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Antioxidant activity of *Moringa oleifera* Lam

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Abstract
The leaves of *Moringa oleifera* Lam were extracted in Methanol, Petroleum ether, and ethyl acetate, and chemical detection of biotic compounds in extracts was carried out. The TLC indicates the presence of quercetin, rutin, and luteolin, and the total flavonoid and antioxidant activity of the extract show high antioxidant activity

Keywords: Antioxidant activity, *Moringa oleifera* Lam, petroleum ether, and ethyl acetate

Introduction
The immune system has a role in the pathophysiology as well as the etiology of many diseases, making medicinal plants a rich source of chemicals that are said to stimulate immunity [1]. In particular, granulocytes, macrophages, natural killer cells, and complement activities are non-specifically immunomodulated by chemicals found in these sources. Due to worries about the adverse effects of conventional medications, phytochemicals are being used as an alternative to conventional medicine to treat a variety of disorders. According to the current study, *Moringa oleifera* L. has immunomodulatory properties Leaves extracts.

Materials and Methods
The Moringa leaves were gathered at Career College Gandhi Nagar Campus in Bhopal in the period between December 2019 and January 2020. Dr. Jagrati Tripathi, Asst. Prof. of Botany at Govt. College Khemlasha (M.P.), then identified and verified the plant, and specimen voucher number 397 was then placed in the P.G. Department of Zoology at Career College Bhopal.

Extraction
The Moringa leaves were cleaned of any adulterants and ground to a fine powder or chopped into little pieces. They were then immersed in petroleum ether solvent for defatting with periodic shaking at room temperature for about 4-5 days. Whatman quality grade 1 filter paper was used after it had been passed through a muslin cloth. The filtrate was mixed and concentrated to a thick pasty material using a vacuum rotary evaporator (RE-111, Buchi, Flawil, Switzerland), a B-700 recirculation chiller, and a water bath model 461. Phytochemical analysing various Moringa leaf extracts. To determine the presence of various primary and secondary metabolites, such as alkaloids, terpenoids, glycosides, and glucosides, in biologically active extract, the crude fractions of ethyl acetate and methanol were examined for preliminary phytochemical screening.

Chromatographic purification of crude
When the matching fractions were run in a particular solvent system, a TLC investigation of *Moringa oleifera* Lam's methanolic extract revealed the presence of several components.

Table 1: Showing chromatographic values of CH_3OH extract of *M. oleifera*

Solvent System	Spot No	Rf values	Colour of Peaks
$C7H_8$:CH_3COOH:CH_2O_2	1	0.08	Blue
	2	0.39	Yellow
	3	0.43	Brown
	4	0.71	Green
	5	0.74	Green

The crude fractions of the methanolic extract of *Moringa oleifera* Lam were further purified using a silica gel 'G' glass column, and gradient elution was used to carry out the elution from non-polar to polar solvents. For *Moringa oleifera* Lam, various fractions including 0, 4, 3, 7, 3, 5, and 5 were eluted. However, fractions 0, 4, 3, 7, 3, 5, and 5 produced no residue or very little following evaporation and were therefore discarded. One spot-on TLC was observed in the fractions eluted with chloroform: ethyl acetate (50:50), which provided the compound (F-I) (6.1% w/w,

Table 2: Separation of constituents from methanolic extract of *Moringa oleifera* L.

S. No.	Eluting Solvent	Ratio (%)	Fractions Collected	% Yield of Isolated Compounds
1.	n-Hexane	100%	0	No Residue after evaporation
2.	Chloroform	100%	4	Very less quantity was isolated
3.	Chloroform and ethyl acetate	75%/25%	3	Very less quantity was isolated
4.	Chloroform and ethyl acetate	50%/50%	7	Fr-I (R_f=0.37) (6.1% w/w, 2.275 gm)
5.	Chloroform and ethyl acetate	40%/50%	3	Very less quantity was isolated
6.	Chloroform and methanol	50%/50%	5	Very less quantity was isolated
7.	Methanol	100%	5	No Residue after evaporation

Spectrophotometric Quantification of Total Phenolic Content (TPC)

Gallic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE). The variation of mean absorbance with varied amounts of gallic acid is shown by the line of regression from gallic acid, which was utilised to estimate an unknown phenol content. The Folin Ciocalteu reagent was used to calculate the gallic acid equivalent of total phenols. The phenolic content of extracts of *Moringa oleifera* L. made in Petroleum ether, ethyl acetate, and methanol varied significantly leaves. The comparison of the total phenolics present in petroleum ether, ethyl acetate, and methanol extract may be seen in the amounts of phenolics present in each extract, respectively. The quantitative analysis of TPC in the extracts showed that the largest amounts of TPC (140.23 mgGAE/gm) were found in the methanol extract, which was followed by the ethyl acetate extract (133.41 mgGAE/gm), while only moderate amounts were found in the petroleum ether extract (81.98 mgGAE/gm).

Table 3: Absorbance v/s concentration of Gallic acid

S. No.	Concentration of Gallic acid (µg/ml)	Absorbance
1.	10	0.102±0.000577
2.	20	0.113±0.000577
3.	30	0.133±0.001155
4.	40	0.16±0.004714
5.	50	0.183±0.000577

Values are expressed as MEAN±SD

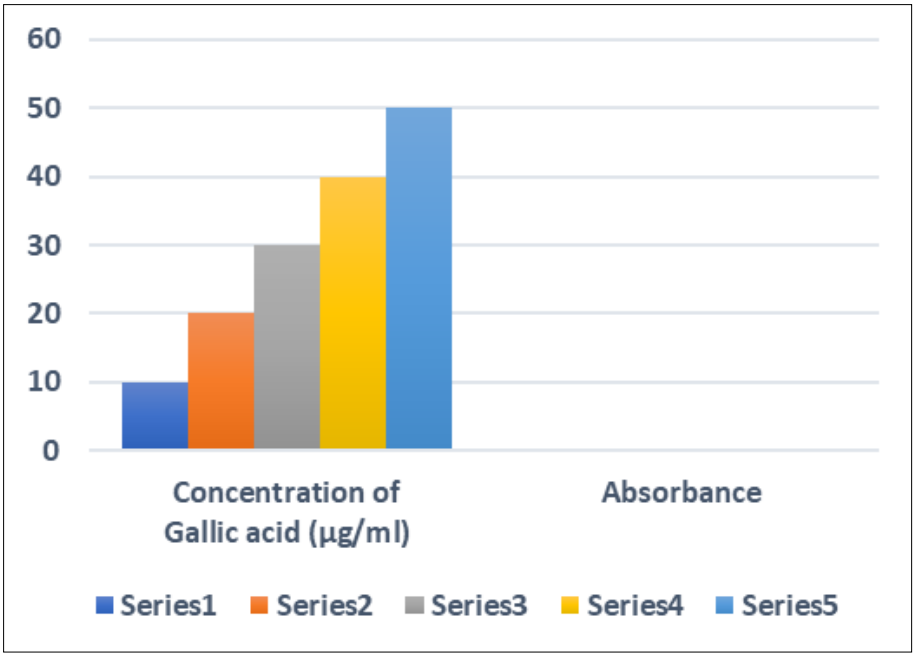

Fig 1: Absorbance v/s concentration of Gallic acid

Spectrophotometric Quantification of Total Flavonoid Content (TFC)

The measurement of total flavonoid was done using rutin as a reference component. Rutin was present in it in the form of mg/g dry weight (mg/g DW). The fluctuation in mean absorbance with varied concentrations of rutin is shown by the line of regression from rutin that was used to estimate an unknown flavonoid content. There were varying concentrations of phenolics in the petroleum ether, ethyl acetate, and methanol extracts. compares the amounts of flavonoids in extracts made from petroleum ether, ethyl acetate, and methanol. The quantitative measurement of TFC in extracts showed that petroleum ether contained relatively little TFC (15.86 mgRE/gm), whereas methanol extract contained the maximum quantity (107.64 mgRE/gm), followed by ethyl acetate (86.97 mgRE/gm).

According to the outcomes of the techniques, methanol extract has the highest level of antioxidant activity.

Determination of *In vitro* Antioxidant activity of crude extracts of Leaves of *Moringa oleifera* L.

DPPH free Radical Scavenging assay

One of the most reliable and frequently used techniques for assessing antioxidant activity is the DPPH (2, 2-diphenyl-1-picryl hydrazyl radical) assay. In order to determine the DPPH of petroleum ether, ethyl acetate, and methanol, ascorbic acid was utilized as a reference. Using a non-linear regression technique, the percentage inhibition was determined, and the IC_{50} values were inferred from the percentage inhibition vs concentration plot. The highest ability of the extracts to serve as DPPH radical scavengers is shown by the higher % inhibition of DPPH and lowest IC_{50}. illustrates the % inhibition of standard ascorbic acid and three extracts at various doses, as well as the regression lines, IC_{50} values, and percentage inhibition of standard ascorbic acid that were used to calculate the values.

Methanol extract contained 495.63 g/ml, ethyl acetate contained 507.33 g/ml, and petroleum ether contained 978.41 g/ml. The IC_{50} value for the methanol extract was 495.63 g/ml, indicating that it has the greatest capacity to scavenge DPPH radicals.

Table 4: Evaluation of isolated bioactive fractions from successive methanolic extract of *Moringa oleifera* for antioxidant activity.

Conc. µg/mL	% Inhibition (µg/mL)					
	EF-I	EF-II	BF-I	BF-II	AQF	ASA
25	20.02±0.25	4.32±0.49	2.01±0.22	13.01±0.33	1.22±0.55	31.23±0.31
50	39.83±0.31	13.99±0.34	8.39±0.35	30.33±0.42	5.91±0.35	48.71±0.34
75	52.89±0.27	21.54±0.61	19.01±0.27	46.39±0.23	13.01±0.56	62.03±0.42
100	75.37±0.43	29.23±0.31	23.02±0.32	66.18±0.39	21.51±0.41	79.11±0.49
125	88.80±0.41	36.01±0.56	29.27±0.31	75.23±0.36	26.89±0.38	91.21±0.46

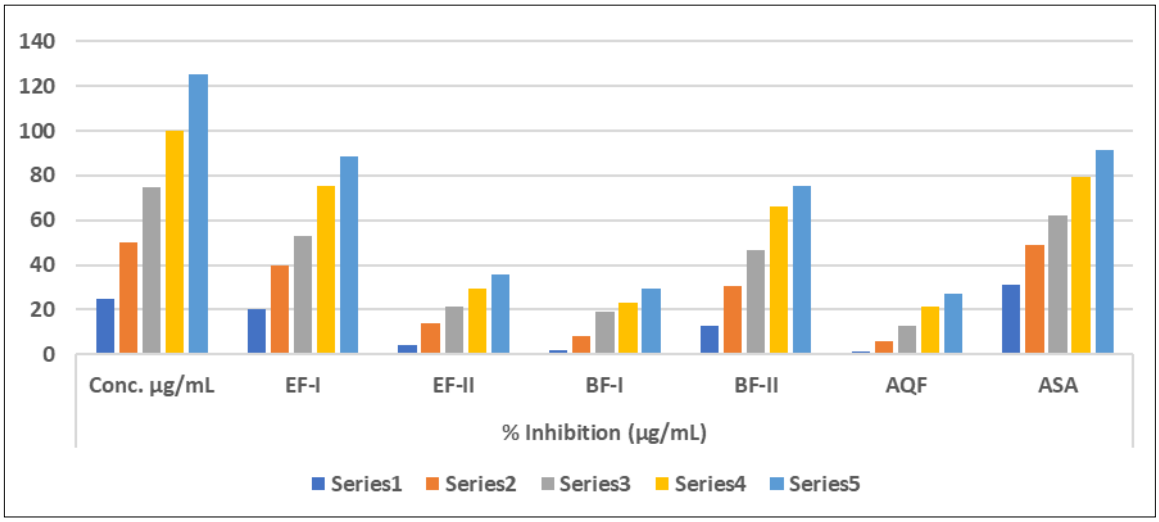

Fig 2: % Inhibition (µg/mL)

Total phenolic content of methanolic extract of leaf of *Moringa oleifera* was estimated by Folin-Ciocalteu assay. The results showed that M3 showed highest content of phenolics (275.35 mg/g equivalent of Gallic acid)

Reducing power assay

An important indicator of the antioxidant activity is the reducing power test. Ascorbic acid served as the benchmark. When doing a reducing power experiment, the greater the reaction mixture's absorbance, the higher the reducing power would be calculated using the absorbance of three extracts at various doses.

Comparing methanol extract to ethyl acetate and petroleum ether, a greater absorption was seen. The reducing power was discovered to be in the following order: petroleum ether extract (PE) > methanol extract (ME) > ethyl acetate extract (EAE) (PEE).

Methanol extract demonstrated the maximum antioxidant activity in both the DPPH and the reducing power assays, according to the *in-vitro* antioxidant activity data.

Table 5: IC_{50} values (µg/ml) of different parts of *Moringa oleifera* using DPPH assay.

S. No	Sample Code	Extract	IC_{50}
1	Z 1	MeoH Extract	371.46
2	Z3	CHCl3 fraction	602.00
3	Z5	MeoH Extract	536.64
4	Standard	Ascorbic acid	10.22

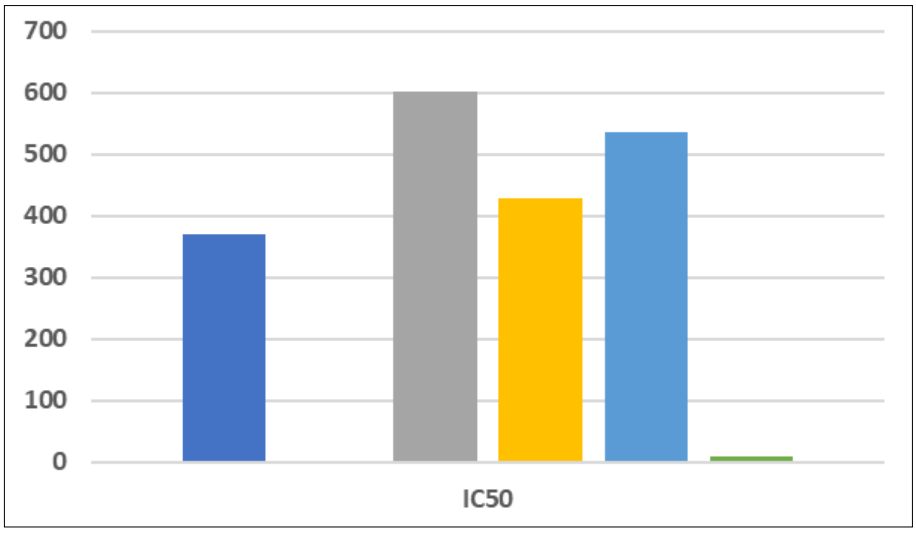

Fig 3: IC_{50} values (µg/ml) of different parts of *Moringa oleifera* using DPPH assay

Table 6: Concentration Vs Absorbance curve of Gallic acid

S. No.	Conc. µg/mL	Absorbance
1	10	0.103
2	20	0.196
3	30	0.274
4	40	0.313
5	50	0.368
6	60	0.418
7	70	0.466
8	80	0.500
9	90	0.573
10	100	0.629

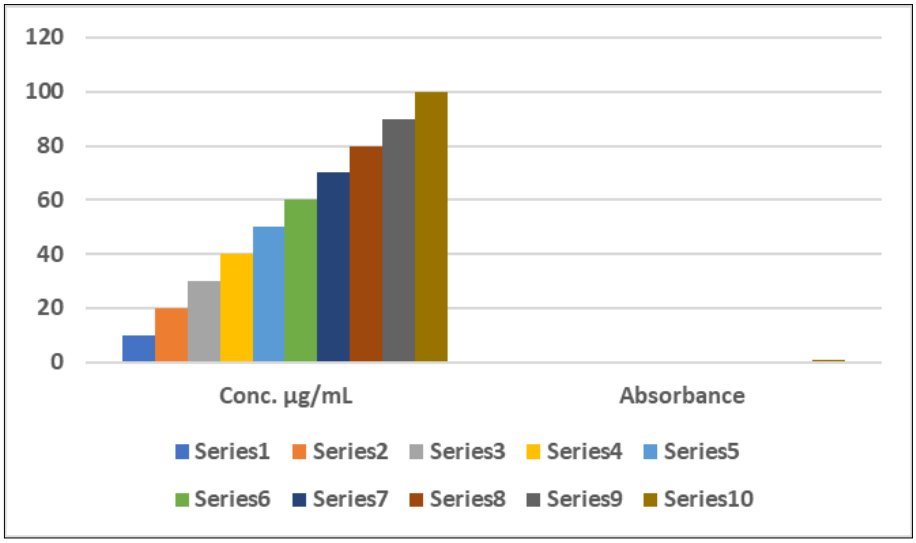

Fig 4: Concentration Vs Absorbance curve of Gallic acid

Table 7: Total Phenolic content in Fractions expressed in mg/g equivalent to Gallic Acid.

S. No.	Fraction	Absorbance (Mean±SD)	Conc. of Fraction	Total Phenolic Content mg/g equivalent to Gallic Acid (Mean±SD)
1	F-I	0.813±0.002	0.01 mg/mL	146.4±2.83
2	F-II	0.792±0.003	0.01 mg/mL	81.6±2.11
3	F-I	0.489±0.006	0.01 mg/mL	142.2±2.26
4	F-II	0.367±0.008	0.01 mg/mL	57.4±1.47
5	AQE	0.113±0.003	0.01 mg/mL	6.4±0.2

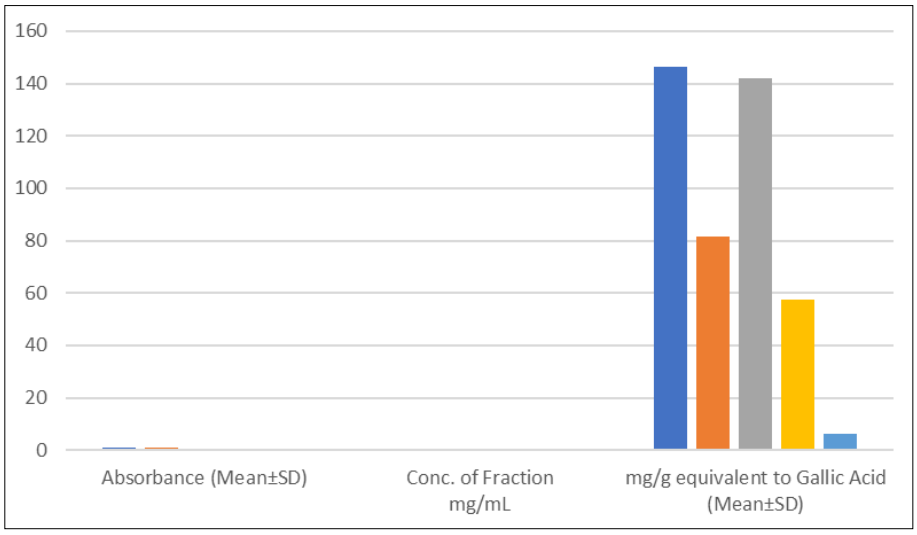

Fig 5: Total Phenolic content in Fractions expressed in mg/g equivalent to Gallic Acid

Table 8: Concentration Vs Absorbance curve of Rutin

S. No.	Conc. µg/mL	Absorbance
1	10	0.117
2	20	0.152
3	30	0.166
4	40	0.169
5	50	0.291

Result and Discussion

Because herbal medicine has been shown to be safe and to have fewer side effects than manmade drugs, there has been a growing resurgence of interest in the use of meditation herbs in poor nations in recent years. With varied degrees of success, many techniques are used to evaluate the antioxidant properties of natural substances in foods or biological systems. Plants are a source of medications for preventative, therapeutic, protective, or beneficial purposes. Antioxidants act as a defence system that protects against oxidative stress damage and contains substances to remove or repair injured molecules. Numerous crude extracts of *Moringa oleifera* L. have antioxidant activity. The study of extracts was conducted using the most used technique, DPPH. DPPH, also known as diphenyl picryl hydrazyl, is a nitrogen-based free

The DPPH oxidative assay [1], which has been adopted, is used all over the world to quantify radical-scavenging capacity. The degree of an organism's ability to combat oxidation can be determined by how well it can scavenge the DPPH radical. The DPPH alcohol solution has an absorption peak at 517 nm, which vanishes after the reactive system contains a radical scavenger and once the odd electron of the nitrogen in the DPPH is paired. The rate and peak value of DPPH disappearance have an impact on both the reactive rate and the capacity of the radical scavenger. The DPPH test offers many advantages over other alternative procedures, including good stability, simplicity, reliable sensitivity, and practicability.

The antioxidant capacity of several crude extracts of *Moringa oleifera* L. was examined in the current study was examined using the DPPH assay.8 The samples' antioxidant activity was indicated as IC_{50} values. The amount of a medicine or reference material (measured in g/ml) needed to prevent the formation of DPPH radicals by 50% is known as the IC_{50} value. The minimal IC_{50} indicates the extracts' strong capacity to act as DPPH radical scavengers [9] IC_{50} values were calculated using a non-linear regression technique from the percentage inhibition versus concentration plot. By having lower IC_{50} values of 384.35 g/ml, methanol crude extract demonstrated the strongest capacity to act as DPPH radical scavengers.

Based on the aforementioned findings, it was determined that ZN2 has the best antioxidant potential and a lower IC_{50} value, indicating a strong capacity to act as a DPPH radical scavenger.

According to our research, because methanol extract has a stronger antioxidant capability and contains the bulk of phytocompounds, it was utilised for chromatographic purification to isolate the purified compound and for *in-vivo* and *in-vitro* tests of anticancer activity. The findings imply that Lam. *Moringa oleifera* have promising immunomodulatory abilities [10]

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