



Evaluation of Antimicrobial and Antioxidant Activity of different Ancient Herbal Plants

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Abstract

Traditional remedies, as is well known, have a long history in the world and continue to give valuable and appropriate techniques for treating a variety of disorders. The present study was carried out to evaluate the antibacterial activity and antioxidant activity of four different ancient plants Argemone mexicana, Aegle marmelos, Phaseolus vulgaris and Moringa oleifera extracted by using the ethanolic extracts were screened for the antimicrobial and antioxidant activity. The organisms used for antimicrobial activity were Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Bacillus subtilis, Salmonella typhi, and Staphylococcus aureus. Using the 1-1-diphenyl-2-picrylhydrazyl (DPPH) bleaching techniques, the antioxidant activity (AOA) was measured. The total phenolic content (TPC) in the extract was determined spectrophotometrically by the Folin CioCalteus method, Ferric reducing antioxidant power (FRAP), ABTS assay and Total Flavonoids Content (TFC). The plant shows a high amount of phenolic and flavonoid compounds. Within that plant has high antioxidant activity.

Keywords: Antimicrobial activity; Anticancer activity; Herbal Plants; Ethanolic extract; Phenolic; DPPH

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INTRODUCTION

Medicinal herbs are the foundation of various indigenous traditional medical systems around the world. The importance of medicinal plants as a possible source of bioactive chemicals has been recognised in pharmacological investigations[1]. Phytochemicals from medicinal plants are used as leading molecules in drug research and design. In this regard, obtaining a

scientific foundation for the potential use of herbal medications to treat diseases looks beneficial. The results of studies evaluating the antibacterial and antioxidant activities of selected Indian medicinal plants with previously established pharmacologic effects are presented here[2]. Medicinal plants remain essential medicinal assistance in the treatment of human illnesses. The desire for health and longevity for treatments to



relieve pain and discomfort drove the early man to explore his immediate natural environment, resulting in the development of several therapeutic agents using a variety of plants, animal products, minerals, and other natural resources. There is a renewed interest in traditional medicine nowadays, as well as a growing desire for more medications derived from plants. The present prevalent notion that "Green Medicine" is safer and more trustworthy than expensive synthetic medications, many of which have negative side effects, has sparked renewed interest in plant-derived drugs[3]. Plants are used by about 80% of the world's population in developing countries to treat a variety of ailments such as infections, pain management, wound healing, reproductive disorders, skin infections, digestive troubles, and more. The desire for herbal products over synthetic medicine is growing day by day due to the negative consequences of chemical entities. In both developing and developed countries, cancer is one of the main causes of mortality. Its economic losses are growing throughout the planet. Chemotherapy's side effects, such as nausea, vomiting, and baldness, necessitate the search for new candidate plant species or pharmaceutical substances that are less hazardous to normal cells but more toxic to cancerous cells. Plants and their derivatives may be useful in the treatment of cancer[4]. In 2012, the globe saw 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million individuals living with cancer (within five years of diagnosis). There will be 26 million new cancer diagnoses and 17 million cancer deaths every year by 2030, according to estimates. In 2018, 18.1 million new instances of cancer were diagnosed, and 9.6 million people died from the disease[5]. Approximately 60% of current cancer treatments are derived from a natural compound with the plant kingdom serving as the most important source[6]. These plants were chosen based on ethnobotanical knowledge, evidence of continued widespread use, and local availability[2]. **Moringa oleifera** plant is beneficial for food, medicine, cosmetics, or water purification, it is known as the world's

most valuable multifunctional and miraculous tree[7]. **Phaseolus vulgaris** is a common leguminous item in Indian cuisine; these seeds provide roughly 22 g of vegetable protein per 100 g[8]. Since Charak (1500 B.C.), **Bael (Aegle marmelos)** has been considered one of India's most valuable medicinal plants. More than 100 phytochemical substances, including phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins, have been isolated from various portions of the plant[9]. **Argemone mexicana (Argemone mexicana L)** is well-known for its use in the treatment of malaria, warts, cold sores, skin illnesses, itches, and a variety of other ailments. Leaf extracts of A. Mexicana was tested for antibacterial activity against a variety of pathogenic bacteria[10].

MATERIAL AND METHODS

Plants Collection

The fresh leaves of *Moringa oleifera* and *Aegle marmelos* were collected from Bala Ji nursery Gr. Noida. *Argemone mexicana* were collected in February 2022 from the wastelands of the village Burari, North district of New Delhi, India. *Phaseolus Vulgaris* were collected from farmer's land in Burari village.

The identification was done by Mr Ajeet Singh, a Faculty Scientist at Helix Biogenesis Pvt. Ltd, Noida.

Extraction of Plant Material

Fresh plant leaves were washed with tap water, rinsed with distilled water[9] and dried for three days in a hot air oven at 25°C[11]. The dried leaves of each plant were pulverised in a pestle mortar to produce a powdered form, which was stored at room temperature in airtight glass containers until used[12]. 5gm of powdered sample was placed in a conical flask containing 70% ethanol solvent[13] and left to stand with intermittent shaking on a rotatory shaker at 200rpm for 24 hours. The preparation was filtered using sterilised Whatman No.1 filter paper before being concentrated to dryness using a rota evaporator under a vacuum at 40°C. The resulting dried extract was sterilised overnight using UV before being kept at 4°C in labelled sterile bottles[1], [14].

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ANTIMICROBIAL TEST

The antibacterial activity was tested using three Gram-positive bacteria *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, and three Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. Staining, biochemical and molecular properties were used to identify bacterial strains. The discovered isolates were suspended on nutrient agar slants, refrigerated at 4°C and sub-cultured regularly[1], [15], [16].

Antimicrobial Susceptibility Test (AST)

The antibacterial activity of aqueous ethanol extract was used to determine by Kirby-Bauer agar diffusion methods. Mueller Hinton Agar (MHA) was put into Petri plates, which were then allowed to solidify before the culture organism was dispersed across them. To construct wells, a sterile cork borer (7mm diameter) was pierced into the cultured solidified plates. Which were filled with 0.03ml of the extracts. In an incubation chamber, the plates were incubated at 37°C for 24 hours. The inhibitory zone was measured and recorded. The diameter of the inhibition zone around the well (in mm) including the diameter of the well was used to compute the zone of growth inhibition[13], [17].

Observation of microbial activity

For all three replicates, antimicrobial susceptibility test (AST) values were taken perpendicularly, and the average value was tabulated.

Minimum Inhibitory Concentration (MIC)

Bacillus subtilis, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis* were tested using a 70% ethanol extract of *Moringa oleifera*, *Phaseolus vulgaris*, *Aegle marmelos*, *Argemone mexicana*[10], [18]. The susceptibility probe should be carried out[8], [19]. The MIC assay uses an agar or broth dilution method to determine the lowest effective concentration of an antimicrobial drug that suppresses the observable growth

of a bacteria of interest under defined test conditions. Minimum inhibitory concentration was first determined by (Baker and Breach1980; Mckane & Kandel 1986)[15]. MIC, which is the lowest concentration of antimicrobial agent that precludes observable development of a microbe. Aqueous-ethanol extracts were utilised on the susceptible microorganism to determine the minimum inhibitory concentration. To reach a concentration of 100mg/ml, 200mg of each extract was diluted in 1ml Dimethyl sulfoxide (DMSO). Two-fold serial dilution was made of obtain 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, and 0.19mg/ml. These concentrations were placed in wells drilled with the susceptible microbe as described previously[16], [20]. Prepare two-fold serial dilutions (up to 7) of the test compounds and one quality control (QC). The inoculum was made by removing a few colonies from an agar plate using a sterile swab, making a Mcfarland standard and diluting the Mcfarland standard into media. The microtiter plate was then placed in an incubator at 37°C for 24 hours after the MIC value was determined. The MIC value is the lowest concentration of a compound at which no growth is observed.

ANTIOXIDANT ACTIVITY DETERMINATION

DPPH Method: 2,2-diphenyl - picryl - hydrazyl - hydrate(DPPH) radical scavenging activity was measured by a spectrophotometric method in which a stock solution of DPPH was prepared.

Protocol for DPPH free radical scavenging Activity: The sample's stock solution's preparation was measured. 15mg of DPPH was dissolved in 10ml of methanol after 5mg of extract was diluted in 1ml of ethanol[21]. The mixture was incubated at 37°C for 30 minutes with a 0.004% DPPH solution in ethanol and 5mg/ml of plant extract and the absorbance of the combination was measured at 517nm[22]. To protect the solution from light, it was wrapped in aluminium foil. The percentage of DPPH radical inhibition was estimated by comparing the results to those of the control (non-treated with extract)[22]. The decrease in DPPH radical absorbance



caused by antioxidants was due to the radical being scavenged by hydrogen donating. It was usually noticeable as a colour change from

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Estimation of Total polyphenolic content(TPC)

The polyphenolic assay is based on Folin – Ciocalteus. The FCR contains a phosphomolybdic/phosphotungstic acid complex where the maximum absorption depends on the concentration of the phenolic compound. The carbonate buffer was employed to modify the pH in the F-C experiment.

Protocol of Total Phenolic control: The following procedure determined the total phenolic content of ethanolic extracts.

5mg extracts were dissolved in ethanol to obtain a 5mg/ml concentration of extracts[26].0.5N FCR reagent was mixed with distilled water. 20% NaNO₃ was used as a buffer solution to adjust pH and gallic acid was used as a standard solution[27], [28].Mixing of plant extract, 100µl F-C reagent, 20% NaNO₃ and gallic acid the solution was to stand at room temperature 37°C for 30min. The total phenol content (TPC) was determined by spectrophotometry[29]. The absorbance of the reaction mixture was measured at 760nm[30].

Determination of Total Flavonoid Content(TFC)

TFC was determined with aluminium chloride(AlCl₃) using Quercetin as a standard[31]. In this method, aluminium chloride forms a complex with hydroxy groups of flavonoids present in the sample. 10% AlCl₃ was mixed with distilled water, and 10 ml of distilled water was mixed with 1 ml of potassium acetate (981.5mg). Extracts were prepared to mix 5mg/ml in ethanol. Then prepared 1mg/ml quercetin stock solution in different concentrations ranging from 10µg/ml to1000 µg/ml and 200µl AlCl₃ 200 µl potassium acetate added to prepared ethanolic extracts. Incubate it at room temperature for 30min. Absorbance

purple to yellow[23]. Control with 1 mL pure water and 1 mL DPPH was also tested[24], [25].

was measured at 420nm by using UV-Vis spectrophotometric.

Ferric Reducing Antioxidant Power(FRAP)

The protocol to evaluate the antioxidant capacity of compounds by the FRAP method is based on the reduction of Fe₃⁺(Ferric) to Fe₂⁺ (Ferrous) by antioxidant molecules forming the blue-coloured Fe₂⁺ TPTZ. Here, different concentrations of standard solution were prepared of concentrations 10, 50, 100, 150, 200 up to 450 µg/ml and 300µl of FRAP solution were added in each prepared solution. Ferrous sulphate(FeSO₄) was used as standard. Extracts were prepared in ethanol at 5mg/ml. Covered all prepared solution with foil paper and incubate at 37°C for 30min in dark. The absorbance was measured at 594nm using a UV-Vis spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power[31], [32].

ABTS Decolorization Assay of Antioxidant

The ABTS radical scavenging assay (2, 2 - azinobis -3-ethyl benzothiazoline -6- sulphonic acid) is based on the scavenging of light by ABTS radicals. An antioxidant with the ability to donate a hydrogen atom will quench the stable free radical a process that is associated with a change in absorption[33].

7nm ABTS of 3.84mg dilute in 1ml of ultrapure water and 2.45mM APS(Ammonium persulphate) of 5.59mg dilute in 10µl of ultra water. Add APS to the ABTS solution so that the final APS concentration is 2.45mM. This step is necessary to generate the ABTS radical. Incubate it overnight at room temperature in dark[34]. Check the concentration of the ABTS radical stock solution at 734nm. Dilute 10µl of ABTS solution in a final volume of 10ml. Then read it at 734nm by using spectrophotometry. Prepare an ABTS solution that absorbs~ 0.700 at 734nm.The extracts were then prepared in DMSO at a concentration of 5mg/ml (Dimethyl sulfoxide). Gallic acid was used as a



standard and 190µl ABTS was used in the stock solution. The absorbance was measured at 734nm using UV-Vis spectrophotometry.

RESULT

In the course of our screening for antimicrobial and antioxidant activity, several ancient traditional medicinal plants were evaluated from different locations. A total of four plants extract were used. Antimicrobial activity of ethanol extract of Moringa oleifera, Phaseolus vulgaris, Argemone mexicana, and Aegle marmelos leaf against six pathogenic microorganisms was carried out by Antimicrobial susceptibility test(AST) and Minimum inhibitory concentration (MIC) methods. It was found that the plant extract showed good inhibitory activity on almost all the pathogenic microbes tested. The result of

the antibacterial assay of different extracts is described as follows.

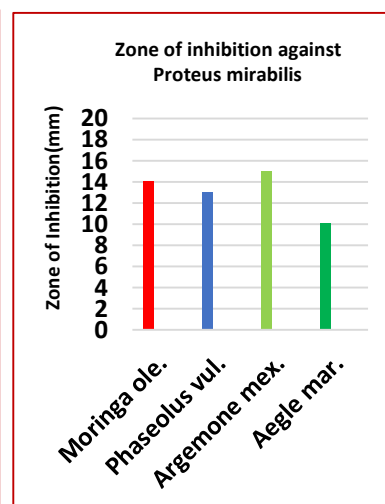
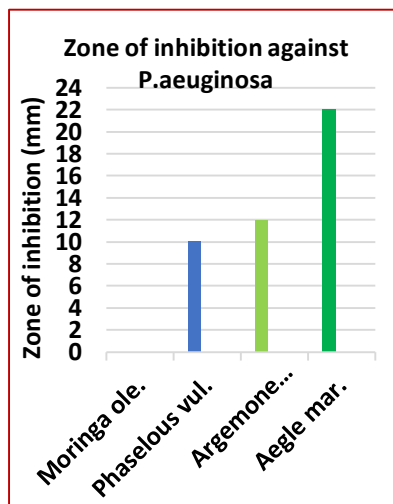
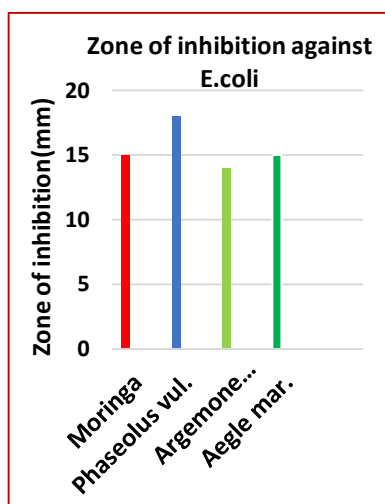
The data table's perusal reveals that all ethanolic plants' leaves extract possessed antibacterial activity against all six pathogenic bacteria. The ethanolic leaf extract of Aegle marmelos was found to be the most effective against Salmonella typhi(25mm), and Pseudomonas aeuginosa(22mm). In Moringa olifera against Staphylococcus aureus(18mm) and Phaseolus Vulgaris against Escherichia coli. Moringa olifera showed no zone of inhibition against Pseudomonas aeuginosa. In MIC Proteus mirabilis, Pseudomonas aeuginosa and Bacillus subtilis were less sensitive pathogens that survived up to 25mg/ml. Salmonella typhi and Staphylococcus aureus have the most sensitive pathogens that survived up to 3.12mg/ml.

Table 1: Antibacterial potential of ethanolic leaves extracts against six Human pathogenic Bacteria. Using an Antimicrobial susceptibility test.

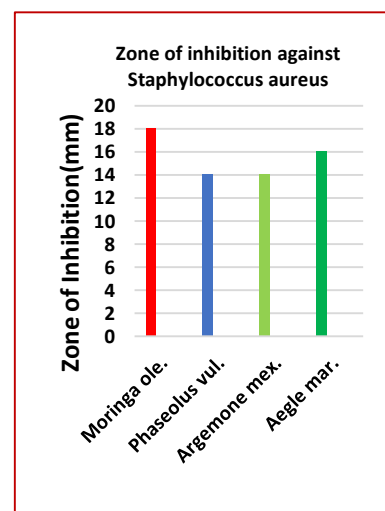
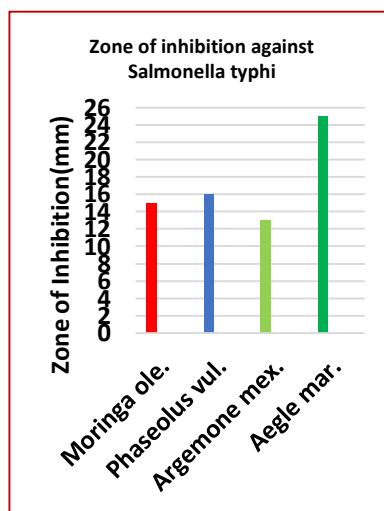
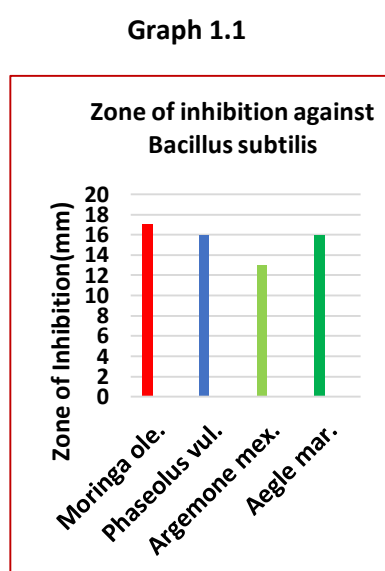
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Tested microorganism	Zone of Inhibition (mm) AST			
	Moringa olifera	Phaseolus vulgaris	Argemone mexicana	Aegle marmelos
<i>Escherichia coli</i>	15	18	14	15
<i>Pseudomonas aeuginosa</i>	-ve	10	12	22
<i>Proteus mirabilis</i>	14	13	15	10
<i>Bacillus subtilis</i>	17	16	13	16
<i>Salmonella typhi</i>	15	16	13	25
<i>Staphylococcus aureus</i>	18	14	14	16





Graphs of Zone of Inhibition



Graph 1.2

Graph 1.3

Graph 1.4

Graph 1.5

Graph 1.6

Table 2: Minimum inhibitory concentration (MIC) for antimicrobial activity using series

two-fold dilution of ethanolic plant leaves extracts against tested microorganisms.

Tested microorganism	Minimum inhibitory concentration value (mg/ml) (MIC)			
	Moringa olifera	Phaseolus vulgaris	Argemone mexicana	Aegle marmelos
<i>Escherichia coli</i>	6.25	12.5	12.5	12.5
<i>Pseudomonas aeuginosa</i>	25	25	12.5	12.5
<i>Proteus mirabilis</i>	6.25	6.25	25	12.5
<i>Bacillus subtilius</i>	25	6.25	25	6.25
<i>Salmonella typhi</i>	6.25	3.12	6.25	3.12



<i>Staphylococcus aureus</i>	3.12	6.25	6.25	12.5
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Antioxidant activity

Free Radical Scavenging Activity Measured by 1,1-Diphenyl-2-picryl-hydrazil(DPPH)

Table:3

S.No	Plant extracts sample	OD	Control OD	% Inhibition
1.	Moringa oleifera	0.410	0.737	44.37%
2.	Phaseolus Vulgaris	0.434		41.11%
3.	Argemone mexicana	0.176		76.12%
4.	Aegle marmelos	0.645		12.48%

Plant extracts sample free radical scavenging activity are given in Table 3. Where the highest % inhibition is 76.12% of Argemone mexicana, the lowest % inhibition is 12.48% Of Aegle marmelos and DPPH control OD is 0.737.

Determination of total phenolic content(TPC)

S.No	Plant extracts	TPC Determination
1.	Moringa oleifera	63.02 µg/ml
2.	Phaseolus Vulgaris	27.42 µg/ml
3.	Argemone mexicana	21.42 µg/ml
4.	Aegle marmelos	6.02 µg/ml

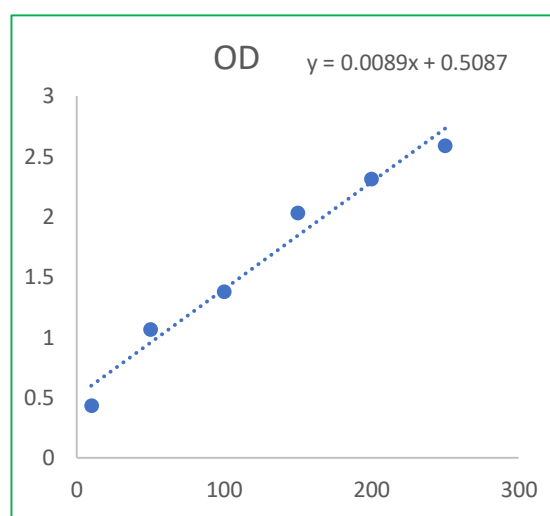


Table: 4 Graph:4.1

In all four types of plant, leaves extract contained a higher amount of total phenolics. The total phenolic content of Moringa oleifera was the highest among all plant extracts while Aegle marmelos had the lowest. The order of total phenolic content in the whole leaves extracts was Moringa oleifera > Phaselous vulgarise > Argemone mexicana > Aegle marmelos. However gallic acid is used as a standard drug from 100µg to 1000µg.

Determinatio of total flavonoid content(TFC)

S.No	Plant extracts	TFC Determination
1.	Moringa oleifera	180.3 µg/ml
2.	Phaseolus Vulgaris	51.71 µg/ml
3.	Argemone mexicana	77 µg/ml
4.	Aegle marmelos	135.7 µg/ml

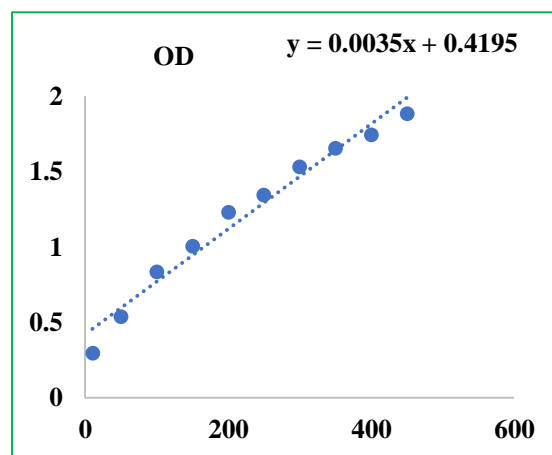


Table 5 Graph 5.1

All plant leaves extracts, flavonoid content is given in Table 5. Moringa oleifera contains a high amount of flavonoid and Argemone mexicana had the lowest, Potassium acetate was used as a reference drug and approximately 200µg/ml in each sample extract.

Determination of ferric reducing antioxidant power(FRAP)

S.No	Plant extracts	TFC Determination
1.	Moringa oleifera	39.86 µg/ml
2.	Phaseolus Vulgaris	38.71 µg/ml
3.	Argemone mexicana	22.43 µg/ml
4.	Aegle marmelos	37.57 µg/ml

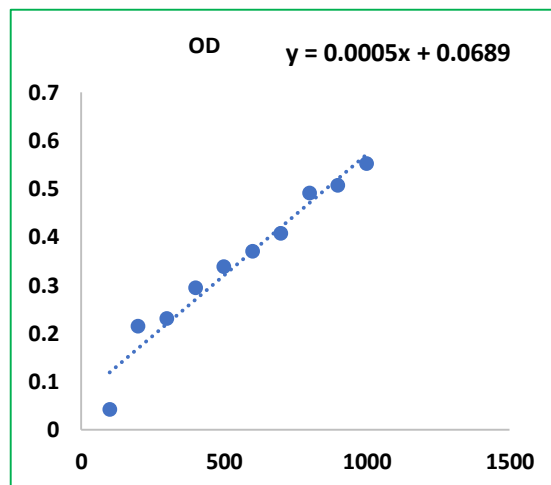


Table 6 Graph 6.1

The plant leaves extract produced a concentration-dependent increase in antioxidant power. FRAP was used as a reference drug and 300µg/ml in each sample, showing that Moringa oleifera has high antioxidant power (Table 6)

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Determination of ABTS Decolorization Assay of Antioxidant

Table:7

S.No	Plant extracts sample	OD	Control OD	% Degradation
1.	Moringa oleifera	0.136	0.227	40.08%
2.	Phaseolus Vulgaris	0.137		39.65%
3.	Argemone mexicana	0.128		43.61%
4.	Aegle marmelos	0.147		35.24%

Plant extracts sample ABTS Decolorization assay of antioxidant are given in Table 7. Where the highest % degradation is 40.08% of Moringa oleifera, the lowest % degradation is 35.24% Of Aegle marmelos and DPPH control OD is 0.737.

DISCUSSION

The phytoconstituents of plant material are utilised as a therapeutic agent in a variety of infections and are known as active components. Many of the studies revealed that ethanol extracts from the plants had positive results, indicating that ethanol is the optimum solvent for extraction. A variety of phytoconstituents are utilised to treat cancer. Leaf extracts are high in flavonoids and

phenols, according to phytochemical analysis. The majority of the phytochemicals discovered had both pharmacological and physiological action. Secondary metabolites, such as polyphenols, are bioactive chemicals that regulate numerous processes such as reproduction, parasite resistance, and environmental stress tolerance. Flavonoids have a wide range of biological functions. Many polyphenols have been discovered to



be effective antioxidants, anticancer agents, antithrombotics, and antihypertensives. Antimicrobial activity is the inhibition of bacterial growth caused by phytochemical action. A plant extract employed as an antibacterial agent has significant therapeutic promise because it has fewer adverse effects than synthetic antimicrobials, which are common. Antimicrobial action was demonstrated by a change in chemical composition. The growth of multidrug resistance against dangerous germs in humans and animals and unwanted antibiotic side effects. Both traditional and modern medicines can be obtained from medicinal plants. Herbal remedies are effective; over time, about 80% of the rural population has relied on them for primary health care. The World Health Organization advised that countries should interact with traditional medicines to discover and exploit components that provide safe and effective cures for disorders of both microbial and non-microbial origin.

CONCLUSION

Medicinal plants are the most important source of life for providing non-side effects of medicinal remedies for the world's people. Plants have remained essential therapeutic agents for the treatment of human illnesses. Early man explored the immediate natural resources against numerous maladies in search of defence mechanisms, lifespan, and cures to relieve pain and discomfort. Today, there is a resurgence of interest in traditional medicine, which is driving up demand for more pharmaceuticals derived from plants, because green medicines are safer, cheaper, and have fewer side effects than synthetic treatments. Since all of the plant extracts tested were highly effective against pathogenic bacteria and showed antimicrobial activity. If the phytoactive components are isolated and an appropriate dosage for proper administration is identified, the antibacterial and anticancer activity can be increased.

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