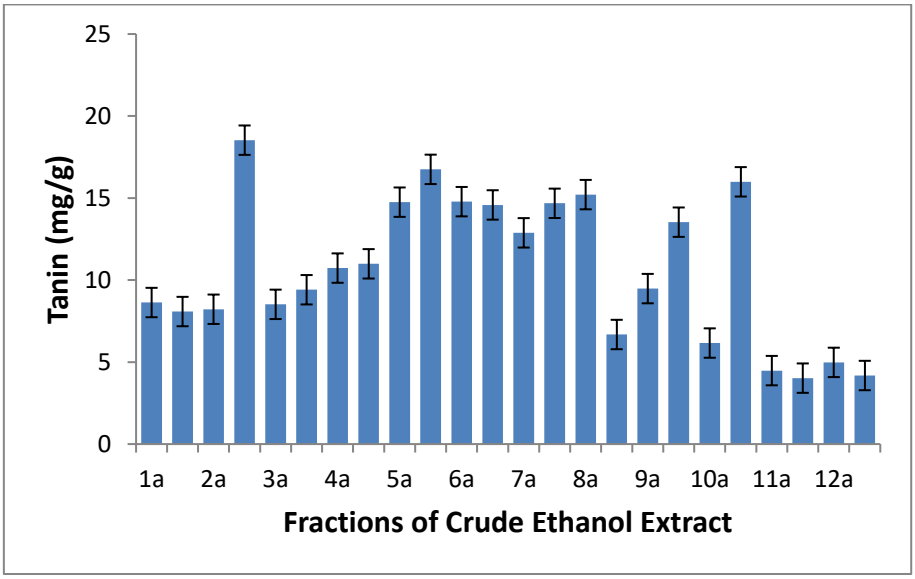


Figure 4: Tannin Contents of Fractions of *Chrysophyllum albidum* Leaf Extract

F1a and b = hexane-100; F2a and b = hexane : chloroform- 50:50; F3a and b = chloroform- 100 F4 aand b = chloroform : ethylacetate- 50:50; F5a and b = ethylacetate - 100; F6 aand b = ethylacetate/ethanol = 50:50; F7a and b = ethanol- 100; F8a and b = ethanol : methonol- 50:50; F9 aand b = methanol- 100; F10a and b = methanol : water- 50:50; F11a and b = water- 100; F12a and b = water : hexane- 50:50.

Understanding in Light of Past Results

The results are in line with what is already known about chromatographic separation, especially for saponins, which are known to be more abundant in the more polar fractions. The unanticipated distribution of flavonoids, particularly their prevalence in non-polar fractions, necessitates additional examination. This indicates that the crude

ethanol extract possesses a substantial fraction of lipophilic or less polar flavonoid types, possibly reflecting a distinctive attribute of this particular plant or its extraction methodology. The diverse distribution of phenolics and tannins reinforces the extract's diversity and the existence of these chemicals across several polarities.

CONCLUSION

The phytochemical analysis of the separated *Chrysophyllum albidum* leaf extract gives a full picture of what chemicals are in it. The results show that the fractionation procedure worked well to separate phytochemicals based on how polar they are. The amount of saponins in the polar fractions is in line with what they are made of. However, the substantial amount of flavonoids in the non-polar fractions shows that there are less prevalent, non-polar flavonoid structures. These results are essential for directing forthcoming bioactivity-guided isolation and characterisation investigations. Concentrating on the less polar fractions (F1 and F2) may facilitate the identification of distinctive non-polar flavonoids, whilst the polar fractions (F7-F11) continue to be attractive sources for the isolation of polar chemicals such as saponins.

Fractionation Profile; it is expected that the gradient elution will segregate molecules based on how polar they are. The less polar fractions (F1-F3), which were eluted with hexane and chloroform, should have more non-polar molecules such terpenoids and fatty acids. The intermediate polarity fractions (F4-F7), which have chloroform, ethyl acetate, and ethanol in them, probably have more moderately polar chemicals in them, such as flavonoids and some phenolic compounds. The most polar fractions (F8-F11), which were eluted with methanol and water, should be full of very polar chemicals such saponins, tannins, and polar phenolics. The last fraction (F12) probably has polar molecules that haven't been eluted.

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