PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (12B Supplementary) 4321-4328 Dec. 2024

Evaluation and Optimization of Yield and Antioxidant Activity of *Moringa oleifera* Flower Extract

[1]UKPE, RA; [2]UDO, II; [3]UDOFIA, PG

*[1]*Department of Chemistry, Federal University Otuoke, Bayelsa State, Nigeria*
[2]Department of Chemical Sciences, Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene, Akwa Ibom State, Nigeria
[3]Department of Food Technology, Akwa Ibom State Polytechnic, Ikot Osurua, Akwa Ibom State, Nigeria

Correspondence author Email: ukpera@fuotuoke.edu.ng
ORCID: https://orcid.org/0000-0002-1010-4933
Tel: +2348035495115

Co-Authors Email: udoidongesit19@gmail.com; kesitpatrick1@gmail.com

ABSTRACT: The local population consumes ethanol extracts of *Moringa oleifera* as antioxidant but without scientific evidence. This work assessed the effect of concentration of ethanol, drying time, and time of harvest of the plant part on its antioxidant activity. The basic free radicals, 1, 1, diphenyl-2-picryl-hydrazyl (DPPH) and the ferric reducing power (FRAP) assays assessed the antioxidant power of the extracts with the help of ascorbic acid as a standard antioxidant. The response surface design uses reiterated values of the dependent variables, one at a time to assess the dependent variables. Result from the experiments revealed that antioxidant power of the extracts associated positively with concentration of the extraction solvent. Optimization analysis on the experimental data indicated that 100% solvent showed 7.40% yield, 32.20 amino acid equivalent per gram (AAE/g) of antioxidant capacity, 6.46 mg/100g of total phenolic content, 2.80 mg/100g of total flavonoid content, and desirability of 55.60%. FRAP assay on 59.11% solvent concentration, 43 hours drying time, and a 10:00 a.m. harvest time showed 52.94 g/100g yield, 52.40% oxidation inhibition, 16.42 AAE/g of IC_{50}, 4.94 mg/100g phenolic acid content, and 3.02 mg/100g flavonoid content, at 90% desirability. The values were comparable to ascorbic acid, therefore the ethanol extract *Moringa oleifera* could serve as a natural antioxidant.

DOI: https://dx.doi.org/10.4314/jasem.v28i12.47

Cite this Article as: UKPE, R. A; UDO, I. I; UDOFIA, P. G (2024). Evaluation and Optimization of Yield and Antioxidant Activity of *Moringa oleifera* Flower Extract. *J. Appl. Sci. Environ. Manage.* 28 (12B Supplementary) 4321-4328

Dates: Received: 22 October 2024; Revised: 20 November 2024; Accepted: 08 December 2024; Published: 31December 2024

Keywords: *Moringa oleifera*; antioxidant; neuro-degeneration; phyto-antioxidant

Plants' extracts are important items in the local health system (Udofia *et al.* 2023). Natives in the study area consume plant extracts to prevent or delay cases of inflammation, neurodegenerative disorders (Debebe *et al*., 201), malaria, and nutrition (Rajkumar *et al.*, 2022). However, the strength of the claim is not sufficiently supported by convincing scientific evidence (Fahey, 2020). Many tissue degenerative problems and food spoilage are most often linked to the accumulation of free radicals, reactive oxygen species, and reactive nitrogen species. Free radicals are unstable atoms and molecules with unpaired electrons making them highly reactive (Costagliola *et al.*, 2022). They oxidize intact macromolecules in the living cells, including oxidative stress, inflammation (Sahin *et al*., 2004). Inversely, antioxidants are substances that can inhibit, delay, prevent, or treat damage to cells caused by free radicals. Antioxidants protect bioactive molecules from oxidation and affect their functionality (Sahin *et al*., 2004). Antioxidants may be obtained externally, including butylated hydroxytoluene, butylated hydroxyanisole, ascorbic

*Correspondence author Email: ukpera@fuotuoke.edu.ng
*ORCID: https://orcid.org/0000-0002-1010-4933
Tel: +2348035495115

acid, and vitamins A and E, they are used in production and preservation of (Ukom *et al*., 2023). Synthetic antioxidants have a history of toxicity to man and the environment, especially in situations of misapplication and abuse (Singh *et al*., 2011). Plant products rich in phenolic, flavonoid, and carotenoid are preferred. These molecules have nutraceutical advantages over their synthetic counterparts; moreover, they are renewable, affordable, and have a good history of safety (Lakshmipriya *et al*., 2016). The antioxidant power of plant extracts is a function of the quantity of the available phytochemicals in the biomass extract. Additionally, the quality and quantity of phytochemicals extracted depends on the concentration of the extraction solvents, extraction coefficient of a solvent, time of immersion of the plant sample in the solvent, particle size, and temperature of the extraction system (Saalu *et al*., 2012; Ukom *et al*., 2020). *Moringa oleifera* is an interesting plant in the study of phyto-antioxidant to replace the synthetic counterparts (Udofia *et al.*, 2022). This plant belongs to the Brassica order, *Moringaceae* family. The plant is a native of the Indian sub-continent, flourishing in the tropical and sub-tropical regions of the world (El-Sohaimy *et al*., 2015). All parts of the plant exhibit relative antioxidant and nutritional potency *in vivo* and *in vitro*. Additionally, it is rich in phytochemicals, including phenolics and flavones, carotenoids, minerals, and vitamins (Valdez-Solana *et al*., 2015). Response surface methodology studies the effect of different levels of independent variables on the dependent variable(s) of new substances (Udofia *et al*., 2020). Consequently, the objectives of this work were to evaluate and optimize the yield and antioxidant activity of ethanol extract of the *Moringa oleifera flower* using the response surface methodology.

MATERIALS AND METHODS

Plant material: *M. oleifera* flower was harvested from Utu Ikot Ukpong village, Nigeria, between 8 to 10 a.m. on the 5th of January, 2023. The flower was dried in the shade to brittleness before being powdered. The powder was extracted with iterated concentrations (0-100%,) of ethanol for 74 hours. Each extract was evaporated to a dark flaky substance and stored for subsequent analyses.

Chemicals: Chemicals used included 95% ethanol, rutin, Folin–Ciocalteus phenol reagent, potassium ferrocyanide, 1,1-diphenyl-2-picry-hydrazyl (DPPH), aluminum chloride, sodium hydroxide, and sodium nitrite.

Response surface design: Response surface methodology is a statistical design that investigates the relationship between independent and response variables (Montgomery *et al.*, 2016); the design assumes equation 1.

$$Y_n = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 \quad \ldots 1$$

Where Y_n is any predicted response, β_0 is the offset term, β_1 is a linear effect, β_{12} is the interaction effect, and β_{11} is the quadratic effect, the main effects. X_1, X_2, and X_3 represent averages of results of changing one factor at a time from its low to high levels. The interaction terms (X_1X_2, X_1X_3, and X_2X_3) are the response when three factors vary simultaneously, and the polynomial terms X_{12}, X_{22}, and X_{32} investigate nonlinearity.

Values of the parameters were calculated using the full linear and second-degree models and building quadratic models. The small option of the design with (20) experimental runs was generated using the Design-Expert Version 13 of Stat-Ease (Inc., 2021 East Hennepin Avenue Suite 480 Minneapolis, MN 55413, USA). The design incorporated the concentration of the extraction solvent (X_1), drying time of the flower (X_2), and time of harvest (X_3), Eqn. 3 to 9.

The second-order coefficients were generated with regression analysis, and the backward elimination option was used. Responses were initially fitted for the individual factors by the partial least square regression analysis. The fit of the model was assessed by the coefficient of determination (R^2) and analysis of variance (ANOVA). The insignificant variables ($p>0.05$), and their terms were eliminated from the final evaluation.

Layout of the experimental design According to the table, each factor varies over 5 levels: - ∝, -1, 0, +1, and +α. The small option of 10 center points, 15 runs, and ∝ value of 1.414, without replication and 1 star was deployed.

Table 1: Rotatable Central Composite 5^3 Design (RCCD)

	Unit	-∝	-1	0	1	+∝
Conc. (X_1)	%	20.71	0	50	100	120
Drying time (X_2)	Hr.	20.73	24	50	240	284
Harvest time interval (X_3)	Hr.	5.90	7	6.5	13	13

Meyers *et al.* (2016)

Determination of total flavonoid content of crude ethanol extract of the M. oleifera flower: The total flavonoid content of the extract was determined according to the method of Baba and Malik, (2015). We diluted 0.1 mL of the extract with 0.3 mL of distilled water, followed by 0.03 mL of 5% sodium nitrite ($5\%^{w}/_{v}$ $NaNO_2$). Subsequently, the mixture was incubated at 25^{o}C for 5 min and treated with 0.2 mL of 1 mM of NaOH. Next, the reaction mixture was diluted to 1 mL with distilled water, and the absorbance was measured at 512 nm. The total flavonoid content was calculated from a routine standard curve (20–100) µg/100g of the extract. A blank was prepared with distilled water instead of the extract.

Determination of total phenolic content of crude ethanol extract of the M. oleifera flower: Assessment of the total phenolic content of the extracts was carried out according to the method of Singleton and Rossi, (1965) and Kahlil *et al.* (2016) in where a suitable volume of 1 mg/mL of the extract was mixed with 10 mL of water and 1.5 mL of Folin–Ciocalteus' reagent in a 25 mL of volumetric flask. After 6 min, 5 mL of 20% sodium carbonate solution was added, and the volume was adjusted to 25 mL with distilled water. Next, the absorbance of the mixture was read at 765 nm after 30 min. Lastly, the percent total phenolics was calculated from the calibration curve of garlic acid (40–260 µg) plotted by using the same procedure, and total phenolics were expressed as % garlic acid equivalent.

The 1, 1, diphenyl-2- picrylhydrazyl assay: The free radical scavenging power of ethanol crude extracts of 0–100% on DPPH was carried out according to the method of Zheng *et al.* (2012). First, 1 mL of 0.1 mM solution of DPPH was added to 3 mL of the plant extracts at 20–80 µg/ml. Next, the mixture was shaken to mix well and allowed to rest at 27±1^{o}C for approximately 30 min. Lastly, the absorbance of the mixture was read at 518 nm. Absorbance was inversely proportional to the scavenging activity of test samples.

$$\%\ \text{inhibition of DPPH} = \frac{(Abo - \text{Abt})}{(\text{Ao})}\ x\ 110 \quad (2)$$

Where Ab_o is the absorbance of the control reaction mixture, and Ab_t is the absorbance of the test substance on DPPH (Zheng *et al*., 2012; Sahin *et al.*, 2004).

Ferric reducing/antioxidant power (FRAP) assay: The $ferric\ reducing/antioxidant\ power$ of the extracts was carried out according to the method adopted by Ukom *et al*. (2023). First, 20–80 µg/ml of the extract was mixed with 2.5 mL of 10% potassium ferrocyanide. Afterward, the mixture was incubated at 50^{o}C for 20 min, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3,000 rpm for 10 min. Lastly, 2.5 mL of the supernatant was mixed with 0.5 mL of ferric chloride and 2.5 mL of distilled, and the absorbance was measured at 700 nm.

RESULTS AND DISCUSSION

Table 2 presents the randomized 20 experimental (units) runs obtained from the pure and interaction of X_1, X_2, and X_3. The responses are presented as percentage oxidation inhibition and IC_{50} on DPPH and FRAP assays. The total phenolic (TPC) and flavonoid contents (TFC) and the yield are also presented as the total volume of slurry from different concentrations of ethanol.

The model of the oxidation inhibition power of the extracts on DPPH was significant ($p<0.05$, R^2 = 47.46, mean = 55.19%), and the FRAP model was significant as well ($p<0.05$, R^2 = 56.00, mean = 49.93%) in Eqn. 3 and 4 elucidate the observation.

$$\text{In}\ (\%) = 66.74+0.32X_1+ 0.034X_2+0.15X_3 \quad (3)$$

$$\text{In}\ (\%) = 46.74 + 0.38X_1 + 0.034X_2 - 2.13X_3 \quad (4)$$

It can also be seen from the table that the parameter increased in value according to the concentration of the extraction solvent, as shown in Fig. 2. The trend of observations in the study agrees with the values reported by Udofia *et al.* (2022) and Ukom *et al*. (2023). The actual values of the parameter are different due to the experimental design, handling of the plant material, and age of the sample. The ability of plant extracts to scavenge free radicals indicates how much of the antioxidants present in the samples can neutralize free radicals in the human system, preventing them from damaging cells. Free radicals are unstable and very reactive biochemical moieties. Plants and their parts, which are proven to contain or intuitively consumed to provide antioxidant effects, are often included in the menu (Rajkumar *et al.*, 2022). IC_{50} model of the extracts on DPPH and FRAP was significant ($p<0.05$, R^2 = 69.60, mean = 59.00% (eqn 5) and $p < 0.05$, R^2 = 61.97, mean = 32.19%, respectively (eqn 6).

$$IC_{50}\ (\text{AAE/g}) = 106.16 - 0.89X_1-0.38X_3 - 4.22X_3 \quad (5)$$

$$IC_{50}\ (\text{AAE/g}) = 101.16 - 0.89X_1 - 0.38X_2 - 4.22X_3 \quad (6)$$

The model of the parameter on both free radicals was higher than the values of ascorbic acid 27.70 on DPPH and FRAP (55.0%AAE/g and 49.95 AAE/g, respectively (eqn 3 and eqn 4), meaning that the extract could provide some level of antioxidant if consumed in good quantities. IC_{50} is the inverse of the free radical scavenging activity of a test substance (Figs. 1 and 1a); therefore, ascorbic acid showed a higher value activity than the extracts of DPPH and FRAP. The trend of the values is similar to that reported by Pooja and Mondi (2015), who found IC_{50} of 51.51 AAE/g and 13.66 AAE/g for DPPH and FRAP, respectively against ascorbic acid with a value of 24.70. Overall, the results indicate that the extracts have antioxidant properties comparable to the standard.

Table 2: Mean influence of X_1, X_2, and X_3 on % Inhibition, IC_{50}, on DPPH(FRAP) assays, TPC, TFC, and yield of the flower extract

Run	X_1	X_2	X_3	% In	LD_{50}	TPC	TFC	Yield
1	100	240	7	58(55)	55.12(50.00)	25.10(25.11)	7.10(7.10)	5.00(4.62)
2	50	132	5	73(67)	12.50(30.11)	12.10(22.20)	3.50(3.50)	2.50(2.11)
3	0	24	12	27(11)	79.00(53.20)	5.10(51.00)	2.10(2.10)	0.50(0.44)
4	50	-50	10	30(9.40)	12.70(49.20)	23.10(23.10)	4.21(4.21)	2.25(2.25)
5	100	240	12	60(71.10)	54.90(41.20)	25.20(25.20)	7.12(7.12)	5.40(4.50)
6	50	132	10	65(0.10)	13.00(51.20)	23.10(25.20)	4.11(4.11)	2.50(2.90)
7	50	132	10	70(69.90)	12.60(32.10)	5.11(5.11)	5.01(5.01)	2.90(3.02)
8	50	132	10	72(70.10)	12.67(60.20)	5.73(5.75)	4.99(4.99)	2.90(2.22)
9	-34	132	10	12(59.00)	100.00(70.20)	4.50(4.90)	3.21(3.21)	2.22(2.44)
10	0	240	7	30(70.10)	120.00(72.11)	5.22(3.21)	1.25(1.25)	0.50(0.94)
11	50	132	14	60(59.00)	59.00(52.20)	11.22(1.25)	3.25(3.25)	2.30(2.92)
12	100	24	7	45(29.00)	55.23(60.20)	25.11(3.25)	7.11(7.11)	5.00(4.52)
13	0	24	7	30(68.00)	90.20(80.20)	5.20(7.11)	2.11(2.17)	0.50(0.11)
14	134	132	10	72(57.00)	12.90(33.20)	26.12(2.17)	7.21(5.21)	5.30(0.77)
15	0	240	12	31(37.00)	8200(62.00)	5.20(5.21)	3.21(4.12)	6.00(0.51)
16	50	314	10	75(71.00)	128.00(43.11)	12.10(4.12)	1.25(3.60)	0.51(2.49)
17	50	132	10	66(27.00)	43.20(43.20)	13.01(3.60)	3.25(3.47)	2.49(4.57)
18	100	24	12	72(66.00)	57.22(37.10)	24.11(3.47)	7.11(8.12)	5.10(2.17)
19	50	132	10	78(68.00)	12.89(42.17)	11.90(8.12)	2.11(3.50)	2.17(2.79)
20	50	132	10	78(77.00)	13.67(40.21)	12.01(3.40)	7.21(3.12)	2.79(2.79)

Source: Experiment: key: X_1 = Concentration of extraction solvent (%); X_2 = Drying time of the flower (Hr); X_3 = Harvest time of the flower interval (am); In = Reduction inhibition of the free radical (%); IC_{50} = Mean concentration to reduce 50% of the free radicals; (AAE/g); TPC = Total phenolic content of the sample (mg/100g); TFC = Total flavonoid content of the sample (mg/100g)

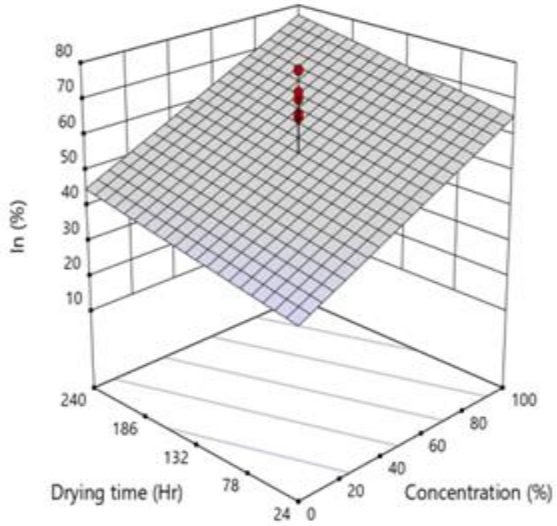

Fig 1: Linear free radical scavenging power of extract

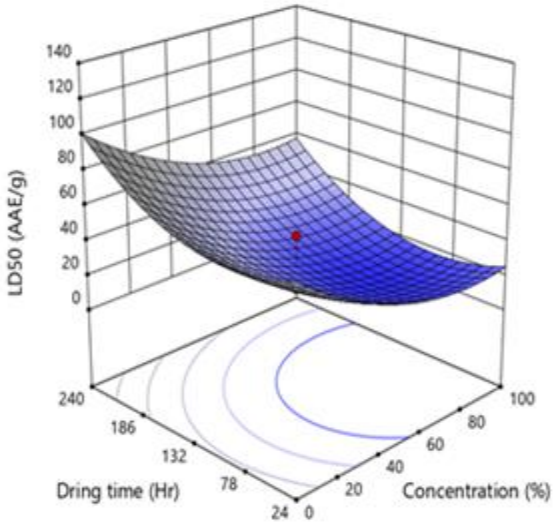

Fig 1a: inverse of free radical scavenging power of the extracts

The antioxidant property of the extract is primarily influenced by the increasing concentration of the extraction solvent, while drying time and harvest time interval have a lesser effect on this parameters. The IC_{50} values observed indicate that the extracts are good antioxidants; therefore, the crude extracts of the *M. oleifera* flower may be a source of effective antioxidants.

The model of TPC was significant ($p<0.05$, R^2 = 0.9113, mean = 5.41 GAE/100g; (Eqn.7).

$$TPC\ (mg/100g) = 14.86-0.17X_1- 0.078X_2 + 0.002X_3 \quad (7)$$

The model of TFC was not significant ($p = 0.5128$, $R^2 = 0.1815$, mean = 2.94 GAE/100g; Eqn. 8.

$$TFC\ (mg/100g) = 5.57-0.17X_1- 0.0027X_2 - 0.15X_3 \quad (8)$$

Both parameters are a function of the concentration of the extraction solvent, consistent with the report of Rajkumar et al. (2022). The harvest time interval and drying time of the samples were not significant in the model. This may mean that the plant sample remains stable in terms of biochemical characteristics. The TPC and TFC are fractions of the total plant. These parameters are the chemical content of plants; they contain phytochemicals that can protect the body against the damaging effects of free radicals. Ukom *et al.* (2023) reported that TPC and TFC could delay, prevent, or control inflammatory diseases. In this study, the TPC 5.41 showed higher content in the plant material (eqn. 8). Overall, the results suggest that both parameters may not necessarily be equal in plants to be active, but they could be in synergy.

The yield of the plant sample is a composite of TPC, TFC, and related molecules. In this study, the yield increased with the concentration of the extraction solvent, which could be related to the antioxidant activity of the extracts. Uchechukwu *et al.* (2022) reported that both parameters correlate to the antioxidant power of test substances. Intuitively, the local population uses a very high concentration of ethanol to obtain maximum yield. In our study, the antioxidant parameters did not show a clean relationship to the concentration of ethanol; lower concentrations showed better antioxidant values (Table 2). Impurities are observed in yields obtained from high concentration of solvent extract. Sheemasharma *et al.* (2015) optimized TPC TFC in germinated foxtail millet for enhanced antioxidant power.

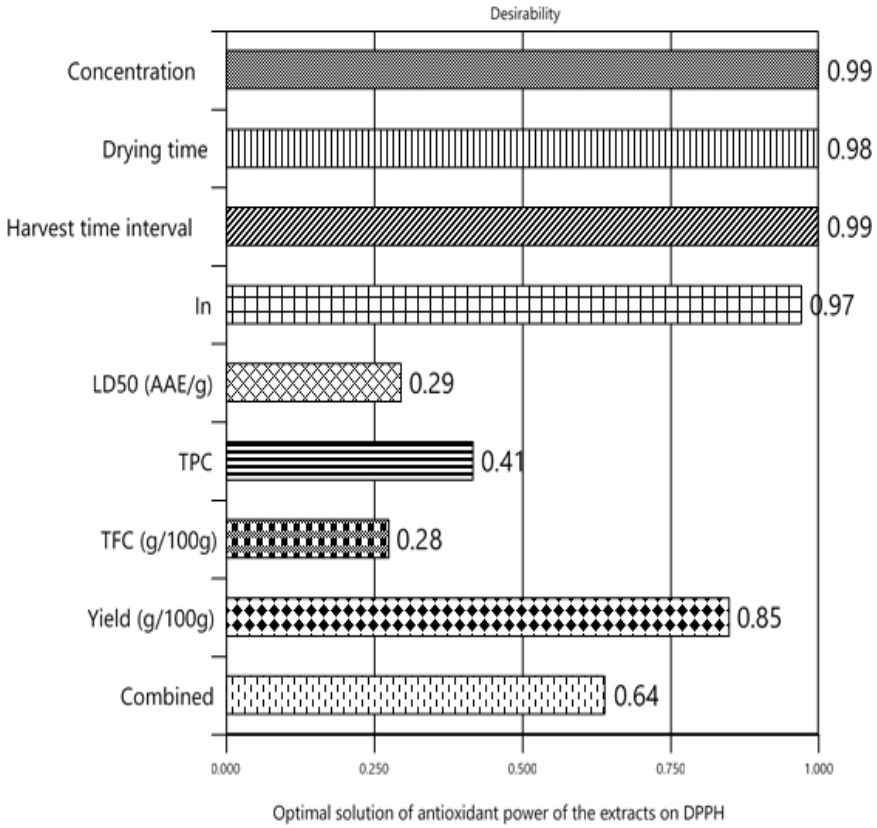

Fig. 3: Pareto chart of extracts on DPPH

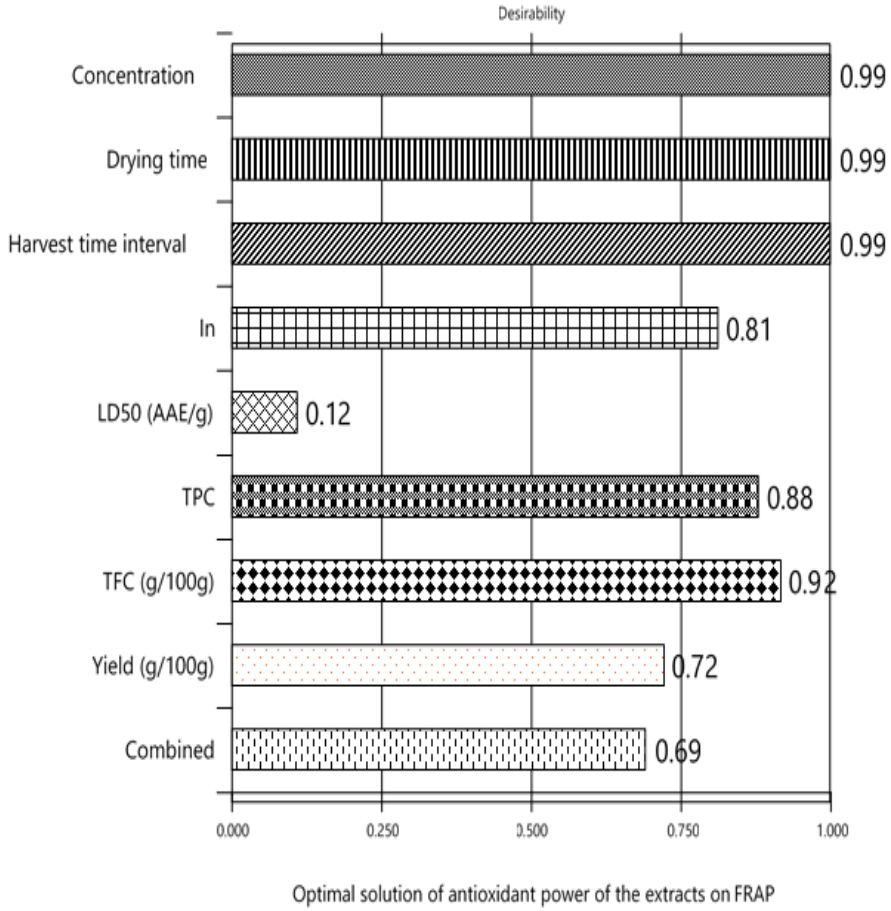

Fig. 4: Pareto chart of extracts on FRAP

	Model	p-value	R^2	Mean	Equations
DPPH	In (%)	0.014	0.4749	55.19	3
	IC_{50} (AAE/g)	0. 0008	0.6960	59.35	4
FRAP	In (%)	0.0242	0.560	49.95	5
	IC_{50} (AAE/g)	0.0171	0.6197	32.19	6
AA	In (%)	0.0011	0.9968	68.81	7
	TPC (mg/100g)	0.002	0.9113	5.41	8
	TFC (mg/100g)	0.5128	0.1815	2.94	9
	Yield (mg/100g)			…	10

Table 3: Models of the extracts on DPPH and FRAP assays, TPC, TFC, and nature of the design
Key: X_1 = Concentration of extraction solvent (%); X_2 = Drying time (Hr.); X_3 = Harvest time (a.m.)

Table 4: Optimization of percentage inhibition, IC_{50}, TPC, TFC yield of extraction

ASSAY	X_1	X_2	X_3	% In	IC_{50}	TPC	TFC	Yield	Der.
DPPH	100	24	7.00	7.40	32.20	6.46	2.80	4.88	0.556
FRAP	59.11	42.34	9.72	52.94	25.57	16.42	4.94	3.02	0.900

DPPH = 1,1, diphenyl-2-picrylhydrazyl assay
FRAP = Ferric antioxidant-reducing assay

The yield of crude plant extract is the total extract obtained from a unit quantity of a plant sample. The parameter may indicate the percentage of TPC and TFC and their contribution to the antioxidant activity of the plant extracts. Although IC_{50} and percentage oxidation inhibition may evaluate the antioxidant capacity of plant extracts, TFC and TPC should be used. According to Uchechukwu *et al.* (2022), TPC

and TFC exhibit a positive correlation with antioxidant activity. In this study, the yield of the plant sample varied mainly with the concentration of the extraction solvent (Eqn. 8 and 9)

Conclusions: In conclusion, the response surface design revealed that concentration of ethanol and varyingly in synergy with drying time and time of harvest influenced amount of total phenolic and total flavonoid compounds plant part. Statistical analysis of the data showed that the antioxidant values of the extracts were proportional to concentration of the extraction solvent as indicated by the IC_{50}. Further research should investigate the synergistic effects of the extract with other natural antioxidants, evaluate its bioavailability and metabolic fate, and develop saleable, cost effective extraction methods.

Declaration of conflicts: The authors declare no conflict of interest.

*Acknowledgemen*t
The Tertiary Education Trust Fund (TetFund) is appreciated for partial funding of the work

Data Availability Statement: Data are available upon request from the first author or corresponding author or any of the other authors

REFERENCES

Anthony, U; Nkeiru, N; Daniella, O (2020) Effect of Drying methods on the chemical composition and anti-nutritional properties of Cocoyam (*Xanthosoma maffafa* Schott) Tuber flour and leaf powder. *EAS. J. Nutr. Food. Sci.* 2(45-51). DOI:10.36349/easjnfs.2020.v02i04.004.

Ashwani K.; Nirma. P; Mukul, K.; Anina. J; Vidisha, T; Emel, O; Charalampos, P; Maomao, Z; Tahra, E; Sneha, K; Fatih, O (2023) Major Phytochemicals: *Recent Advances in Health Benefits and Extraction Method*. (2023). *Molecules* 28(2):887, DOI:10.3390/molecules28020887.

Boongad, V; Pongtip, S; Supch, M; Suchintra, T; Yuvadee, W; Wandee, G. (2012) Maximizing total phenolics , total flavonoids contents and antioxidant activity of Moringa oleifera extract by the appropriate extraction method. *Ind. Crops. Prod.* 44:566-571. http://dx.doi.org/10.1016/j.indcropcrop.2012.021.

Costagliola, C; Mariano, M; D'andrea, L; Napolitano, P; D'Alessandro, A.G. (2022) Free radical properties, source and targets. *Oxygen*.2: 48–78. https://doi.org/10.3390/oxygen2020006.

Debebe, M; Afework, M; Eyasu, M; Asfaw, D; Bekesno, G; Negero, G. (2017) Evaluation of biochemical, hematological and histopathological parameters of sub-chronic administration of ethanol extract of *Albizia gunmmifera* seed in albino Wistar Rat. *J. Clinical. Toxicol.* 7:1 *https://doi.org/10.4172/2161-0495.1000337.*

El S; Sobhy A; Hamad, GM; Mohamed, SE; Amar, Mohamed H. Al- Hindim, Rashad R. (2015) Biochemical and functional properties of Moringa oleifera leaves and their potential as a functional food. *Global Adv. Res. J. Agric. Sci*. 4(4). 188-199, .http://garj.org/garjas/index.htm

Lakshmipriya, G; Kruthi, D; Devaai, SK (2016) Moringa oleifera: A review on nutritive importance and its medicinal application. *Food. Sci. Human Wellness*, 5(2015): 49-56.

Rajkumar, R; Sanjeevirayar, A; Shanmugam, A. (2022) In Vitro antioxidant and Free Radical Scavenging Activity of *Curcuma longa*, *Acorus calamus* and *Camellia sinensis*. *Food. Nutrit. Sci.* DOI: 10.4236/fns.2022.138054.

Saalu, L. C; Ogunlade, B; Ajai, G. O; Oyewopo, AO; Akunna, GG; Ogunmodede, OS. (2012). The Hepato-Protective Potentials of Moringa oleifera Leaf Extract on alcohol-induced Hepatotoxicity in Wistar Rat. *Am. J. Biotechnol. Mole. Sci.* 2159-3701.

Sahin, FC; Kantar, F (2004) Sugar beet and barley yields in relation to inoculation withN2-fixing and phosphate solubilizing bacteria. *Plant. soil.* 265(1-2):123-129.

Seema S; Dharmesh, CS; Saxena; Charanjit, SR. (2015) Antioxidant Activity, Total Flavonoid, Falvonoids and Antinutritional Characteristics of Germinated Foxtail millet (*Setana italica*). *Cognet. Foods. Agric.* 1(1) *http://doi.org/101080/23311932.2015.1817281*.

Shoib AB; Malik, SA (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *J. Taibah University for Sci.* 9(4):449-454

Singh, C., Saini, K., Manda, H., Singhal, M, Sachdeva, K. (2011) Evaluation of Antioxidant Activity of Termitalia arjuna Leaves Extract. *Pharmacologyonlinne*, 1:49-59.

Singleton, V; Rossi, J. (1965) Colorimetry of Total Phenolic Compounds with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Viticulture*, 16, 144-158.

Uchechukwu, EO; Vivian, UO; Jing, W; Hai-Jun, Z; Guang-Hai, QQ; Shu-Geng, W (2022). Potential Implications of Natural Antioxidants of Plant Origin on Oxidative Stability of Chicken Albumen during Storage: A Review. *Antioxidants.* *11*(4): 630; https://doi.org/10.3390/antiox11040630.

Udofia, PG; Ojimelukwe, PC, Olaoye, AO; Ukom, N; Ekanem, MC. (2020) Evaluation of antioxidant activity of ethanol extract of shade-dried leaf of m. oleifera grown in utu Ikpe, Ikot Ekpene Local Government Area. *Nigeria j. Chem. Soc. Nig.* 47(1):101 – 114.

Zheng, C; Riccardo, B; Gugliel, F. (2012) EC_{50} Estimation of Antioxidant Activity in DPPH assay using Several Statistical Programs. *Food Chem.* 138(2013):414-420. http://dx.doi.org/10.1016/j.foodchem. 2012.11.001.\