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Original Article

PHYTOCHEMICAL SCREENING AND TLC STUDIES OF *MORINGAOLEIFERA* EXTRACT: THEIR ANTIBACTERIAL AND ANTI-OXIDANT ACTIVITIES

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ABSTRACT

Objective: India being a rich and varied flora of medicinal plants since the Vedic period. The present study deals with the phytochemical screening and thin-layer chromatographic studies of *Moringa Olifera* leaf extract belonging to family Moringaceae.

Methods: Phytochemical screening determination by some chemical tests and thin layer chromatographic study was carried out by using various solvent system of varying polarity of hexane, chloroform, ethyl acetate, acetone and methanol extracts.

Results: Phytochemical screening reflects the presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, flavonoids, quinones and terpenoids shows different types of results in different solvents extracts. Thin layer chromatographic studies of the *Moring olifera root* extracts constituted different colored phytochemical compounds with different Rf values The result obtained from DPPH Assay, ABTS assay also confirmed *MoringaOlifera* as a rich source of natural antioxidants, and Anti-bacterial activity studies revealed that it exerted maximum effect against the few pathogenic and non-pathogenic bacterial strain.

Conclusion: Thus it provides evidence that solvent extract of *Moringa Olifera* contains medicinally important bioactive compounds and this justifies the use of plant species as a traditional medicine for treatment of various diseases.

Keywords: *Moringa Olifera,* Moringaceae, DPPH, ABTS, TLC, Antibacterial and Antioxidant Activity.

INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. Medicinal plants are great importance to the health of individuals and communities in general. Medicinal plants would be the best source to obtain a variety of newer herbal drugs. For centuries plants have provided mankind with useful, sometimes life-saving drugs. Modern pharmaceutical in cases where the correlation between chemical structure and biological activities were noted, emperical science began to give way to rational drug design. This emerging approach to identify and develop potential new drug is largely successful, due to the intellectual cooperation of chemistry (medicinal). Therefore such plants should be investigated to understand better their properties safety and efficacy. The use of drugs derived from plants has been in practice for a very long time. Using plants for the medicinal purpose is an important part of the culture and the medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants. They also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes as reported by Okwu, D. E. and Hill A. F. [1, 2, 3]. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Different parts of the plants like bark, roots, leaves, exudates, etc. are used as per medicinal properties proposed by Perumal Samy R. and Gopala Krishnakone P. [4]. *Moranga Olifera*is an important medicinal plant, found throughout tropical India as a common tree in fields and wasteland [5]., *Moringa Olifera*is a monogeneric family, the *moringaceae* that is native to the sub-Himalayans regions of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree, also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, Baijhan, Mulangay, Sajna or Ben oil tree, was utilized by the ancient Romans, Greeks and Egyptians. It is now widely cultivated in the tropical and subtropical regions. Because of its reputation in folk medicine, it has become the subject of intense pharmacological and chemical studies for the last 30 y. traditionally, they are used as sap for eye complaints, an infusion is given to cure diarrhoea, kidney stone and in snake bite treatment. In addition to the traditional uses, the plant is reported for a number of pharmacological activities viz., antihelminthic, demulcent, anti-inflammatory in [6], diuretic in [7] expectorant, hepatoprotective in [8] and nephro protective in [9], anti-diabetic in [10], anti-hyperglycaemic, antimicrobial, cytotoxic in [11], urolithiatic, hypoglycaemic, anti-hyperlipidemic, anti-parasitic and anti-helmenthic activities. In order to identify the bioactive compounds responsible for the above pharmacological activities, phytochemical studies have been carried out by several researchers with the report of phenolic compounds [12]. In our paper, we had discussed about phytochemicals presenting in the leaves extract and their potent antibacterial and antioxidant activity.

MATERIALS AND METHODS

Collection of plant

Moringaoliefera leaves were collected from the Kollampalyam, Erode district of Tamil Nadu, India.

Preparation of plant extract

Leaves were collected in bulk, washed, shade dried, macerated and extracted with hexane, chloroform, ethyl acetate, acetone, and methanol. The extract was filtered and it was finally dried at low room temperature under pressure in a rotary vacuum evaporator (Thermotech, buchi type model th-012). The extracts were concentrated, percentage yield calculated and then subjected to phytochemical screening and TLC profiling studies [13]. The dried extract was properly stored in the desiccators for further experiment and analysis.

Phytochemical screening

Chemical tests for the screening and identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, proteins, amino acids,

flavonoids, and tannins, in the medicinal plants under study were carried out in extracts by using the standard procedure in [14].

Thin layer chromatographic studies

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at a distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system Hexane: Acetic acid (9:1) solvent system I, In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1) used. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used tom detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples.

$$\text{Retention factor} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Antibacterial studies

The antibacterial activity of ethanolic extract of *Moringaoliefera* was tested on non-pathogenic bacteria viz. *Escherichia coli*, *Bacillus subtilis* and *Bacillus megatherium* and pathogenic bacteria viz., *Salmonella typhii*, *Vibrio cholera* by standard agar well diffusion method and the microbes were obtained from MTCC Chandigarh, India. 1 ml broth culture of test organisms were inoculated on nutrient agar medium and poured into sterilised Petri dishes. Wells of 5 mm diameter were made on the nutrient agar using a sterile cork borer. The cut agar discs were carefully removed by the use of sterilised forceps. To each well, 20 µL of plant extracts were loaded with the help of micropipette under aseptic conditions. The plates containing the test organism and extracts were incubated at 37 °C for 24 h. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a meter ruler and expressed in millimetre. [15] Control experiments comprising inoculums with gentamicin antibiotic discs were setup and the plates were incubated at 37 °C for 24 h. The zones of inhibition were then recorded and compared.

Antioxidant studies

DPPH assay

Free radical scavenging activity of *Moringa Olifera* was determined by using a rapid, simple and inexpensive method involving the use of the free radical, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) 11. DPPH is a free radical of violet colour. The antioxidants present in the sample scavenge the free radicals and turn it into yellow colour. The colour change from violet to yellow is proportional to the radical scavenging activity. Briefly, sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 50, 100,200, 600, 800, 1000 µg/ml, in ethanol. One ml of a 0.3 mM DPPH ethanolic solution was added to 2.5 ml of sample solutions of different concentrations and allowed to react at room temperature. After 30 min, the degree of reduction of absorbance was recorded in UV–Vis spectrophotometer at 518 nm [16] (Shimadzu UV–Vis 2450).

ABTS Assay

The ABTS+assay was based on the procedure described by ABTS+radical ′[2,2 -azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] was freshly prepared by adding 5 ml of 4.9 mM ammonium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with ethanol (99.5%) to yield an absorbance of 0.70±0.02 at 734 nm and the same was used for the assay. To 950 µl of ABTS radical solution, added 50 µl of extract solutions (25-500 µg/ml) and the reaction mixture was vortexed for 10 sec. After 6 min the absorbance was recorded at 734 nm and compared with the control ABTS solution.

RESULTS AND DISCUSSION

Percentage of yield extract

The amount obtained from hexane, chloroform, ethyl acetate, acetone and methanol extracts are 4.00 gm, 3.020 gm, 2.682 gm, 1.630 gm, and 4.750 gm respectively, and their yield percentage were 2.12%,1.98%,1.45%,0.84% and 2.56%.

Phytochemical screening

The present study carried out in the *Moringafera Olifera* revealed the presence of active medicinal constituents. The active phytochemical compounds of *Moringaoliefera* were qualitatively analysed for roots and the results are presented in table 1. In these screening process alkaloids, glycosides, saponins, phenolic compounds, tannins, carbohydrates, proteins, amino acids, flavanoids, quinones and terpenoids shows different types of results in different solvents extracts. Among these phytochemical screening, Alkaloids, Saponinis, Tannins, Amino acids, Flavanoids and Terpenoids were present in all solvent extracts where as Phytosterols are absent in all extracts, Phenolic compounds are in Ethyl acetate and methanol extracts, proteins and carbohydrates were present in ethyl acetate and methanol extracts, Quinones were found in hexane, acetone, and methanol extracts, Glycosides are absent in all solvent extracts.

Thin layer chromatographic studies

A large number of solvent systems were tried to achieve a good resolution. Finally, the solvents hexane: ethyl acetate: acetic acid was used [17]. Thin layer chromatographic studies of the hexane extract of *Moringa Olifera* Solvent system I Hexane: Acetic acid (9:1), 3 spots were visible Rf values 0.22, 0.35 and 0.50. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 1 spot detected Rf value 0.94. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 1 spot detected Rf value 0.92. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible Rf values 0.7 and 0.81. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 3 spots were obtained having Rf of 0.9, 0.82 and 0.93. TLC studies of the Chloroform extract of Solvent system I Hexane: Acetic acid (9:1), 2 spots were visible Rf values 0.12 and 0.41. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 3 spots were detected Rf values 0.11, 0.83 and 0.90. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 2 spots were detected Rf values 0.5 and 0.92. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible Rf values 0.9 and 0.75. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having Rf of 0.16 and 0.92. TLC studies of the Ethyl acetate extract of *Aervalanata*. The solvent system I Hexane: Acetic acid (9:1), 2 spots were visible Rf values 0.12 and 0.42. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 2 spots were detected Rf values value 0.84. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible Rf values 0.7 and 0.81. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having Rf of 0.3 and 0.94.

Antibacterial activity

The ethanolic extract of *Moringa Olifera* showed significant antibacterial [18-20] activity against pathogenic organisms than non-pathogenic ones. They exerted high toxicity against non-pathogenic bacteria like *Escherichia coli*, *Bacillus subtilis* and low effect against *Bacillus megaterium.* In pathogenic bacteria, they exerted high toxicity against *Salmonella typhii* and *Vibrio cholerae.* The zones of inhibition for the sample against pathogenic and non-pathogenic organisms are established in table 2.

Antioxidant activity

DPPH ASSAY

The reduction of DPPH absorption is indicative of the capability of the sample to scavenge free radicals fig. 1 and table 3 illustrates the DPPH radical scavenging ability of *Moringaolifera* and it showed excellent DPPH radical scavenging activity that was enhanced with increasing concentration. The IC_{50} value is found to be 60 mg/ml.

Table 1: Phytochemical constitute of the root extract of *Moringa Olifera*

S. No.	Phytoconstituents	Tests	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
1	Alkaloids	+ve	+ve	+ve	+ve	+ve	+ve
2	Glycosides	-ve	-ve	-ve	-ve	-ve	-ve
3	Saponins	+ve	+ve	+ve	+ve	+ve	+ve
4	Phenolic compounds	-ve	-ve	+ve	-ve	-ve	-ve
5	Tannins	+ve	+ve	+ve	+ve	+ve	+ve
6	Phytosterols	-ve	-ve	-ve	-ve	-ve	-ve
7	Carbohydrates	-ve	+ve	-ve	+ve	+ve	+ve
8	Protiens	-ve	+ve	-ve	+ve	+ve	+ve
9	Aminoacids	+ve	+ve	+ve	+ve	+ve	+ve
10	Flavanoids	+ve	+ve	+ve	+ve	+ve	+ve
11	Quinones	+ve	+ve	+ve	+ve	+ve	+ve
12	Terpenoids	+ve	+ve	+ve	+ve	+ve	+ve

Table 2 Antibacterial activity of *MoringaOlifera*

Bacterias		Zone of inhibition (mm)					
Non-pathogenic							
1.	*B. subtilis*	14	ne	11	ne	12	4
2.	*B. megaterium*	11	4	10	5	13	3
3.	*E. coli*	4	ne	1	ne	14	5
Pathogenic							
4.	*S. typhii*	11	1	4	3	11	3
5.	*V. cholera*	5	1	5	2	9	1

Ne–no effect

Table 3: DPPH assay

Concentration(mg/ml)	% Inhibition
20	30
40	45
60	50
80	51
100	67

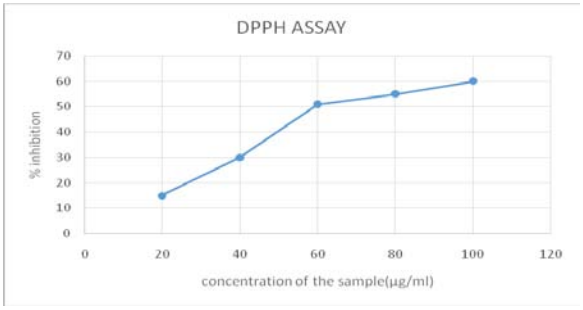

Fig. 1: DPPH assay for different concentration of Leaves extract

ABTS assay

Leaves extract of Moringa Oliferais known to scavenge the free radicals generated by ABTS by donating a hydrogen atom indicating Moringa Olifera a potent anti-oxidant. Decolorization of ABTS is observed which expressed IC_{50}value of 60µg/ml and it was depicted in table 4 and fig. 2.

Table 4: ABTS assay

Concentration(mg/ml)	% Inhibition
20	15
40	30
60	51
80	55
100	60

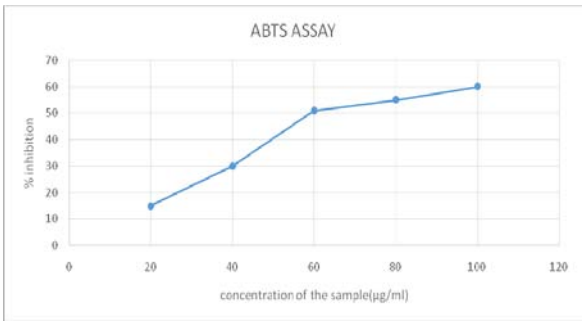

Fig. 2: ABTS assay for different concentration of leaves extract

CONCLUSION

The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. These findings suggested that *Moringa Olifera* could be a potential source of natural antioxidant having great importance as a therapeutic agent and preventing oxidative stress-related degenerative diseases. They can provide lead molecules which could be a useful substrate for the synthesis of new broad-spectrum antibiotics for the treatment of infections caused by the organisms.

CONFLICT OF INTERESTS

Declare none

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