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Anticytotoxic and antioxidant activities of ethanolic extract of dried flowers of *Moringa oleifera*

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Key words: *Moringa oleifera*, FRAP, FTIR, anticytotoxic, Antioxidant, Extraction

Abstract

Moringa oleifera is a common plant and known for various medicinal properties. The research work was conducted to investigate the anticytotoxic and antioxidant activity of dried flower powder extract of leaf *Moringa oleifera*. The ethanol extracts from flower of moringa plants contain severalphytochemicals such asalkaloids,amino acids, quinones, cardiac glycosides, flavonoids, phenols, saponins, tannins, terpenoids, coumarins and triterpenoids.The antioxidant activities of different concentrations of ethanol extracts of the leaves were determined by the three assay techniques i.e., DPPH radical scavenging assay, Ferric reducing ability power (FRAP). The type of chemical bonds is identified through FTIR analysis. The results obtained in the present study indicate that the leaves of Moringa oleifera are a potential source of anticytotoxic and antioxidants.

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Introduction

Moringa oleifera (MO) grows owing to its nutrient-rich seeds, edible leaves and flowers that can be used as food, medication, cosmetic oil, or livestock feed. The height varies between 5 to 10 meters. Different experiments have been demonstrated positive effects on health. MO is also used in developing countries as a source of fruits, medicinal plants, and edible oil. It is an essential nutrient- rich vegetative plant and is commonly considered as a versatile food that can be eaten in all sections (*Bharali et al.,* 2003). *Moringa* claimed as a nutrient-rich due to its anti-ulcer, anti-diabetic, hepatoprotective, diuretic and cholesterol lowering capacity. It has also been used in skin and hair care products (Brown *et al.,* 1998). *Moringa oleifera* Lam., also known as the drumstick tree.' It is mostly found in areas having warm and dry and moist. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds (Caceres *et al.,* 1992). Different parts of this plan contain a profile of important minerals, and a good source of protein, vitamin, a carotene, amino acids and various phenolics. In the tropics, it is used as forage for livestock, and in many countries, it is used as a micronutrient powder to treat various ailments (Clarke Hans Thacher, 2007). *Moringa oleifera* has several medicinal properties and has potentiality to cure many diseases (Donli *et al.,* 2003). It is used to treat diseases such as diabetes, heart disease, anaemia, arthritis, respiratory problems, skin, liver problems, paralysis, sterility, rheumatism, digestive disorders and many more (Eilert *et al.,* 1981). The anticancer result of *Moringa* has been tested for its chemo-protective properties and has been shown to prevent the development of various human cancer cells (Fahey, 2005). *M. oleifera* has several bioactive compounds with antitumor activity.

Materials and methods

Plant Material

All the chemical reagents used in this experiment were of analytical grade purchased from Loba chemicals, India. Fresh *Moringa oleifera* flower were collected in and round Kanchipuram, Tamil Nadu, India.

Preparation of ethanol extract

10 g of dried flower powder was extracted with 200 millilitres of ethanol for 18 cycles using the Soxhlet apparatus (Özcan *et al.,* 2019). After 3 hours, the extract was concentrated in a hot air oven at temperature ranging from 60-80°C. The concentrates were dried in vacuum desiccators and the dried extract was stored at 4°C.

Preliminary screening

A preliminary phytochemical screening of *Moringa oleifera* was carried out. The presence of alkaloid (Dragendorff'stest (Adams *et al.,* 1996), Wagner's test (Jha *et al.,* 2012) and Mayer's reagent (Verma *et al.,* 2010)), Amino acids (Yemm *et al.,* 1995), Quinones (Bitenc *et al.,* 2020), Cardiac Glycosides (Ebana*et al.,* 1991), Flavonoids (Sankhalkar *et al.,* 2016), Phenols (Santhi *et al.,* 2016), Saponins (Sharma *et al.,* 2013), Tannins (Teixeira *et al.,* 2014), Terpenoids (Santhi *et al.,* 2016), Coumarins (Kasolo*et al.,* 2010) and Triterpenoids (UC *et al.,* 2013) were analysed.

Estimation of total phenol compounds

The amount of phenol was estimated by Folin-ciacalteau method (Singleton *et al.*, 1999).

Estimation of flavonoids

The standard Quercetin solution was added followed by the addition of 0.2ml of homogenate the Solutions were made to equal volume using 3ml of 95% Ethanol. Then 0.1ml of Aluminium Chloride hexahydrate and 1M potassium Acetate was added and incubated at room temperature for 40 minutes and the absorbance is read at 430nm.

Evaluation of radical scavenging and antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of extracts, based on scavenging (Marinova *et al.,* 2011) or hydrogen/electron transferring ability against the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined the method defined by Koleva *et al.* (2002). 0.1ml of extract (25-125µg/ml) was mixed with 3ml of freshly prepared 0.001M

DPPH in methanol. After 30 minutes of incubation, the absorbance of the sample was measured at 517 nm, using a UV-visible spectrophotometer (Shimadzu, UV-2450). The radical scavenging activity was calculated as

% Inhibition= [(Ao - Ae)/Ao] × 100

Ao = Absorbance without extract

Ae = Absorbance with extract/ Standard

ascorbic acid was used as a standard antioxidant compound

Determination of ferric reducing ability power (FRAP)

The reducing power (Nobossé*et al.,* 2018) of plant extract was determined, according to the technique of Oyaizu (1986). Plant extract (5-100 µg) in 1ml of distilled water was mixed with phosphate buffer [2.5 ml, 0.2 M, pH 6.6 and 2.5 ml of 1% potassium ferricyanide (K_3Fe $(CN)_6$)]. The mixture was incubated at 50°C for 20 minutes. 2.5ml of 10 % tri-chloroacetic acid was added to the mixture, and the mixture was centrifuged at 1800 RPM for 10 minutes. Then 2.5 ml of supernatant was mixed with 2.5ml of distilled water and 0.5 ml of 0.1 % $FeCl_3$. Absorbance was measured at 700 nm, using a UV-visible spectrophotometer (Shimadzu, UV-2450). The reducing activity was calculated as % inhibition using the formula:

% Inhibition = [(Ao - Ae)/Ao] × 100 Ao = Absorbance without extract

Ae = Absorbance with extract/Standard

Ascorbic acid was used as a standard antioxidant compound.

Fourier transforms infrared spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different solvent extracts of each plant materials was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm 1 with a resolution of 4 cm.

GC-MS analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min–1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 230 °C; ion source temperature 230 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC- MS NIST (2008) library.

Treatment of Cancer cell line with Plant extract

The cell lines were treated with crude extracts (Steenkamp *et al.,* 2006), 1ml of MEM with Sodium pyruvate & 10% FCS was added to the dissolved *Calotropis gigantea* aqueous extracts, Aqueous extracts were filtered through Whatman syringe filter (pore size-0.2µm). The filtered crude extracts were made up of 1ml stock. 100µl of diluted *T. populnea* aqueous extracts were added in the 96 well plates. The 96 well plates were kept in 5% CO_2 incubator at 36°C and observed after 72 hours.

Results and discussion

Preliminary phytochemical constituents of Moringa oleifera flower extracts

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Analysis of the plant extracts revealed the presence of different phytochemicals. Qualitative results of the flower extract of *Moringa oleifera* Lam. are mentioned in the Table 1.

The ethanol extracts from flower of Moringa plants contained several phytochemicals such as alkaloids, flavonoids, glycoside, phenols, saponins, steroids and tannins. The presence of flavonoids indicates the natural occurring phenolic compound, with beneficial effects in the human diet as antioxidants and neutralizing free radicals. Tannins are group of polymeric phenolic compound and cause local tumours. Terpenoids and steroids were detected in *Moringa pterygosperma* which were reported to be active against antibacterial activity. Saponins have the properties of precipitating and coagulating red blood cells, anti-inflammatory. Alkaloids are used in medicines for reducing headache and fever (Shaw *et al.,* 2010).

Total phenolic and flavonoid content

Quantitative estimation of important secondary metabolites was shown. *Moringa oleifera* plant material having higher phenol than the flavonoid. Flavonoids were one of the polyphenolic secondary metabolites extensively disseminated in plants with recognized possessions, which comprise inhibition of hydrolytic and oxidative enzymes, free radical scavenging activity, and anti-inflammatory activity.

Various reports have shown that existence of phenolics in diets is predominantly vital for oxidative steadiness and antimicrobial defence. Tannins are widely distributed in almost all plant foods. The tannin containing remedies are used as antihelminthic, antioxidants, antimicrobial agents, and anti-viral agents. The selected plants showed significant amount of various secondary metabolites which might be responsible for the wound healing activities (Barik *et al.,* 2008) (Table 2).

Table 1. Preliminary phytochemical constituents of *Moringa oleifera* flower extracts

SL	Phytochemicals	Ethanol extract
1	Alkaloids	+
2	Flavonoids	+
3	Carbohydrate	+
4	Saponin	+
5	Tannins	+
6	Terpenoids	+
7	Proteins	+
8	Anthraquinone	-
9	Polyphenol	+
10	Glycosides	+

+ Indicate present - Indicate absent

Table 2. Total phenolic and flavonoid content

Extract	TPC	TFC
Ethanol Flower Extract	28.92	13.46

Table 3. Free radical scavenging activity of DPPH

Conc in µg/ml	Std. Ascorbic acid	Ethanol flower extract
5	20.00%	15.38%
10	38.46%	29.23%
25	55.38%	36.92%
50	72.31%	44.62%
100	90.77%	53.85%

Table 4. Determination of ferric reducing ability power *Moringa oleifera*

Conc in µg/ml	Std. Ascorbic acid	Ethanol flower extract
5	0.03	0.02
10	0.06	0.03
25	0.09	0.06
50	0.12	0.07
100	0.15	0.1

Table 5. FTIR analysis of flower extracts of *Moringa oleifera*

Peak number	Compound	Peak value	Functional group
1	Alkane	2973.32	C-H Stretching
2	Alkane	2920.28	C-H Stretching
3	Aldehydes	2848.91	C-H Stretching
4	Ester	1765.10	C=O Stretching
5	Alkene	1454.35	C-H Bending
6	sulphate	1415.78	S=O stretching
7	Aliphatic ether	1086.91	C-O Stretching

DPPH free radical scavenging activity

The DPPH assay has been widely used to estimate the antioxidant capacity of plant extracts.

When an antioxidant scavenges the free radicals by hydrogen donation (HAT– hydrogen atom transfer), the purple colour of the methanolic DPPH solution changes to a pale yellow and a characteristic absorbance is seen at 517 nm. In this study, the plant extracts were found to be an effective scavenger of DPPH radicals. This antioxidant action may be attributed to the presence of phenolic phytochemicals such as flavonoids found to be present in the extract.

Table 6. GC-MS analysis of *Moringa oleifera*

GC-MS analysis

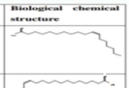
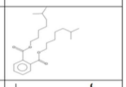
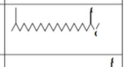
S. No.	Compound name	RT	Molecular weight	Molecular formula	Probability	Biological chemical structure
1.	cis-Vaccenic acid	14.241	282	$C_{18}H_{34}O_2$	16.3%	
2.	cis-13-Octadecenoic acid	16.028	282	$C_{18}H_{34}O_2$	16.7%	
3.	6-Octadecenoic acid	16.458	282	$C_{18}H_{34}O_2$	7.85%	
4.	Oleic Acid	18.137	282	$C_{18}H_{34}O_2$	6.36%	
5.	6-Octadecenoic acid	18.808	282	$C_{18}H_{34}O_2$	3.63%	
6.	cis-Vaccenic acid	19.918	282	$C_{18}H_{34}O_2$	23.7%	
7.	11-Eicosenoic acid, methyl ester	20.546	282	$C_{18}H_{34}O_2$	18.5%	
8.	11-Eicosenoic acid, methyl ester	24.243	324	$C_{21}H_{40}O_2$	22.8%	
9.	Eicosanoic acid, methyl ester	26.286	326	$C_{21}H_{42}O_2$	59.4%	
10.	: cis-11-Eicosenoic acid, methyl ester	28.929	324	$C_{21}H_{40}O_2$	21.3%	
11.	19-methyl-eicosanoate	31.053	340	$C_{22}H_{44}O_2$	81.3%	
12.	: Docosanoic acid, methyl ester	45.258	351	$C_{23}H_{46}O_2$	62.0%	

Table 7. Anti-cancer activity of *Moringa oleifera*

Concentration in µg/mL	Doxorubicin	EMO
50	26.81	15.89
100	65.17	24.71
150	78.86	29.90
200	86.38	39.12
250	96.23	43.39

Plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. The role of antioxidants from plant extracts in wound healing has been widely researched and it has been found that free radical scavenging properties enhance wound healing (Vincent *et al.,* 2004) (Table 3).

Determination of Ferric Reducing Ability power (FRAP)

Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential. The reduction ability, i.e., "Fe^{3+} to Fe^{2+} transformation" (in terms of increase in absorbance at 700 nm), was found to increase with increasing concentration of the extract. A maximum absorbance of 0.15 was obtained at a concentration of 100 µg of extract. Gallic acid was used as positive control which gave maximum absorbance of 0.1 at a concentration of 100 µg (Table 4)

FTIR Analysis of flower extracts of Moringa oleifera

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in. The peak at 2973.32 indicated Alkane. The peak at 2920.28 indicates C-H Stretching of Alkane. The peak at 2848.91 indicates C-H Stretching of Aldehydes. The peak at 1765.10 indicates C=O Stretching of Ester. The peak at 1454.35 indicates C-H Bending, the peak at 1415.78, 1086.91 and 879.56 indicates, S=O stretching, C-O Stretching and C-C Bending of, sulphate, Aliphatic Ether, and Alkene (Pari *et al.,* 2005).

FTIR absorption spectra of soluble extract reduction of Ag ions. Absorbance bands in the region of 500 - 4000 cm-1 are 3287, 2902, 1776, 1732, 1701, 1624, 1525,1361,1338,1209,1189 and 497 cm-1 .These absorbance bands are known to be associated with the stretching vibrations for O-H carboxylic acid, C-H alkane stretching, $(RC(O))_2O$ unhydride stretch, C=C alkenes stretch, C=O unhydride and ester stretch, CH_3- C(=O)-CH_3 ketone stretch, No_2 nitro stretch , O-H stretching for alcohols and phenols, R–O–R ether stretch and monosubstituted benzene stretching. The total disappearance of this band the bio reduction since mainly responsible for the reduction of Ag ions, whereby they themselves to leading abroad peak at 3287 cm^{-1} (Heim *et al.,* 2002) (Table 5).

GC-MS analysis of flower extracts of Moringa oleifera

Studies of organic compounds from plants and their activities are increasing, mainly as they are

storehouses for novel drugs. Gas chromatography–mass spectrometry (GC-MS) has proven to be a valuable tool for the dependable identification of bioactive components in plant studies (Pari *et al.,* 2005).

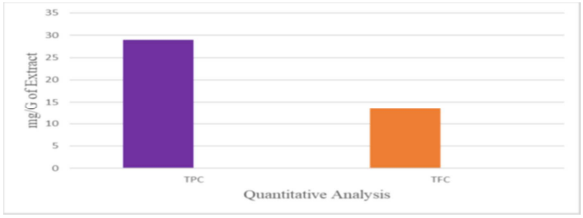


Fig 1. Total phenolic and flavonoid content

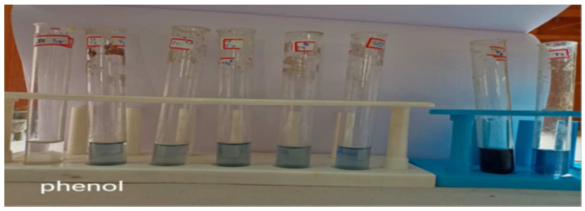


Fig 2. Total phenolic content of ethanolic flower extract of *Moringa oleifera*

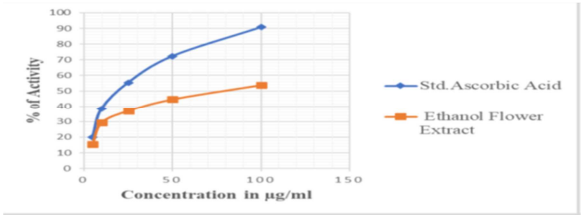


Fig 3. Free radical scavenging activity of DPPH

Fig 4. Free radical scavenging activity standard ascorbic acid

Fig 5. Free radical scavenging activity of ethanolic flower extract of *Moringa oleifera*

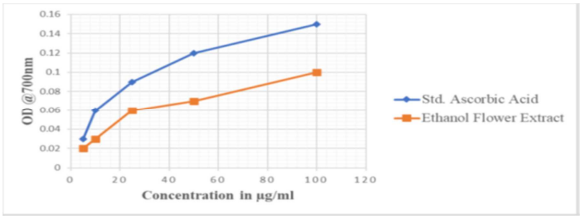


Fig 6. Determination of Ferric Reducing Ability power *Moringa oleife*

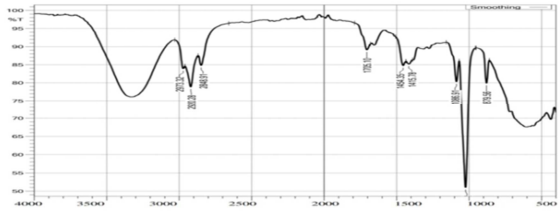


Fig 7. FTIR Analysis of flower extracts of *Moringa oleifera*

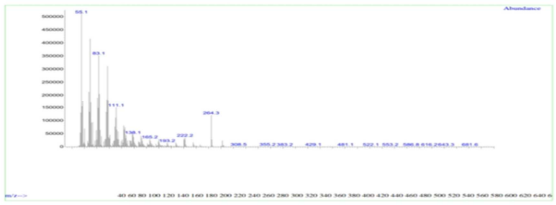


Fig 8. GC-MS Analysis of flower extracts of *Moringa oleifera*

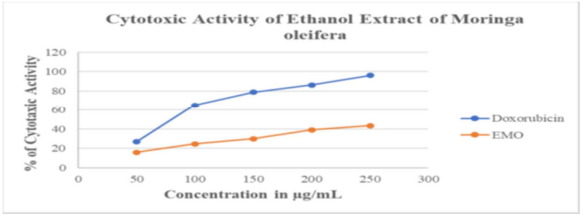


Fig 9. Anti-cancera ctivity of *Moringa oleifera*

However, some of the identified compounds were like the ones earlier documented: butanoic acid, 1,5-heptadiene, 3,3, dimethyl-(E) and 2-propanoic acid, 2 propenyl ester in leaf and Squalene, 1-hexanol, 2-ethyl- 2-propyl, 1, 2-benzenedicarboxylic acid, hexane dioic acid, heptane, heptanoic acid, and iso octanol from the Flower of *Moringa concanensis*. These organic compounds identified could be accountable for the antimicrobial, anti-cancer, analgesic, hepatoprotective and anti-inflammatory properties which support its wide use as health aid by trad medicinal practitioners. However, gave credence to

the findings of this study when they reported that organic solvents like methanol extracts of parts of *Moringa oleifera* exhibited strong *in vitro* and *in vivo* antioxidant activities (Picton *et al.,* 2001) (Table 6).

Anti-cancer activity of Moringa oleifera

To evaluate the cytotoxic effect of the sequentially prepared extracts of *Moringa oleifera* flower extract on liver cancer cell line (HepG2), the cells were treated with different concentrations of drug ranging from 12.5 to 100 µg/ml for 72 h of incubation, and the cell viability was determined by MTT assay. Both aqueous and ethanol extract inhibited the viability in all the tested cell lines in a dose-dependent manner.

Previous studies conducted by Boonsri *et al.* (2008) evaluated the cytotoxic properties of *Moringa oleifera*. In their study, the heartwood and wood were separately extracted from *Moringa oleifera* in dichloromethane and these extracts were shown to induce a potent cytotoxic ability against different cancer cell lines, including HepG2 cells. In addition, extracted four quinones, namely mansonone-D, mansonone-H, thespone and thespesone from the flower of *T. Populnea*and evaluated their cytotoxicity in HepG2 liver cancer cells. Mansonone-D and Thespone showed enhanced cytotoxic effects than the other two quinones, mainly owing to generation of superoxide anions, which the authors speculate to be responsible for the cell killing effect. It is interesting to observe the presence of several natural occurring cytotoxic compounds in *Moringa oleifera* and one of compounds, most elaborately studied is Gossypol. Further studies involving clinical trials showed minimal adverse effects, however the response rates of the receiving patients were low. Hence additional studies need to be performed to explore either a new extract or a synergistic combination with an additional compound (Table 7) and (Fig. 1 -9).

Conclusion

The results obtained from the present study shows that the flower extracts of *Moringa oleifera*. possesses potent anti-oxidant and cytotoxic activities. The claim by traditional healers that the flower extract of *Moringa oleifera* is partially validated in the present study by identifying it has higher apoptotic activity than the ethanolic extract. If some of the compounds are structurally identified and characterized, they may be candidates for further anticancer drug development.

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