UJMR, Conference Special Issue Vol. 9 No. 3. June, 2024, pp. 464 - 474

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.2493.054

Received: 17th March, 2024 Accepted: 18th June, 2024

Assessment of Proximate, Vitamins, In Vitro Antioxidant, and Functional Properties of Some Polyherbal Formulations as Prospective Botanical Candidates for Pharmaceutical Applications

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Abstract

Conventional drugs have numerous side effects, toxicity, and microbial resistance, making them unsafe for consumption. Polyherbal formulations are popular due to their potency, low cost, clinical efficacy, safety, and success in chronic conditions. The study aims to evaluate the proximate, vitamin, functional, and antioxidant activity of polyherbal formulations of Turmeric, Coriander, and mint (TCM) leaves as potential botanical candidates for pharmaceutical screening. Thus, addressing these issues and improving access to drugs for individuals by focusing on polyherbal formulations (PHF) is a potential solution. All analyses conducted were determined according to standard assay guidelines. The proximate analysis shows no significant difference among the formulations ($p > 0.05$), with TCM showing a nutritional profile shift because of the combination of the herbs rather than single herbs. In the vitamin composition, Vitamin A displays a pattern among the formulations, with TC and CM showing higher values than TM and TCM, which are, in turn, better than the single herbs. Vitamin C fluctuates across the formulations. TM displays higher, while T shows the lowest. Vitamin E content remains relatively stable among the formulations with a difference from the single herbs. In the functional properties, GAC displays notable variations among the formulations. TCM exhibits the highest GAC at 14.7 %, indicating its superior ability to absorb glucose. TC, CM, TM, and the single herbs show a comparatively lower GAC value. WAC also varies among the formulations. TCM displays lower WAC compared to TC, TM and CM. The single herbs show the lowest WAC. OAC shows intriguing trends across the formulations. TCM and TC exhibit similar OAC, while CM shows a slightly higher OAC, and TM displays better than single herbs, showing no significant difference ($p > 0.05$). In the antioxidant Activity, TCM shows lightly higher scavenging activity in 40, 80 and 120 mg/mL concentrations than other formulations. The outcome of this study has led us to conclude that employing safe combinations of these herbal formulations for the production of nutraceuticals is recommended.

Keywords: Turmeric, Coriander and Mint Leaves, polyherbal formulation, proximate,

INTRODUCTION

Conventional drugs and or orthodox drugs show a variety of side effects, which is of great concern to the health of individuals (Aini *et al.*, 2019). Also, the toxicity of these drugs makes them sometimes not safe for the consumption of individuals (Vaibhav, 2019). Microbial drug resistance to several drugs is of great concern and a challenging issue upon consumption of conventional drugs (Wanda, 2018). Poverty and expensiveness of drugs decrease the availability or access to many drugs by many individuals (Vincent, 2020). The problems mentioned above make this research an important prospect that will address or reduce the problems mentioned.

Nutraceuticals play an important role in preventing the onset of chronic disease and reducing the complications involved (Jain *et al.*, 2022). The notion of polyherbal formulation (PHF) is gaining popularity as the obstacles to standardization are addressed by current science and technology (Sadaf *et al.*, 2022). Polyherbal formulation treatments are widely accepted due to their potency, low cost, easy availability, clinical efficacy, safety, patient tolerance, and success in chronic conditions (Joshi *et al.*, 2020). The active phytochemical constituents of individual plants are inadequate to attain the desirable therapeutic effects (Sarita *et al.*, 2019). Polyherbal formulation is a combination that serves as a combination therapy against drug resistance (Mussarat *et al.*, 2021). Plant food provides nutrients that help maintain the body's functions. Nowadays, nutritional information is increasingly concern for many reasons, for example, to meet nutritional requirements or to prevent nutritional deficiency disease (Aunyachulee and Suksaard 2020). Plants like turmeric (Curcuma longa), Coriander (Coriandrum sativum), and mint leaves (Mentha spicita) are found to be more prominent in their nutritional and medicinal properties.

Turmeric (Curcuma longa) is a flowering plant of the ginger family with a long history of medicinal use due to its potent anti-inflammatory properties (Khalil *et al.*, 2023). Its most active constituent is curcumin, which has been widely studied (Tanvir *et al.*, 2017). However, curcumin has extremely limited bioavailability (Hussain, 2022). It has been used for centuries in Chinese and Ayurvedic medicine for the treatment of dyspepsia and epigastric pain (Joseph, 2018). Coriander is recognized for its medicinal properties, and in traditional medicine, it has been used as a carminative to treat dyspepsia, flatulence, and diarrhea to relieve respiratory and urinary problems (Samir *et al.*, 2023). The therapeutic properties and nutritional values of coriander seeds are due to the presence of many bioactive compounds, such as fatty acids, sterols, tocols, and many volatile compounds (Bochra *et al.*, 2015). Coriander seeds have been actively investigated for their chemical composition and biological activities, including anti-microbial, antioxidant, hypoglycemic, and anti-cancer activities, among others (Ashraf *et al.*, 2020). Mint varieties are common in different regions of the world and are used as aromatic and medicinal ingredients in cosmetics, drugs, food, and drinks (Muhammad *et al.*, 2018). Many volatile compounds have been identified in spear mint, including flavonoids, thymonin, carvone, and limonene (Muhammad and Rawshdeh, 2018). The non-volatile composition of mentha species has beneficial effects on human health due to its anti-microbial, antioxidant, anti-inflammatory, and anti-allergic properties (Majid *et al.*, 2021).

The study aims to evaluate the proximate, vitamin, functional, and antioxidant activity of polyherbal formulations of Turmeric, Coriander, and mint leaves as potential botanical candidates for pharmaceutical screening.

MATERIALS AND METHODS

Collection of Samples and Preparation

Turmeric and Coriander were purchased from Chake local Market on July 3, 2023. Mint leaves were collected from a farm in Kofar Sauri, Katsina, Katsina State. The samples were washed properly with tap water and dried at room temperature. The dried samples were then ground to powder.

Experimental Design

The research is designed as follows:

F1 (T): Turmeric

F2 (C): Coriander

F3 (M): Mint

F4 (TC): Turmeric + Coriander (1:1 W/W)

F5 (TM): Turmeric + Mint (1:1 W/W)

F6 (CM): Coriander + Mint (1:1 W/W)

F7 (TCM): Turmeric+ Coriander + Mint (1:1:1 W/W)

Proximate Analysis

All the formulations were subjected to proximate analysis in accordance with the following established standard laboratory procedures (AOAC, 1990; AOCS, 2000). The proximate contents analyzed were moisture, crude protein, total ash, crude fiber, fat, and carbohydrate.

Determination of Vitamin Composition

The analyzed liquid was measured, KOH solution was added, heated, cooled, and centrifuged. The extracted extract was collected, separated, and measured for absorbance A1. The extract was then irradiated using UV light for 30 minutes, and absorbance A2 was measured.

The concentration of vitamin A was calculated using the formula.

$$Ax = (A1 - A2) \times 22.23$$

Three centrifugal test tubes were used to analyze a sample, standard vitamin C, and distilled water. PR-reagent was added, mixed, and incubated for 30 minutes. The sample was centrifuged, and absorbance was measured. Vitamin C concentration was calculated in µM using the formula:

Cx = Absorbance of sample × Concentration of standard (56.8 µM)

Absorbance of standard

The experiment involved preparing sample extract, standard, and water in centrifuge tubes, adding ethanol, water, xylene, and 2, 2'-dipyridyl reagent. The mixture was then pipetted into spectrophotometer cuvettes, and absorbance was measured at 460 nm. After 15 minutes, a ferric chloride solution was added, and the amount of vitamin E was calculated using the formula.

Vitamin E (µg/g) = (ΔA520nm - ΔA460nm) x conc. [S] x 0.29 x Total volume

ΔA520nm x Vol for experiment x Weight of sample

Functionality Test (AOAC, 2005)

Water Absorption Capacity (WAC) Measurement

A weighed sample is placed in a glass beaker, diluted with 10ml of water, stirred, and allowed to stand for 30 minutes. Excess water is poured, and the absorption capacity is calculated using the formula:

WAC= [(weight after absorption - initial weight of sample)/initial weight of Sample] × 100

Oil Absorption Capacity (OAC) Measurement

The steps above for WAC will be repeated, but food-grade vegetable oil will be used as the absorbing liquid instead of water. The oil absorption capacity will be calculated using the same formula as for WAC.

Glucose Absorption Capacity (GAC) Measurement

A 5 % w/v glucose solution is prepared by dissolving glucose in distilled water. A sample is weighed, added to the solution, stirred, and allowed to stand for 1 hour. The sample is separated, and glucose concentration is measured using a spectrophotometer. The glucose absorption capacity will be calculated using the formula:

GAC = [(initial glucose concentration - final glucose concentration) /initial glucose concentration] × 100

Determination of Antioxidant Activity (Siddharth *et al*., 2020)

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Scavenging Assay

A stock solution of 24 milligrams of DPPH was prepared by dissolving it in 100 mL of methanol. The mixture was then combined with 100 µL of sample extract in a test tube, and the absorbance was determined at 517 nm.

The following formula was used to compute the percentage of antioxidants or RSA

% of antioxidant activity= [(Ac−As) ÷ Ac] × 100

where: Ac—Control reaction absorbance; As—Testing specimen absorbance

RESULTS

Proximate Compositions

Table 1 Provides a comprehensive overview of the proximate compositions of various formulations (%) of *Curcuma longa* (Turmeric), *Coriandrum sativum* (Coriander), and *Mentha spicita* (Mint leaves)., offering insights into the macronutrient composition of the different herbal formulations. The data showcases several key differences and trends among the formulations T, C, M, TC, TM, CM, and TCM, each comprising various combinations of turmeric, Coriander, and mint leaves.

Moisture content varies notably across the formulations. TC displays the lowest moisture content at 9.26%, while M exhibits the highest at 11.75 %. It's interesting to note that TCM, which includes all three ingredients, demonstrates slightly higher moisture content (10.64 %) than TC but lower than M (11.75 %), potentially influenced by the combination of these components.

Moreover, the combination of ingredients appears to influence the protein, fat, and carbohydrate content. When turmeric is combined with coriander and mint leaves in the TCM formulation, there's an increase in both protein and fat content compared to the individual components, TC, TM, and the single herbs. However, the carbohydrate content decreases in TCM. This suggests a shift in the nutritional profile when all three ingredients are combined, potentially offering a formulation with a more protein-rich and less carbohydrate-heavy profile compared to the individual ingredients.

ANOVA (Analysis of Variance) tests conducted on these values indicate that there are no significant differences among the formulations for these nutrients (P > 0.05). The ANOVA results, suggesting no significant differences among the formulations, imply that while there are observable variations in the nutritional composition, these differences are not statistically significant. The trend of increased protein and fat content with the addition of mint leaves and Coriander to turmeric might offer a more balanced nutritional profile, although not significant based on this statistical test.

Table 2 presents the vitamin composition of the herbal formulations, providing insights into the variations in vitamin content across different combinations of turmeric, Coriander, and mint leaves. The data reveals distinct trends in the presence of vitamins A, C, and E among the formulations T, C, M, TC, TM, CM, and TCM.

Vitamin A content displays patterns among the formulations. TC and CM show relatively higher Vitamin A content compared to TM, TCM, and single herbs. The variation suggests that the inclusion or exclusion of specific ingredients, such as Coriander or turmeric individually, may significantly impact the Vitamin A levels. Vitamin C content fluctuates across the formulations without a clear, consistent trend. TM displays the highest Vitamin C content, followed by CM, TCM, and TC, while the singles show the lowest. Vitamin E content remains relatively stable across all formulations, displaying minimal variations.

The ANOVA results suggest that there are no significant differences (P > 0.05) among the formulations for all three vitamins. The lack of significant differences in vitamin composition among these formulations, as indicated by the ANOVA results, suggests that the variations observed in vitamin content for A, C, and E are likely due to inherent variability or natural differences in the ingredients themselves, rather than the combinations in the formulations. This implies that the specific combinations of turmeric, Coriander, and mint leaves might not have a significant impact on the overall vitamin content, at least within the parameters of this analysis.

Table 1: proximate Compositions of Polyherbal Formulation of *Curcuma Longa* (Turmeric), *Coriandrum Sativum* (Coriander), and *Mentha Spicita* (Mint Leaves).

PT	T	C	M	TC	TM	CM	TCM
MC	10.5±0.02	9.3±0.05	11.75±0.02	9.26±0.01	10.7±0.025	10±0.04	10.64±0.05
AC	3.04±0.02	8.55±0.01	5.3±0.02	5.8±0.01	5.7±0.03	8.2±0.01	6.1±0.04
CP	6.54±0.01	4.56±0.04	3.31±0.04	2.54±0.03	3.76±	6.38±	6.56±0.04
FC	6.24±0.03	19.3±0.02	5.03±0.01	10.3±0.02	14.4±0.03	16.2±0.02	18.1±0.03
CF	14.14±0.04	25.01±0.05	19.05±0.01	16.1±0.02	18.4±0.03	20.10±0.04	24.3±0.03
CB	59.5±0.01	33.2±0.02	55.56±0.02	56±0.04	47.04±0.05	39.1±0.04	34.3±0.03

Key: PT (Parameter)**, MC** (Moisture Content)**, AC** (Ash Content)**, CP** (Crude Protein)**, FC** (Fat Content)**, CF** (Crude Fiber)**, CB** (Carbohydrate)**,** T (Turmeric); C (Coriander); M (Mint); TC (Turmeric: Coriander); TM (Turmeric: Mint); CM (Coriander: Mint); TCM (Turmeric: Coriander: Mint). Values were expressed as mean± standard error of mean (SEM) P> 0.05

Table 2: Vitamin Composition of Turmeric, Coriander and Mint Formulations

	Formulation						
V (mg/ml)	T	C	M	TC	TM	CM	TCM
V. A	3.45±0.02	3.08±0.05	3.91±0.04	6.46±0.03	4.38±0.04	9.49±0.04	4.91±0.03
V. C	3.25±0.01	4.55±0.03	5.67±0.02	6.42±0.03	9.64±0.02	9.17±0.04	6.25±0.04
V. E	9.45±0.01	9.5±0.02	8.96±0.02	10.83±0.02	10.77±0.04	10.78±0.03	10.85±0.03

Key: V (Vitamin), T (Turmeric); C (Coriander); M (Mint); TC (Turmeric: Coriander); TM (Turmeric: Mint); CM (Coriander: Mint); TCM (Turmeric: Coriander: Mint); VIT= Vitamin. Values were expressed as mean± standard error of mean (SEM) P> 0.05

Figure 1, 2 and 3 focuses on the functional analysis of the herbal formulations, providing essential data on glucose absorption capacity (GAC), water absorption capacity (WAC), and oil absorption capacity (OAC) across the formulations T, C, M. TC, TM, CM, and TCM, composed of turmeric, Coriander, and mint leaves in different combinations. Glucose absorption capacity (GAC) displays notable variations among the formulations. TCM exhibits the highest GAC at 14.7 %, indicating its superior ability to absorb glucose. On the other hand, TC, TM, and CM show comparatively lower but relatively consistent GAC values, suggesting that the individual components or their combinations might affect the formulations' ability to absorb glucose to varying extents.

Water absorption capacity (WAC) also varies among the formulations. TCM displays a lower WAC compared to TC and CM, while TM shows the lowest water absorption capacity. Oil absorption capacity (OAC) showcases intriguing trends across the formulations. TCM and TC exhibit similar oil absorption capacities, while CM shows a slightly higher OAC, and TM displays the lowest.

Despite the variations in individual components' properties, the overall ANOVA results suggest no significant differences among the formulations in their water, oil, or gas absorption capacities. This implies that from a functional perspective, these herbal mixtures might be somewhat interchangeable based on these specific criteria.

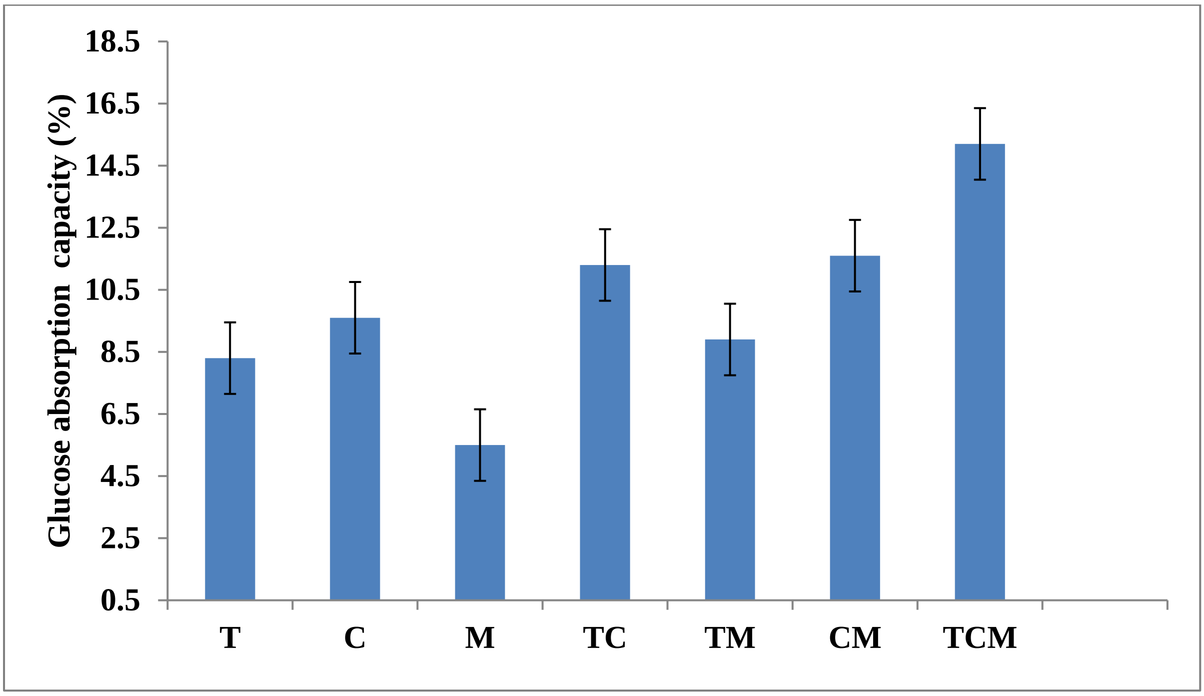

Figure 1: Glucose Absorption Capacity of T, C, M, TC, TM, CM and TCM
Key: T (Turmeric); **C** (Coriander); **M** (Mint); **TC** (Turmeric: Coriander); **TM** (Turmeric: Mint); **CM** (Coriander: Mint); **TCM** (Turmeric: Coriander: Mint). Values were expressed as mean± standard error of mean (SEM) P> 0.05

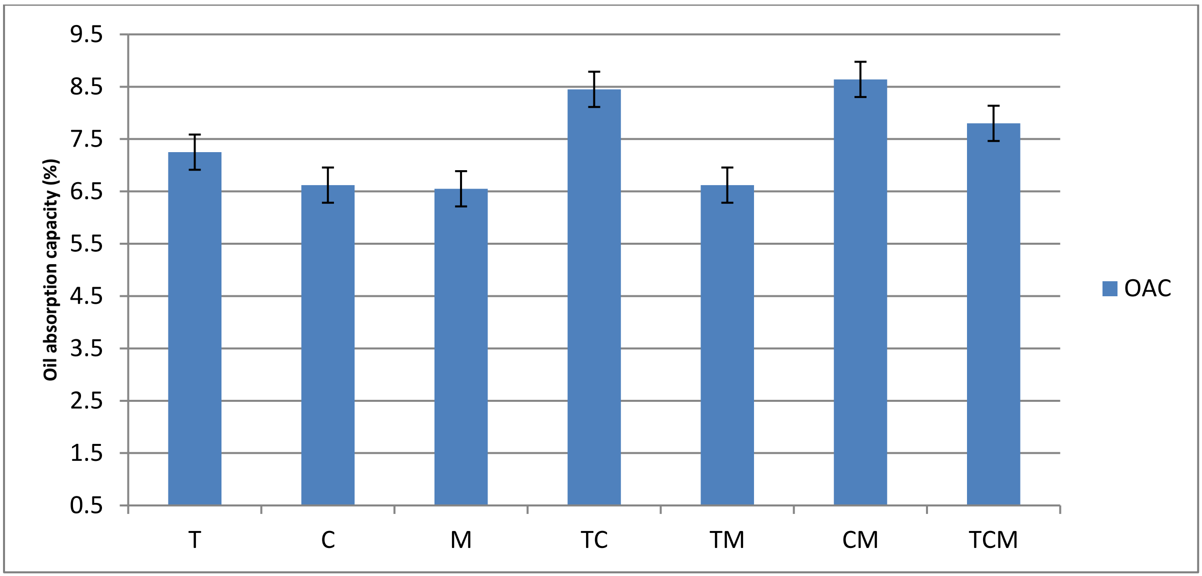

Figure 2: Oil Absorption Capacity of T, C, M, TC, TM, CM, and TCM.
Key: T (Turmeric); **C** (Coriander); **M** (Mint); **TC** (Turmeric: Coriander); **TM** (Turmeric: Mint); **CM** (Coriander: Mint); **TCM** (Turmeric: Coriander: Mint). Values were expressed as mean± standard error of mean (SEM) P> 0.05

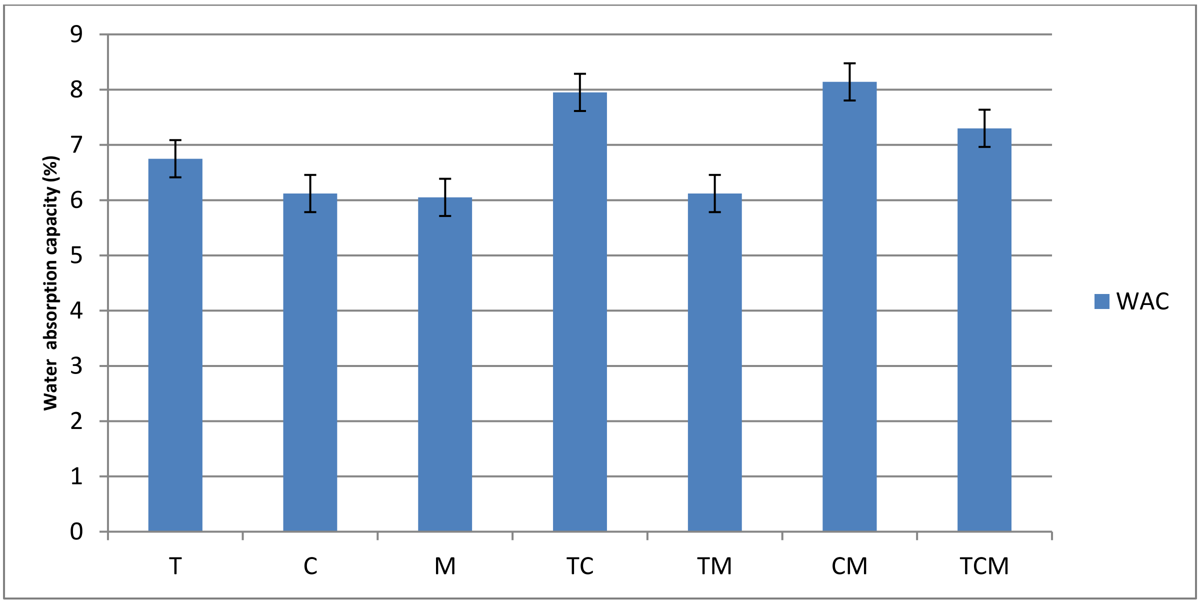

Figure 3: Water Absorption Capacity of T, C, M, TC, TM, CM, and TCM.
Key: T (Turmeric); **C** (Coriander); **M** (Mint); **TC** (Turmeric: Coriander); **TM** (Turmeric: Mint); **CM** (Coriander: Mint); **TCM** (Turmeric: Coriander: Mint). Values were expressed as mean± standard error of mean (SEM) P> 0.05

The DPPH assay results reveal that there's no significant difference in antioxidant activity among the formulations. While there are minor fluctuations in the scavenging activity, the ANOVA indicates that these differences are not statistically significant. Despite the variations, the formulations as a whole exhibit similar antioxidant activities. This suggests that the combinations of turmeric, Coriander, and mint leaves, whether individually or together, contribute similarly to the overall antioxidant potential of the formulations. The minor differences in antioxidant activity might be attributed to the individual properties of the herbs and their interactions within the formulations. While TCM shows slightly higher scavenging activity in all the concentrations, it doesn't drastically surpass the antioxidant potential of TC, TM, or CM. This similarity in the antioxidant capabilities across the formulations suggests that the combination of turmeric, Coriander, and mint leaves doesn't substantially alter the overall antioxidant activity measured by the DPPH assay. The ANOVA results indicate no significant difference (P > 0.05) the consistently slightly higher values in TCM warrant further exploration.

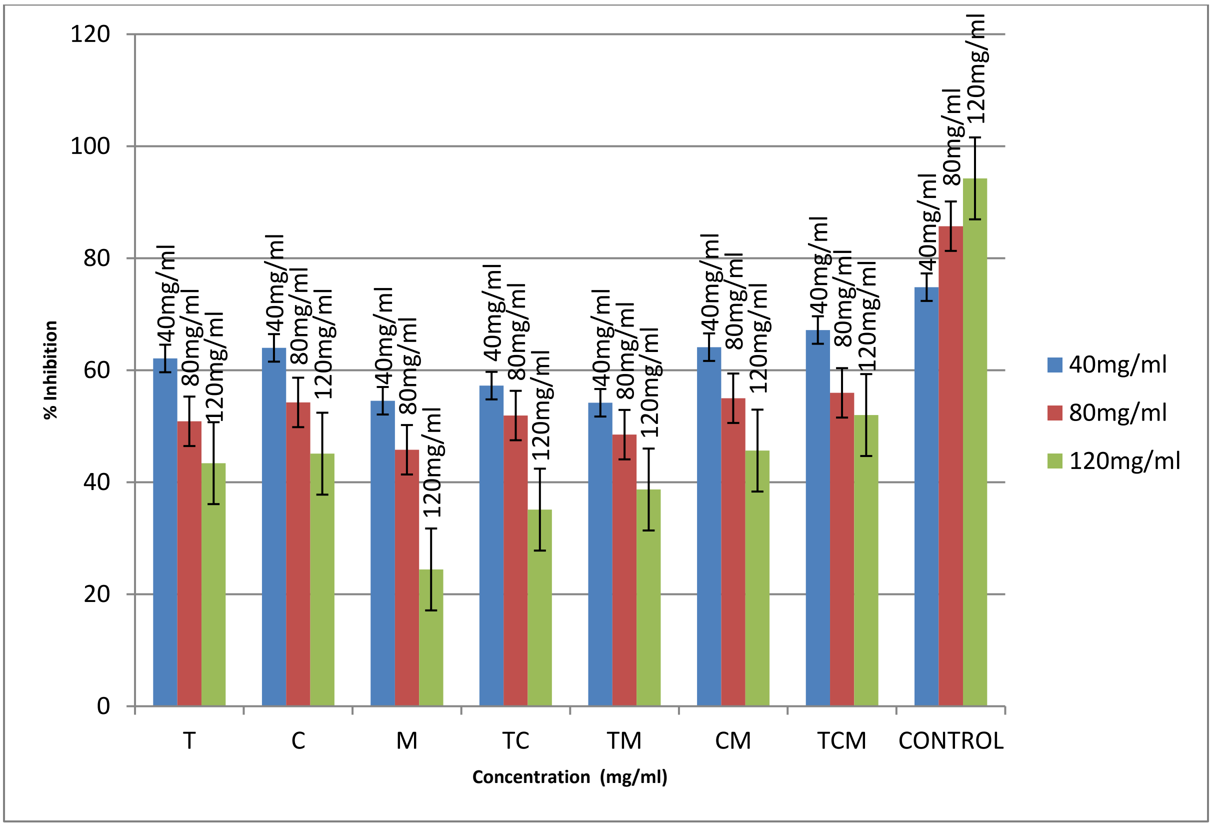

Figure 4: % inhibition of DPPH scavenging activity of T, C, M, TC, TM, CM and TCM
Key: T (Turmeric); **C** (Coriander); **M** (Mint); **TC** (Turmeric: Coriander); **TM** (Turmeric: Mint); **CM** (Coriander: Mint); **TCM** (Turmeric: Coriander: Mint). Values were expressed as mean± standard error of mean (SEM) P> 0.05

DISCUSSION

In the proximate analysis, food deterioration is primarily determined by moisture content. It is known that reduced moisture content in food samples lowers microbial activity and enhances the shelf life of food products (Ismaila *et al.*, 2018). The highest moisture is recorded in M (11.7%) and the lowest in TC (9.26 %). TCM offers a formulation with a more protein-rich and less carbohydrate-heavy profile compared to the individual ingredients.

Water is important in almost every diet, serving not only as a filling material and solvent but also as an essential means of maintaining macromolecules and cell activities (Awuchi, 2019).

Ash content indicates the total inorganic components after the oxidation of organic materials in the furnace (Iwe *et al.*, 2016).

Vitamins play an important role in a variety of metabolic and physiological processes (Ferraro et al., 2016). Vitamin deficiencies or excess can contribute to disease conditions by limiting normal cell growth. Thus, thorough and precise vitamin analysis is critical for a typical balanced diet (Awuchi *et al.*, 2020). Vitamins A, C, and E are antioxidants, thus making them effective therapeutic agents for the forestallment of a variety of ailments (Ali *et al.*, 2020).

In the Functional Properties evaluation of the formulations, Glucose Absorption Capacity (GAC) was studied because it serves as a measure of fibers ability to absorb glucose, thereby lowering the glucose concentration in the body (Ismaila *et al.*, 2018). The highest GAC was observed in TCM (14.7%). This indicates that fiber could aid glucose retention in an in-vitro setting. This is similar to Abubakar *et al.* (2022) research work on Tomato, Garlic, and Carrot, where T: G and T: G: C formulations had the highest GAC.

Water Absorption Capacity (WAC) is useful for predicting a fiber material fecal bulking ability, where as Oil Absorption Capacity (OAC) is used for predicting fiber materials lipophilic behavior. WAC is solely dependent on the availability of hydrophilic groups that attract water molecules as well as the macromolecules ability to form gels (Hannington *et al.,* 2020). WAC is significant in foods because it affects emulsification, adhesion, solubility, and other functions (Obiegbuna *et al*., 2019).

The free radical DPPH, which is widely used to evaluate the ability of compounds to operate as free radical scavengers and hydrogen suppliers, is a rapid, simple, and inexpensive method for testing antioxidant activities (Sridhar and Charles, 2018). In vitro, several plants have been shown to neutralize DPPH radical scavenging activity (Aini *et al.,* 2019) (Kurniawan *et al*., 2021) (Amrulloh *et al*., 2021) (Lalrinzuali *et al*., 2015). TCM shows the highest % inhibition in all three concentrations.

CONCLUSIONS

The present study has established the proximate analysis, vitamin composition, functional properties, and antioxidant activities of turmeric, Coriander, and mint leaves and their formulations.

The proximate analysis shows no significant difference among the formulations (p>0.05), with TCM showing a nutritional profile shift because of the combination of the herbs rather than single herbs.

In the vitamin composition, Vitamin A displays a pattern among the formulations, with TC and CM showing higher values than TM and TCM, which are, in turn, better than the single herbs. Vitamin C fluctuates across the formulations. TM displays higher, while T shows the lowest. Vitamin E content remains relatively stable among the formulations with a difference from the single herbs.

In the functional properties, GAC displays notable variations among the formulations. TCM exhibits the highest GAC at 14.7 %, indicating its superior ability to absorb glucose. TC, CM, TM, and the single herbs show a comparatively lower GAC value. WAC also varies among the formulations. TCM displays lower WAC compared to TC, TM and CM. The single herbs show the lowest WAC. OAC shows intriguing trends across the formulations. TCM and TC exhibit similar OAC, while CM shows a slightly higher OAC, and TM displays better than single herbs. There is no significant difference (p>0.05).

In the antioxidant Activity, TCM shows lightly higher scavenging activity in 40, 80 and 120 mg/mL concentrations than other formulations.

The results of this study suggest that the combination of turmeric, Coriander, and mint leaves TCM may be a viable option for the development of nutraceuticals. Further research is needed to determine the optimal ratios and processing methods for this combination (TCM) to achieve the desired nutrient content.

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