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THE EFFECT OF TEMPERATURE, EXTRACTION TIME, AND SOLVENTS ON THE EXTRACTION OF BIOACTIVE COMPOUNDS FROM GALACTOGENIC PLANTS USED IN THE REPUBLIC OF BENIN

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ABSTRACT

Optimizing extraction processes is essential to ensuring the quality and efficacy of active ingredients intended for therapeutic and nutraceutical uses. This study aims to analyze the influence of temperature, extraction time, and solvent type on the extraction of compounds with galactogenic potential, in order to identify the most favorable conditions for their valorization. The study focused on three medicinal plants known for their galactogenic properties: *Carica papaya L.*, *Momordica charantia L.*, and *Vernonia amygdalina* Del. Extracts were obtained by maceration, infusion, and decoction. A qualitative phytochemical analysis, based on precipitation and color tests, revealed different groups of secondary metabolites. The total flavonoid content was determined using the aluminum trichloride ($AlCl_3$) method, while the total polyphenol content was determined using the Folin-Ciocalteu method. Tannin content was determined using the vanillin method. Extraction yields ranged from 5% to 28.33%. Analyses revealed the presence of alkaloids, tannins, and polyphenols, associated with potential galactogenic activities, as well as anthocyanins, saponins, triterpenoids, steroids, and coumarins. Polyphenol levels ranged from 2.10 to 922.65 mg GAE/g, flavonoid levels from 161.13 to 345.02 mg QE/g, and tannin levels from 140.00 to 443.66 mg CE/g extract. Among the techniques evaluated, maceration proved to be the most effective at concentrating bioactive compounds, although it exhibited a low extraction yield. These results highlight the pharmacological potential of these plant species to support lactation, provided that extraction parameters are rigorously optimized to maximize their bioavailability and efficacy.

KEYWORDS: Optimization, extraction methods, phytochemical screening, galactogenic plants.

I- INTRODUCTION

Lactation is a physiological process essential for infant survival and development, particularly in developing countries where breastfeeding is the primary source of infant nutrition.[1,2] However, many women experience difficulties related to a lack of or insufficient milk production, often referred to as hypogalactia or agalactia.[3] In a context of limited resources and inaccessibility to sometimes costly conventional treatments, the use of galactagogue plants remains a widespread practice, particularly in West Africa and Benin, where traditional medicine plays a significant role in primary healthcare.[4,5]

The galactagogue effects of these plants are mainly attributed to their richness in secondary metabolites, especially phenolic compounds, flavonoids, and tannins.[8-10] These compounds exhibit antioxidant, anti-inflammatory, and hormone-regulating properties that can stimulate prolactin secretion and improve mammary gland function.[11] Flavonoids, for example, are known for their ability to modulate endocrine pathways involved in lactation, while tannins contribute to the protection of mammary tissues against oxidative stress.[12,13] Polyphenols, meanwhile, improve the bioavailability of nutrients necessary for milk synthesis.[9, 14]

However, the therapeutic efficacy of these plants depends heavily on the extraction methods used, which influence the nature and quantity of the bioactive compounds responsible.[15,16] Parameters such as temperature, solvent polarity, and extraction time play a crucial role in the recovery of secondary metabolites.[17] For example, organic solvents like ethanol are often more effective at extracting phenolic compounds, while aqueous methods can favor the extraction of other constituents.[18] Thus, poor control of these parameters can lead to a significant loss of the biological activity of the extracts.

In the context of promoting local plant genetic resources and improving phytotherapy practices, optimizing extraction conditions to maximize the concentration of galactogenic active ingredients is crucial. This optimization not only increases the efficacy of traditional preparations but also ensures their reproducibility and safety.[19]

This study therefore aims to evaluate the influence of temperature, extraction time, and solvent on the extraction of active ingredients from some Beninese galactogenic plants (*Carica papaya* L., *Momordica charantia* L., and *Vernonia amygdalina* Del.) in order to propose optimal extraction conditions. This approach is part of the ongoing effort to optimize the efficacy of galactogenic extracts and contribute to the scientific development of plants used to stimulate lactation.

II- MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials

The plant Materials consists of the Leaves of *carica papaya, Momordice charantia, de Vernonia amygdalina* collected early in the morning in the surrounding forests of the communes of Natitingou in the department of Atacora and the communes of Za-Kpota, Covè, Zagnanado, Houinhi and Zogbodomè in the department of Zou in the Republic of Benin.

***Carica papaya* L.** ***Momordice charantia* L.** ***Vernonia amygdalina* Del**

Picture 1: Leaves of *carica papaya* L., *Momordice charantia* L. *et de Vernonia amygdalina* Del

These plants were identified at the National Herbarium of Benin of the University of Abomey-Calavi.

Table 1: Plant Identification Numbers.

Identification Numbers	Scientifique Names	families
YH 1119/HNB	*Carica papaya* L.	Caricaceae
YH 1120/HNB	*Vernonia amygdalina* Delile	Asteraceae
YH 1121/HNB	*Momordice charantia* L.	Cucurbitaceae

2.1.2. Chemical Materials

The chemical materials consisted specifically of chemicals such as methanol, ethanol, hydrochloric acid, gallic acid, and quercetin, marketed by Sigma-Aldrich and Acros-Organics. Various types of glassware were also used in this experiment.

2.2. METHODS

2.2.1. Preparation of Powders

The leaves of the plants mentioned above were carefully washed and then dried at room temperature in the laboratory for two weeks. They were then ground into a powder using an electric grinder and stored in a glass jar protected from light.[20,21]

2.2.2. Preparation of Extracts

Four extraction methods were used to extract the active ingredients from the plants used in this study.

2.2.2.1. Maceration

Maceration (solid-liquid extraction) is a simpler process, which consists of leaving the plant material (ground) in the solvent to extract the active ingredients. This method allows for the conservation of solvent volume. It also allows for the extraction of bioactive compounds at low temperatures. During this process, 600 ml of 96% ethanol were added to 60 g of powder from each plant. After 72 hours of maceration in the dark, the mixture was filtered and then evaporated at 40 °C under low pressure, followed by oven drying at 35 °C.[20,21]

2.2.2.2. Decoction

Decoction is an extraction technique that involves boiling the solute (solid) and solvent mixture for a few minutes.[22] This method allows for the extraction of heat-sensitive compounds and is suitable for hard plant parts (roots and bark).[22] For this purpose, 600 ml of distilled water were added to 60 g of powder from each matrix. After 30 minutes of heating at 100°C, the mixture is filtered and then evaporated at 40°C under low pressure and then dried in an oven at 35°C.

2.2.2.3. Infusion

Infusion is an extraction technique that involves heating distilled water for a few minutes at a temperature between 60 and 100°C and then pouring it over the solute (solid).[22] This method is easy to perform without sophisticated equipment. It requires only hot water. Thus, 600 ml of hot distilled water at 100°C is added to 60 g of powder of each plant. After 24 hours of infusion, the mixture is filtered and then evaporated at 40°C under low pressure and then dried in an oven at 35°C.[21]

The yield was calculated using the following formula: R = 100 x Mass of dry powder obtained / Mass of plant material. Each extract obtained is packaged in a glass jar, protected from light, and stored in a refrigerator at 4°C.

2.2.3. Qualitative Analysis of Phytochemical Groups

The qualitative analysis of the extracts allowed for the identification of the major phytochemical families present through solubility tests, colorimetric reactions, precipitation tests, and ultraviolet light examinations using the Houghton and Raman method (1998).[23]

2.2.4. Quantitative Analysis of Total Polyphenols

The polyphenol content was determined using the Folin-Ciocalteu reagent. This reagent is reduced upon oxidation of the phenols to a mixture of blue tungsten and molybdenum oxides. The resulting blue color has a maximum absorption at approximately 750 nm. Absorbance, measured against a standard curve obtained with a phenolic acid (gallic acid), allows the determination of the total phenol content in the extract, expressed as mg gallic acid equivalent per g of extract.[24]

2.2.5. Quantitative analysis of flavonoids

The total flavonoid content of plant extracts is estimated using the aluminum trichloride (AlCl3) method. Quercetin is used as a reference compound to create the calibration curve.[24] The polyphenol and flavonoid contents are determined using the following formula: $T = (C \times Vr) / (Vp \times Cp)$

T = Compound content; C = Concentration obtained from the calibration curve

Vr = Reaction volume; Vp = Volume of extract sampled; Cp = Concentration of the extract solution.

2.2.6. Quantitative Analysis of Tannins

The total condensed tannin content of plant extracts is determined by the vanillin-hydrochloric acid colorimetric method. This method is based on the reaction of condensed tannins with vanillin in an acidic medium (hydrochloric acid), producing a red complex. The intensity of this color is measured spectrophotometrically at a wavelength of 500 nm.[25]

Quantification is performed using a calibration curve obtained with a reference compound such as quercetin or catechin. The results are expressed as standard equivalents per gram of extract.

2.2.7. Data treatment and analysis

The spreadsheet Microsoft Excel version 2013 has been used for the capture and encoding the data.

III- RESULTS AND DISCUSSION

3.1. Nature of the Extracts

Table 2 presents the different methods used to obtain extracts from the three medicinal plants studied: *Carica papaya*, *Momordica charantia*, and *Vernonia amygdalina*. These three plants were selected after an ethnobotanical survey combined with data from the literature. Four extraction techniques were applied (ethanolic maceration, aqueous decoction, hydroethanolic maceration, and hot water infusion), yielding a total of twelve extracts identified by specific codes ranging from A1 to C4. This diversity of methods aims to extract different groups of bioactive compounds depending on the polarity of the solvents and other extraction conditions (temperature and extraction time).

Table 2: Extraction Methods, Codes, and Nature of Extracts from the Three Medicinal.

Methods	Extract Codes	Nature of Extracts
Ethanolic maceration	A1	Ethanolic extract of *Carica papaya* L.
	B1	Ethanolic extract of *Momordica charantia* L.
	C1	Ethanolic extract of *Vernonia amygdalina* Del.
Aqueous decoction	A2	Decoction extract of *Carica papaya* L.
	B2	Decoction extract of *Momordica charantia* L.
	C2	Decoction extract of *Vernonia amygdalina* Del.
Hydroethanolic maceration	A3	Hydroethanolic extract of *Carica papaya* L.
	B3	Hydroethanolic extract of *Momordica charantia* L.
	C3	Hydroethanolic extract of *Vernonia amygdalina* Del.
Hot water infusion	A4	Infused extract of *Carica papaya* L.
	B4	Infused extract of *Momordica charantia* L.
	C4	Infused extract of *Vernonia amygdalina* Del.

The methodical approach used allows for the extraction of different types of bioactive compounds in varying proportions, as the nature of the solvent, the extraction technique, and the extraction time can all influence the solubilization of metabolites present in the plants. The differences observed below in extraction yields and phytochemical compositions for each method serve as indicators to validate or refute the influence of the processes employed and to identify the most effective method for the search for galactogenic active ingredients.

3.2. Extraction Yields

Figure 1 presents the extraction yields (%) obtained using different extraction methods applied to the three medicinal plants (*Carica papaya*, *Momordica charantia*, and *Vernonia amygdalina*). The yields obtained range from 5.00 ± 0.47% to 28.33 ± 2.35%, demonstrating that extraction efficiency depends on both the method used and the plant species.

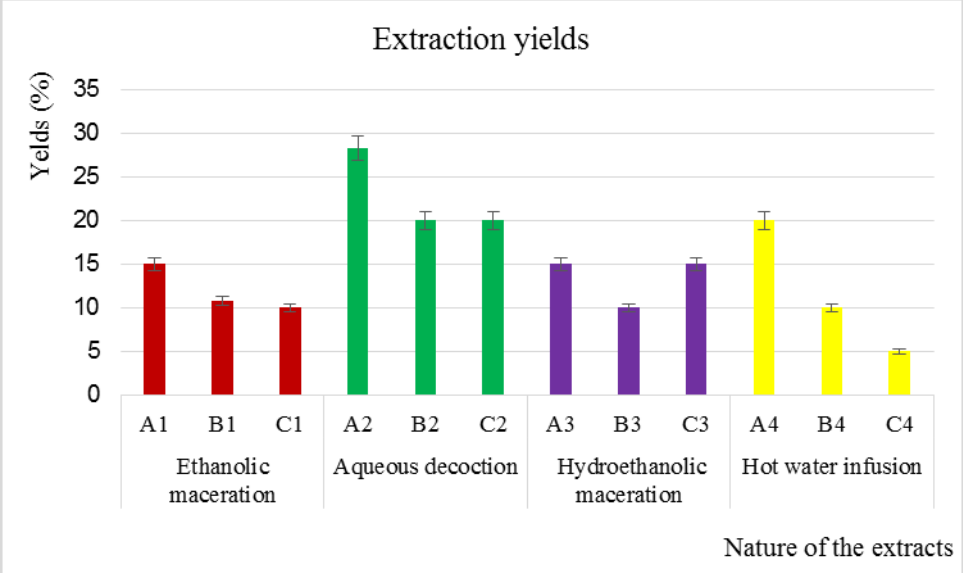

Figure 1: Extraction Yields (%) Obtained Using Different Extraction Methods.

Considering the same species, we observe that for *Carica papaya*, the highest yield is obtained by aqueous decoction (A2: 28.33 ± 2.35%), followed by infusion (A4: 20.00 ± 1.02%), while ethanolic and hydroethanolic maceration show almost identical yields of around 15.00%. For *Momordica charantia*, decoction (B2: 20.00 ± 0.78%) also gives the highest yield, while the other methods give values close to 10%. In the case of *Vernonia amygdalina*, decoction (C2: 20.00 ± 0.78%) and hydroethanolic maceration (C3: 15.00 ± 0.43%) showed higher yields than infusion (C4: 5.00 ± 0.47%).

When comparing the same solvents, aqueous decoction (A2, B2, C2) generally gave the best yields, which could be explained by the effect of heat promoting the diffusion of water-soluble compounds. According to Altemimi et al. in 2017, the temperature and polarity of the solvent strongly influence extraction efficiency.[26] Similarly, Singleton et al. in 1999 found that hot aqueous solvents can improve the extraction of many bioactive compounds.[27]

3.3. Qualitative Analysis of Phytochemical Groups in Extracts

Table 3 shows that the extraction method strongly influences the phytochemical profile of the extracts obtained from the plants used in this study. Overall, alkaloids, polyphenols, gallic tannins, and reducing compounds are present in virtually all fractions (all methods, all extracts).

Table 3: Phytochemical Screening of Plant Extracts.

Extraction Methods / Phytochemical Compounds		Ethanolic maceration			Aqueous decoction			Hydroethanolic maceration			Hot water infusion		
		A_1	B_1	C_1	A_2	B_2	C_2	A_3	B_3	C_3	A_4	B_4	C_4
Alkaloids		+	+	+	+	+	+	+	+	+	+	+	+
Polyphenols		+	+	+	+	+	+	+	+	+	+	+	+
Tannins		+	+	+	+	+	+	+	+	+	+	+	+
Catechic tannins		+	+	+	-	-	-	+	+	+	+	+	+
gallic Tannins		+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids		+	+	-	+	+	-	+	+	+	+	+	+
Anthocyanins		+	+	+	+	+	-	+	+	+	+	-	-
Leucoanthocyanins		+	-	+	-	+	-	+	+	+	+	-	-
Quinone derivatives		+	-	+	-	-	-	+	+	+	+	+	-
Saponins		-	-	+	-	+	+	-	-	+	-	-	+
Triterpénoids		+	+	+	-	-	-	+	+	+	-	-	-
Stéroids		+	+	+	-	-	-	-	-	-	-	-	-
Cyanogenic derivatives		-	-	-	-	-	-	-	-	-	-	-	-
Mucilages		-	+	-	-	-	+	+	+	+	-	-	-
Coumarins		+	-	+	+	-	+	+	-	+	+	-	+
Reducing compounds		+	+	+	+	+	+	+	+	+	+	+	+
Free anthracenic compounds		-	+	-	+	+	+	-	-	-	-	-	-
Combined anthracenic compounds	O-heterosides	-	+	-	-	-	-	-	-	-	-	-	-
	O-heterosides (reduced genins)	+	+	+	+	+	-	-	-	+	-	-	-
	C-heterosides (genins)	+	+	+	+	+	+	+	+	+	+	+	+

+ = Present; **-** = Absent

Most biomolecules are relatively easy to extract regardless of the temperature and solvent used (ethanol, water, or hydroethanol). This corroborates previous findings highlighting the broad extraction of polyphenols by various polar solvents.[28]

In contrast, variations appear for families such as flavonoids, anthocyanins, leucoanthocyanins, and saponins, which exhibit method-dependent presence. For example, flavonoids are absent in some hydroethanolic and aqueous extracts, which may be explained by the lower solubility of certain flavonoids in less polar mixtures or depending on the temperature. This phenomenon has been described in studies on the extraction of antioxidants from African plants, where the composition depended on the solvent polarity and extraction time.[29]

The variable presence of coumarins, mucilage, or quinone derivatives depending on the method highlights that compounds more sensitive to heat or solvents are better preserved or released by certain techniques. For example, hot decoction and infusion can degrade some heat-sensitive compounds, which is consistent with studies on aqueous extracts of *Vernonia amygdalina*. Conversely, the use of moderate heat can prove more effective in some cases.[30]

This variability means that the choice of extraction method must be adapted to the objective (antioxidant, antimicrobial, anti-inflammatory). For example, the use of ethanol or a hydroethanolic mixture optimizes the extraction of many fat-soluble bioactive compounds, which has been confirmed in phytotherapy studies in Benin.[30]

3.4. Quantitative Analysis of Total Polyphenols

Figure 2 shows the total polyphenol content of extracts from three plant species, namely *Carica papaya*, *Momordica charantia*, and *Vernonia amygdalina*, obtained by different extraction methods. The contents are expressed as mg gallic acid equivalent per gram of extract (mg GAE/g). The results show significant variations depending on the plant species and the solvent used, reflecting the influence of the extraction methods on the recovery of phenolic compounds. The polyphenol contents of the extracts generally range from 2.1017 ± 0.0001 to 922.6565 ± 0.0001 mg GAE/g of dry extract.

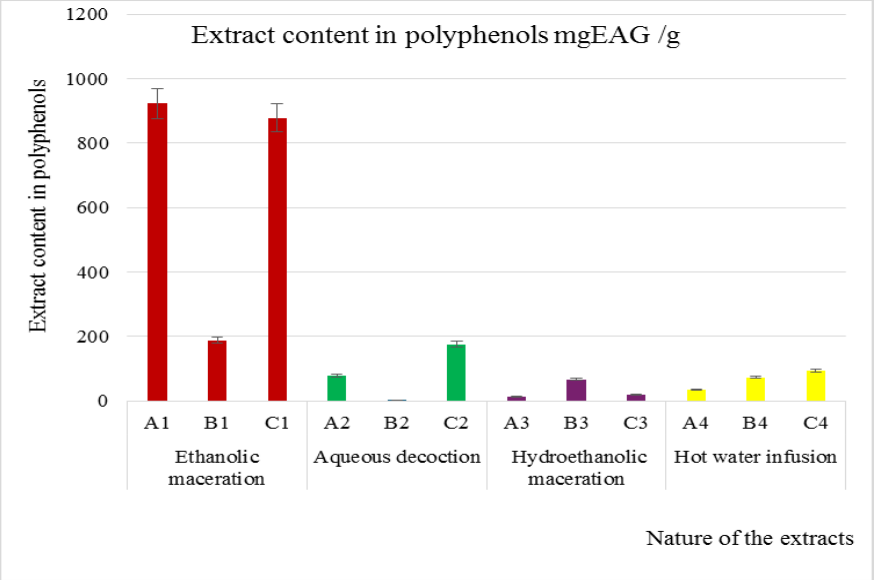

Figure 2: Polyphenol Content of Extracts.

Analysis of the results shows that, for Carica papaya, the ethanolic extract (A1) has the highest polyphenol content (922.6565 mg GAE/g), followed by the decoction (A2), the infusion (A4), and the hydroethanolic extract (A3). This effectiveness of ethanol is explained by its ability to solubilize phenolic compounds of intermediate polarity, as reported by Altemimi et al.[26]

A similar trend is observed in *Momordica charantia*, where the ethanolic extract (B1) predominates, while the decoction (B2) has the lowest content (2.1017 mg GAE/g). This decrease could be related to the thermal degradation of polyphenols, as heating conditions promote their oxidation.[32] In *Vernonia amygdalina*, the ethanolic extract (C1) remains the richest, confirming the effectiveness of organic solvents for polyphenol extraction, as previously reported in the literature.[33]

Overall, the ethanolic extracts (A1, B1, C1) exhibit the highest concentrations, with variability linked to the specific composition of the species.[33] In contrast, aqueous extracts (decoction, infusion) yield lower and variable results depending on the thermal stability of the compounds.

Thus, these results confirm the influence of the solvent, temperature, and plant species on polyphenol extraction, with ethanol appearing as the most efficient solvent.[26]

3.5. Quantitative analysis of flavonoids

Figure 3 shows the variation in total flavonoid content (mg Quercetin Equivalent/g extract) according to the extraction methods and plants studied. The values obtained during the assay of flavonoids vary overall between 161.13 and 345.02 mg EQ/g of dry extract when considering all fractions.

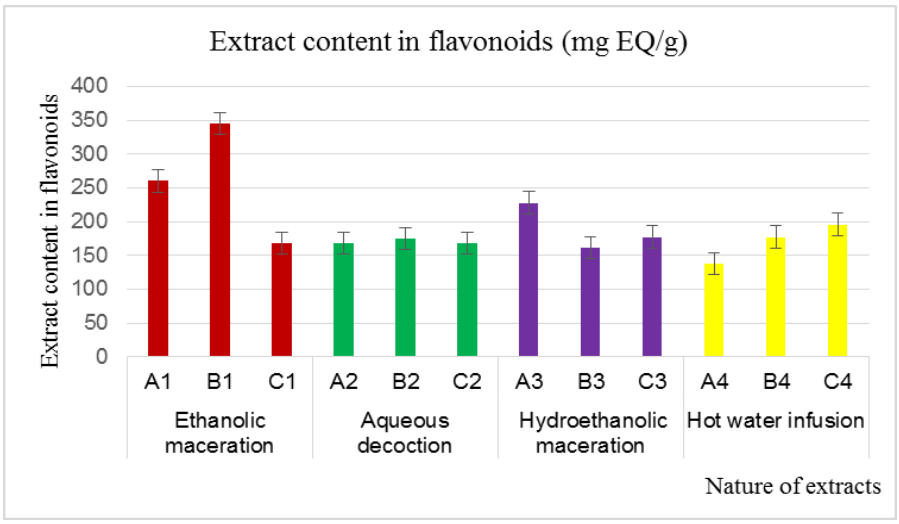

Figure 3: Flavonoid content of extracts.

Flavonoids are phenolic compounds associated with antioxidant, anti-inflammatory, and antimicrobial activities.[33] Analyzing each plant individually, we observe that in *Momordica charantia*, the ethanolic extract (B1) has the highest content (345.02 mg EQ/g), significantly higher than that obtained by infusion (177.22 mg EQ/g). For Carica papaya, the ethanolic maceration (A1: 259.97) is also richer than the other extracts, while in Vernonia amygdalina, the values remain more moderate, particularly for the decoction (C2: 168.03) and the hydroethanolic extract (C3: 176.99).

Considering the extraction methods, alcohol-based processes generally yield the highest concentrations, confirming the effectiveness of ethanol for extracting polyphenols due to its intermediate polarity.[11] Ethanol maceration thus appears to be the most efficient, unlike aqueous decoction, which gives lower concentrations (A2 and C2: 168.03), probably due to thermal degradation of flavonoids.[34] Hydroethanolic extracts show intermediate values, reflecting moderate efficiency of mixed solvents.[35] Finally, infusion shows low to medium concentrations (A4: 138.14; B4: 177.22), suggesting limited extraction or thermal alteration.[36]

3.6. Quantitative analysis of tannins

Figure 4 shows the tannin content of the different extracts of the three medicinal plants: *Carica papaya*, *Momordica charantia*, and *Vernonia amygdalina*. The concentrations are expressed in milligrams of catechin equivalent per gram of extract (mgEC/g). The results show that the tannin concentration varies according to the plant species and the extraction method used, highlighting the influence of solvent polarity and temperature on the extraction of these compounds.

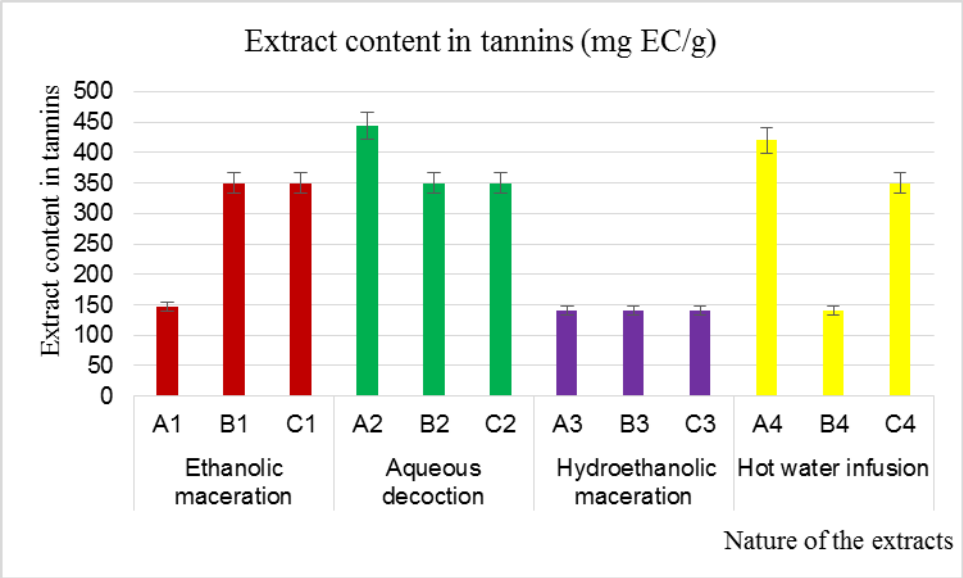

Figure 4: Concentrated Tannin Assay.

Analysis by species shows that, in *Carica papaya*, the aqueous decoction (A2) has the highest tannin content (443.661 mg EC/g), followed by the infusion (A4: 420.000 mg EC/g), while the ethanolic (A1: 147.880 mg EC/g) and hydroethanolic (A3: 140.000 mg EC/g) extracts have significantly lower content. This trend reflects the greater solubility of tannins in hot water, linked to their high polarity.[37] Furthermore, the increased temperature enhances the diffusion of the compounds and the disruption of cell walls, facilitating their extraction.[11]

In *Momordica charantia*, the ethanolic (B1) and decoction (B2) extracts have identical concentrations (350,000 mg EQ/g), while the hydroethanolic (B3) and infusion (B4) extracts have lower concentrations (140,000 mg EQ/g). This indicates that ethanol and hot water are effective extraction systems for this phenolic-rich species.[38]

For *Vernonia amygdalina*, the ethanolic, decoction, and infusion extracts (C1, C2, C4) have similar concentrations (350,000 mg EQ/g), confirming the good extractability of tannins, unlike the lower concentration of the hydroethanolic extract (C3).

Regarding the methods, the results show that processes using polar solvents, particularly hot water (decoction, infusion) and ethanol, are the most effective for extracting tannins. Hot water promotes extraction by thermal diffusion, while ethanol optimizes the solubilization of phenolic compounds of intermediate polarity. Conversely, the hydroethanolic mixture appears less effective under these experimental conditions, probably due to a less optimal polarity compromise. These results confirm the major influence of the solvent, temperature, and the chemical nature of the tannins on extraction efficiency.

IV- CONCLUSION

Carica papaya, *Momordica charantia*, and *Vernonia amygdalina* contain several phytochemical groups such as alkaloids, polyphenols, tannins, gallic tannins, and flavonoids, which are responsible for stimulating milk production. Indeed, traditional methods such as maceration, infusion, and decoction can be used to extract these phytochemical groups from medicinal plants. These methods have advantages and disadvantages depending on the type of active ingredient being sought. Furthermore, although aqueous decoction offers the highest extraction yields, maceration in ethanol appears to be the most relevant method for concentrating galactogenic principles.

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