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Phytochemicals Screening and Antioxidant Potential of Ant Nest Plants From the Nabire Forest With Different Solvents

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ABSTRACT*: Ant nests (Myrmecodia pendans), characteristic epiphytic flora of Papua, are recognized for harboring diverse bioactive constituents, including phenolics, flavonoids, tannins, saponins, alkaloids, and triterpenoids, which contribute significantly to antioxidant and pharmacological properties. Nonetheless, the concentrations of these secondary metabolites exhibit inconsistency, primarily attributable to variations in solvent polarity and environmental factors in cultivation regions. This investigation sought to profile and characterize secondary metabolites from ant nests sourced from the Nabire forest, employing solvents of varying polarities—ethanol, methanol, and n-hexane—to evaluate the efficacy of bioactive compound extraction and antioxidant potential. Extraction procedures involved maceration over a 72-hour duration with the aforementioned solvents. The data obtained were then analyzed quantitatively descriptively with an average followed by a standard deviation. Phytochemical screening revealed that all three extracts contained phenolics, flavonoids, tannins, and triterpenoids; however, ethanol and methanol extracts demonstrated robust positive responses for saponins and alkaloids, whereas the n-hexane extract yielded solely triterpenoids and steroids. Quantitative assessments indicated that the 96% ethanol extract exhibited the highest total phenolic content (84.52 ± 2.31 mg GAE/g) and flavonoid content (61.47 ± 1.28 mg QE/g), coupled with the most potent antioxidant activity (IC_{50} = 78.65 ± 2.11 ppm). The methanol extract displayed moderate efficacy, while n-hexane showed the weakest performance. These findings underscore the substantial influence of solvent selection on the yield of active compounds. Consequently, ethanol is advocated as the optimal solvent for isolating phenolic and flavonoid compounds from Nabire ant nests. Future investigations are proposed, incorporating advanced extraction techniques such as ultrasonic-assisted methods or ohmic heating, alongside detailed profiling via LC-MS to pinpoint predominant bioactive entities.*

Keywords: *antioxidant activity, myrmecodia pendans; phytochemicals,; secondary metabolites; solvents;*

1. INTRODUCTION

Indonesia stands out as a nation exemplifying global megabiodiversity, characterized by its vast array of tropical vegetation and serving as a repository of secondary metabolites with applications in pharmaceutical, nutraceutical, and functional food industries. Among the species indigenous to Eastern Indonesia and possessing notable ethnopharmacological significance is *Myrmecodia pendans*, commonly referred to as the ant nest, an epiphytic organism that engages in a symbiotic association with ant communities and has been employed traditionally by Papuan populations to address diverse pathological states, encompassing inflammation, infectious ailments, and metabolic imbalances (Dirgantara et al., 2022). The pharmacological efficacy of this plant is linked to its repertoire of bioactive constituents, including phenolics, flavonoids, tannins, saponins, alkaloids, and triterpenoids (Ogbuagu et al., 2022), which contribute to mechanisms such as free radical scavenging, induction of apoptosis in neoplastic cells, and modulation of immunological responses (Thaeabteh et al., 2019). Consequently, M. pendans holds considerable potential as a reservoir of natural bioactive agents to facilitate the advancement of contemporary pharmaceuticals derived from biological resources.

Nonetheless, empirical investigations into the secondary metabolite profiles of M. pendans reveal considerable heterogeneity and an absence of uniform protocols, attributable to disparities in cultivation environments and the methodologies employed for compound isolation. Geographically, the Nabire forest biome in Papua exhibits distinctive ecological attributes, including elevated moisture levels, pronounced solar irradiance, and a diverse array of symbiotic host organisms, which diverge markedly from those observed in locales such as Jayawijaya or Biak. Such environmental parameters are poised to influence the biosynthetic pathways of secondary metabolites, thereby engendering a distinctive phytochemical signature that remains inadequately characterized in extant literature (Guerriero et al., 2018). The dearth of exhaustive data pertaining to the chemical variability of M. pendans originating from Nabire constitutes a pivotal lacuna in scientific understanding, particularly in light of this species' status as a vital component of indigenous biodiversity, poised to substantially bolster the advancement of domestically sourced pharmaceutical precursors.

From a methodological standpoint, the choice of extraction solvent constitutes a pivotal determinant in governing the spectrum of secondary metabolites amenable to dissolution and subsequent identification. Polar solvents, exemplified by methanol and ethanol, demonstrate superior efficacy in isolating hydrophilic constituents such as phenolic compounds and flavonoids, whereas nonpolar solvents like n-hexane prove more adept at sequestering lipophilic entities, including triterpenoids and steroids. (Tzanova et al., 2020). Such divergences in solvent polarity engender variability in the outcomes of phytochemical evaluations, thereby mandating a structured methodology to pinpoint the most efficacious solvent formulation for deriving a holistic chemical depiction. Additionally, the preponderance of antecedent research endeavors predominantly delivers qualitative findings, eschewing the delineation of molecular configurations for the pharmacologically active substances. (Najmi et al., 2022). This limits the understanding of the mechanisms of action and pharmacological potential of these bioactive compounds.

In this study, various quantitative tests were conducted to assess the levels and biological functions of secondary metabolites in M. extracts. The provided text lacks context or complete sentences, making the paraphrasing process difficult. Please provide more complete and clear sentences or text so that it can be paraphrased properly. Total phenolic (TPC) and total flavonoid (TFC) measurements were performed to estimate the amount of bioactive compounds

successfully extracted by each solvent type. Furthermore, IC_{50} values serve as a quantitative measure of antioxidant activity, allowing for evaluation of the extract's effectiveness in scavenging free radicals.

To gain more comprehensive insights, this study employed a comparative analysis of solvent effectiveness, which involved comparing extraction performance based on their degree of polarity. The analysis included comparing TPC, TFC, and IC_{50} data across various solvents, enabling the identification of the most efficient solvents for extracting bioactive secondary metabolites. This method emphasizes not only quantitative measurements but also provides a clear picture of the differences and variations in solvent capacity in isolating chemical substances from plants. By integrating quantitative measurements and comparative analysis, this study was able to generate more accurate data on the contribution of each solvent to the extract's composition and biological function. This will strengthen the scientific basis for determining the appropriate solvent choice for secondary metabolite extraction.

This study's suggested method combines structural identification and phytochemical screening with solvents of different polarity, specifically methanol, ethanol, and n-hexane. This approach makes it easier to gather thorough information on the secondary metabolite classes in M. pendans from Nabire. The most efficient solvent fractions for the extraction of particular active chemicals will be identified by means of methodical analysis in this study, which will also serve as a foundation for further extraction process optimization. This strategy will also help with the creation of standardized extracts as raw materials for phytopharmaceuticals, which is a significant obstacle in Indonesian natural product research (Khademi, 2024).

This research is innovative in a number of important ways. Initially, it employed M. pendans samples from the Nabire forest, an area with peculiar and little-studied ecological circumstances that may produce novel metabolite profiles that are different from those seen elsewhere. Second, it conducted a systematic comparison of the effects of polar and nonpolar solvents on the composition of secondary metabolites—a technique that has rarely been included in prior research. Third, it is anticipated that the findings will offer reliable preliminary information for the standardization of phytochemicals and form the basis for additional studies, including the separation of pure compounds, assessment of biological activity, and creation of natural medicines and functional foods. Thus, our study supports national efforts to promote medication discovery and the creation of health goods based on Indonesia's natural resources in addition to advancing our understanding of Nabire's biodiversity.

2. MATERIALS AND METHODS

- *Materials: A*nt nest from Nabire, destilated water, ethanol, methanol, n-hexane, $NaNO_2$, $AlCl_3$, NaOH, Gallic acid, Folin-Ciocalteu, HCl, Na_2CO_3, DPPH, chloroform, H_2SO_4, Mayer's, Wagner's, and Dragendorf reagents, Mg, $FeCl_3$.
- Tools: volume pipette, rotary evaporator, spectrophotometry, test tube, measuring cup, analytical balance, Erlenmeyer flask, measuring flask, beaker glass, vacuum pump,
- *Methods*: The ant nest plants used in this study originated from the Nabire forest. These plants were analyzed in the Chemistry and Food Analysis Laboratory of the Universitas Muhammadiyah Gresik. This study used phytochemical screening and secondary metabolite analysis of methanol, ethanol, and n-hexane extracts from the ant nest plants.

2.1.Ant nest plant preparation

The ant nest plants, which come from the Nabire forest in Papua, were employed because of their hollow stems. Based on factors such as being neither too young nor too old, having good or intact shape, being brown, and having wrinkled or unwrinkled

skin, the ant nest plants were chosen as samples. The texture of the ant nest plants was compressed.

2.2.Preparation of the sample

The intact ant nest tubers were washed, drained, and cleared of any physical soil. The water was drained, then dried and thinly cut. The sun was used for indirect drying. After that, they spent three hours being dried in a drying oven. After drying, the ant nest hollow stems were chopped into tiny pieces and blended into a fine powder. After being sieved, the final product was put in a sterile container (Prayitno & Utami, 2024).

2.3 Extraction of the ant's nest plant

A maceration vessel containing 400 g of the ant's nest plant was filled with the corresponding solvents, ethanol, methanol, and hexane. After that, it was placed in a water bath set at 40°C for 72 hours with a lid on. A rotary evaporator was then used to filter and evaporate the material, producing a thick or concentrated extract (Prayitno et al., 2025).

2.4.Phytochemical analysis of extracts

Phytochemical screening was carried out to identify several compounds, including flavonoids, terpenoids, tannins, steroids, alkaloid and saponins (Prayitno & Utami, 2024).

2.5.Total Flavonoid Analysis (TFC) (Loizzo & Sanches-silva, 2021).

Using a modified method, 1 ml of the extract sample was added to a test tube containing 4 ml of distilled water and 0.3 ml of 5% $NaNO_2$, homogenized, and then incubated for 5 minutes in order to quantify the total flavonoids in the ant nest plant extract. 0.3 ml of $AlCl_3$ was added after 5 minutes, and the mixture was incubated for 6 minutes. Next, 10 ml of distilled water and 2 ml of 1 M NaOH were added, and the mixture was homogenized. At 510 nm, the absorbance of the sample was measured. Quercetin concentrations of 20, 40, 60, 80, and 100 ppm were the standards that were employed. The formula for TFC was mg QE/g dry weight.

2.6.Total Phenol (TPC*)* (Loizzo & Sanches-silva, 2021)

Gallic acid was employed as a standard in TPC analysis. A modified Folin-Ciocalteu spectrophotometry method was employed in this analysis. The extract was serially diluted five times as part of the analytical process. A 10 ml volumetric flask was filled with a 0.4 ml sample. The Folin-Ciocalteu reagent (0.4 ml) was then added and mixed thoroughly. Four milliliters of 7% Na_2CO_3 were added after five minutes. To make a total volume of 10 ml, add distilled water and mix thoroughly. After that, incubate at 23°C for 90 minutes. The absorbance was measured with a UV-visible spectrophotometer at λ 760 nm. Gallic Acid standards with concentrations of 50, 100, 150, 200, and 250 ppm were employed. TPC was determined using the gallic acid calibration curve and expressed as mg GAE/g of the material's dry weight.

2.7.Antioxidant Activity (DPPH) (Seiquer & Palma, 2021)

The DPPH scavenging assay, with a few modifications, was used to evaluate the antioxidant activity of each plant extract under test. The DPPH technique was employed in the Antioxidant Activity (IC_{50}) test. 50, 100, 150, 200, and 250 ppm were the concentrations that were employed. After adding 1 milliliter of a 200 µM DPPH solution to 2 milliliters of the extract sample in a test tube, the mixture was homogenized and incubated for 30 minutes at 30°C. The wavelength at which the sample absorbance was measured was 517 nm. The proportion of DPPH scavenging activity was determined using the formula below:

$$\text{DPPH inhibition (\%)} = \frac{\text{AC-AS}}{\text{AC}} \text{ x } 100\% \quad (1)$$

AC : Absorbance of the control
AS : Absorbance of the sample

3. RESULTS AND DISCUSSION

Total phenols (TPC), total flavonoids (TFC), and total antioxidants by DPPH assay (ppm) are among the quantitative studies in this study, while qualitative analysis in the form of screening is the other. The bioactive chemicals found in ant nest extract differ depending on the solvent used, specifically methanol, ethanol, and n-hexane, according to the results of phytochemical screening. This discrepancy is directly tied to the polarity of the solvent, which dictates how well it can extract particular secondary metabolites.

Table 1. Results of phytochemical screening

Phytochemicals	Extract		
	MeOH	EtOH	N-hex
Phenols	+	+	-
Tannin	+	+	-
Saponin	+	+	-
Flavonoid	+	+	-
Steroid/Triterpenoid	-	-	+
Alkaloid:			
Meyer	+	+	-
Wagner	+	+	-
Dragendrof	+	+	-

MeOH: methanol; EtOH:Ethanol; N-hex: N-Hexane

3.1. Phenolic and Flavonoid Compound Content

Phenolic and flavonoid chemicals were detected in methanol and ethanol extracts, but not in n-hexane. Due to their polarity, phenolic and flavonoid chemicals are easily soluble in polar solvents like ethanol and methanol (El Mannoubi, 2023). Flavonoids are natural antioxidants that can stop lipid oxidation and stop oxidative stress-induced cell damage, while phenolic substances serve as electron donors that can lower free radicals (Usman et al., 2020). Thus, these results strengthen the indication that polar extracts have higher antioxidant potential than nonpolar extracts.

3.2. Tannin and Saponin Content

Only the methanol and ethanol extracts showed the presence of tannins and saponins; n-hexane did not. Polyphenolic substances called tannins have antibacterial and anti-inflammatory properties (Doughari & Saa-Aondo, 2021), whereas saponins can improve the permeability of microbial cell membranes and decrease cholesterol (Kholif, 2023). This demonstrates that polar solvents, especially ethanol, which is recognized to be more environmentally acceptable for use in food and medicine, may more readily extract these two chemicals.

3.3. Steroid/Triterpenoid Content

In contrast to polar molecules, n-hexane extract tested positive for steroid/triterpenoid chemicals in phytochemical assays, although methanol and ethanol did not. This is in line with these compounds nonpolar characteristics, which make them more soluble in nonpolar solvents like n-hexane (Roslizawaty et al., 2023). Triterpenoids and steroids have significant biological effects, including immunomodulatory, anti-inflammatory, and anticancer effects (Dembitsky et al., 2021).

3.4. Alkaloid Content

The methanol and ethanol extracts tested positive in alkaloid tests with Meyer, Wagner, and Dragendorff reagents, however n-hexane tested negative. This suggests that the alkaloids found in ant nests fall into the semi-polar to polar chemical category. Alkaloids are known to possess a number of biological properties, including antibacterial, anticancer, and analgesic properties (Yan et al., 2021). The existence of these compounds is shown by the colored precipitates that are produced when the nitrogenous organic bases of the alkaloids react with Meyer and Wagner reagents.

3.5. The Relationship between Solvent Polarity and Extraction Effectiveness

The three solvents varying patterns of phytochemical ingredient detection suggest that solvent polarity is a major factor in extraction selectivity. High-polarity substances like methanol and ethanol efficiently dissolve bioactive substances like

alkaloids, phenolics, flavonoids, saponins, and tannins. On the other hand, nonpolar n-hexane works better in removing lipophilic substances like triterpenoids and steroids. These findings are in line with studies that found that increased antioxidant activity was produced by extraction using polar solvents because of the higher amount of phenolic compounds (Aghoutane et al., 2023).

The highest overall phenol and flavonoid content and the best antioxidant activity (lowest IC_{50}) were obtained from the extraction process using 96% ethanol. This is because polar and semi-polar substances like flavonoids, tannins, and phenolics, all of which are prevalent in ant nests, can be dissolved by the ethanol solvent.

3.6. Total Phenol Content (TPC)

The extraction with ethanol yielded the highest total phenol concentration (84.52 ± 2.31 mg GAE/g), followed by methanol (76.34 ± 2.08 mg GAE/g) and n-hexane (18.27 ± 0.94 mg GAE/g). This discrepancy suggests that the solvent polarity has a significant impact on its capacity to extract phenolic chemicals. Because phenolic compounds have carboxyl (-COOH) and hydroxyl (-OH) groups, polar solvents like ethanol and methanol can more readily extract them (Autor et al., 2022).

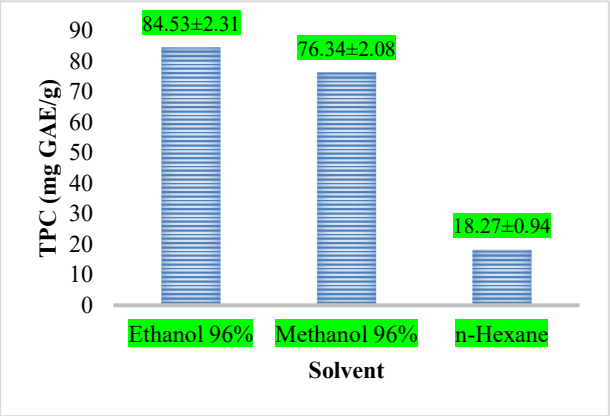

Fig 1. *Total Phenol Content (TPC)*

The total phenolic content (TPC) of the 96% ethanol extract was higher than that of the 96% methanol, even though both solvents are polar. This is because ethanol's moderate polarity better matches the semi-polar nature of phenolic compounds, thus improving solubility and the effectiveness of the extraction process. Ethanol is also better at penetrating cell walls and triggering tissue swelling, which ultimately accelerates the release of aromatic phenolic metabolites. Furthermore, 96% ethanol offers a perfect balance between water and solvent for the specific extraction of phenolic compounds without drawing out many non-phenolic components. The stability of phenolic compounds is also better in ethanol than in methanol, thus reducing the risk of damage during extraction. This combination of elements makes ethanol superior in extracting total phenolics, resulting in higher TPC values.

Because of its polarity and viscosity, which enable more stable solubilization of polar and semi-polar compounds during the extraction process, ethanol typically yields a higher phenolic content than methanol (Nisca & Tanase, 2025). Furthermore, ethanol exhibits greater selectivity for medium molecular weight phenolic chemicals, including catechol, gallic acid, and ferulic acid, which are prevalent in ant nests *(Myrmecodia pendans)* (Dirgantara et al., 2022). Only lipophilic substances, such as sterols, oils, and terpenoids, can be extracted by the non-polar solvent n-hexane, which lowers the amounts of phenol (Simões et al., 2022).

Compared to non-polar extracts, the nest extract with a polar solvent exhibited more biological activity and phenolic content (Mushtaq et al., 2021). Because ethanol is safer than methanol, it might be suggested as the best solvent for separating phenolic chemicals from natural materials, particularly for functional food and medicinal applications.

3.7.Total Flavonoid Content (TFC)

Ethanol produced the highest flavonoid concentration, measuring 61.47 ± 1.28 mg QE/g, followed by methanol at 55.83 ± 1.74 mg QE/g and n-hexane at 12.41 ± 0.53 mg QE/g, according to an analysis of the total flavonoid content. The data for total phenol

concentration and this pattern exhibit a similar tendency since flavonoids are derived from phenolic chemicals, which are likewise polar (Ayele et al., 2022). Flavonoids are soluble in polar solvents due to the presence of hydroxyl and carbonyl groups in their structure.

The difference in total flavonoid content (TFC) between 96% ethanol and 96% methanol, despite both being polar solvents, stems from variations in polarity, the solvent's capacity to penetrate plant structures, and the solvent's compatibility with the chemical characteristics of flavonoids. Ethanol has moderate polarity, making it more suited to the semi-polar nature of flavonoids, which can improve solubility and extraction efficiency compared to methanol, which has a higher polarity. Furthermore, ethanol is more effective in loosening cell walls, allowing for optimal flavonoid release. The low water content in 96% ethanol also results in more specific extraction conditions for flavonoids, while methanol tends to extract polar, non-flavonoid compounds, which reduces the effectiveness of flavonoid extraction. Consequently, although both are polar, differences in effective polarity, solvent diffusion capacity, and selectivity toward semi-polar compounds result in ethanol providing a higher TFC value than methanol.

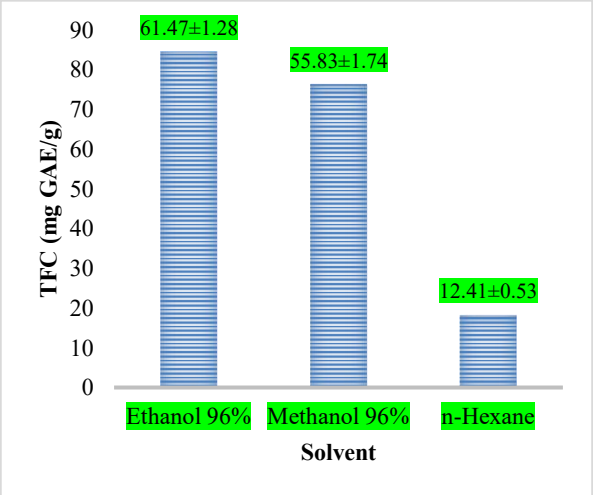

Fig 2. *Total Flavonoid Content (TFC)*

Solvent polarity, extraction temperature, and solvent-to-material ratio all affect how well flavonoids are extracted. (Ramesh et al., 2024). Because it can extract monomeric and glycosylated flavonoids without triggering heat degradation, ethanol produces the maximum yield (Antony & Farid, 2022). Additionally, during the extraction process, ethanol can stabilize the structure of flavonoids, particularly flavonols and flavanones, which are heat-sensitive (Dias et al., 2021).

Numerous flavonoids, including luteolin, quercetin, and apigenin, which contribute to antioxidant and antibacterial action, are found in ant nests (Dirgantara et al., 2022). Since these flavonoids often dissolve well in polar solvents, ethanol extracts contain the largest quantities of them (Yati et al., 2022). On the other hand, because the compound polarity is incompatible with n-hexane, extraction with this solvent results in extremely low quantities of flavonoids (Tzanova et al., 2020).

These findings thus support the use of ant nests as a possible natural source of bioactive flavonoids for development in functional food products and reinforce the fact that polar solvents like ethanol are more successful for extracting flavonoids from natural goods (Krisnaningsih et al., 2024).

3.8.Antioxidant Activity (DPPH, IC_{50})

Ant nest extracts' IC_{50} values revealed that ethanol had the highest antioxidant activity (78.65 ± 2.11 ppm), followed by methanol (92.11 ± 2.64 ppm) and n-hexane (310.26 ± 4.32 ppm). Stronger capacity to scavenge free radicals is indicated by a low IC_{50} value. These findings are consistent with the highest amounts of flavonoids and total phenols in ethanol, suggesting a positive relationship between antioxidant capability and phenolic compound content (Muflihah et al., 2021).

As reducing agents, phenolic and flavonoid chemicals neutralize DPPH free radicals by contributing hydrogen atoms or electrons (Parcheta et al., 2021). The DPPH method for determining antioxidant capacity

is based on these processes, which are referred to as hydrogen atom transfer (HAT) and single electron transfer (SET). It has been demonstrated that ethanol works best in dissolving potent phenolic substances with strong antioxidant potential, like gallic acid and catechins (Lohvina et al., 2022).

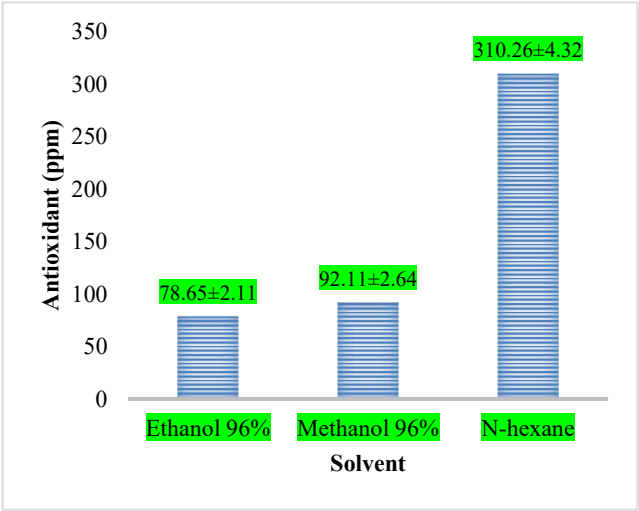

Fig 3. *Antioxidant capacity*

On the other hand, the n-hexane extract high IC_{50} value suggests that it has little antioxidant action. The low concentration of phenolic and flavonoid chemicals that were effectively extracted using non-polar solvents is the cause of this. Other lipophilic substances like tocopherols and carotenoids may be present in the non-polar fraction, but their concentrations are minimal and their contribution to overall antioxidant activity is negligible (Dirgantara et al., 2022).

Antioxidant activity and phenolic and flavonoid concentration in different Indonesian medicinal plant extracts have a linear relationship (Manuhara et al., 2022). As a result, ethanol solvent is thought to be the best option for extracting high levels of antioxidant activity from ant nests. It may also find use as a functional element in natural supplement formulations or as a way to reduce oxidative stress.

4. CONCLUSIONS AND RECOMMENDATIONS

Studies on the impact of solvent type on the antioxidant activity, total phenol, and total flavonoid content of ant nest (*Myrmecodia pendans*) extracts demonstrate that solvent type is a key factor in determining how well bioactive chemicals are extracted. Compared to methanol and n-hexane, ethanol had the highest total phenol content (84.52 ± 2.31 mg GAE/g) and total flavonoid content (61.47 ± 1.28 mg QE/g), as well as a greater antioxidant activity (IC_{50} value of 78.65 ± 2.11 ppm).

The solubility of bioactive chemicals varies depending on the polarity of the solvent. Because they are polar, ethanol and methanol dissolve phenolic and flavonoid molecules better than non-polar n-hexane. Total phenol and flavonoid content and antioxidant activity have a positive connection, suggesting that these two chemical groups play a significant role in the ant nest extracts' capacity to scavenge free radicals.

Optimizing the extraction parameters (material to solvent ratio, temperature, time, and extraction techniques like ohmic heating extraction or ultrasonic-assisted extraction) is advised for future research in order to increase the recovery of phenolic compounds without compromising their stability. Additionally, additional characterization by chromatographic analysis (HPLC or LC-MS) is advised in order to determine the primary active compounds that are responsible for antioxidant activity.

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