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A Comparative Analysis of Flavonoid and Phenolic concentration from Diverse Varieties of *Moringa oleifera* Leaves Utilizing Methanol and Ethanol

Oluewu, M. M [a*], Walker, L. T [a], Ogutu, S. [a] and Koko, C. O [a]

[a] Department of Food and Animal Science, Alabama Agricultural and Mechanical University, Normal, Alabama, USA.

Authors' contributions

This work was carried out in collaboration among all authors. Author OMM and WLT designed the study, performed the statistical analysis, and wrote the protocol. Authors KCO and OMM wrote the first draft of the manuscript. Authors OMM, WLT and OS managed the analyses of the study. Authors OMM, WLT, and KCO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Having its leaves abundant in nutrients such as vitamins and minerals, the moringa plant is highly regarded because of its nutritious and industrial usefulness. Despite its widespread utilisation, little is understood concerning the way various *Moringa oleifera* cultivars vary with regard to their phenolic and flavonoid concentration, particularly when extracting the compound utilising various solvents. To attempt to throw insight into the ways that various solvent types and environment variables affects the phytochemical compounds of *Moringa oleifera* leaves, this research investigation compared the efficacy of ethanol and methanol in removing phenolics as well as

**Corresponding author: E-mail: festusmercy@yahoo.com;*

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flavonoids from the leaves. The information gathered through this research could assist to identify the most appropriate methods for extraction that will maximize the medical and nutritional advantages associated with *Moringa oleifera*. New leaves of five different types of *Moringa oleifera* had been bought from the Winfred Thomas Agricultural Research Station in Alabama A&M University. Upon being pulverised and dried, the resulting leaves had been preserved at room temperature. The leaves were immersed in a mixture of 70% ethanol as the solvent and 80% methanol all through the extraction method. The extracted samples had been then stirred, filtered, and freeze-dried. Following that, High-performance liquid chromatography was the technique employed to know the overall concentration of phenolic and flavonoid compounds. Descriptive and statistical analysis were performed using box plots, scatter plots, bar plots, and t-tests to evaluate the extent or degree to which both solvents removed phenolic and flavonoid compounds and estimated the total quantity of phenolic and flavonoids in the different cultivars. The findings from the descriptive study revealed the Nigerian variety had an increased phenolic content when extracted using methanol—roughly 822.3 µg/ml—than when removed using ethanol (80.6 µg/ml). The Indian type, on the contrary, had phenolic concentrations of 814.3 µg/ml as compared with 647.3 µg/ml in ethanol, revealing an important distinction in preference for methanol. The Nigerian cultivar possessed an increased extraction effectiveness of 1253.12 µg/ml using ethanol as opposed to methanol (1083.52 µg/ml) for flavonoids. The t-test results proved that though the difference was not statistically significant ($t(28) = 0.608$, $p = 0.382$), the average phenolic content obtained with methanol ($M = 798.60$, $SD = 23.567$) was slightly more than that obtained via ethanol ($M = 790.60$, $SD = 45.183$). In a similar vein extraction with methanol showed a smaller average flavonoid concentration ($M = 1068.40$, $SD = 128.641$) compared to the extraction with ethanol ($M = 1129.40$, $SD = 166.018$); nonetheless, this difference was not statistically significant ($t(28) = 1.125$, $p = 0.334$). The scatter diagram revealed a significant linear connection with both phenolic and flavonoid content, having a significant positive correlation (Pearson correlation coefficient = 0.95). Although some modifications depending on the variety, the research reveals that methanol and ethanol serve as suitable solvents for phenolic and flavonoid compound extraction from *Moringa oleifera* leaves. The usefulness of these developments in enhancing methods of extraction in manufacturing industries needs to be explored deeper.

Keywords: Flavonoid and phenolic concentration; Moringa oleifera leaves; vitamins; minerals.

1. INTRODUCTION

The great nutritional and financial benefits of the moringa tree are making it increasingly popular (Patil et al, 2022; Boopathi & Raveendran, 2021). Among vegetables, its leaves are exceptional since they are a rich source of protein, vitamins, and minerals (Covington, 2021; Fernández-López et al, 2020). Along with being nutritive, the leaves also have therapeutic qualities and can be eaten fresh, dried, in tea, capsule form, or as an instant soup. Peñalver et al. [1] and Islam et al. [2] report using them as green fertilizers and as a supplement for hens, goats, and cows. Though non-toxic and bearing anti-carcinogenic attributes, Elhawary et al. (2024) describe moringa the Miracle Tree given its exceptional nutritional content and broad cultivating. The significant quantity of total anti-oxidative polyphenols in the leaves may minimize the risk of sickness in humans and animals [3]. Flavonoids and phenolic compounds range in concentration with respect to the variety and cultivation region [4-6]. A greater amount may be witnessed in places under drought stress. Also, latitude, precipitation, and cultivation area could all have a major effect on the total flavonoids and phenolic acids that concentrate in Moringa leaves [6-8]. Exploring variability regarding the phenolic and flavonoid content of leaves of *Moringa oleifera* among five countries was the ultimate objective of the study. This investigation would shed insight into the implications of environmental conditions on the phytochemical makeup of moringa leaves. It was previously found that Moringa leaves possess an abundance of phytochemicals and beneficial compounds, yet little has been established regarding the way high-performance liquid chromatography can be made use of in distinguishing between the phenolic and flavonoid content of different types.

2. MATERIALS AND METHODS

2.1 *Moringa oleifera* Leaves

2.1.1 Chemicals

Gallic acid, Catechin, Folin & Ciocalteu's phenol reagent, Methanol, Trolox, ABTS salt, Aluminium

Chloride, Sodium Hydroxide, Sodium Nitrite, Sodium Carbonate, Acetic acid, Ethanol, Potassium Persulfate, Hydrochloric acid, TPTZ (tripyridyl-S-triazine), DPPH (2,2-diphenyl-1-picrylhydrazyl), Iron Chloride were purchased.

2.2 Sample Extraction

These leaves received treatment in methanol and ethanol in order to remove the extracts. For three hours at room temperature, the combination was agitated via a magnetic stir bar with a VMR Standard Multi-Position Stirrer. All of the samples had been filtered with Whatman filtering paper No. 4, and the resultant filtrate subsequently dried at 50 degrees Celsius via Buchi Rotavapor at minimal pressure. After soaking the specimens in deionised water, they were left frozen for a whole night at -80°C. For 48 hours, the samples had been preserved in the freezer drier. For further investigation, the freeze-dried contents had been well-preserved at room temperature.

2.3 Statistical Analysis

The variation of phenolic and flavonoid content in the extracts was represented and reported in the current investigation effort through box graphs, scatter diagrams, and bar graphs. In order to find out if there existed a significant difference between both phenolic and flavonoid compound methods of extraction making use of 70% ethanol and 80% methanol, the T-test was performed.

3. RESULTS

3.1 Patterns and Trends in Phenolic Content Concentrations

Fig. 1 displays the way all of the phenolic content varies among the leaves of five distinct types of *Moringa oleifera*: Ghana, Ghana, Haiti, India, and Tusk. It emerged the Nigerian species of Moringa leaves had a phenolic content of about 753.3 µg/ml when it was collected via 70% ethanol as the solvent along with 792.2 µg/ml when removed with 80% methanol. The results indicate a minor difference in the phenolic content of the two solvents; the methanol solvent brings about a slightly higher phenolic content than the one produced by ethanol [9,10]. Comparing to the ethanol solvent, with a value of about 806.3 µg/ml, the phenolic content of the methanol in the Ghanaian variant is considerably greater, at almost 822.3 µg/ml. These values [11-13] suggest that the quantity of phenolics that can be extracted out of each solvent differs significantly. With only little variations between the solvents, the Haitian variety's phenolic content hovers around 812.3 µg/ml in methanol and 791.3 µg/ml in ethanol. The phenolic content of methanol is a bit greater [12]. However, according to Morales-Olán et al. [14], methanol has a significantly greater phenolic content than ethanol, with 814.3 µg/ml in methanol and 647.3 µg/ml in ethanol. This means the phenolic content of the Indian variety varies drastically depending on the solvent. The phenolic content of the tusk strain is nearly 763-0.0 µg/ml in methanol and 796.3 µg/ml in ethanol. According to the average found in other varieties, the phenolic content of ethanol in this case is notably greater when compared to that of methanol [15,16].

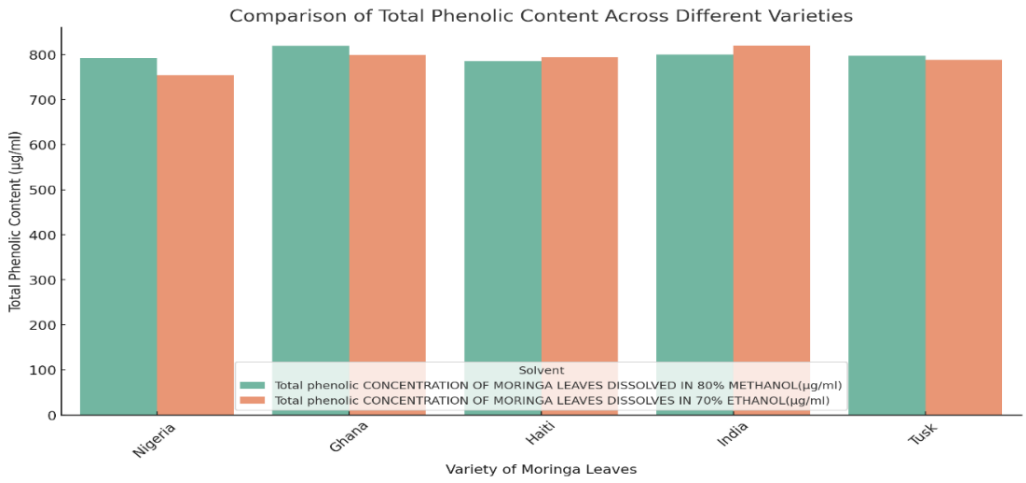

Fig. 1. Bar chart showing phenolic distribution with the two solvents

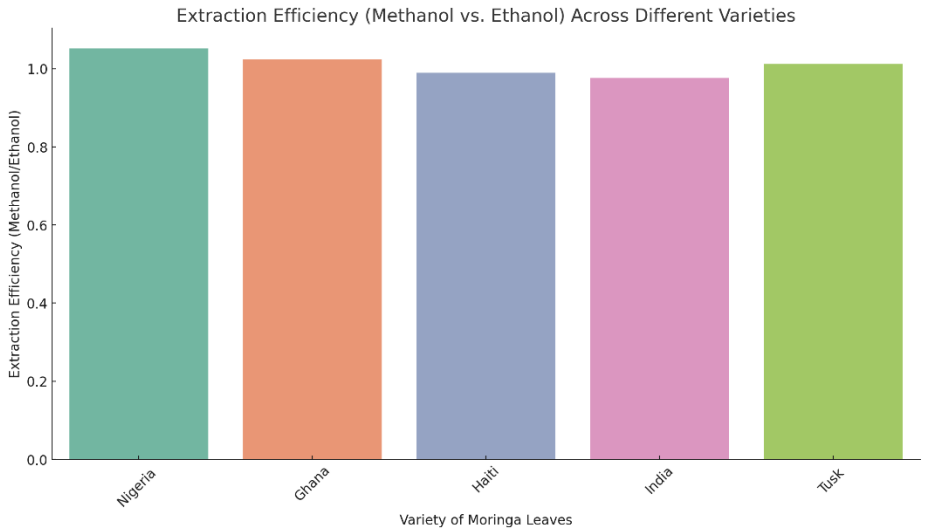

Fig. 2. Bar chart showing the extraction efficiency between Methanol and Ethanol

The methanol against ethanol extraction efficiency for each variety of *Moringa oleifera* leaves is presented in Fig. 2 as a bar plot. The proportion of the quantity of phenolic content obtained by methanol to that extracted by ethanol is employed to calculate the extraction efficiency. The Nigerian cultivar of moringa leaves has a little greater rate of extraction with methanol as compared with ethanol, yielding a ratio that is slightly greater than 1, as reported by Hikmawanti et al. [17] and Tzanova et al. [18]. On the contrary, the Ghanaian strain revealed near the same effectiveness for both solvents, with methanol revealing a minimal benefit [19,20], judging by a ratio with a value nearly exactly 1. The Haitian species similarly showed a little higher efficacy with methanol, reflected by a ratio that was slightly more than 1 [21,22]. A ratio considerably greater than one points out that the Indian strain has an advantage in maintaining a far superior methanol extraction efficiency [23,24]. The Tusk cultivar possessed a ratio just below 1, revealing that ethanol is a far better extractant of phenolic compound than methanol in this particular case [25,26].

Fig. 3 indicates the total phenolic amount of *Moringa Oleifera* leaves taken with two distinct solvents: 80% methanol and 70% ethanol, depicted in a box plot. The amounts of each compound are shown in Fig. 3 in two separate colours: green for 80% methanol and red for 70% ethanol. Both of the extraction solvents could possibly be immediately distinguished from each other because of the colour differentiation. Based on 70% ethanol, the horizontal line in the red box shows the median phenolic concentration, which is nearly 775 mg/g. The interquartile range (IQR) is displayed by the box, which covers the center 50% of the data and fluctuates from nearly 725 mg/g to 800 mg/g. Considering an outlier with approximately 650 mg/g, a specific variety may have had a noticeably lower phenolic concentration when extracted with 70% ethanol. The median for methanol with a concentration of 80% is slightly higher just in excess of 800 mg/g. The IQR, spanning between about 790 mg/g to 815 mg/g when methanol has been utilised to assess the phenolic content, has a smaller variability among the various types instead of when ethanol extract is utilised [27]. A wider spectrum of phenolic contents in the ethanol extracts compared to the methanol extracts can be seen by the whiskers of the red box (70% ethanol) stretching farther compared to that of the green box (80% methanol) [16]. Accordingly, the quantity of phenolic content removed with 70% ethanol as a solvent could vary greatly based on the particular variety of *Moringa oleifera* leaves. The slightly increased median concentration and lowered variation in the extracts produced with methanol reveal that methanol at a concentration of 80% could serve as a more accurate and effective solvent rather than 70% ethanol for removing phenolic compounds from *Moringa Oleifera* leaves [28,29,30]. The broader variety of the ethanol extract and its presence of an outlier show that various types of *Moringa oleifera* may react different ways to the use of ethanol [31-33].

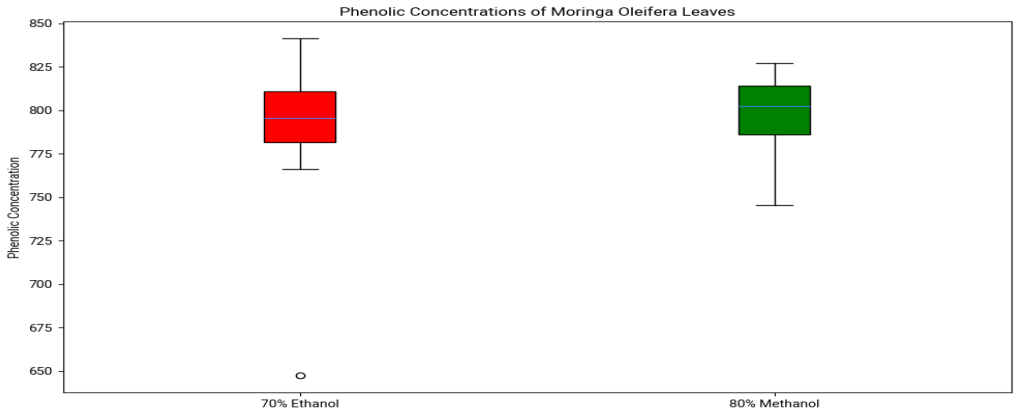

Fig. 3a. Box plot

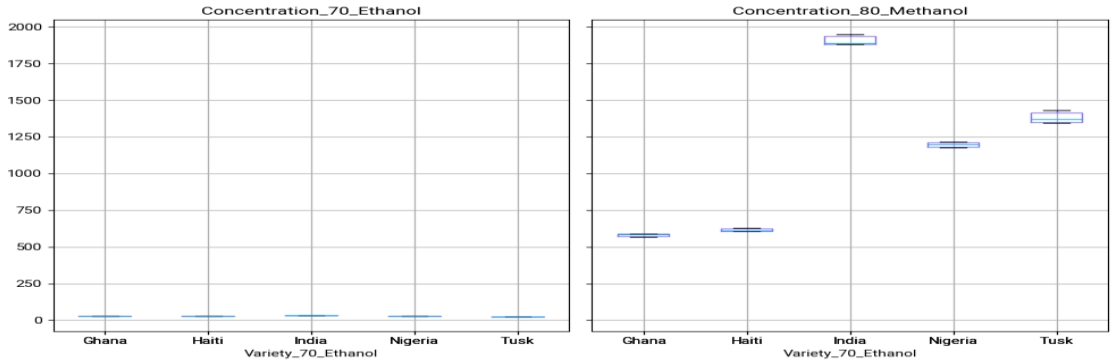

Fig. 3b. Box plot

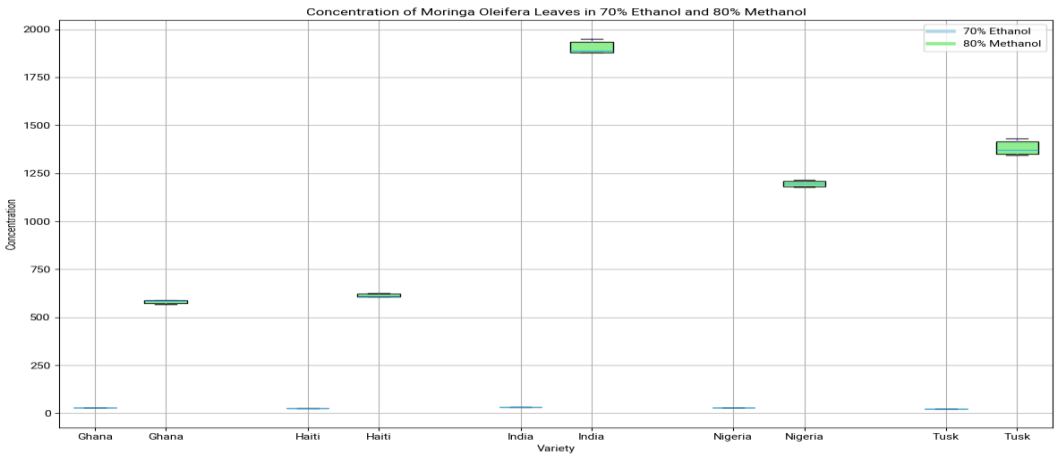

Fig. 3c. Box plot

Fig. 3. Box plot showing total phenolic amount of *Moringa Oleifera* leaves taken between two distinct solvents

3.2 Patterns and Trends in Flavonoid Content

A bar graph using 80% methanol and 70% ethanol solvents is shown in Fig. 4 to examine the total flavonoid content of leaves from five different varieties of *Moringa oleifera* (Nigeria, Ghana, Haiti, India, and Tusk). A straightforward comparison is provided by the bars, each of revealing the flavonoid concentration for specific

type and solvent. The flavonoid content with methanol in the Nigerian variety is roughly 1083.52 µg/ml, while the flavonoid content of ethanol amounts to about 1253.12 µg/ml. This implies that ethanol is more effective in removing flavonoids from this variety than methanol [34,35]. As reported by Xu et al. [36], the two solvents remove approximately the same quantity of flavonoids from the Ghana cultivar, with ethanol extracting slightly greater quantities. In the study by Megakallu et al. [37], the Haitian variation has comparable outcomes, with methanol doing slightly better. Ethanol is evidently more effective in removing flavonoids from the Indian type [38]. The Tusk variety reveals similar outcomes likewise, though ethanol extracts flavonoids more efficiently.

In order to show the comparative efficacy of methanol and ethanol in the extraction of flavonoids from several *Moringa oleifera* leaves types, Fig. 5 utilised a bar chart. As noted by Bui et al. [39] and Tzanova et al. [18], the Nigerian variety exhibits a bit smaller extraction efficiency ratio, signalling that ethanol is slightly more efficient than methanol for flavonoid extraction in this one particular variety. The Ghanaian type possesses a ratio with a value roughly equal to 1, meaning that ethanol has only a slight edge above the other solvent in terms of performance. The ratio for the Haiti cultivar is equally lower than one, reflecting that ethanol extracts flavonoids with greater efficiency than methanol. The ratio is substantially below one in the instance of the Indian variety, implying that ethanol is significantly much better than methanol at collecting flavonoids from this specific type [18]. The Tusk cultivar shows a ratio that is substantially higher than 1, pointing that methanol is a slightly more successful flavonoid extraction method than ethanol in this case.

The total flavonoid contents of *Moringa Oleifera* leaves removed using both distinct solvents—70% ethanol and 80% methanol—are represented in a box plot in Fig. 6. Plot analysis revealed the range of flavonoid concentrations released with 70% ethanol are around 850 and 1363 µg/ml, with a median score of nearly 1129 µg/ml. On the opposite hand, quantities achieved with 80% methanol have a median of around 1068 µg/ml and fluctuate between roughly 870 µg/ml to 1322 µg/ml. In juxtaposition with extracts made with 80% methanol, ones made with 70% ethanol had more median flavonoid concentration [40]. The findings reveal that a concentration of 70% ethanol could prove slightly quicker in extracting flavonoids from *Moringa oleifera* leaves under the current situation. 70% ethanol proved to be the superior solvent for collecting more flavonoids in *Moringa Oleifera* leaves compared with 80% methanol. Modifications in the polarisation of the solvent or the manner in which it reacted with the plant matrix could be an explanation for this. It is equally essential to take into account that sample heterogeneity occurs less for 80% methanol extractions as compared to 70% ethanol extractions due to the interquartile range is smaller. The use of 70 percent ethanol proved to obtain more flavonoids on average, although methanol with a concentration of 80% seemed to produce greater consistency results.

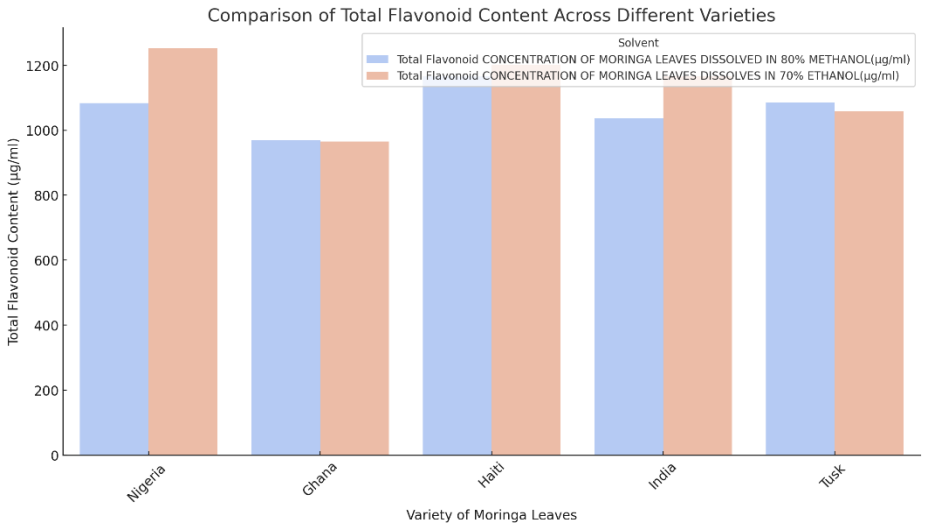

Fig. 4. Bar chart showing the distribution of flavonoid

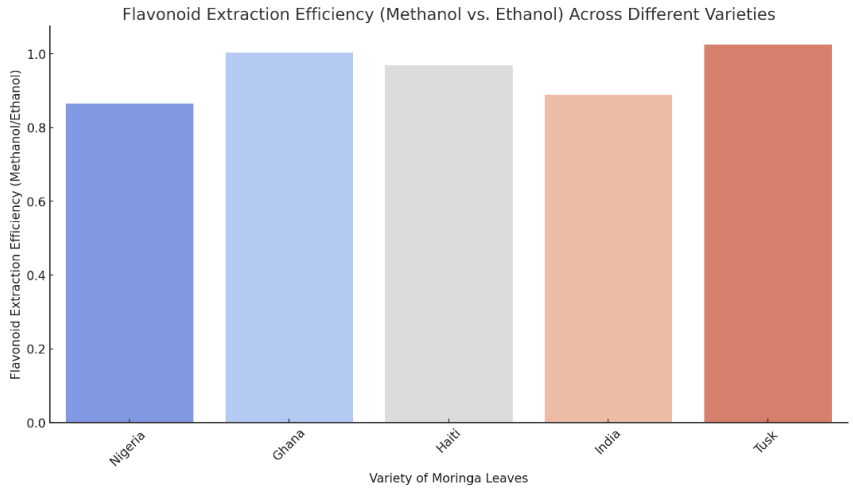

Fig. 5. Bar plot showing flavonoid extraction efficiency

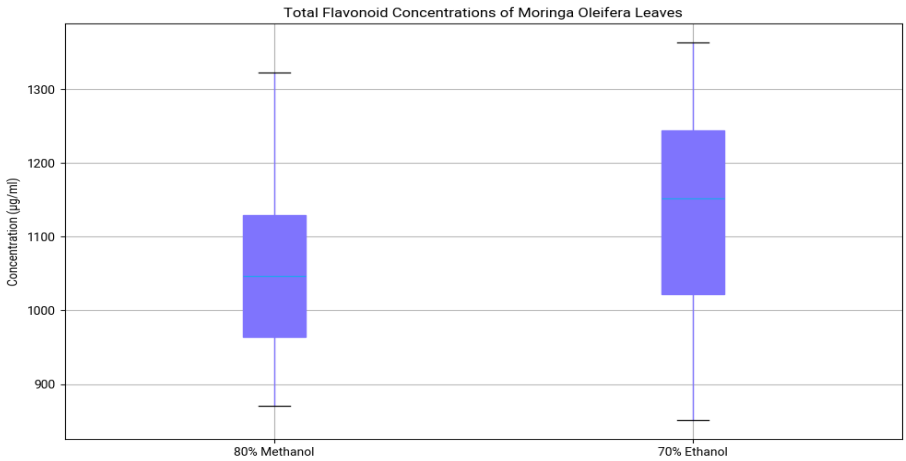

Fig. 6a. Box plot for flavonoid

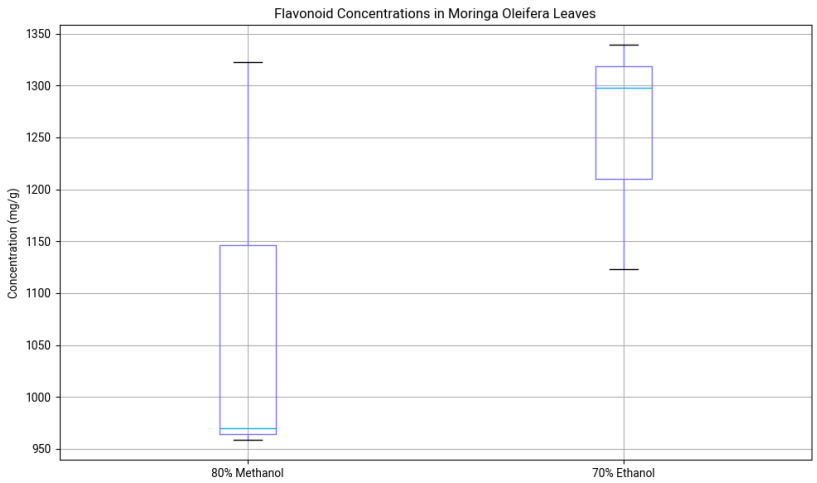

Fig. 6b. Box plot for flavonoid

Fig.6. Box plot showing total flavonoid contents of *Moringa Oleifera* leaves removed using both distinct solvents

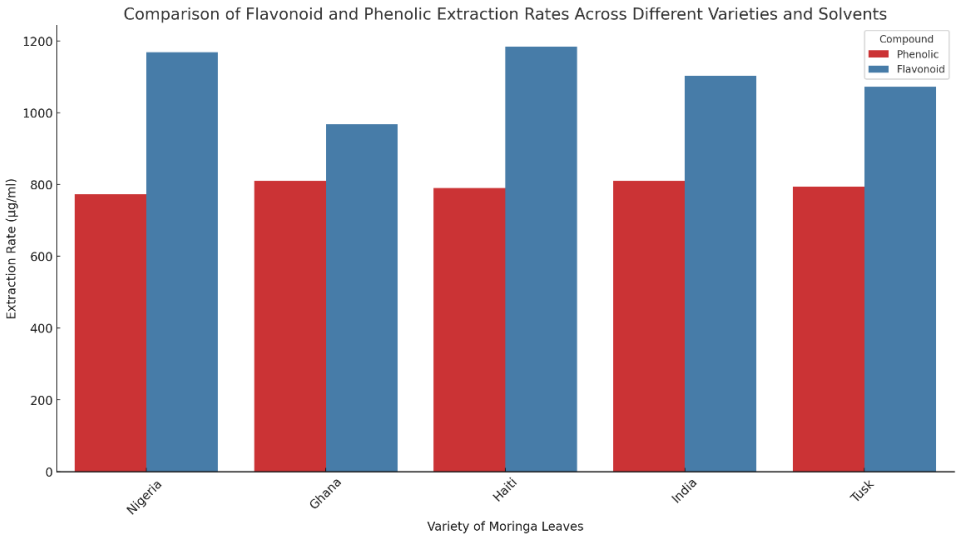

Fig. 7. Bar plot showing extraction rate

3.3 Comparison of Phenolic and Flavonoid Extraction

The bar graph in Fig. 7 indicates the way various *Moringa oleifera* leaf varieties' flavonoid and phenolic extraction rates differ. A grasp of the efficiency of the method of extraction for each variety could be acquired simply by looking at the extraction rates for both categories of compounds. The flavonoid extraction rate of the Nigerian cultivar of Moringa leaves is estimated to be 1253 µg/ml, and this is considerably greater compared to the phenolic extraction rate that is roughly 792 µg/ml. It could mean that the Nigerian variety possesses a more quantity of flavonoids or the solvent extraction had been carried out with greater efficiency [41]. Compared to the Nigerian variety [42,43], even though with a minor difference, the Ghanaian type supports flavonoid extraction with a slightly greater flavonoid extraction rate of 982 µg/ml as compared to its phenolic extraction rate of 806 µg/ml. The Haitian species shows a close extraction concentration of 851 µg/ml for flavonoids and 822 µg/ml for phenolics, suggesting an evenly distributed extraction of compounds. The Indian variety, on the opposing hand, varies markedly from the Nigerian variety because it displays a flavonoid extraction rate of near 1293 µg/ml and a phenolic extraction rate of 814 µg/ml, implying greater efficacy in flavonoid extraction. The Tusk variety showed greater effectiveness in extracting flavonoids, with a flavonoid extraction rate of around 921 µg/ml, relative to a phenolic extraction rate of 763 µg/ml [44].

Fig. 8 presents a bar chart that depicts the overall extraction efficiency of phenolics and flavonoids in multiple types of *Moringa oleifera* leaves by applying methanol and ethanol solvents. We computed the combined extraction efficiency for each type by adding the extraction rates of both compounds then contrasting the results in order to obtain a greater grasp of the overall extraction efficiency. The Nigerian variety proves that ethanol is far more effective in extracting both compounds from it over methanol, with a total extraction efficiency that is approximately 2045 µg/ml with ethanol compared to approximately 1875 µg/ml with methanol [29]. It shows that both solvents are quite effective for this variety given that the Ghanaian variety shows a slightly greater overall extraction efficiency with ethanol at around 1788 µg/ml, as opposed to around 1727 µg/ml using methanol. With nearly 1672 µg/ml for both methanol and ethanol, the Haitian cultivar shows equal extraction efficiency, confirming that the two solvents perform equally effectively for this type. Having an extraction efficiency of close to 1940 µg/ml in ethanol as well as 1689 µg/ml in methanol, the Indian strain possesses the greatest overall extraction efficiency, confirming that ethanol is the preferred solvent for this variety. Furthermore, the Tusk type showed more extraction efficiency with ethanol, at around 1718 µg/ml, compared to methanol, at almost 1566 µg/ml. This indicate that ethanol works more effectively in general for this variety during extraction.

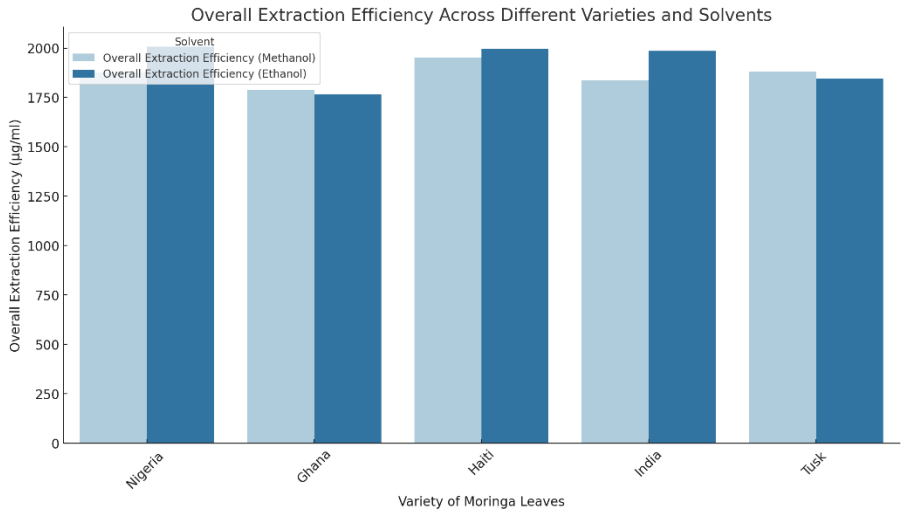

Fig. 8. Bar plot showing overall extraction efficiency

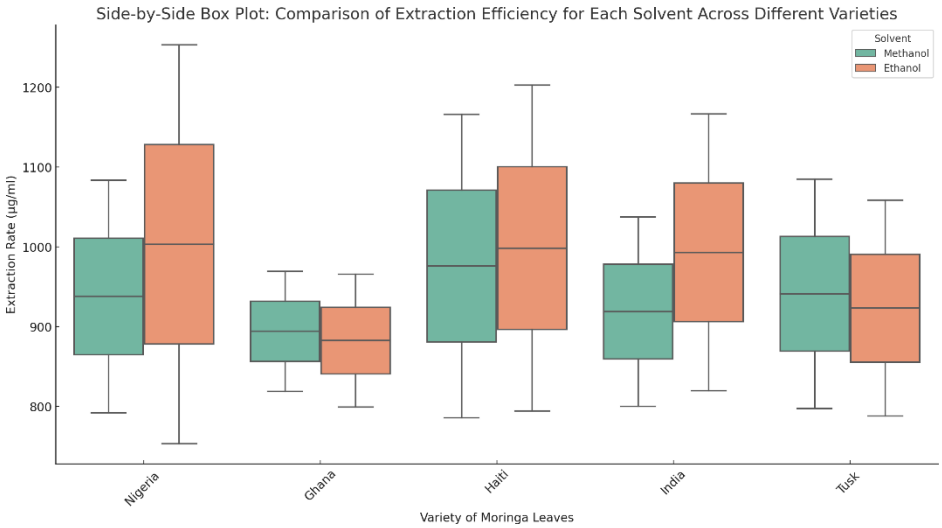

Fig. 9. Box plot showing overall extraction efficiency

More comprehensive details concerning the variation of extraction efficiency across various cultivar and solvents can be obtained through the box plot displayed in Fig. 9. The variation of rate of extraction for total flavonoids and phenolics can be seen on the box of each variety. In juxtaposition to methanol, the ethanol box graph associated with the Nigerian variety reveals a little greater variability but a greater median with flavonoid extraction. Phenolic extraction, on the contrary, remains more suitable with methanol. For the Ghanaian cultivar, the two solvents reveal similar performance, although marginally higher median values for ethanol, especially with regards to flavonoids. As juxtaposed with ethanol, methanol in the Haiti variant exhibits a slightly superior and more consistent extraction efficiency for both compounds. For India, methanol tends to be unchanged and effective in removing phenolics, but ethanol displays greater yet more varied flavonoid extraction efficiency. However, the Tusk variety demonstrates that ethanol usually performs more effectively than methanol for both compounds. This is less apparent in comparison to other types, though. For the most part, specifically in the Nigerian and Indian species, ethanol at a concentration of 70% removes flavonoids with greater efficiency than 80% methanol. This means that some types' flavonoid compounds could either be more easily soluble or simpler to extract using ethanol. In overall, methanol with a concentration of 80% works more effectively or at par with 70 per cent ethanol as far as is used for removing phenolic

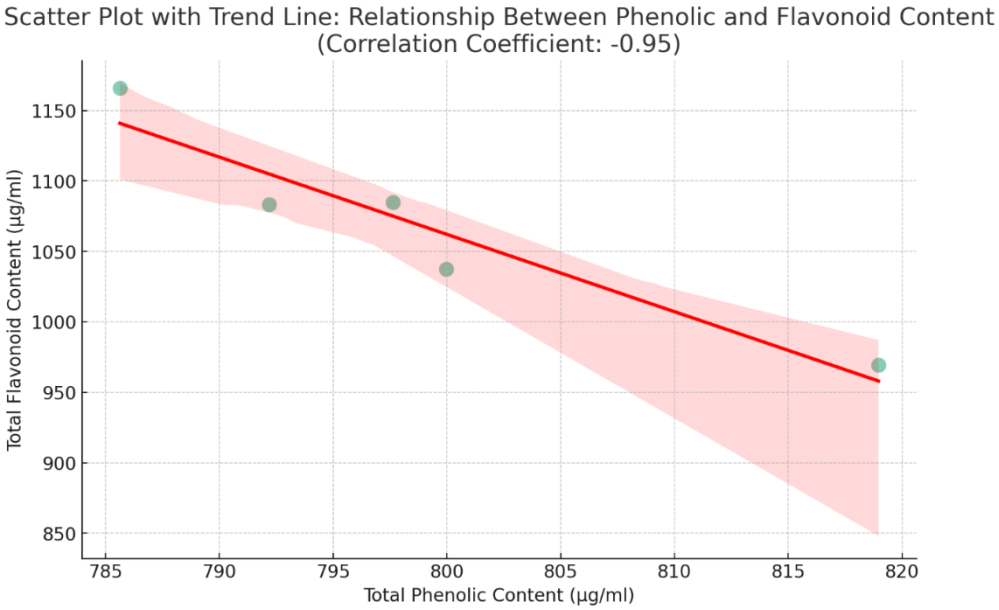

Fig. 10. Scatter plot showing the relationship between phenolic content and flavonoid content

compounds; this can be particularly the case for both the Ghanaian and Indian varieties, hinting that methanol could be more effective at solubilizing or dissolving phenolic compounds.

The link between the total phenolic content and the total flavonoid content of the leaves of *Moringa oleifera* is presented in Fig. 10 as a scatter plot featuring a trend line. With the x-axis of the chart indicating phenolic content and the y-axis being the one representing flavonoid content, every spot on the graph represents an individual type of Moringa leaves. The overall trajectory of the relation between these two compounds has been shown by the red trend line. The sloping upward trend line in the scatter graph reveals a positive correlation between the phenolic and flavonoid contents of Moringa leaves, suggesting that a spike in phenolic content usually comes followed by an increase in flavonoid content. Additionally, there is a notable positive correlation, or a significant linear connection, between both phenolic and flavonoid contents, as shown by the calculated Pearson correlation coefficient of 0.95 for this relationship. The variability in the quantity of phenolic compounds constitutes approximately 95% of the variations in flavonoid content. There exist a few outliers that reflect that other variables could also independently affect flavonoid concentration without regard to phenolic content, even though most of the data points align closely with the linear trend, confirming consistent connections between types. The result matches up with study carried out by Bennour et al. [45], Lin et al. [46], and Xu et al. [47].

3.4 Test of difference of Phenolic and Flavonoid

The results presented in Table 1 reveal that the average phenolic content which was extracted using 80% methanol (798.60 µg/ml) is slightly more than the quantity which was removed with 70 percent ethanol (790.60 g/ml). And based on Table 1's t-test outcome (p-value = 0.382), the difference is not statistically significant. It therefore follows that the phenolic compounds in the moringa leaves are capable of being extracted through both solvents with similar effectiveness.

Table 1. Comparison of phenolic content using 80% methanol and 70% ethanol

Group	M	SD	T	Df	P
Methane (80%)	798.60	23.567	0.608	28	0.382
Ethanol (70%)	790.60	45.183			

M = Mean; SD = Standard deviation

Table 2. Comparison of flavonoid content using 80% methanol and 70% ethanol

Group	M	SD	T	df	p
Methane (80%)	1068.40	128.641	1.125	28	0.334
Ethanol (70%)	1129.40	166.018			

M = Mean; SD = Standard deviation

The results shown in Table 2, the average quantity of flavonoid of the 80% methanol extraction (1068.40 µg/ml) is a bit lower than the comparable quantity of the 70% ethanol extraction (1129.40 µg/ml). Still, Table 2's t-test outcome (p-value = 0.334) reveal that there fails to be a statistically significant difference between the two. These outcomes reflect that both of the solvents have similar effectiveness with regard to phenolic component extraction from moringa leaves [48,49].

4. SUMMARY

The box plot reveals the variability in the concentration of phenol between each of the solvents used for extraction and *Moringa oleifera* leaves cultivars. Unlike that of the Tusk variety, methanol with a concentration of 80% typically removes an increased concentration of phenolic compounds than ethanol with a concentration of 70%. Despite a substantial phenolic quantity in the two solvents, the Ghanaian cultivar stands separate from the other ones as could possess much more antioxidant benefits. Especially with regard to Indian cultivar, the plot shows that methanol usually boasts more effective rate of extraction compared to ethanol. The Tusk variety seems to be an oddity, as ethanol works more efficiently than methanol. The figure reveals that with the vast majority of cultivars, ethanol usually removes an increased or similar flavonoid quantity compared to methanol. As the most noticeable difference benefiting ethanol, the Indian species stood out, indicating that the it possesses particular compounds that render it more readily soluble in ethanol. Ethanol could serve as the more desirable solvent for flavonoid extraction for both the Nigerian and Tusk species, as proven by the increased flavonoid quantity in ethanol. The bar graph depicts that, in overall, ethanol has an effective extraction rate for flavonoids deemed either superior to or similar as that of methanol; the Indian variety displays the most significant distinction in favouring ethanol. Methanol is a little greater in effectiveness for the Tusk cultivar, that is an aberration corresponding to the trend observed in the quantity of phenolic compounds. With respect to the specific purposes of the method of extraction, this assessment could help with identifying the most suitable solvent to remove flavonoids from different Moringa types. Furthermore, utilising both extraction approaches, the test of significance findings proved that there is no statistically significant difference between the phenolic and flavonoid concentrations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. The version of ChatGPT-4 is release by open AI.
2. Used for some part of the Introduction and Methodology
3. Used for the comparison of results with previous studies

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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