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Review Article

Phytochemical Screening and Qualitative Analysis of Secondary Metabolites from *Ibervillea sonorae* Roots

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Abstract

This study aimed to qualitatively identify the main secondary metabolites present in the roots of *Ibervillea sonorae* (wereke) using colorimetric assays and thin-layer chromatography (TLC). Root samples were purchased from the Sonora Market in Mexico City. The powder obtained was characterized for its chemical composition and physical properties, including color. A 100% aqueous extract and a sequential extraction with solvents of increasing polarity (hexane and ethyl acetate, 1:18 g/mL), both assisted by ultrasound, were prepared. Extracts were concentrated using a rotary evaporator and subjected to qualitative phytochemical analysis. Proximate analysis revealed low moisture content (69.26 ± 3.53) and low carbohydrate content (19.38 ± 0.83). Colorimetric screening of the powder indicated the presence of phenols (moderate), flavonoids (low), and phytosterols (low). The aqueous extract showed abundant phenols and triterpenes, along with moderate flavonoids and phytosterols, whereas the sequential extract contained abundant saponins, flavonoids, and phytosterols [2]. TLC confirmed these observations, detecting moderate triterpenes and saponins in the aqueous extract, and abundant phytosterols in the sequential extract. Overall, solvents of different polarity enabled differential recovery of metabolites: aqueous extraction was more effective for phenols and triterpenes, while organic solvents favored saponins and phytosterols. These findings highlight *I. sonorae* as a potential source of functional compounds with possible therapeutic applications.

Keywords: *Ibervillea Sonorae*; Wereke; Root Extract; Secondary Metabolites; Qualitative Phytochemical Analysis

Introduction

Ibervillea sonorae (commonly known as wereke) is a dioecious perennial plant of the Cucurbitaceae family that thrives in the arid regions of northwestern Mexico, particularly in the states of Sonora, Sinaloa, and Baja California Sur. Traditionally, it has been used in folk medicine for the treatment of various ailments, especially for the management of type II diabetes mellitus [1,2]. Scientific interest in this species stems from its high content of secondary metabolites linked to relevant therapeutic effects. The root of I. sonorae contains phenols, flavonoids, triterpenes, saponins, and phytosterols, compounds that have demonstrated antioxidant, hypoglycemic, anti-inflammatory, and antimicrobial activities in diverse models [3]. These bioactives not only underpin the plant's medicinal value but also highlight its potential as a source of novel natural products with pharmacological applications. Despite its traditional

use and reported bioactivity, systematic studies on the occurrence and distribution of these compounds remain limited. Qualitative approaches, such as thin-layer chromatography (TLC) and targeted colorimetric assays, enable the identification of metabolite families based on their functional groups and reactivity to specific reagents. This study aims to qualitatively characterize the major secondary metabolites in I. sonorae root extracts obtained using solvents of varying polarity. The resulting phytochemical profile will expand scientific knowledge of this species and support its potential use in developing functional or therapeutic products of natural origin.

Materials and Methods

Materials

Fresh roots of *Ibervillea sonorae* were sourced from the Sonora Market in Mexico City, Mexico, and authenticated based on morphological characteristics. All chemicals and reagents used for the assays were of analytical grade or higher, ensuring compliance with standard laboratory quality requirements.

Preparation and pre-treatment of plant material

The roots were carefully selected and rinsed with potable water to remove debris and soil. They were oven-dried at 50 °C for 24 h, ground, and sieved to obtain a uniform powder. The powder was stored in sealed polyethylene bags within a desiccator to prevent moisture uptake and preserve its physicochemical properties [4].

Extraction procedures

Aqueous extraction

A 1:10 (w/v) suspension was subjected to ultrasound-assisted extraction (80 kHz, 30 min), followed by vacuum filtration through Whatman No. 1 paper. The filtrate was concentrated (40 °C, 60 rpm) using a rotary evaporator and stored at 4 °C until analysis [4,5].

Sequential extraction

Successive extractions were performed using a plant-to-solvent ratio of 1:10 (w/v), first with hexane and then with ethyl acetate. Each extraction involved ultrasound treatment (80 kHz, 30 min), vacuum filtration, concentration by rotary evaporation (40 °C, 60 rpm), and storage at 4 °C until analysis [4,5].

Color parameters and proximate chemical analysis

Powder color was assessed with a 3nh NR110® colorimeter using the CIELCH system, recording L* (lightness), C* (chroma), and h° (hue) for objective comparison between treatments [6]. Proximate analysis of *Ibervillea sonorae* root powder was also performed to determine moisture, protein, fat, fiber, ash, and carbohydrate contents, following AOAC procedures [7].

Identification of secondary metabolites

Colorimetric tests

The qualitative characterization of secondary metabolites in *Ibervillea sonorae* extracts was performed using classical colorimetric tests [8], commonly employed in preliminary phytochemical screening. Phenols were detected with ferric chloride reagent, which produces a dark brown color in their presence. Saponins were identified by the foam test, considering samples positive when a stable foam exceeding 5 mm formed after vigorous shaking. Flavonoids were detected using a 20% sodium hydroxide solution, where the development of an intense yellow color indicated a positive result. Triterpenoids were determined via the Liebermann-Burchardt test, in which reaction with acetic anhydride and concentrated sulfuric acid produces a characteristic purple color. Phytosterols were detected using the Salkowski test, confirmed by the formation of a red lower phase after adding concentrated sulfuric acid.

Thin layer chromatography (TLC)

Thin-layer chromatography (TLC) was employed to confirm the presence of secondary metabolites [9,10]. Concentrated extracts were applied to silica gel plates and developed using solvent systems selected according to compound polarity. The resulting bands were visualized under UV light at 254 and 356 nm, and their Rf values were calculated. This approach enabled the separation and observation of compounds including phenols, flavonoids, triterpenes, saponins, and phytosterols.

Statistical analysis

All analyses were conducted in triplicate, and results are presented as mean ± standard deviation. Data were subjected to one-way analysis of variance (ANOVA), with differences considered statistically significant at $p < 0.05$ [11].

Results and Discussion

Proximate chemical composition of *Ibervillea sonorae* root

Proximate analysis of the *Ibervillea sonorae* root showed a moisture content of 69.26 ± 3.53%, ash 8.86 ± 0.17%, protein 13.21 ± 0.03%, fat 2.55 ± 0.09%, fiber 25.50 ± 0.69%, and carbohydrates 19.38 ± 0.83% (Table 1). Compared to the results of Sinagawa-García., *et al.* [12], which reported higher moisture (80.03%) and markedly lower protein (2.10%) and fiber (2.65%) contents, these findings suggest that the root analyzed in the present study may provide a more substantial source of protein and dietary fiber, enhancing its potential nutritional and functional value.

Moisture	Ash	Proteins	Lipids	Fiber	Carbohydrates
69.26 ± 3.53	8.86 ± 0.17	13.21 ± 0.03	2.55 ± 0.09	25.50 ± 0.69	19.38 ± 0.83

Table 1: Proximate Chemical Composition (%) of *Ibervillea sonorae* Root.

The differences observed between the proximate composition obtained in this study and the values reported by Sinagawa-García., *et al*. [12] may be attributed to a combination of agronomic, biological, environmental, and analytical factors. In particular, the lower moisture content found in our samples has a concentrating effect on the remaining components, thereby contributing to the discrepancies. From a biological and environmental standpoint, genetic variation among populations of Ibervillea sonorae, together with soil characteristics and climatic conditions (e.g., soil type, fertility, and nitrogen availability), can strongly influence the allocation of carbon and nitrogen to different metabolic compounds. Additional sources of variability include the developmental stage of the plant, the season of harvest, and exposure to abiotic stresses such as drought. Under water deficit, for example, plants often reinforce structural components by synthesizing lignin and polysaccharides, which could account for the higher fiber content observed. Similarly, stress conditions combined with high soil nitrogen availability may stimulate the accumulation of nitrogen-rich compounds in the roots, potentially explaining the elevated protein levels in some samples. Overall, these findings suggest that both environmental pressures and genetic traits of individual populations contribute to the observed variation in fiber and protein content.

Sample	*L**	*C**	*h*°	ΔE	Color palette
Control	49.46 ± 0.02^{a}	12.34 ± 5.77^{c}	80.48 ± 0.05^{a}	0^{c}	#83735B
AE	41.20 ± 0.24^{b}	27.12 ± 0.02^{b}	75.99 ± 0.06^{b}	14.21 ± 0.15^{b}	#785C35
SE	16.06 ± 0.11^{c}	40.53 ± 0.91^{a}	55.75 ± 0.52^{c}	43.03 ± 0.40^{a}	#4C1700

Table 2: Color parameters of *Ibervillea sonorae* root samples.

Means with different superscript letters in the same column differ significantly ($p < 0.05$).

AE= Aqueous Extract

SE= Sequential Extract

Control= Powder

Color parameters

Significant differences were observed among the control powder, the aqueous extract (EA), and the successive extract (ES) of *Ibervillea sonorae* root (Table 2). The *L** parameter decreased progressively, from 49.46 in the control powder to 41.20 in the aqueous extract and reached its lowest value in the successive extract (16.06), indicating a marked darkening of the samples. In contrast, the *C** parameter showed a pronounced increase in color saturation in both extracts compared to the control. The aqueous extract exhibited a chroma value of 27.12, more than twice that of the control (12.34), while the successive extract displayed an even higher value (40.53), reflecting an intensified color. Regarding the

hue angle ($h°$), a progressive reduction was recorded: both the control and aqueous extract exhibited yellowish tones (75.99°), whereas the successive extract shifted towards more reddish-brown hues (55.75°). This trend suggests qualitative changes in the nature and concentration of the compounds responsible for color development.

In the aqueous extract, a significant decrease in luminosity (L^*) was observed, accompanied by an increase in chroma (C^*) and a notable change in hue angle ($h°$). These changes suggest a predominant extraction of water-soluble compounds with chromogenic properties, including simple phenols, glycosylated flavonoids, and condensed tannins. This type of metabolite not only has the ability to absorb light in the visible range, contributing to the darkening of the extract, but can also interact with proteins, polysaccharides, or mineral ions present in the plant matrix, promoting the formation of complexes that intensify the color and modify its hue. Furthermore, the presence of these water-soluble compounds may be related to the stability and intensity of the color during the handling and storage of the extract, thus reflecting their relevance in both phytochemical and functional terms.

On the other hand, the successive extract, obtained through an extraction sequence with solvents of different polarity (hexane followed by ethyl acetate), showed chromatic characteristics that suggest a more diverse and complex chemical composition. The higher presence of less polar compounds, such as terpenoids, lipophilic alkaloids, aglycone flavonoids and quinones, largely explains the differences observed in the color parameters. Many of these metabolites are inherently pigmented or undergo oxidation reactions during extraction, generating byproducts that contribute to intensifying the color of the extract. These chromatic modifications not only reflect the chemical composition of the extract, but also indicate a greater diversity and concentration of secondary metabolites with bioactive potential. Overall, the results show that the choice of solvent type and polarity used directly influences the color profile and the extraction capacity of bioactive compounds, providing valuable information on the chemistry and functional properties of plant extracts [13].

Identification of secondary metabolites

The colorimetric analyses (Table 3) revealed clear differences in metabolite profiles among the samples evaluated. The aqueous extract (AE) exhibited a markedly higher content of phenols, flavonoids, and triterpenes compared to both the powdered root and the sequential extract (SE). Conversely, the sequential extract was characterized by an abundant presence of saponins, flavonoids, phytosterols, and triterpenes, whereas the powdered sample showed only low levels of flavonoids and phytosterols, along with a moderate response for phenols, and a complete absence of other metabolites. These results underscore the role of solvent polarity in determining extraction efficiency: polar solvents such as water preferentially extract hydrophilic compounds with functional groups capable of forming hydrogen bonds, including phenols and flavonoids. This observation aligns with the findings of Hernández Díaz., *et al*. [14], who reported similar trends using ethanol and methanol in the extraction of plant metabolites, as these solvents share polarity characteristics with water.

The prominence of saponins and phytosterols in the sequential extract further confirms that less polar metabolites require additional fractionation steps to achieve efficient recovery. This pattern highlights the practical advantage of stepwise extraction strategies in maximizing the yield of bioactive compounds with varying polarities. Thin-layer chromatography (TLC) analyses corroborated and complemented the colorimetric findings, providing higher sensitivity and specificity for metabolite detection and confirming the distribution patterns observed among the different extracts.

Specifically, the sequential extract diluted in ethyl acetate (SE) exhibited a strong positive reaction for triterpenes, indicative of a high concentration of these lipophilic metabolites. This result is consistent with the use of nonpolar organic solvents, which favor the solubilization and extraction of hydrophobic compounds. In contrast, Alor Chávez [15] reported low triterpene concentrations in hydroalcoholic extracts of *Catharanthus*, suggesting that both the choice of solvent and the botanical source critically influence extraction efficiency.

Regarding saponins, the sequential extract showed an abundant response, in agreement with Nevarez Ramírez [16], who reported similar results using hydroethanolic subfractions of *Ibervillea sonorae*. This consistency supports the notion that saponins exhibit intermediate solubility and can be efficiently extracted in both hydroalcoholic and semipolar organic solvents. Meanwhile, the aque-

	Colorimetric assays			TLC		
Metabolites	**Powder**	**AE**	**SE**	**AAC**	**SE**	**SAE**
Phenols	++	+++	-	++	+++	+
Saponins	-	-	+++	++	++	+++
Flavonoids	+	++	+++	++	+++	+
Triterpenes	-	+++	+	++	++	+++
Phytosterols	+	++	+++	+	+	++

Table 3: Comparative Qualitative Analysis of Secondary Metabolites in *Ibervillea sonorae* Extracts.

Presence: Absent (-) Low (+) Moderate (++) Abundant (+++)

EA = Aqueous Extract

ES = Sequential Extract

AAC = Aqueous Extract diluted in Chloroform

AE = Aqueous Extract diluted in Ethyl Acetate

SAE = Sequential Extract diluted in Ethyl Acetate

ous extract demonstrated strong positive reactions for flavonoids and phenols, confirming the high abundance of polar metabolites. This behavior aligns with the observations of García Cruz [17] in aqueous extracts of *Randia aculeata*, highlighting a general tendency of these compounds to accumulate in polar media despite species-specific differences.

Finally, phytosterols were detected with high intensity in the sequential extract, in contrast with the low levels reported by Arias González [18] in lipid extracts of *Luffa cylindrica*. This discrepancy can be attributed to differences in plant matrix composition and extraction methodology. In this study, the use of successive organic solvent fractionations facilitated the efficient recovery of lipophilic metabolites, underscoring the importance of tailored extraction protocols to target specific classes of bioactive compounds.

Conclusions

The type of solvent employed in the extraction process had a marked influence on the phytochemical composition of *Ibervillea sonorae*, significantly affecting both the qualitative recovery of secondary metabolites and the observed colorimetric properties of the extracts. The aqueous extract demonstrated a pronounced affinity for polar compounds, particularly phenols and triterpenes, reflecting the capacity of water to solubilize hydrophilic metabolites. In contrast, the sequential extraction using solvents of increasing nonpolarity proved more effective in recovering less polar compounds, including saponins, flavonoids, and phytosterols, as confirmed through qualitative assessments and thin-layer chromatography analyses. The observed changes in color parameters-specifically the reduction in luminosity (L*) alongside increases in chroma (C*) and hue angle (h°)-correlated with higher concentrations of bioactive compounds in the sequential extract. These chromatic modifications suggest that metabolite composition not only influences the visual properties of the extracts but can also serve as an indirect indicator of their chemical richness. Collectively, these findings emphasize the critical role of solvent polarity in optimizing the extraction of diverse secondary metabolites from *Ibervillea sonorae*. The study demonstrates that strategic selection and sequencing of solvents can maximize the recovery of bioactive compounds with different chemical characteristics, thereby enhancing both the functional and analytical value of the extracts. Consequently, *Ibervillea sonorae* emerges as a promising source of phytochemicals with potential applications in pharmacology, nutraceuticals, and functional foods. Future studies focusing on quantitative analyses and bioactivity assays are warranted to further explore and harness the therapeutic potential of its metabolites.

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Conflict of Interest

The authors state that there are no conflicts of interest associated with this publication.

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