Comparative evaluation of methanol and water extracts: antioxidant activity and total phenolic content of ten papuan medicinal plants

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Abstract. The use of Papuan medicinal plants as traditional medicine is widespread, but comprehensive and systematic research on their antioxidant activity and phenolic content remains limited. This study aimed to compare the antioxidant activity and total phenolic content (TPC) of 10 medicinal plant species traditionally used in Manokwari, Indonesia. Antioxidant activity was determined using the Ferric Thiocyanate (FTC) method, whereas TPC was quantified using the Folin–Ciocalteu assay in both aqueous and methanolic extracts. Multivariate analysis for the plants' phenolic content or antioxidant activity clustering was performed using principal component analysis (PCA). The methanolic extract of *Myrmecodia jack* exhibited the highest antioxidant activity (97.30%), while the aqueous extract of *Persea americana* showed a comparable potency (96.29%). The highest TPC was recorded in *Areca catechu L.* Statistical analysis revealed that the phenolic content accounted for 83.2% of the antioxidant activity in methanolic extracts and 59.6% in aqueous extracts. The TPC was markedly higher in the methanolic extracts. PCA classified samples into three distinct clusters: high phenolic, high antioxidant, and mixed profiles. These findings highlight the critical role of solvent polarity in bioactive compound recovery and the promising antioxidant potential of Papuan medicinal plants for natural product development.

1 Introduction

Climate change and modern lifestyle patterns have intensified global health challenges, underscoring the urgent need for more effective and sustainable health interventions [1]. Rising pollution levels, which increase human exposure to environmental contaminants, combined with unbalanced modern dietary habits, have made the human body increasingly vulnerable to oxidative stress. This physiological disruption is a critical factor in the development and progression of numerous chronic diseases globally [1]. Within this framework, natural antioxidants have gained considerable attention as an auspicious therapeutic approach because of their remarkable capacity to mitigate oxidative stress, one of the primary etiological factors underlying chronic and degenerative conditions, including diabetes, cancer, cardiovascular disease, and neurodegenerative diseases [2]. Bioactive natural compounds, namely polyphenols, flavonoids, carotenoids, and vitamins, contribute to protective functions by engaging multiple mechanisms of action. These actions include direct neutralization of Reactive Oxygen Species (ROS) to safeguard cellular integrity [3], chelation of pro-oxidative transition metals to limit their participation in harmful reactions, and upregulation of endogenous antioxidant enzymes, as demonstrated by studies on pea peptides [4]. Furthermore, natural antioxidants are currently undergoing intensive therapeutic research because of their potential in managing other health concerns, such as skin disorders, obesity, and metabolic syndrome [5]. For example, turmeric (*Curcuma longa* L.) supplementation has been shown to confer protective effects against metabolic syndrome by strengthening the body's native antioxidant defense mechanisms [6].

The persistent demand for effective health interventions has led to the search for natural antioxidant sources from plant matrices, a global research imperative. This interest is driven not only by their significant therapeutic health benefits but also by their extensive applications across a range of industrial sectors, notable the food [7] and cosmetics industries [8]. Indonesia's Papua region, recognized globally as a biodiversity hotspot, is a crucial area for bioprospecting. This area hosts numerous medicinal plant species with vast potential to yield novel natural antioxidant compounds; however, the majority of its phytochemical wealth remains scientifically unexamined [9].

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In line with this untapped potential, initial phytochemical screening efforts have validated the ethnobotanical use of this region. Several Papuan species, including *Breyniacernua*, *Villebrunearubescens*, *Myrmecodiabeccarii*, *Dodonaeaviscosa*, and *Bridelia spp.*, have demonstrated significant antioxidant capabilities, as measured by DPPH and CUPRAC assays. Notably, the ethyl acetate extract of *Myrmecodiabeccarii* (sarang semut) exhibited superior antioxidant activity, which strongly correlates with its high total flavonoid and phenolic contents [10].

Various studies have unequivocally demonstrated that both the choice of solvent and extraction conditions critically determine the yield and efficacy of phenolic compound recovery, as well as their associated antioxidant activity. Among the various solvents employed, methanol and ethanol, particularly at aqueous concentrations between 30% and 60%**,** have consistently been identified as optimal solvents for the efficient extraction of phenolic compounds and antioxidant constituents from a wide range of plant species. Conversely, solvents such as acetone and ethyl acetate, although occasionally utilized, typically exhibit inferior extraction efficiencies and are considered less effective in maximizing the recovery of these bioactive compounds [11].

Furthermore, a strong and well-established correlation has been demonstrated between TPC and antioxidant activity in diverse plant extracts, where an increase in phenolic concentration typically leads to enhanced antioxidant activity [11]. However, despite this universally recognized relationship, systematic comparative studies of Papuan medicinal plants, particularly those used by local communities in the Manokwari Regency, remain severely limited. Specifically, there is a lack of research that comprehensively compares and evaluates the TPC and antioxidant activity of diverse medicinal species used in ethnomedicine in this region. This critical limitation raises fundamental methodological questions regarding the optimal extraction conditions: whether there are significant differences in TPC and antioxidant activity between methanol and water extracts, and consequently, which indigenous Manokwari species possess the highest potential for further pharmaceutical development.

Therefore, this study was designed to conduct a systematic comparative evaluation of the TPC and antioxidant activity of ten selected medicinal plant species used in ethnomedicinal practices in Manokwari, West Papua, Indonesia. This study employed a comparative experimental approach in which antioxidant activity was assessed using the FTC assay and TPC was measured using the Folin–Ciocalteu method. By directly comparing methanol and water extracts, this investigation will not only contribute to the sustainable utilization of Papuan biodiversity but also establish a robust scientific foundation for future *bioprospecting* efforts and product formulation development, ultimately facilitating the creation of effective, safe, and sustainable natural health interventions.

2 Materials and Methods

2.1 Chemical

The study utilized distilled water, 80% (v/v) methanol, and a 0.1 M phosphate buffer (pH 7.0), 30% m/v ammonium thiocyanate, 50 mM linoleic acid dissolved in 75% ethanol, 20 mM $FeCl_2$ prepared in 3.5% HCl, Folin–Ciocalteu reagent, gallic acid standards at concentrations of 25, 50, 100, 150, and 200 mg/L, and sodium carbonate. All reagents and chemicals used in this study were of analytical-grade purity and procured from Merck (Darmstadt, Germany).

2.2 Sample collection

All plant materials analyzed in this study were obtained from the Arboretum of the University of Papua and the Gunung Meja Nature Tourism Park, in Manokwari, West Papua, Indonesia. The selected medicinal species are those traditionally used by indigenous communities of Manokwari for therapeutic purposes. Species identification was carried out by Mr. Marthen Jitmau, a taxonomist at the Herbarium of the University of Papua. Comprehensive information on the species and the specific plant parts analyzed is presented in **Table 1**.

Table 1. List of plant samples used in the study

Scientific Name (Family)	Plant Part Used	Local/General Name
Zingiber officinale Roxb.	Rhizome	Ginger
Piper betle L.	Leaf	Sirih
Areca catechu L.	Fruit	Pinang
Persea americana Mill.	Leaf	Avocado
Syzygiumpolyantum (Wight) Walp.	Leaf	Salam leaf
Ocimum citriodorum Vis.	Leaf	Kemangi
*Myrmecodia*jack	Stem	Sarang semut
Abelmoschus manihot (L.) Medik.	Leaf	Gedi
Pseuderanthemumscu tellarioides (L.) Benth.	Leaf	Mayana
Ficus septica Burm. f.	Leaf	Awar-awar

2.2 Extract preparation and extraction

The method was carried out following the protocol described in [12], with certain modifications. The plant materials were first dried in a Memmert UN110 oven at 40 °C for 24 h, then ground into a fine, homogeneous powder using a Waring laboratory blender. The powdered samples were then extracted with distilled water and 80% (v/v) methanol. Aqueous extraction was performed by heating 0.500 g of powdered sample with 10 mL of distilled water to boiling for 30 min. For the methanolic extract, 0.500 g of powder was mixed with 10 mL of 80% methanol and incubated at 40°C for 24 h. After extraction, the samples were cooled to room temperature and then centrifuged at 4500 rpm for 15 min. The resulting supernatants were collected and used to determine antioxidant activity and TPC, and all assays were conducted in triplicate.

It should be clarified that the extraction procedures employed in this study represent two distinct extraction strategies rather than a direct solvent-only comparison. The aqueous extraction method reflects the traditional preparation approach commonly practiced by local communities, involving hot water boiling. In contrast, methanolic extraction is a modern laboratory-based protocol designed to optimize the recovery of phenolic compounds under controlled conditions. Consequently, differences in the extraction temperature and duration may influence compound stability and extraction selectivity beyond solvent polarity alone.

2.4 Determination of TPC

TPC was quantified by the Folin–Ciocalteu colorimetric method [13]. A 200 µL aliquot of the sample solution, previously diluted at a ratio of 1:10, was combined with 1 mL of the Folin–Ciocalteu reagent was diluted at a ratio of 1:10 and incubated for 4 min. Subsequently, 800 µL of sodium carbonate solution (75 g/L) was added, and the mixture was incubated at room temperature for 2 h. The absorbance was measured at 765 nm using a UV–vis spectrophotometer, and TPC was calculated from a gallic acid calibration curve (25–200 mg/L) prepared under identical conditions. Each determination was carried out in triplicate, and data are presented as mean values. The TPC value was calculated from absorbance measurements and quantified as mg GAE per gram of dried plant material.

2.5 Antioxidant activity assay (FTC method)

The antioxidant capacity of the samples was evaluated using the Ferric Thiocyanate (FTC) assay, following the protocol described previously [14]. In brief, 1 mL of the sample supernatant was combined with 1 mL of 0.1 M phosphate buffer (pH 7.0), 0.1 mL of distilled water, and 1 mL of 50 mM linoleic acid prepared in 99.5% ethanol. The resulting mixture was incubated at 37°C for six days. Aliquots (50 µL) were withdrawn and mixed with 6 mL of 75% ethanol, followed by the sequential addition of 50 µL of 30% ammonium thiocyanate and 50 µL of 20 mM $FeCl_2$ prepared in 3.5% HCl. The mixture was incubated for 3 min, after which the absorbance was measured at 500 nm using a HACH DR2500 VIS spectrophotometer. Control samples were prepared under identical conditions, with distilled water (DW) substituted for the plant extract. Antioxidant activity was expressed as the percentage inhibition of linoleic acid oxidation relative to the control. The inhibition percentage was calculated by dividing the difference between the slopes of the control and sample by the slope of the control and multiplying the result by 100%. The slopes were obtained from the linear regression of absorbance versus incubation time, with calculations performed according to the following equation: antioxidant activity (%) = $\{(slope_c - slope_s)/slope_c\}\ x\ 100\%$ (1)

2.6 Statistical analysis

Statistical analyses were performed to assess the antioxidant activity and TPC of the medicinal plant species. Data handling and visualization were performed using Microsoft Excel and RStudio. Comparative charts were created in Excel to compare the methanolic and aqueous extracts, and means with standard deviations were computed for each sample. The coefficient of determination (R^2) was calculated to quantify the relationship between the TPC and antioxidant activity.

Additional analyses were conducted using RStudio software. Paired t-tests were employed to evaluate significant differences in phenolic content and antioxidant activity between methanolic and aqueous extracts. Correlation analyses were used to examine the association between phenolic levels and antioxidant capacity. Principal Component Analysis (PCA) was used to cluster plant samples based on their phenolic and antioxidant profiles, and heatmaps were generated to identify groups with similar biochemical traits.

All statistical tests were performed using a significance threshold of $p < 0.05$. Collectively, these analyses clarify how solvent polarity and species-level variation influence the antioxidant potential of Papuan medicinal plants.

3 Results and Discussion

3.1 TPC and Antioxidant Activity of Methanolic and Aqueous Extracts of Ten Medicinal Plant Species from Manokwari

Aqueous extracts demonstrated the highest levels of TPC, with *Areca catechu* exhibiting the highest TPC in both aqueous and 80% methanol extracts. However, the highest antioxidant activity was not recorded in *A. catechu*, but rather in the methanolic extract of *Myrmecodia jack*, which showed a strong antioxidant capacity of 97.30%. This value was not significantly different from that of the aqueous extract of *Persea americana* (96.29%), which also exhibited strong antioxidant activity. Overall, the methanolic extracts tended to yield higher TPC values than the aqueous extracts, indicating that methanol is generally more efficient in extracting phenolic compounds.

However, this comparison should be interpreted within the context of extraction traditions and purposes. The aqueous extraction method used in this study is a traditional preparation method commonly employed by local communities in Manokwari, in which plant materials are boiled in water. This approach reflects real-world ethnobotanical usage, in which thermal treatment may facilitate the release of heat-stable phenolic compounds and other water-soluble antioxidants, while potentially degrading thermolabile constituents. In contrast, methanolic extraction is a modern laboratory-based protocol designed to maximize the recovery of phenolic compounds under more controlled and milder thermal conditions. Therefore, the observed differences between aqueous and methanolic extracts reflect not only solvent polarity, but also fundamental differences between traditional and modern extraction strategies.

The capacity of methanol and water to extract phenolic compounds associated with antioxidant activity differed significantly ($p < 0.10$). However, higher phenolic content does not always correspond to higher antioxidant activity. The analysis further showed no statistically significant difference in antioxidant activity between methanolic and aqueous extracts across the ten medicinal plant species from Manokwari. However, the methanol extracts exhibited slightly higher mean antioxidant activity values.

These findings suggest that their TPC does not exclusively govern the antioxidant activity observed in aqueous extracts but may also be influenced by the presence of other non-phenolic constituents, such as carotenoids, alkaloids, and ascorbic acid. The association between TPC and antioxidant activity is not inherently linear, as both the qualitative and quantitative profiles of bioactive compounds present in each extract strongly influence it. The TPC and antioxidant activity of the aqueous and methanolic extracts are shown in Figure 1**.**

The higher TPC observed in the 80% methanol extract than in the aqueous extract can be attributed to differences in solvent properties and their interactions with phenolic compounds. Although methanol is a polar solvent, its polarity differs from that of water, enabling it to solubilize a broader spectrum of phenolic compounds, including polar and moderately nonpolar constituents. Moreover, methanol exhibits superior penetration into plant tissues, facilitating the disruption of cell walls and the release of phenolic compounds that are less accessible to water. This combination of high solubility and enhanced tissue penetration makes methanol more efficient at extracting phenolic compounds, yielding higher TPC and demonstrating a strong correlation with antioxidant activity.

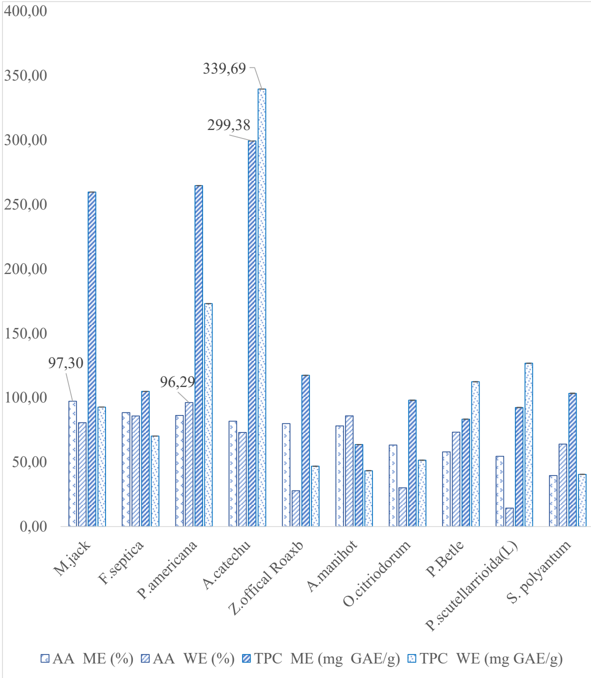

Fig. 1. TPC and antioxidant activity (AA) of methanolic and aqueous extracts of ten medicinal plants traditionally utilized in Manokwari.

Figure 2 illustrates the correlation between antioxidant activity and TPC across the medicinal plant species. A significant difference was evident between aqueous and methanolic extracts. In the methanolic extract, TPC exhibited a strong positive correlation with antioxidant activity, as reflected by the high coefficient of determination (R^2=0.832), suggesting that approximately 83.20% of antioxidant activity can be explained by the TPC. Conversely, the aqueous extract displayed a weaker correlation ($R^2 = 0.596$), indicating that only 59.60% of the antioxidant activity was attributable to the phenolic compounds. This implies that, in aqueous extracts, antioxidant activity may also be modulated by non-phenolic constituents such as carotenoids, ascorbic acid, and alkaloids. Pearson correlation analysis further supported these findings: the methanolic extract showed a statistically significant correlation ($r = 0.744$, $p = 0.013 < 0.05$), whereas the aqueous extract showed a weaker, non-significant relationship ($r = 0.477$, $p = 0.164 > 0.05$). Collectively, these results suggest that their phenolic constituents mainly determine the antioxidant potential of methanolic extracts. In contrast, other molecular classes contribute substantially to aqueous systems. Consequently, it can be inferred that the extraction of phenolic compounds is markedly more efficient using 80% methanol than water, as methanol facilitates greater solubilization of phenolics, thereby yielding extracts with enhanced and more consistent antioxidant activity.

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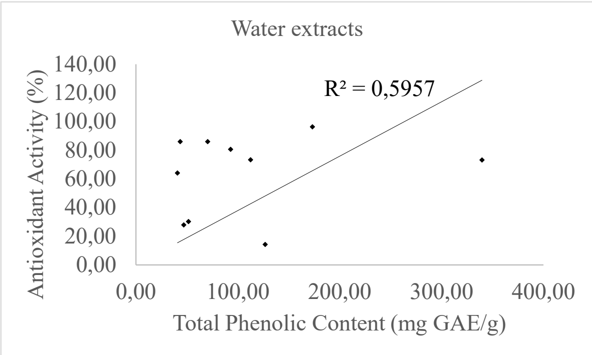
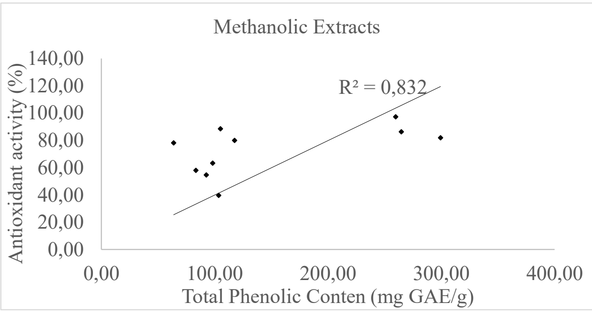


(a)



(b)

Fig. 2. Correlation between TPC and antioxidant activity in water extract (a) and Methanolic extracts of Medicinal Plants

3.2 Pattern of Relationship and Cluster Distribution of Ten Manokwari Medicinal Plants Based on TPC and Antioxidant Activity

As shown in Figure 3, based on the PCA biplot visualization, the relationships between the variables and the distribution of plant samples can be easily understood. The vectors that represent methanol and water as solvents for antioxidant activity were positioned close to each other, indicating a strong positive correlation between the two variables. In contrast, the vectors that represent methanol and water in the extraction of TPC were oriented somewhat opposite to the antioxidant vectors, suggesting that phenolic content does not always directly correspond to antioxidant activity. The angles between the vectors further reflected the strength of the correlations, with smaller angles indicating stronger relationships.

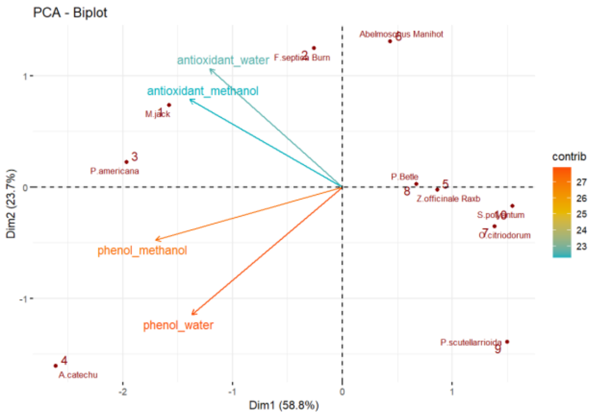
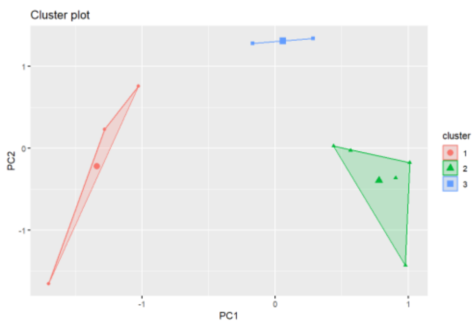
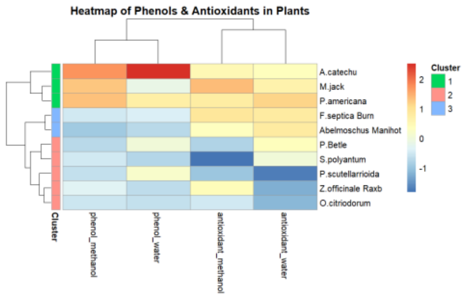


Fig. 3. PCA Biplot visualization

The arrangement of plants in the biplot indicated that species situated near the methanol and water vectors for antioxidant activity generally showed greater antioxidant activity. Conversely, the TPC had a greater impact on those closer to the phenolic extraction vectors. Plants positioned on the opposite side of the biplot displayed characteristics markedly different from those of the other groups. PCA successfully distinguished the plants based on their phenolic content or antioxidant activity, enabling their classification into three clusters: Cluster 1 (high antioxidant activity), Cluster 2 (high phenolic content), and a mixed cluster representing plants influenced by both factors (Figure 4). The combination of PC1 (58.8%) and PC2 (23.7%) accounted for 82.5% of the variance, offering a strong representation of the relationships between variables and plant samples. The biplot shows that the link between TPC and antioxidant activity is not always linear. For example, plants with many phenolic compounds do not always exhibit the highest antioxidant activity. This analysis is useful for identifying plants with similar characteristics and for determining the dominant variables, whether phenolics or antioxidant activity, that contribute to their biological properties.



(a)



(b)

Fig. 4. Profiles of Ten Medicinal Plants from Manokwari Derived from Principal Component Analysis (PCA) and Heatmap Clustering. (a) Cluster Plot; (b) Heatmap Visualization

Heatmap analysis clearly distinguished the tested medicinal plants into three functional clusters, as reflected by their TPC and antioxidant activity, providing clear insights into their chemical profiles. *M. jack, P. americana*, and *A. catechu* formed the high-phenolic cluster, characterized by the highest Methanol- and Aqueous-TPC values, with *A. catechu* notably exhibiting an exceptionally high Aqueous-TPC of 339.69±0.013 mg GAE/g. However, their antioxidant activity was high but not maximal. The mixed/low responder cluster, including *Z. officinale Raxb., O. citriodorum, P. betle, P. scutellarioides,* and *S. polyantum*, displayed low -to moderate TPC and generally low antioxidant activity, with only a few species maintaining moderate potency. In contrast, *F. septica Burn* and *Abelmoschus manihot* comprised a high-antioxidant cluster, exhibiting the strongest antioxidant activity despite relatively low TPC, suggesting that specific phenolic sub-types or non-phenolic secondary metabolites likely drive their bioactivity. These findings collectively highlight that the antioxidant potential of medicinal plants is not solely determined by the TPC, emphasizing the importance of both phenolic and non-phenolic compounds in guiding the selection of species with targeted bioactive properties.

4 Conclusion

This study revealed that the antioxidant activity and TPC of ten medicinal plants from Manokwari exhibited marked variation depending on the solvent system applied. The methanolic extracts possessed TPC values ranging from 63.75±0.08 to 299.38±0.014 mg GAE/g, with the highest level observed in *A.catechu*, whereas the aqueous extracts ranged from 40.63±0.085 to 339.69±0.013 mg GAE/g, with *A. catechu* showing the greatest value. The antioxidant activity of the methanolic extracts (39.62–97.30%) was significantly correlated with TPC, while no such correlation was evident in the aqueous extracts (14.34–96.29%). The data show that 80% methanol is a markedly better choice than water for extracting phenolic compounds, owing to its intermediate polarity and superior solvation capacity.

Principal Component Analysis (PCA) further differentiated the ten plant species into three major clusters: Cluster 1, characterized by high phenolic content (*M. jack*, *P. americana*, and *A. catechu*); Cluster 2, comprising species with relatively low phenolic content and antioxidant activity (*Z. officinale Raxb.*, *O. citriodorum*, *P. betle*, *P. scutellarioides*, and *S. polyantum*); and Cluster 3, consisting of species with pronounced antioxidant activity (*F. septica Burn.* and *A. manihot*). Overall, the results emphasize the pivotal influence of solvent polarity on phenolic extraction efficiency and reveal distinct metabolic profiles across the studied medicinal plants, as reflected in their differential antioxidant activities.

Future studies should employ a combination of complementary antioxidant assays, including DPPH, FRAP, ORAC, and TBARS, along with in-depth metabolomic profiling of secondary metabolites within the clusters exhibiting the highest antioxidant and phenolic levels. This integrated approach would enable a more comprehensive elucidation of the antioxidant mechanisms of action in medicinal plants traditionally used by the local Manokwari community, while also providing critical biochemical insights to support the development of high-value, bioactive natural products with promising therapeutic and commercial applications.

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