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ANALYSIS OF SOME PRIMARY METABOLITES AND FREE RADICAL ACTIVITIES IN A MEDICINAL BLEND OF METHANOLIC EXTRACT OF DOUM PALM (*Hyphaene thebaica*), LEMON GRASS (*Cymbopogon citratus*) AND MINT LEAVES (*Menthe piperita*)

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Abstract

This study is aimed at determining the presence of primary metabolites and free radical scavenging activities in a medicinal blend mixture of (doum palm (D), mint leaf (M) and lemon grass (L) consumed in Northern Nigeria. Several studies have focused on individual blends there is little or no analysis on the blend. Analysis was carried out on a mixture of two different formulations F₁(60%, 20% and 20%) and F₂ (50%, 25% and 25%) of the medicinal blend (D, M, L) were assessed respectively using methanol extracts. The extract of the blends was screened qualitatively and quantitatively for phytochemicals, the concentration of mineral elements and vitamin contents (A, C and E) determined using AAS. Total Phenolic Content (TPC) evaluated using Folin- ciocalteu reagent, and the free radical was determined by 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The results indicated the presence of the phytochemicals; saponins, tannins, flavonoids, cardiac glycosides, phenols, terpenoids, alkaloids while anthraquinones and chalcones were absent, the result show the phyto-chemical content in (F₁) had higher value in alkaloids (48.99mg/g), flavonoids (22.62mg/g), TPC (212.83mg/ml) and tannins (55.39mg/g) than (F₂), had alkaloids (43.33mg/g), flavonoids (22.83mg/g), TPC (197.45mg/g), tannins (38,48mg/g). The antioxidant activity was found to be much higher in (F₁) than (F₂). TPC of extract (F₁) yielded highest amount of TPC compared to (F₂) with (F₁) having 212.83mg/g. DPPH (F₁) showed the highest scavenging activities as evidenced by maximum scavenging of DPPH 82.9% at 1000µg/ml. Also, the results indicate high vitamin concentration in (F₁) 0.333±0.01mg/dl of vitamin A, 8.90 ±0.3mg/dl of vitamin C and 0.166±0.01mg/dl of vitamin E compared to (F₂). The results indicate the plant blends Possess invariably high amount of poly-phenolic compound and antioxidant property thus, could be further assessed for pharmacological application.

Keywords: doum-palm, lemon-grass, mint-leaf, primary-metabolites and antioxidants

INTRODUCTION

Medicinal plants are the preferred primary health care system in many societies, over 60% of the world population and about 80% in developing countries depend on the use of medicinal plants for the treatment of ailments (Birhan *et al.* 2007). This is due to a number of reasons including affordability, accessibility, and cost effectiveness

(Fayiah *et al.*2008). Plants or herbs when used in disease management are highly encouraged due to their low side effect and effectiveness (Bourhia 2019). Thus, a blend of three different plants would be extremely effective. *Hyphaene thebaica* is a common palm tree with edible fruit, commonly known as doum palm is one of the world's most beneficial plants found in Egypt, Sudan and Sub-

Saharan African (Gharb and Fadhel 2015, Inuwa et al. 2023), it is a rich source of macro and micronutrients (Reda and Aamer 2015), and also natural antioxidants. It is used in the treatment of several diseases (Salib et al. 2013). *Menthapiperita L* is one of the ten most widely used plants in the world and is commonly used in the treatment of several diseases (Gulluce, et al. 2007). *Cymbopogoncitratrus* is used as a taste enhancer, known to be potent anti-oxidants (Zou et al. 2016), and is reported for the potential anti-diabetic activity (Agarwal et al. 2020). Phytochemicals are a wide range of biologically active compounds found in plants (Chede 2013) and provide additional health benefits against pathogen (Mathew et al. 2012). Dietary minerals are inorganic elements required by living orgs (Mitchell et al. 2012). They include Ca, Mg, P, Na and Fe etc. Some have roles as cofactors, while others are electrolytes (Nelson et al. 2000). Micronutrient work in correlation with macronutrients to keep the body functioning. They are co-enzymes, deficiencies in vitamins may result in diseases (Shills et al. 2005). Antioxidants are natural chemical compounds found in plants that help protect against and repair damage caused by free radicals, which are harmful molecules that can lead to oxidative stress (Aurelia et al. 2015). Doum palm, lemon grass, and mint leaf are commonly and extensively used for medicinal purposes. Several studies had focused on doum plant (*Hyphaene thebaica*), mint leaf (*Menthe piperita*) and lemon grass (*Cymbopogon citratus*) separately. There is little or no research remunerated on the formulation of lemon grass (*Cymbopogon citratus*) mint leave (*Menthe piperita*) and doum palm (*Hyphaene thebaica*) in a blend. Thus, scientific information on the constituent blend is lacking. Medicinal plants play a significant role in providing primary health care services are used by 80% of the marginal communities around the world (Shinwari et al. 2006). Doum plant, Lemon grass and Mint leaves have been reported to possess many biological and medicinal activities. Although, the chemical constituents have been assessed separately assessing the blended formulation of herbal plants in right combination may provide strong scientific reasoning for the reported biological activities and therapeutic effect. The Objectives of this study were to determine the phytochemical content, mineral

element (Zn, Co, Fe, Ca, Na, Mg, Cu and K), antioxidant activity (TPC, DPPH) and vitamin (A, C and E) from the formulated blend and compare the two different blend formulation in different proportions.

2.0 MATERIALS AND METHOD

2.1 Plant materials

The plant samples were collected from Rimi Market in Municipal LGA, Kano, identification and authentication were done by botanist in the Department of Plant Biology, Bayero University, Kano.

2.2 Preparation of crude extract

The plant samples were washed and dried at room temp for 5 days under hygienic conditions and then pulverized into a fine powder using a mortar and pestle. The different plant samples were then mixed in the proportion, blended formulation1; F1 (doum 60%, lemon 20% and mint 20%) and blended formulation 2; F2 (doum 50 %, lemon 25% and mint 25%) as 100 g portion of the blend was dissolved in 500 ml of methanol. The mixture was shaken vigorously for 2 hrs. using a mechanical shaker and allowed to stand for a day. The solution was filtered using Whatman filter paper. The filtrate was concentration in water bath and stored in a clean airtight container and used for the chemical analysis as at required AOAC (2006).

2.3 Chemicals and reagents

Methanol, chloroform, 1, 1-Diphenyl-2-picrylhydrazine (DPPH), gallic acid, folin ciocalateau phenol reagent and potassium acetate were products of sigma Aldric), Methanol and L-ascorbic acid were from a chemical company (Kermel), while Vitamin E and ferric chloride were from BDH, Chemicals.

2.4 Phytochemical screening

The extract of these two formulations were screened qualitatively and quantitatively for phytochemicals

2.4.1 Qualitative determination

Saponins, tannins, alkaloids, phenols, flavonoids and anthraquinones, terpenoids, chalcones, cardiac glycosides and physterols in the methanolic extract were screened qualitatively using the method as described Trease and Evans (1983).

2.4.2 Quantitative determination

Total phenolics, flavonoids, alkaloids and tannins in the methanolic extract were quantitatively screened

using the method as described Trease and Evans (1983).

2.4.3 Minerals composition

Mineral elements were determined by the method of AOAC (2006) in the methanolic extract analyzed for mineral composition.

2.4.4 Total phenolic composition

Total Phenolic Content (TPC) evaluated using Folin- ciocalteu reagent, and the free radical of these blend methanolic extract was determined by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) according to the method of Rabiu, *et al.*, (2021).

2.4.5 Antioxidant (DPPH) determination

The free radical scavenging activity of methanol extract was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method of (Rabiu 2021). The DPPHscavenging activities of different herbal blend results are presented below.

2.4.6 Vitamin concentration determination

The concentration of Vitamin Content (A, C and E) was determined using atomic absorption spectrophotometer according to the method of AOAC (2023).

2.4.7 Statistical Analysis

All data were expressed as mean $\pm$ standard deviation and were statistical analyzed using the SPSS statistical software version 20 (SPSS). All analysis was done in triplicate ($p < 0.05$).

3.0 RESULT AND DISCUSSION

The Results for the qualitative, quantitative, mineral, total phenolic content and antioxidant analysis for the blended formulation F1 and F2 of Doum palm, lemon grass and mint leaf are presented below. The plants were in the combination F1 (doum 60%, lemon 20% and mint 20%) and blended formulation 2; F2 (doum 50 %, lemon 25% and mint 25%)

3.1 The qualitative analysis

The qualitative analysis of phytochemicals saponins, tannins, alkaloids, phenols, flavonoids and anthraquinones, terpenoids, chalcones, cardiac glycosides and physterols in the methanolic extract screened qualitatively using the method as described Trease and Evans (1983) for the blended formulation F1 and F2.

Table1: Qualitative analysis of chemicals in F1& F2

Phytochemicals	F1	F2
Saponins	+	+
Tannins	+	+
Alkaloids	+	+
Phenols	+	+
Anthraquinone	-	-
Cardiac glycosides	+	+
Terpenoids	+	+
Chalcones	-	-
Flavonoids	+	+
Phytosterols	+	+

(+) Indicates presence of phytochemicals
(-) indicates Absence of phytochemicals

3.2 Quantitative determination. The results for the quantitative analysis for the

Total phenolics, flavonoids, alkaloids and tannins in the methanolic extract quantitatively screened using the method as described by Balamurugan *et al.* 2019 are presented below;

Table 2: Quantitative analysis of phytochemicals in F1 and F2

Phytochemical	F 1 (mg/g)	F 2 (mg/g)
Flavonoids	22.62 $\pm$ 3.68 ^a	22.83 $\pm$ 2.67 ^b
Alkaloids	48.99 $\pm$ 4.01 ^a	43.33 $\pm$ 10.58 ^b
Tannins	55.39 $\pm$ 6.79 ^a	38.48 $\pm$ 8.04 ^b

Values are presented as mean $\pm$ standard deviation values with different superscript are significant ant different. ($P < 0.05$).

F1(doum 60%, lemon 20% and mint 20%) and blended formulation 2; F2 (doum 50 %, lemon 25% and mint 25%).

The results showed that both F1 and F2 are rich in the phytochemicals, saponins, tannins, alkaloid, phenols, cardiac glycosides, terpenoids, flavonoids, and phytosterols while anthraquinone and chalcones were absent. The phytochemicals found in the blend are very crucial in the maintenance of electrolyte level, antioxidant activities and protection against infection. (Zahra'u *et al.*, 2014). F1 has a higher value of total phenolic (212.83 mg/g), flavonoids (22.62 mg/g), a1kaloids (48.99mg/g), tannins (55.39mg/g). This is in line

with previous research carried out on doum palm found to have phytochemicals, flavonoids, total phenolic, tannins and alkaloids all within the following concentration (47.17mg/g), (56.87mg/g), (28.54mg/g) and (24.67mg/g) respectively (Mohamed, 2016). This shows that the result obtained from both F1 and F2 to be of a higher concentration of phytochemicals with the exception of flavonoids than is obtained from concentrations in doum palm separately. Also, previous findings in lemon grass indicate the concentrations of the phytochemical's tannins, flavonoids, phenols, and alkaloids are (29.43mg/g), (24.76mg/g), (16.54mg/g), and (26.87mg/g) respectively (Mata *et al.*, 2007) this shows that both F1 and F2 have higher concentration of the phytochemical than is obtained from previous findings of lemon grass conducted separately. Thus, the blended plants have higher phytochemical activity than their individual plants.

3.3 Minerals composition

The results of the mineral elements determined by the method of AOAC (2006) in the methanolic extract analyzed for mineral composition are presented as follows;

Table 3: Mineral Composition of F 1 and F 2

Elements	F1(mg/l)	F2 (mg/l)
Ca	8.60 ± 0.019 ^a	6.41±0.09 ^b
Co	0.01± 0.014 ^a	0.87±0.006 ^b
Cu	0.02±0.010 ^a	0.65±0.0004 ^b
Fe	0.80±0.001 ^a	0.43±0.001 ^b
K	215.9±0.072 ^a	187±0.043 ^b
Mg	8.10±0.051 ^a	7.50±0.042 ^b
Na	59.10±0.067 ^a	52.78±0.054 ^b
Zn	0.13±0.01 ^a	0.79±0.049 ^b

Values are presented as mean ± standard deviation values with different superscript are significant different. (P<0.05).

F1 (doum 60%, lemon 20% and mint 20%) and blended formulation 2; F2 (doum 50 %, lemon 25% and mint 25%). Mineral elements are naturally occurring inorganic elements which are present in very small amount in plants but are important for the vital processes of life. The Average Mean Concentration of eight essential elements were analyzed in formulation 1 and 2 in Table 3.0. These elements are Ca, Co, Cu, Fe, K, Mg, Na, and Zn. Among these elements Ca, K, Mg, and Na were found to be in higher

concentration from both formulations while Co, Cu, Fe and Zn are found in trace amount from both formulations which are within the standard requirements.

3.4 Total Phenolic Composition

The results for the TPC of the different blends (F1 and F2) were determined using the gallic acid equivalents (mg/g) and the results are gallic acid standards as the calibration curve summarised as follows;

Table 4: Total phenolic contents for different blend of herbal extract (mg/ml)

SAMPLE	TPC
F1	212.83±22.01 ^a
F2	197.45±15.67 ^b

Values are mean±standard deviation with different superscript (P<0.05),

The total phenolic contents of different methanolic extracts of doum palm, mint leaves and lemon grass were evaluated using folin-ciocalteu method. The results of this finding were found to be 212.83mg/g. Thus, the results of the F1 formulation indicate a higher TPC. The results of previous findings according to Kaur and Kapoor (2002) also showed that lemon grass have TPC of 67mg/g, while Fahad al-juhaimi and Ghafoor (2011) also indicated that mint leave to have a TPC of 1.24mgGAE/100ml. This implies that the combination of the whole of the blend for both formulation of extract with 212.83mg/g to have a higher TPC when compared to various findings of herbal extracts carried out separately. This indicates that the plant formulation in combination have higher activity of TPC than separately.

From the investigation, it was observed that TPC of various extract F1 yielded highest amount of TPC than F2. The slight variations observed in the different blends can be attributed to the higher concentration of the doum palm. Phenolic compounds are aromatic secondary metabolites that have been extensively investigated in medicinal plants, fruit and vegetable (Djeridane *et al.* 2006). Phenolic compounds are associated with colour, sensory qualities, nutritional and

antioxidant properties (Robbins 2003).

3.5 Antioxidant (DPPH) determination

The free radical scavenging activity of methanol extract was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method of (Rabiu 2021). The DPPH scavenging activities of different herbal blend results are presented below

The results indicate high scavenging free radical activities in the different herbal blend. The lower the IC₅₀ the higher the scavenging activities these implies that F1 with a concentration of 12.01 ± 1.61% at 1000 µg/ml to have higher antioxidant activities than F2 at 15.15 ± 4.1 % at 1000 µg/ml. Thus, indicating a slightly higher scavenging activity in F1 blend with a higher doum content than F2 blend. Previous finding of doum palm according to Zheng and Wang (2001) also showed that at 1500 µg/ml extract exhibited 80% antioxidant activity. While, also findings in lemon grass according to Rozynska and Gwiazdowska (2020) also showed that at 50 µl it scavenges 26.3 ± 1.60, and that of mint leave was according to Al-Juhaimi and Ghafoor (2011) at a concentration of 34.21%. The results from previous studies implies that the combination of the three-plant blend yielded high antioxidant activities as a blend compared to individual plant. The antioxidant properties were enhanced when at the F1 combination was found to be slightly more than their F2 combination, indicating that the effectiveness of its antioxidant potential would have a slightly better advantage. 2,2-Diphenyl-1-picrylhydrazyl is a sustainable diamagnetic molecule that has gained an extra electron. The reduction in absorption at 517 nm caused by antioxidants was used to identify DPPH. The effectiveness of the phenolic components in the doum fruits extracts to donate hydrogen improves the DPPH scavenging property Kolla *et al.* (2021) when utilizing DPPH to evaluate the antioxidant property.

3.6 Vitamin Concentration

The concentration of Vitamin Content (A, C and E) determined using atomic absorption spectrophotometer according to the method of AOAC (2006).

The antioxidant activity of vitamin A, C and E was carried out and confirmed high antioxidant activity

for vitamin A, C and E in the blend of plants. Also, the F1 blend was found to have a higher antioxidant activity in vitamin A, C and E than F2, with vitamin A having 0.333 ± 0.015, vitamin C having 8.9 ± 0.3 mg/dl and vitamin E having 0.166 ± 0.005 mg/dl. The slight difference in primary metabolites and free radical scavenging activity can be attributed to an increased concentration of doum

Table 5.0 IC₅₀ in (µg/ml) of DPPH Radical scavenging activity

F1	F2	Ascorbic Acid
12.01 ± 1.61 ^c	15.15 ± 4.16 ^b	0.01 ± 0.00 ^a

Values are presented as mean ± standard deviation of triplicate readings n=3 values within the same row bearing different superscripts letters are significantly (p<0.05) different.

palm in the F1 blend. Doum palm is a rich source of primary metabolites, which has been proven to have high phytochemical and antioxidant activity which increased in combination with other plants lemon grass and mint leaf to have tremendous efficacy.

3.0 CONCLUSION

In conclusion, the herbal blends of different plant extracts of doum palm, lemon grass and mint leaf was found to have high concentration of primary and free radical scavenging activities. The concentration of phytochemicals (Alkaloids, Flavonoids, Tanins and Phenolic compounds), minerals (Ca, K, Mg), and Na, TPC (212.83 mg/ml), vitamin activity at 0.333 ± 0.01 mg/dl of vitamin A, 8.90 ± 0.3 mg/dl of vitamin C and 0.166 ± 0.01 mg/dl and free radical scavenging activity (12.01 ± 1.61^c µg/ml) was found to be much higher in the F1 when compared to the F2 blend. Thus, concentration obtained from this study showed that high composition of doum palm is related to high scavenging activities and can be used pharmacologically.

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Table 6.0 Vitamin Concentration (F1 and F2) (mg/dl)

Vitamin	F1	F2
A	0.333±0.015 ^a	0.323±0.013 ^b
C	8.900±0.300 ^a	6.100±0.36 ^b
E	0.166±0.005 ^a	0.012±0.003 ^b

Values are presented as mean ± standard deviation of triplicate readings n=3 values within the same row bearing different superscripts letters are significantly (p<0.05) different.

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