International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Physiochemical and Antioxidant Evaluation of Moringa Oleifera Leaf Extract Followed by Phytochemical Screening and Antibacterial Activity

Muthu Tanya[1], Swayam P Mishra[2], Ankita P Das[3], Baishali Acharya[4], Dharitri Priyadarsini[5], Rajat Kumar Nayak[6]

[1]MSc. Student, Biochemistry and Bioinformatics, GITAM University, Telangana.
[2]M.Sc. Student, Biotechnology, MITS School of Biotechnology, Bhubaneswar, Odisha.
[3,4]M.Tech. Student, Biotechnology, GIET University, Gunupur, Odisha.
[5,6]Senior Research Associate, SALT Bioscience, Bhubaneswar, Odisha.

Abstract

Phytomedicines are believed to have benefits over conventional drugs and are regaining interest in current research. Moringa oleifera is a multi-purpose herbal plant used as human food and an alternative for medicinal purposes worldwide. An important factor that accounts for the medicinal uses of Moringa oleifera is its very wide range of vital antioxidants, antibiotics and nutrients including vitamins and minerals. Almost all parts from Moringa can be used as a source for nutrition with other useful values. Physiochemical and antioxidant evaluation of moringa leaves yielded 6.59 % moisture content, 9.64 % total ash, 6.38pH (1 % solution), 5.81 pH (10 % solution), 0.07 % acidity (1% solution), 0.31 %acidity (10%solution), 2.54 mg GAE/100g total phenolic contents (TPC), 3.68 mg QE/100g total flavonoids contents (TFC) and 71.98 DPP Hradicals scavenging activity. Phytochemical screening of ethanol extracts showed the presence of coumarine, terpenoids, polyphenols, glycosides, tannins, flavonoids and alkaloids. Antibacterial assay revealed that the highest antibacterial activity of 1 mg/ml and 0.5 mg/ml of ethanolic extracts were found to be against E. coli (18.4 mm, and 9.1 mm respectively) followed by S. aureus (12.9 mm, and8.2mm respectively), B.subtilis (10.3 mm, and 6.5 mm respectively) and P.aeruginosa (9.3 mm, and 5.2 mm respectively). Moringa extractal so inhibited the development of certain bacteria, demonstrating it santi-microbial potential. This study verifies the presence of important phytochemicals in moringa leaf extracts, leading to the conclusion that moringa leaves can be utilized to curea variety of ailments.

Keywords: Moringa oleifera, flavonoid, DPPH, E. coli, S. aureus, B. subtilis, P. aeruginosa, tannin, alkaloids.

1. Introduction:

Plants which have medicinal values are effective for treating many human diseases because they contain several bioactive compounds.Herbalplantsareconsideredasnaturalchemicalfactorieswith therapeutic sources of biomolecules that can produce an effect physiologically on human system properties **[1]**.

Diabetes is a disorder that occurs when there is a little or no insulin production from the pancreas and pancreatic α-lipase expression is vital in developing obesity. Phytochemicals such as quercetin, flavonoids, and polyphenols are known to inhibit pancreatic lipase. There is a need to adopt the use of herbs because these are easily available, minimize the side effects caused by synthetic drugs, and also reduce the cost of drug purchases. Moringa oleifera is a small, fast-growing evergreen or deciduous tree, having soft and white wood with corky and gummy bark and grows up to a height of 12 meters**[2]**.

Aromatic and medicinal plants and their products offer a wide range of therapeutic properties and chemical elements that have been utilized for medical reasons across the world. The phytocompounds found in plants work together to strengthen the immune system, improve cognition, and alleviate stress and weariness**[1]**.

Compounds like flavonoids and phenolics, which are found in a wide range of aromatic and medicinal plants and have significant pharmacological potential which can help avoid oxidative stress. Superoxide dismutase, thioredoxin, catalase, glutathione, uric acid, and ascorbic acid are antioxidant defence systems that help the body to remove the free radicals and stop the chain reactions they start**[3]**.

Research shows that moringa has vitamin C seven times higher than oranges, vitamin A 10 times higher than carrots, calcium 17 times higher than milk**[4]**, protein 9 times higher than yoghurt**[4]**, potassium 15 times higher than bananas**[5]**, and iron 25 times higher than spinach**[6]**. Moringa's phytochemicals include tannins, sterols, saponins, phenolics, alkaloids, flavonoids (quercetin, isoquercetin, kaempferol, isothiocyanates), and glycosides**[2]**.

M. oleifera possess antispasmodic, expectorant, diuretic and stimulant activities. Whole plant can be used as cardiac circulatory tonic and antiseptic. Pods possess anthelmintic; diabetes and antipyretic effect. The root juice is utilized as cardiac tonic, antiepileptic, nervous debility, treat hepatic and spleen enlargement, asthma, detox toxins, inflammation and as a good diuretic agent. It is also using as anti-paralytic, anti-cholesterolemic, mosquito larvicidal activity**[7]**.

Several research groups have reported that the leaves have been a rich source of natural antioxidant compounds**[8]**. In the Philippines, its leaves are used to treat a wide range of medical conditions like: healing skin infections, anxiety, asthma, wounds, fever, diarrhea, sore throats, HIV/AIDs symptoms, bronchitis, ulcers, and malaria**[9, 10, 11, 12, 13]**. Thus, this work was aimed at elucidating the mechanism of action and thereby validating the use of M. oleifera ethanolic extract for the prevention and treatment of various diseases by the study of antibacterial activity, total phenolic content, total flavonoid content from leaves.

2. Materials and Methods:

2.1. Sample Collection: Moringa leaves were collected from the houses near Sundarpada, Bhubaneswar, Odisha. Fresh leaves of drumstick plants were used for phytochemical analysis and antimicrobial activity.

2.2. Preparation of Plant Extract: The leaves of the moringa plant were collected**.** Then dust and soil particles were removed by washing under running tap water followed by distilled water.The samples

were then dried under shade at room temperature for about 20 days and crushed to fine powder and kept in air tight container for future studies.

2.3. Preparation of Solvent Extract of Sample: The extract of sample was prepared by soaking 25 g of powdered leaf in ethanol of 200 mL and shaken well. The solution was left at room temperature for 48 hours and then filtered with the help of Whatman filter paper. The filtrate of the sample was taken and used for further phytochemical analysis and antimicrobial activity.

2.4. Qualitative Phytochemical Screening:

The drumstick leaf extracts were tested for phytochemical through various test methods.

2.4.1. Test for Alkaloids:

2.4.1.1. Wagner's Test: To the 0.5 ml extract, 2-4 drops of Mayer's reagent were added and observed the formation of reddish-brown precipitate (or colouration) (Kokate *et al.*, 2001).

2.4.1.2. Hager's Test: 1 ml of extract were treated with 5 drops of Hager's reagent. A yellow precipitate was formed**[14]**.

2.4.2. Test for Flavonoids:

2.4.2.1. Alkaline Test: 1.5 ml of extract was treated with 4-6 drops of 20% sodium hydroxide solution shows yellow colouration which disappeared on addition of dilute hydrochloric acid (Khandewal, 2008).

2.4.2.2. Shinod's Test: 20 drops of dilute hydrochloric acid and a piece of magnesium were added to 2 ml of extract which gives red or orange colouration**[15]**.

2.4.3. Test for Tanins:

2.4.3.1. Lead acetate test: To 2 ml of extract, 4 drops of 1% lead acetate solution was added. The formation of orange or red precipitate indicates the presence of tannins.

2.4.3.2. Braymer's Test: 2.5 ml extract and 2.5 ml of 10% ferric chloride solution was mixed, which gives bluish-green or black precipitation.

2.4.4. Test for Saponins:

2.4.4.1. Froth Test: To 5 ml of ethanol extract add 2 drops of sodium bicarbonate solution and left to rest for 5 minutes after shaking vigorously. Formation of a honey comb like froth indicates the presence of saponins.

2.4.4.2. Foam Test: Distilled water of 5 ml was added in 1.5 ml of extract. The mixture was shaken thoroughly and observed for the formation of constant foam (Dubey and Sushma, 2014).

2.4.5. Test for Terpenoids:

2.4.5.1. Salkowski's Test: 2 ml of chloroform was added to 2 ml of extract and few drops of concentrated sulphuric acid were added. A reddish brown precipitate produced immediately after the mixture was shaken well (Mir *et al.*, 2013).

2.4.5.2. Horizon Test: Red colour precipitate was observed after 2.5 ml of trichloroacetic acid was added to 1.5 ml of extract.

2.4.6. Test for Cardiac Glycosides:

2.4.6.1. Keller Kelliani Test: 1.5 ml of extract was treated with glacial acetic acidand 3-5 drops of 5% aqueous ferric chloride solution. To this mixture 0.5 ml ofconcentrated sulphuric acid was added and observedfora reddish-brown ringattheinterface (Kumar *et al*., 2013).

2.4.6.2. Legal Test: To 1 ml of the extract, 0.5 ml of pyridine and 0.5 ml of sodium nitroprusidewere added.The formation ofpinkorredindicates the presence ofcardiacglycosides.

2.4.7. Test for Phenols:

2.4.7.1. Ferric Chloride Test: 1 ml of extract were treated with 0.5 ml of aqueous 5% ferric chloride and observed for the formation of deep blue or black colouration (Hema *et al.*, 2012).

2.4.7.2. Lead Acetate Test: 2 ml of extract and 1.5 ml of10% lead acetate solution were mixed. A bulky white precipitate indicates the presence ofphenols.

2.4.8. Test for carbohydrate:

2.4.8.1. Molish's Test: The extracts of 2 ml added to 10 drops of Molisch's reagent, along with 1.5 ml of concentrated sulphuric acid down the side of the test tube. Then allow the mixture to stand for 2-3 minutes. Then the formation of red or dull violet colour at the interface of the two layers is a positive result**[1]**.

2.4.8.2. Fehling's Test: 2 ml of Fehling's solution A and B were mixed with 1 ml of extract and boiled in water bath for 5 minutes. The brick-red precipitate formation shows positive result.

2.4.9. Test for Proteins:

2.4.9.1. Ninhydrin Test: 2 ml of extract and 5 drops of 1% ninhydrin solution were added and placed in a boiling water bath for 2-5 minutes and observe for the formation of purple colour (Singh *et al.*, 2013).

2.4.10. Test for Resin:

2.4.10.1. Sulphuric Acid Test: To 1 ml of extract 5 ml of acetic anhydride added and dissolves gently by heating. After cooling add 0.5 ml of sulphuric acid which gives bright purple colour.

2.5. Physiochemical Evaluation of Moringa Leaves:

The following parameters were analyzed as described by AOAC, 2000**[16]**.

2.5.1. Moisture (%):

$$\text{Moisture (\%)} = \frac{W1-W2}{W1} \times 100$$

Where W1 is weight of sample before drying and W2 is weight of sample after drying.

2.5.2. Total Ash (%):

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

2.5.3. pH Measurement:

1% and 10% (w/v) solution of moringa leaf powder was prepared and subjected to pH measurement using pH meter**[17]**.

2.5.4. Titratable acidity:

$$\text{Titratable acidity (\%)} = \frac{\text{ml of NaOH X normality of NaOH X mileq factor}}{\text{Weight of sample}} \times 100$$

2.6. Antioxidants and Radical Scavenging Activity:

2.6.1. Extract preparation:

1 gm of moringa leaf powder was added in 40 ml of ethanol (80%) and concentrated to 10 ml of its original volume using rotary evaporator at 55℃. The concentrate was used to evaluate the total phenolic content, total flavonoids content, and DPPH- Radical scavenging activity**[18]**.

2.6.2. Total phenolic content (mg GAE/100g DW):

The total phenolics of the extracts were determined using the Folin and Ciocalteu reagent, following the method described by Singleton and Rossi, with slight modifications. Sample and standard readings were made using a spectrophotometer at 765 nm against the reagent blank**[19, 18]**.

2.6.3. Total flavonoid contents (mg QE/100g DW):

0.3 ml sodium nitrate was added to 1 ml of already prepared sample (5%). For 5 minutes, this combination was left alone. After that, 0.6 ml of aluminum chloride was added. 2 ml of 1 M NaOH was added after 5 minutes. Finally, using a spectrophotometer, the absorbance was measured at 510 nm. Quercetin was used as a reference to determine the total flavonoids content **[20]**.

2.6.4. Free Radical scavenging activity (%):

Radical scavenging activity expressed as the inhibition percentage and calculated using the following formula:

$$RSA\ (\%) = \frac{AC - AS}{AC} \times 100$$

Where AC and AS are the absorbance of control and sample solutions respectively**[21]**.

2.7. Antibacterial Activity Assay:

Antibacterial assay of ethanolic extract was performed. Four different strains of bacteria were selected on the basis of their suitability and functionality, which were made up of two gram positive bacteria (Staphylococcus aureus and Bacillus subtilis) and two gram negative bacteria (Escherichia coli and Pseudomonas aeruginosa)**[22]**. These were cultured on selective agar media, while antibacterial assay was carried out on nutrient broth media. The microorganisms used were cultured at SALT BIOSCIENCE, Bhubaneswar, Odisha. Inoculation of the culture was carried separately in each nutrient broth and agar. Wells of 5 mm in diameter were prepared on inoculated agar and filled with different concentrations of extract**[23]**. It was then incubated at 37℃ for 24 hours and the clear zone of inhibition was measured after 24 hours**[24, 25]**.

3. Results and Discussion:

3.1. Qualitative Phytochemical Analysis:

It is clearly from the data that major phytochemicals present in moringa leaves are identified from ethanolic extracts which was positive for coumarine, phytosterols, polyphenols, glycosides, tannins, flavonoids and alkaloids.

Previous study explained that aqueous extract of moringa leaves showed presence of phenols, alkaloids, flavonoids, tannins, carbohydrates, and saponins, while chloroform extracts only showed positive results of carbohydrates. The difference might be due to the geographical variation and growing conditions, which played a significant role in difference of bioactive compounds in moringa samples. A highly diversified profile of secondary metabolites was exhibited by the polar fraction when compared to those of the other fractions of moringa extract**[26]**.

It was already suggested that the use of polar solvent for extraction through maceration released such diversified secondary metabolites. Therefore, it can be suggested that sonication of aqueous samples influences the extraction of phytochemicals. All above mentioned factors impacts the ability of aqueous extracts of moringa leaves to exhibit better phytochemical profile followed by ethanol as solvent. However, chloroform facilitated the extraction of cardiac glycosides and steroids**[27]**.

The current investigation was carried out for the qualitative phytochemical analysis of M. oleiferaleaf extracts in ethanol used as solvents is shown in Table 1.

Table.1: Qualitative phytochemical analysis of ethanol solvent extract of leaf of *M. oleifera*

PHYTOCHEMICALS	TEST NAME	ETHANOL EXTRACT
ALKALOID	Wagner's Test	+ve
	Hager's Test	+ve
FLAVONOID	Alkaline Test	+ve
	Shinod's test	+ve
TANNIN	Lead Acetate Test	+ve
	Braymer's Test	+ve
SAPONIN	Froth Test	-ve
	Foam Test	-ve
TERPENOID	Salkowski's Test	+ve
	Horizon Test	+ve
CARDIAC GLYCOSIDE	Keller Kelliani Test	+ve
	Legal Test	+ve
PHENOL	Ferric Chloride Test	+ve
	Lead Acetate Test	+ve
CARBOHYDRATE	Molish's Test	-ve
	Fehling's Test	-ve
PROTEIN	Ninhydrin Test	-ve
RESIN	Sulphuric Acid Test	-ve

3.2. Physiochemical Analysis of Moringa Leaves:

Physiochemical analysis reveals the identification and quality of the plant material especially in concern with food and pharmacological application**[28]**. The results presented in table 2 showed a decline in pH value (6.38 to 5.81) and increase in acid contents (0.07% to 0.31%) with increasing concentration of moringa in aqueous solution.

The moisture content of moringa was analyzed as 6.59% on average basis which is facilitated by the average temperature during drying around 23 + 3℃. Moringa is considered as best source of mineral and exhibited 9.64% ash content in this study.

Table.2: Physiochemical attributes of moringa leaves

PHYSIOCHEMICALPARAMETERS	QUANTITATIVEANALYSIS
pH(1%solution)	6.38 ±0.02
pH(10%solution)	5.81 ±0.03
Acidity(1%)	0.07 ±0.005
Acidity(10%)	0.31 ±0.09
MoistureContent(%)	6.59 ±0.4
Ash(%)	9.64 ±0.35
Thevaluesare givenasmean (n=3)±standard error(SE)	

3.3 Antioxidant Properties of Moringa Leaves:

The data presented in table 3 indicated that concentrations of 100 mg/ml ethanolic extracts contained higher amount of TPC (2.54 mg GAE/ml) while no difference was observed in TFC of both ethanolic (3.82 mg QE/ml) and aqueous extracts (3.75 mg QE/ml). RSA of both extracts were determined by using DPPH which acts as a free radical which reduces in presence of antioxidants and become colorless from violet. As moringa extracts at the concentration of 100mg/ml were green colored complex due to the presence of chlorophyll, a notable anomaly in determination of RSA was observed. The original green color masked the reduction in DPPH color complex, therefore RSA (%) at this concentration was not determined. However, when the concentration of moringa extracts were reduced considerably to 5 mg/ml (colorless solution obtained), DPPH-RSA was determined as 72.034% and 72.31% for ethanolic and aqueous extracts respectively. The results from this study support the greater antioxidant potential of moringa leaves to reduce the oxidative damage.

Table.3: Assessment of antioxidant potential of moringa leaves

ANTIOXIDANTS	ETHANOLICEXTRACTS	
	100mg/ml	5mg/ml
TPC (mg GAE/100gDW)	2.54 ±0.168	0.168 ±0.06
TFC (mg QE/100gDW)	3.68 ±0.12	1.2 ±0.025
RSA(%)	71.97 ±0.92	71.98 ±0.92

3.5 Antibacterial Properties of Moringa Leaf Extract:

Data regarding antibacterial properties of ethanolic extract of moringa leaves is presented in table 4. It was observed that higher the concentration of extract used the greater is the antibacterial activity which might be due to the difference in concentration of secondary metabolites among tested concentration. From the data, it also depicted that ethanolic extracts showed higher activity in case of E. coli and S. aureus which might be due to the presence of high levels of terpenoids and tannins in ethanolic extract. This antibacterial activity was attributed the ability of tannins to inhibit cell wall synthesis and the ability of terpenoids to weaken the membranous tissues creating dissolution of cell wall of microorganism. The highest antibacterial activities of 1 mg/µl and 0.5 mg/µl of ethanolic extracts were found to be against E. coli (18.4 mm, and 9.1 mm respectively), followed by S. aureus (12.9 mm, and8.2mm respectively), B.subtilis (10.3 mm,and6.5mm respectively) and P.aeruginosa (9.3 mm, and5.2mmrespectively).

Table.4: Antibacterial activity of ethanolic extract of moringa leaves

MICROORGANISM	CONCENTRATIONS/ ML OF ETHANOLICEXTRACT	ZONE OF INHIBITION (MM)
Bacillussubtilis	1 mg	10.3 ±0.7
	0.5 mg	6.5 ±0.59
Staphylococcus aureus	1 mg	12.9 ±1.55
	0.5 mg	8.2 ±0.55
Pseudomonasaeruginosa	1 mg	9.3 ±0.66
	0.5 mg	5.2 ±0.78
Escherichiacoli	1 mg	18.4 ±1.55
	0.5 mg	9.1 ±0.55
Thevaluesare givenasmean (n=3)±standard error(SE)		

4. Conclusion:

Previous studies proven that the Moringa oleifera leaf possesses a wide range of medicinal and therapeutic properties. In this paper, it views the general nutrition contents of the Moringa up to several specific remedial properties including its anti-fibrotic, anti-inflammatory, anti-microbial, anti-hyperglycemia, anti-oxidant, anti-tumor and anti-cancer properties. The further studies should emphasis on probable mode of action of the isolates and possible structural-activity relationship as the chemical constituents of moringa leaves are very well investigated and documented. In conclusion, Moringa oleifera has numerous applications in medicinal field. The ethanol is the best solvents to isolate phytochemicals and their extracts are most effective against Escherichia coli, followed by Bacillus subtilis, Staphylococcus aureus, and Pseudomonas aeruginosa. These natural bioactive compounds and M. oleifera powdered leaves can likewise beincluded in foods and used as drugs for weight loss management.Clinical trial studies can be explored further tomaximize the gains in the use of M. oleifera leaves in humans as well.

5. Acknowledgment:

We are grateful to SALT Bioscience for supporting us for well-established laboratory facilities and helping us in paper writing.

6. References

1. Tanya, M., Priyadarsini, D., & Nayak, R. K. (2022). Screening of Bioactive Compounds from Madagascar Periwinkle and Their Importance in Drug Discovery. IJFMR-International Journal For Multidisciplinary Research, 4(6).
2. Asghar, N., Aziz, A., Azhar, M.F., El-Sharnouby, M., Irfan, U., Rafiq, I., Farooq, H., Rehmani,M.I.A., Sakran, M., Altalhi, A. and Alzuaibr, F.M., (2022). Assessment of PhytochemicalAnalysis,Nutritional Composition and Antimicrobial Activity of Moringaoleifera. *Phyton-international journal of experimental botany*, *91*(8),pp.1817-1829.

3. Elangovan,N.M.,Dhanarajan,M.S.andElangovan,I.,(2015).Preliminaryphytochemicalscreening and HPTLC fingerprinting profile of leaf extracts of Moringa oleifera andPhyllanthusemblica.*InternationalResearchJournalofPharmaceuticalandBioscience*,*2*(2), pp.32-40.
4. Iqbal,S.andBhanger,M.I.,(2006).EffectofseasonandproductionlocationonantioxidantactivityofMoringaoleiferaleavesgrowninPakistan.*JournaloffoodCompositionandAnalysis*,*19*(6-7), pp.544-551.
5. Kumar Bargah, R., (2015). Preliminary test of phytochemical screening of crude ethanolic andaqueousextractofMoringapterygospermaGaertn.*Journalofpharmacognosyandphytochemistry*,*4*(1).
6. Fahal, M.E., Rani, B.M.A., Aklakur, M.D., Chanu, T.I. and Saharan, N., (2018). Qualitative andquantitativephytochemicalanalysisofMoringaoleifera(Lam)Pods.*Internationaljournalofcurrent microbiologyand applied sciences*, *7*(5), pp.657-665.
7. Putri,G.E.,Rilda,Y.,Syukri,S.,Labanni,A.andArief,S.,(2021).HighlyantimicrobialactivityofceriumoxidenanoparticlessynthesizedusingMoringaoleiferaleafextractbyarapidgreenprecipitationmethod. *JournalofMaterialsResearchand Technology*,*15*,pp.2355-2364.
8. Mehmood,A.,Naveed,K.,Azeem,K.,Khan,A.,Ali,N.andKhan,S.M.,(2018).Sowingtimeandnitrogenapplicationmethodsimpactonproductiontraitsof Kalonji(*Nigellasativa*L.).*Pureand Applied Biology (PAB)*, *7*(2), pp.476-485.
9. Shahzad,Q.,Sammi,S.,Mehmood,A.,Naveed,K.,Azeem,K.,AhmedAyub,M.H.,Hussain,M.,Ayub, Q. and Shokat, O., (2020). Phytochemical analysis and antimicrobial activity ofadhatodavasicaleaves. *PureandApplied Biology(PAB)*, *9*(2), pp.1654-1661.
10. Mehmood,A.,Naveed,K.,Jadoon,N.,Ayub,Q.,Hussain,M.andHassaan,M.,(2021) b.Phytochemical screening and antibacterial efficacy of black cumin (*Nigella sativa* L.)seeds.*FUUASTJournalof Biology*, *11*(1), pp.23-28.
11. Wiltshire, F.M.S., de França Santos, A., Silva, L.K.B., de Almeida, L.C., dos Santos Freitas, L.,Lima,A.S.,Fricks,A.T.,Dariva,C.andSoares,C.M.F.,(2022).Influenceofseasonalityonthe physicochemical properties of Moringa oleifera Lam. Seed oil and their oleochemicalpotential.*Food Chemistry: Molecular Sciences*, *4*,p.100068.
12. Siyanbola, T.O., Ajanaku, C.O., Akinsiku, A.A., Adedapo, E.A., Aladesuyi, O., Olanrewaju, I.O.and Jokotagba, O.A., (2015). Nutritional and Physico-Chemical Evaluations of Moringaoleifera Seedlings and Oil. *Journal of Advance Research in Applied Science*, *2*(7), pp.01-04.
13. Syeda, A.M. and Riazunnisa, K., (2020). Data on GC-MS analysis, in vitro anti-oxidant and anti-microbial activity of the Catharanthus roseus and Moringa oleifera leaf extracts. *Data inbrief*, *29*, p.105258.
14. Maqsood,M.,Qureshi,R.,Arshad,M.,Ahmed,M.S.andIkram,M.,(2017).Preliminaryphytochemical screening, antifungal and cytotoxic activities of leaves extract of MoringaoleiferaLam. from Saltrange, Pakistan.*Pak.J. Bot*, *49*(1),pp.353-359.
15. Lawan,F.A.,Shehu,B.B.,Benisheikh,A.A.G.,Dige,A.M.,Jidda,H.A.andAbdulkadir,A.,(2020).Evaluation of antimicrobial potential and phytochemical screening of Moringa oleiferaleaveextract:AnEmergingfolkloricmedicinalplantinMaiduguri,Bornostate,Nigeria.*WorldJournalof BiologyPharmacyandHealthSciences*,*4*(3), pp.043-047.

16. Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J. and Bertoli, S., (2015). Cultivation,genetic,ethnopharmacology,phytochemistryandpharmacologyofMoringaoleiferaleaves:Anoverview.*Internationaljournalofmolecularsciences*,*16*(6),pp.12791-12835.
17. Bekoe,E.O.,Jibira,Y.andAgyei,G.A.,(2020).Physicochemical,phytochemical,heavymetalandmicrobiologicalanalysisofMoringa oleifera Lam. leaves. *PharmacognosyJournal*,*12*(6s).
18. Falowo, A.B., Mukumbo, F.E., Idamokoro, E.M., Lorenzo, J.M., Afolayan, A.J. and Muchenje,V., (2018). Multi-functional application of Moringa oleifera Lam. in nutrition and animalfoodproducts: A review.*Food research international*, *106*,pp.317-334.
19. Afzal, M.I. and Iqbal, M.A., (2015). Plant nutrients supplementation with foliar application ofallelopathic water extractsimproves wheat (*Triticum aestivum*L.)yield.*Advance inAgricultureand Biology*, *4*, pp.64-70.
20. Manilal, A., Sabu, K.R., Shewangizaw, M., Aklilu, A., Seid, M., Merdekios, B. and Tsegaye, B.,(2020).Invitroantibacterialactivityofmedicinalplantsagainstbiofilm-formingmethicillin-resistantStaphylococcusaureus:efficacyofMoringastenopetalaandRosmarinusofficinalisextracts.*Heliyon*, *6*(1), p.e03303.
21. Tang, X., Xu, C., Yagiz, Y., Simonne, A. and Marshall, M.R., (2018). Phytochemical profiles, andantimicrobialandantioxidantactivitiesofgreatergalangal(*Alpiniagalangal*)flowers.*Food chemistry*,*255*, pp.300-308.
22. Mehmood, A., Naveed, K., Ayub, Q., Alamri, S., Siddiqui, M.H., Wu, C., Wang, D., Saud, S.,Banout,J.,Danish,S.andDatta,R.,(2021) a.Exploringthepotentialofmoringaleafextractasbiostimulantforimprovingyieldandqualityofblackcuminoil.*Scientificreports*,*11*(1), pp.1-10.
23. Van Bergen, M. A., & Snoeijer, W. (1996). *Revision of Catharanthus G. Don: Series of Revisions of Apocynaceae XLI*. Wageningen Agricultural University.
24. Schneider, M. J., Abdel-Aziz, H., & Efferth, T. (2014). Phytochemicals for the treatment of inflammatory bowel diseases. *Phytochemistry reviews*, *13*(3), 629-642.
25. Dubey M (2014). Phytochemical Status of Some Selected Medicinal Plants (Eclipta Alba, Cathranthus Roseus and Swertia Chirata). Pelagia Research Library Asian Journal of Plant Science and Research 4(5): 28–34.
26. Kokate CK, Purohit AP, Gokhale SB. (2001). Carbohydrate and derived Products, drugs containing glycosides, drugs containing tannin Hs, lipids and protein alkaloids. Text book of Pharmacognosy, 7, edition, 2: 133-166.
27. Khandelwa,l K.R. (2008).Practical Pharmacognocy. 19th edition, Nirali Prakashan, Pune, p. 149-159.
28. Singh S, Kaur R and Sharma SK (2013). Pharmacognostical standardization of the roots of *Rumex hastatus* D. Don. *Asian. J. Pharm. Clin. Res*., 6: 126-128.