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Influence of Extraction Method and Solvent Selection on Phytochemical Composition of Melia azedarach Root and Bark

J.K. Ponsian[1*], K.P. Sibuga[1], I.S. Selemani[2]

[1]Department of Crop Science and Horticulture, Sokoine University of Agriculture, Morogoro, Tanzania.
[2]Department of Animal, Aquaculture and Range Sciences, Sokoine University of Agriculture, Morogoro, Tanzania.

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***Corresponding author**
J.K. Ponsian,
Department of Crop Science and Horticulture, Sokoine University of Agriculture, Morogoro, Tanzania
Email: kwesigajeruphina@gmail.com

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Abstract

Melia azederach (chinaberry tree) is one of the plants with high abundant in phytochemicals, that can be extracted, purified, and packaged for developing bioherbicides. Despite the reported effectiveness of chinaberry extracts in controlling invasive weeds such as Kongwa weed, its bioactive compounds remain understudied. A study was conducted using factorial arrangement in a Completely Randomized Design to evaluate the phytochemical composition of the root and barks of chinaberry tree through soxhlet and maceration extraction techniques using three different solvents; methanol, ethanol and distilled water. The extraction processes were carried out following standard extraction methods to analyses concentration of phytochemicals in the plants. Qualitative analysis confirmed the presence of alkaloids, flavonoids, phenolics, saponins, tannins, glycosides, terpenoids, and steroids. Ethanol was the most effective solvent for extracting a broad spectrum of phytochemicals based on qualitative assessment, particularly from bark samples. On the other hands, quantitative analysis indicated that methanol provided the highest overall extraction efficiency across multiple phytochemical classes, with notable effectiveness for saponins and terpenoids. Water showed superior performance in extracting alkaloids and flavonoids, especially when Soxhlet extraction was employed.

Maceration and Soxhlet extraction methods both yielded notable results, with ethanol-macerated bark exhibiting the highest levels of phenolics and tannins, and distilled water Soxhlet extraction yielding maximum flavonoid and alkaloid content. These findings confirm the potential of the chinaberry tree in on pesticide production. The study emphasizes the need for further research into compound isolation and bioactivity evaluation to harness the full therapeutic and industrial potential of this plant.

Keywords: *Melia azedarach*; phytochemical analysis; extraction methods; solvents; Kongwa weed.

Introduction

Melia azedarach commonly known as chinaberry tree, is a quick-growing deciduous tree with widespread distribution with several common names such as white cedar, pride of India, and Indian lilac (Ferreiro *et al*., 2010). This plant is a member of the Meliaceae family, native to tropical and subtropical regions and has been widely recognized for its diverse biological activities (Forrester *et al*., 2020).

Chinaberry tree is less utilized despite of its high potential for bioherbicide development (Pretorius and Watt, 2011). It is known to be rich in many bioactive components such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which can be derived in their specific parts such as leaves, flowers, bark, seeds, fruits, and root (Nxumalo *et al*., 2021; Yadav and Agarwala, 2011; Shah, 2010). These bioactive components are responsible for imparting pesticidal properties to the plant and are thus useful as a pesticide. Their composition and

concentration are affected by genetic factors, environmental conditions, geographic location, seasonal changes, growth stages, and post-harvest handling (Munuo, *et al.,* 2025).

The use of bioherbicides derived from natural sources, particularly from plants such as chinaberry tree, has become increasingly common due to their eco-friendly nature (Abdullah and Zahoor, 2023; Khursheed *et al*., 2022). These bioherbicides are biodegradable, pose minimal risks to non-target organisms, and have a lower risk of causing resistance development in weeds (Hasan *et al.,* 2021). Synthetic herbicides are reported to have detrimental effects to the environment such as contamination of soil and water bodies, harm to non-target organisms, disruption of ecosystems, and the proliferation of herbicide-resistant weed species has increased and become a public concern (Das *et al*., 2024; Abdullah and Zahoor, 2023). Therefore, environmentally friend bio-herbicides extracted from plants are highly recommended (Ustuner *et al*., 2020).

Chinaberry tree products have been found to be very effective as herbicides, fungicides, bactericides, and as bioactive compounds against various ailments (Maqsood *et al*., 2023; Mwalongo *et al*., 2020). As an insecticide, leaf aqueous and methanolic extracts have shown comparable effects against adult whiteflies. This discovery has suggested chinaberry tree as a possible source for controlling the sweet potato whitefly *Bamisia tabaci* (Al-Rubae, 2009). Also, the extracts have been found to be effective against different orders of destructive insects such as Coleoptera, Lepidoptera and Orthoptera (Carpinella *et al*., 2003; Chiffelle *et al*., 2009). The activity of ethanolic leaves, seed and fruit extracts from Chinaberry tree have been reported to control pathogenic fungus such as *Aspergillus flavus*, *Fusarium monitiform*, *Microsporum canis* and *Candida albicans* (Al-Rubae, 2009). Furthermore, leaf extracts of Chinaberry tree were found to be effective in managing weed species such as Kongwa weed as bio- herbicides ((Mwalongo *et al*., 2020).However, various studies have investigated the phytochemical composition of chinaberry tree, the focus has predominantly been on its leaves while roots, barks and flowers, in particular, have not been extensively studied for their chemical composition or medicinal potential, and comparative studies of the entire plant are scarce (Sultana *et al*., 2012).

Despite its potential as a natural herbicide, the effectiveness of chinaberry tree depends largely on the method used to extract its bioactive compounds, plant materials used and solvent polarity ((Akacha *et al.,* 2017; Sharma *et al*., 2021)Different extraction techniques yield varying concentrations and profiles of phytochemicals, influencing their bioactivity (Castro-López *et al*., 2017; Bhadange *et al*., 2024)Traditional methods such as maceration and soxhlet extraction are widely used for their simplicity and reliability (Bitwell *et al*., 2023; Gupta *et al.,* 2012).This study focused on evaluating the phytochemical contents of chinaberry root and bark by employing maceration and soxhlet extraction techniques with various solvents, in order to determine the most efficient combination of plant part, extraction method, and solvent for isolating its bioactive compounds."

Materials and Methods

Collection of Plant Samples

Fresh stem bark and root samples (including both tap and lateral roots) of the Chinaberry tree were collected from Njoge village in Kongwa District, located in central Tanzania (5° 57' 0" S and 36° 40' 59" E). This site was selected due to the high abundance and dominance of the Chinaberry tree in the area. The area falls within a semi-arid region characterized by rainfed agriculture, making it ideal for research on natural resources and sustainable practices.

Ten mature chinaberry plants were randomly selected from the study population based on morphological characteristics commonly associated with maturity, including plant height, stem diameter, and the presence of reproductive structures such as flowers or fruits. All plants meeting these maturity criteria were first identified within the study area, and from this subset, ten individuals were randomly selected using the lottery method to ensure unbiased sampling.

Bark samples were collected from the main trunk at breast height (approximately 1.3 meters above ground level), specifically from the outer bark layer after removing any loose debris or surface growth. Root samples were taken from lateral roots located near the soil surface, at a depth of approximately 10–20 cm and within a 1-meter radius of the trunk, ensuring that structural roots were sampled rather than fine feeder roots. From each plant, one root and one bark sample were obtained, resulting in a total of 20 individual samples (10 root and 10 bark).

To ensure consistency and prevent cross-contamination, all samples were collected using sterilized tools. Following collection, root and bark samples from the ten plants were separately pooled (bulk sampled) to create two homogenized composite samples, one for root and one for bark.

Preparation of Plant Extracts

Fresh bark and root samples of chinaberry tree were transported in an ice-packed cooler to the chemistry Laboratory of Sokoine University of Agriculture (SUA), Morogoro, Tanzania. The samples were air-dried in the shade for 7 days to prevent degradation of the bioactive compounds. After drying, the plant materials were ground into a fine powder using a mechanical blender. The powdered samples were sieved through a wire mesh with a pore size of 0.001 mm to ensure uniform particle size. The resulting powder was stored in airtight containers at 4°C to preserve its phytochemical content until further analysis.

Study Design

A factorial study arranged in a Completely Randomized Design (CRD) was conducted, comprising a total of 12 treatment combinations derived from three factors: Factor A (plant part: root and bark), Factor B (solvent type: methanol, ethanol, and distilled water), and Factor C (extraction technique: maceration and Soxhlet extraction). Each treatment combination was replicated three times, resulting in a total of 36 experimental units (12 combinations × 3 replicates). Randomization was carried out by assigning the 12 treatment combinations to the 36 experimental units using a random number table, ensuring that each treatment was randomly and evenly distributed across the experimental units to avoid bias."

Extraction Procedure

A laboratory analysis was done to evaluate the phytochemical content of chinaberry tree extracts using two extraction methods: maceration and soxhlet extraction. Ninety-two grams (92 g) of powdered bark and root) were each weighed and distributed into three labeled beakers. About 600 mL and 200 mL, of the methanol, ethanol and distilled water for the maceration and Soxhlet method was added respectively to each labeled beakers containing the root or bark samples, which were then sealed with aluminum foil to prevent solvent evaporation. Samples were kept at room temperature for 72 hours and at 60–70°C for 24 hours for maceration and Soxhlet method respectively. while in for maceration methods, mixtures were filtered through Whatman No. 1 filter paper, and the filtrates concentrated by evaporation to yield crude extracts, the soxhlet extracts were concentrated on a hot plate at 30–40°C, followed by solvent removal under reduced pressure using a rotary evaporator to preserve volatile and heat-sensitive phytochemicals.

Phytochemical Screening

The phytochemical screening of the plant's secondary metabolites was performed following established protocols as outlined in previous research (Youl *et al*., 2023; Alamgir and Alamgir, 2018; Morsy, 2014; Shaikh and Patil, 2020; Harbone, 1998)

Phytochemical Quantification

Quantitative analysis of key phytochemicals, including alkaloids, flavonoids, steroids, tannins, phenolics, glycosides, saponins, and terpenoids, was carried out using standard colorimetric and spectrophotometric methods. Alkaloid content was determined following the protocol of Alamgir and Alamgir (2018), while flavonoids were quantified using the method described by Shraim *et al*. (2021) and Ramos *et al.* (2017). Steroids were analyzed according to the procedure involving acetic anhydride and sulfuric acid-based colorimetric detection. Tannin content was measured using the egg albumen precipitation method followed by Folin-Ciocalteu analysis, as described by Makkar *et al*. (2017), Sowjanya *et al.* (2017), and Verma *et al*. (2012). Total phenolic content was estimated using the Folin-Ciocalteu reagent method as outlined by Madaan *et al*. (2011). Glycosides were quantified based on the lead acetate precipitation and Baljet reagent method reported by Morsy (2015). Saponins were determined using the vanillin-sulfuric acid method following the procedure of Makkar and Becker (1996), while terpenoids were measured using the linalool-based method as described by Edeoga *et al.* (2005). Results were expressed as milligram equivalents per gram of crude extract (mg/g).

Data Analysis

Statistical analyses were performed to evaluate the effects of solvent type, extraction method, and plant part. Analysis of variance (ANOVA) was conducted using Genstat statistical software to assess significant differences in phytochemical concentrations among the different solvents, plant parts, and extraction techniques. Where significant differences were detected, means were separated using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p < 0.05$.

Observational analysis was primarily employed to evaluate the intensity of reactions between various phytochemicals and reagents, categorizing the results as present (+), slightly present (++), heavily present (+++), or absent (-), based on color changes observed during laboratory testing.

Results

Phytochemical Composition of Chinaberry Tree Extracts

The phytochemical composition varied depending on the solvent and extraction method used (Table 1). Phytochemical analysis of the bark and root extracts revealed varying levels of phytochemical contents depending on the extraction method and solvent used. Alkaloids and flavonoids were largely absent across all samples, with only trace amounts of alkaloids detected in some bark extracts. Phenolic compounds were moderately presence, particularly in ethanol extracts of the bark and root. Saponins were present at low levels in ethanol and methanol extracts, but showed high levels in distilled water extracts, especially when Soxhlet extraction was used. Tannins were mostly absent except for strong presence in ethanol extracts of both bark and root. Glycosides were detected at low levels in several bark extracts, particularly with ethanol and methanol solvents. Terpenoids appeared predominantly in the bark, with the highest levels observed in ethanol maceration. Steroids were notably abundant, especially in ethanol and distilled water extracts of both bark and root, indicating a high presence of these compounds under maceration and Soxhlet extraction methods. Overall, ethanol proved to be the most effective solvent for extracting a broad range of phytochemicals, particularly from the bark samples.

Table 1: Phytochemical Composition of Bark and Root of Chinaberry Tree From Different Extraction Methods with Different Solvents

Extraction method	**Plant sample**	**Solvent used**	***Alkaloid***	**Flavonoid**	***Phenolics***	***Saponin***	***Tannin***	***Glycoside***	***Terpenoid***	***Steroids***
Maceration	Root	Methanol	-	-	-	-	-	-	+++	-
		Ethanol	-	-	-	-	-	-	-	+++
		DW	-	-	-	++	-	-	-	-
	Bark	Methanol	-	-	+	-	+	-	+	-
		Ethanol	-	-	++	+	+++	+	-	+++
		DW	-	-	-	+	-	-	-	+++
Soxhlet	Root	Methanol	-	-	-	-	-	-	-	+++
		Ethanol	-	-	+++	+	+++	-	-	+++
		DW	-	+	+	+++	-	-	-	-
	Bark	Methanol	+	-	-	+	-	+	-	-
		Ethanol	-	-	-	+	-	+	-	+++
		DW	+	-	+	+++	+	+	-	+
Method used			*Pew's test*	*Ferric Chloride test*	*Aqueous NaOH* test	*Foam Test*	*Salkows ki test*	*Burchard test:*	*Ellagic acid test*	*Meyer's test*

Note; + =Present, ++ =slightly present, +++ = Heavily present, - =Absent
DW means distilled water

Quantitative Analysis of Phytochemicals

Table 2 presents the concentrations of key phytochemicals based on extraction method, plant part, and solvent type. The quantitative analysis provided insights into the concentration of bioactive compounds across different sample of plant parts, extraction methods and solvents. A factorial analysis of variance (ANOVA) revealed statistically significant differences ($p < 0.001$) in the concentrations of all tested phytochemicals, including alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, tannins, and terpenoids. The analysis evaluated the effects of solvent type, extraction method, and plant part. The significance level was set at $p < 0.05$, and where significant effects were observed, treatment means were further compared using Tukey's Honestly Significant Difference (HSD) test.

Among plant parts, bark extracts consistently yielded higher concentrations of most phytochemicals, particularly phenolics (up to 31.42 mg/g), flavonoids (8.69 mg/g), and tannins (15.97 mg/g), indicating that the bark is a richer source of bioactive compounds than roots.

Regarding solvents, methanol generally performed best across various compound types. It was particularly effective in extracting saponins and terpenoids, while water excelled in extracting alkaloids and flavonoids, especially when used with soxhlet extraction. Ethanol extracted all phytochemicals in moderate concentrations, without outperforming methanol in any specific group. While ethanol was effective overall, its extraction efficiency was slightly lower than methanol, particularly for saponins and phenolics, indicating reduced solubility or interaction compared to methanol.

When comparing extraction methods, maceration yielded higher concentrations of phenolics and tannins, whereas Soxhlet extraction was more effective for alkaloids and flavonoids, particularly in water-based extractions.

Table 2: Phytochemical Composition Concentration by Extraction Method, Plant Part, And Solvent Type.

Method of extraction	Plant parts	Solvent used	Alkaloids	Flavonoids	Glycosides	Phenolic	Saponin	Steroids	Tannin	Terpenoids
Maceration	Barks	Ethanol	0.01ef	2.70f	1.20a	31.42a	0.24bcd	0.01bc	15.97a	0.34bcde
Maceration	Barks	Methanol	0.03de	7.22bcd	1.30a	30.88a	0.25bcd	0.03abc	14.28abc	0.57a
Maceration	Barks	Water	0.02def	8.13ab	1.30a	21.98d	0.26bcd	0.02abc	7.99e	0.52ab
Maceration	Roots	Ethanol	0.02def	5.478e	0.65cd	28.34bc	0.40ab	0.02bc	11.67d	0.19e
Maceration	Roots	Methanol	0.04b	3.03f	0.84bc	27.40bc	0.47a	0.03ab	12.07cd	0.43abcd
Maceration	Roots	Water	0.04bc	5.89de	0.83bc	10.35f	0.31abc	0.04a	5.37f	0.54ab
Soxhlet	Barks	Ethanol	0.02def	7.40abc	0.82bc	28.00bc	0.12d	0.02abc	13.00bcd	0.34bcde
Soxhlet	Barks	Methanol	0.02def	8.62ab	0.66c	26.00c	0.21cd	0.02bc	14.64ab	0.48abc
Soxhlet	Barks	Water	0.01f	8.69a	1.38a	29.13ab	0.19cd	0.01c	5.57f	0.38abcde
Soxhlet	Roots	Ethanol	0.03cd	3.02f	0.46d	12.49f	0.32abc	0.03abc	5.48f	0.24de
Soxhlet	Roots	Methanol	0.02def	5.14e	0.46d	16.50e	0.36abc	0.02bc	6.31ef	0.38abcde
Soxhlet	Roots	Water	0.22a	6.00cde	0.92a	22.69d	0.24bcd	0.02 abc	4.46f	0.29cde
P-Value			<.001	<.001	<.001	<.001	<.001	0.01	<.001	<.001
GM			0.04	5.94	0.90	23.77	0.28	0.02	9.73	0.39
SE(m)±			0.02	0.28	0.04	0.48	0.04	0.00	0.44	0.04
CV (%)			4.9	5.7	4.1	2.2	4.6	7.4	2.9	7.3

Discussion

Phytochemical analysis of chinaberry tree extracts confirmed the presence of diverse secondary metabolites, which are known to exhibit a broad spectrum of medicinal and physiological activities. These compounds have long been recognized for their biological functions, including antimicrobial, antiviral, and antifungal properties, thereby contributing to plant defense mechanisms (Pagare *et al*., 2015, Qureshi *et al*., 2021). Their presence provides a scientific basis for the traditional use of medicinal plants in ethnobotany and natural pest control.

The qualitative screening identified key classes of phytochemicals, including alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenols, and steroids. Quantitative analysis further revealed notable variations in the concentration and distribution of these compounds based on the plant part used (bark or root), the solvent (ethanol, methanol, or distilled water), and the extraction method (maceration or Soxhlet). These findings underscore the phytochemical richness and pesticidal potential of chinaberry tree as a natural source of bioactive compounds applicable in weed management.

Effects of Plant Part (Root and Bark) on Phytochemical Yield

Comparative analysis between bark and root extracts showed that bark contained higher concentrations of phytochemicals across most treatments regardless of the extraction method or solvent used. Bark extracts consistently yielded higher concentrations of most phytochemicals, particularly phenolics, flavonoids, and tannins. This enrichment may be attributed to the bark's protective role in the plant, where secondary metabolites tend to accumulate in higher quantities as a defense against environmental stressors and herbivory. This finding aligns with previous studies that reported greater secondary metabolite accumulation in bark tissues due to their protective role against abiotic stresses and biotic threats such as microbial invaders (Kumar *et al*., 2003; Sharma and Paul, 2013). The elevated phenolic, flavonoids and tannin content in bark extracts is particularly notable, which are very useful as well-documented for their antioxidant, antimicrobial, and pesticidal properties. For instance, several studies, including Anwar *et al*. (2021) and Balasundram *et al*. (2006) have reported that phenolic compounds exert phytotoxic effects by inhibiting nutrient absorption, disrupting respiration and photosynthesis, and impairing enzymatic functions in affected plant species. Therefore, phenolic compound extracted from chinaberry plants can be used to inhibit growth of noxious plants such as Kongwa weeds. In addition, tannins are known to have pesticidal effect in pest control. For example, a study by Petchidurai *et al*. (2023) and Divekar, *et al.,*2022, found that tannins inhibit larval development, reduce fecundity, and disrupt the growth cycle of various insect pests, thereby

suppressing their population in the environment. The less phytochemical extracts from roots suggesting a tissue-specific distribution of bioactive compounds which might be associated with low level of disturbance, which may lead to lower production or storage of secondary metabolites. This pattern aligns with previous findings that plant tissues with greater environmental exposure often exhibit higher concentrations of secondary metabolites as a protective adaptation (Pant *et al*.,2021)

Effect of Extraction Method (Soxhlet and Maceration) on Phytochemical Yield

When comparing extraction methods, maceration yielded higher concentrations of phenolics and tannins, whereas Soxhlet extraction was more effective for alkaloids and flavonoids, particularly in water-based extractions. The superior performance of maceration for phenolics and tannins may be attributed to its low-temperature conditions, which help preserve thermolabile compounds. This aligns with the study conducted by Ahmetović *et al*., (2025) who reported that maceration remains suitable for the extraction of thermosensitive compounds due to the mild nature of the process. Additionally, the method is, cost-effective, and does not require complex equipment, making it suitable for both traditional herbal preparations and low-resource research settings.

In contrast, Soxhlet extraction was more effective for alkaloids and flavonoids, particularly in water-based extractions. Flavonoids and alkaloids are notable for their pesticidal activities, with flavonoids known for their effective free radical scavenging and alkaloids widely recognized for their cytotoxic properties. This may be attributed to continuous cycling of heated solvent in Soxhlet apparatus, maintaining a strong concentration gradient that facilitates the diffusion of target compounds. This finding is supported by the study conducted by Kamarudin *et al*., (2016) and Azwanida (2015), who reported that Soxhlet extraction enhances flavonoid yield, likely due to the application of heat and continuous solvent circulation, which improve the extraction of thermally stable compounds. In addition, Soxhlet extraction requires a longer processing time and consumes a larger volume of solvent, making it more resource-intensive. These findings highlight the importance of selecting extraction methods based on the specific phytochemical groups, particularly in pesticidal applications, where maintaining the chemical stability and bioactivity of the compounds is critical to their effectiveness.

Influence of Solvent (Methanol, Ethanol, Distilled Water)

Qualitative analysis revealed that ethanol effectively extracted a broad spectrum of phytochemicals, underscoring its versatility as a solvent capable of targeting multiple compound classes. This versatility can be attributed to ethanol's intermediate polarity, which enables it to dissolve both polar and nonpolar substances efficiently. Ahmetović et al. (2025) similarly found that ethanol's balanced polarity contributes to its effectiveness in extracting diverse natural products. In contrast, methanol exhibited superior extraction of specific phytochemicals such as saponins, tannins, and terpenoids, while distilled water was most efficient at extracting alkaloids and flavonoids. These differences are likely due to the distinct polarity profiles and solvent properties of methanol and water, which facilitate the solubilization of highly polar compounds. This observation is consistent with established chemical principles, where solvents with higher polarity preferentially dissolve hydrophilic phytochemicals through mechanisms such as hydrogen bonding and dipole interactions, thereby enhancing the recovery of antioxidant and antimicrobial secondary metabolites (Sharma et al., 2021; Truong et al., 2019). Overall, these findings highlight the critical role of solvent polarity and chemical compatibility in optimizing extraction methods, with methanol and water being more suitable for isolating specific polar compounds, while ethanol provides broader extraction capabilities in qualitative assessments.

Distribution Of Other Phytochemicals

Other secondary metabolites, such as saponins, glycosides, terpenoids, and steroids, also exhibited variable concentrations across treatments. Saponin are known to exert anti-inflammatory and antimicrobial effects. In the current study, saponins were most abundant in macerated roots extracted with methanol (0.47 mg/g), followed by ethanol (0.40 mg/g), supporting previous findings that polar solvents are effective in extracting amphiphilic compounds (Sharma *et al*., 2021).

The highest terpenoid content (0.57 mg/g) was observed in methanol-extracted bark using maceration, which also aligned with qualitative findings. Terpenoids play a key role in the pesticidal activity of chinaberry tree, as they interfere with insect feeding, reproduction, and development, acting as natural insect growth regulators (Maqsood *et al*., 2023; Kar *et al.*, 2022).

Implications And Future Directions

The results of this study validate the pesticidal use of chinaberry tree in pest management such as weed and highlight its potential for the development of plant-based bioactive products. These findings emphasize the importance of optimizing extraction parameters such as solvent type, extraction method, and plant part selection to maximize phytochemical yield and biological efficacy.

Conclusion

This study demonstrates that chinaberry is a rich source of diverse phytochemicals with significant pesticidal potential. Both qualitative and quantitative analyses revealed the presence of key secondary metabolites such as phenolics, flavonoids, alkaloids, tannins, terpenoids, saponins, and

steroids across various plant parts and extraction treatments. Bark extracts, particularly those obtained using ethanol maceration and Soxhlet extraction with distilled water, consistently yielded higher concentrations of bioactive compounds, indicating that the bark is a more potential in phytochemical reservoir than the root.

The effectiveness of different solvents and extraction methods underscores the importance of optimizing these parameters to enhance the yield and efficacy of plant-derived compounds. These findings provide strong scientific support for the traditional use of chinaberry tree in weed management and highlight its potential for development into natural biopesticides, antimicrobial agents, and pharmaceutical formulations. Future research should focus on the isolation and structural elucidation of individual compounds, as well as the evaluation of their specific biological activities through targeted assays.

Author Contributions

J.P.K, K.P.S and I.S.S designed the study, J.P.K experimented, collected data, conducted data analysis, and drafted the manuscript. K. P.S. and I.S.S reviewed and edited the study concept. Final form of manuscript was approved by all authors.

Conflict of Interest

The authors declare no conflict of interest with the present research.

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