IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Analysis Of The Coagulation Property In *Moringa Oliefera* Leaves In Poultry Blood

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Abstract: Along with elevated cholesterol and triglycerides, blood clotting has become one of the most dangerous adverse effects linked to the coronavirus. Therefore, it is now essential to use physiologically safe medicinal plants that contain anti-clotting components. The phytochemical compositions, properties, and potential applications of these extracts in a range of industries, such as food, cosmetics, and others, have been the subject of more recent research. In order to assess the impact of the aqueous extract of Moringa oleifera leaves on blood clotting activity in the chicken blood sample by measuring prothrombin time (PT), the aqueous and methanolic Moringa leaf extracts in this study were subjected to phytochemical analysis using gas chromatography-mass spectrometry (GCMS) and to analyze the anti-coagulating effect. According to the quantitative study, the aqueous extract of Moringa oleifera contains 52.08 ± 0.21 µg/g of total phenol (µg/g GAE), 116.93 ± 1.22 µg/g of total flavonoid (µg/g QE), and 86.91±0.42 µg/g of total tannin (µg/g). Twenty major compounds were found in the extract of Moringa oleifera leaves, with 2-(3,4-dihydroxyphenyl)-trihydroxychromen-4-one, 3TMS derivative having the highest quantum (38.9 percent), followed by 1H-Indene, 2-butyl-5-hexylocta hydro (15.29 percent), and 3-(2-aminoethyl)-1H-indol-5-ol (7.46 percent). It helps with blood coagulation, bone health, sleep, sexual function, mood regulation, and memory. The data analysis qa1 shows that the group with a concentration of 100 percent Moringa oleifera leaf extract had the shortest blood coagulation time, with notable differences from other treatment extracts leading to a lower average blood coagulation time.

Index Terms - Moringa oleifera, Phytochemical test ,GCMS and Anti- Coagulant activity

INTRODUCTION`

For thousands of years, nature has been a fantastic supply of medicinal remedies, and a startling number of modern medicines have been derived from natural sources. Since the beginning of time, plant extracts have been highly valued for their vast medical potential. More recently, researchers have focused on learning more about the phytochemical compositions, qualities, and possible uses of these extracts in a variety of contexts, including foods, cosmetics, and other industries. (Nitesh Bhalla *et al.*, 2021)

The widespread use of plants is still very significant today. According to the World Health Organisation 80 Percentage of the world's population, , uses traditional medicine to manage their health issues because they often do not have access to modern medicinal products, and also these medicinal plants can be highly effective (Novais J. 2004). In fact, most modern medications are derived from or based on their models (active ingredient synthesis or chemical semisynthesis. Herbal medicine has developed into a brilliant science where the active ingredient is found directly in the plant. The usage of traditional herbal remedies can be explained by sociocultural norms, the high expense of pharmaceuticals, the absence of necessary medications, and inadequate health care [Atchade S. Pascal, *et al.*, 2018].

It is important to emphasize that using medicinal herbs as an alternative for modern medicine is appropriate in such a scenario. It is vital and ongoing to understand, advance, and value the usage of therapeutic plants. In this regard, *Moringa oleifera* is a drought resistant fast-growing perennial plant belong to the family Moringaceae. It is one of the plants used in treatment for various parts such as leaves, roots, seed, fruits, flowers and immature pods act as circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory (Kumar AK *et al*., 2009). The Moringa tree contains the majority of the phytochemicals. Indeed, it is generally believed that the presence of these phytochemicals is responsible for varies range of biological activities and disease-prevention potential of moringa. Therefore, it is quite promising that future studies will be able to use the chemical diversity of Moringa phytochemicals to treat illnesses and promote better health. (S.K. Bohn *et al*., 2012).In southern Asia it also utilized as an indigenous of medicine system for treatment of antiulcer, antispasmodic diuretic, antihypertensive, cholesterol lowering antioxidant, antidiabetic, hepatoprotective [Shunmugapriya K *et al*., 2017]].Plant extracts are immediately used in traditional medicine as food, tonics , external application to improve the immunity and vigor since ancient time.(R. Ullah *et al*., 2020)

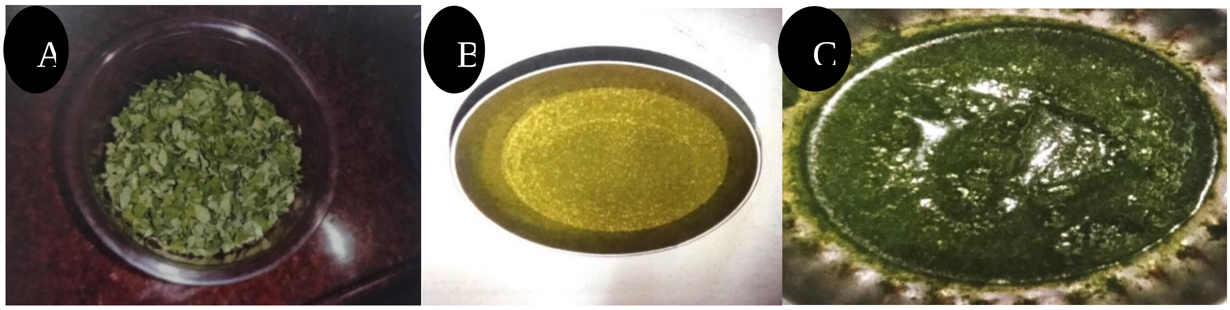
In fact, using the vital power of nature is crucial to prevent rapidly growing proliferating diseases such as cancer, heart attacks, diabetes, and rapid ageing of the skin, as well as emerging forms of alarming new health concerns like COVID-19, (Nitesh Bhalla *et al*., 2021).Because of their impact on biological processes, plants may offer up novel possibilities for the creation of cutting-edge anticoagulant drugs. Medicinal drugs made from plants have a wide range of activity with a focus on preventive action, in addition to being easy to use and effective. There is compelling scientific evidence that indicates the consumption of dietary anticoagulants or phytochemicals with anticoagulant activity gradually reduce blood clotting or eliminate the risk of thromboembolic diseases. (Al-Saadi N. 2011).

Anticoagulants are a class of medicines that prevent he formation of a clot in the blood vessels by affecting platelets and blood coagulation factors' availability or activation. However, life-threatening adverse effects such thrombocytopenia, bleeding episodes, and osteopenia are often brought on by these medications (S. Kumar *et al*. 2011). Consequently, the hunt for novel, alternative agents derived from natural sources has been sparked by the concerning limitations of the anticoagulants already in use. (Manicam C. *et al*., 2010) The present work was conducted to identify and quantify the presence of various compound by using GCMS in Moringa leaves for anti-coagulant and to evaluate the effect of aqueous *Moringa oleifera* leaves extract on blood clotting activity in the chicken blood sample by the determination of prothrombin time (PT)

MATERIALS AND METHODS

Plant Collection and Extract Preparation

The plant utilized in this study was analysed first to distinguish that the leaves were without a doubt *Moringa oleifera*. After the assessment and recognizable proof, the leaves were brought to the research centre to be handled. *Moringa oleifera* leaves were softly eroded in deionized water by which the dust particles were removed. The leaves were dried to a constant weight at 18°C in an enclosed air-conditioned research laboratory for 72 hours. Dried leaves were grounded using mortar and pestle. After the process of grinding, the leaves powder was sieved to get very fine particles of uniform size. The powdered (100gm) was extracted by cold percolation method with 300 ml of distilled water. After Maceration cycle of powder was dissipated utilizing the rotating evaporator gadget, creating glue structure extricate. The *Moringa oleifera* leaves glue separate was then put away at -20°C and store till use for the further process



A) Collected and air dry of *Moringa oleifera* leaves in room temperature B) Dried leaves were Grounded into powder C) Aqueous Extract of *Moringa oleifera*

Plate 1 : Preparation of *Moringa oleifera* leaves aqueous extraction

Phytochemical Screening Preliminary: Phytochemical screening was carried out on the crude aqueous extracts of *Moringa oleifera* leaves using standard procedures as described by Trease and Evans 1989; Sofowora 1993; Ushie *et al*.,2016; and Santhi *et al*.,2016.

Detection of Alkaloids The test sample extracts were dissolved in dilute hydrochloric acid and then filtered. The filtrates were used to test the presence of alkaloids. **Mayer's Test.** The Filtrates samples were treated with Mayer's reagent. Formation of a yellow cream precipitate signify the presence of alkaloids..

Detection of Flavonoids by Lead **Acetate:** The test samples extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate imply the presence of flavonoids.

Detection of Phenols using Ferric Chloride Test 10 mL of each extract was treated with few drops of ferric chloride solution and evolve the bluish black color indicates the presence of phenol

Detection of Tannins 1 mL of each of the extracts was mixed with water and heated on a water bath. The mixture was distilled and ferric chloride was added into it. Then formation of a dark green color reveals the presence of tannins.

Detection of Saponins Foam Test 1 mL of test sample extracts was shaken with 5 mL of the distilled water. Formation of stable persistent foam express the presence of saponins.

Detection of Glycosides: Take 0.5 mL of each extract was dissolved in 1 mL of water and then aq NaOH solution was added. Formation of yellow color imply the presence of glycosides.

Detection of Steroids 2 mL of acetic anhydride was added to 0.5 mL of each extract in a test tube, followed by the addition of 2 mL of sulfuric acid. The color changes from violet to blue or violet to green indicates the presence of steroids.

Detection of sterols Chloroform and sulfuric acid test Add 5 ml of chloroform to 2 ml of plant extract, then carefully add 1 ml of concentrated sulfuric acid along the walls of the tube. A reddish-brown color in the lower layer indicates the presence of sterols

Detection of Anthraquinones 2 ml of each of the plant extracts was boiled with 10 ml of sulfuric acid (H2SO4) and was filtered while hot. 2 ml of chloroform were shaken with the filtrate. One ml of diluted ammonia was introduced to a test tube containing the chloroform layer using a pipette. Colour variations in the resultant solution were monitored.

Detection of Carbohydrates: Add equal volume of Fehling's (A & B) solution to 2 mL of extract, then heat for five minutes. A dark red precipitate reveals the presence of carbohydrates.

Detection of Amino acid: A few drops of the 2Percentage ninhydrin solution must be added to the test solution. The test tube must be kept in a warm water bath for approximately 5 minutes. The development of a deep blue or violet colour indicates the presence of amino acids.

Detection of Terpenoids Salkowski s Test 0.5 mL of each extract was mixed with 2 mL of chloroform, and 3mL of concentrated H2SO4 was carefully added to form a layer. An appearance of a reddish-brown color interface indicated the presence of terpenoids.

Detection for Reducing Sugars 1 ml of the analyte sample must be mixed with 2 ml of Benedict's reagent and heated in a bath of boiling water for 3 to 5 minutes. The development of a brick-red coloured precipitate of cuprous oxide confirms the presence of reducing sugars in the analyte.

Quantitative Analysis of the Phytochemicals

Quantitative analysis of plant extracts is a method for determining the amount or concentration of phytochemicals in a plant sample. It can be used to study drug discovery, standardize herbal drugs, and determine toxicity levels in plants.

Estimation of Tannins : According to Rahman Gu *et al*., 2017 the tannins were determined by Folin-Ciocalteu method) .A volumetric flask (10 ml) with 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent, 1 mi of 35Percentage sodium carbonate solution, and 10 ml of distilled water was diluted with approximately 0.1 ml of the sample extract. After giving the mixture an adequate shake, it was left at room temperature for a half-hour. Tannic acid reference standard solutions (20, 40, 60, 80, and 100 µg/ml) were made using the same technique earlier mentioned. Using a UV/visible spectrophotometer, the absorbance of the test and standard solutions was measured at 700 nm in relation to the blank and repeat the process in triplet and calculate tannin.

Total phenolic content The Folin-Ciocalteu (FC) reagent was used to calculate the total phenolic content.(1983, Mallick EP, Singh MB) Two millilitres of 20Percentage Na2CO3 were added after the plant extract (0.5 mL) and 0.5 mL of FC reagent (1:1 diluted with distilled water) were combined and incubated for five minutes at 22°C. After 90 minutes of additional incubation at 22°C, the mixture's absorbance at 650 nm was determined. Gallic acid served as a standard in the calculation of the total phenolic content (mg/mL).

Total flavonoid content *The aluminium chloride (AlCl3) technique was used to calculate the total flavonoid content (mg/mL). (Riya Kadia* et al., *2022). The assay mixture, consisting of 0.3 mL of 5Percentage NaNO2,* *0.5 mL of distilled water, and 0.5 mL of the plant extract, was incubated for 5 min at 25°C. Following that, 0.3 mL of 10Percentage $AlCl_3$ was added immediately. After adding 2 ml of 1 M NaOH to the reaction mixture, the absorbance at 510 nm was calculated. The standard used was quercetin.*

GCMS analysis

The analysis of extracted phytochemicals of *Moringa oleifera* leaves were done using GC-MS Agilent Technologies-7820A GC system. Gas Chromatogram coupled with Mass Spectrometer of Agilent Technologies-5977MSD equipped with an Agilent Technologies GCMS capillary column HP-5MS (30 m 0.25 mm ID 0.25m) composed of 5Percentage diphenyl 95Percentage Dimethyl polysiloxane. An electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.99Percentage) was used as the carrier gas at constant flow rate 1 mL/min and an injection volume of 1 ml was employed at split ratio of 50:1, injector temperature was at 60C and ion source temperature was at 250C. Mass spectra were recorded using voltage of 70 eV. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas, software of GC-MS Mass Hunter used for spectra and chromatograms analysis

Blood Plasma Preparation:

The whole blood sample of the chicken was collected and immediately transfer into two other blood tubes, each holding 10 mL of 3.2Percentage anticoagulant trisodium citrate at a 9:1 ratio (Adcock D. M., *et al.*,1997).). To avoid blood clotting, the blood and trisodium citrate were well mixed by constantly flipping the blood tubes. Using a refrigerated centrifuge, cells are extracted from plasma by centrifuging at 1,000–2,000 rpm for 10 minutes. The plasma sample's platelet content is reduced by centrifugation for 15 minutes at 3,000 rpm. The resulting supernatant is designated as Pure Platelet Plasma (PPP). After centrifugation, it is essential to quickly put the liquid component (plasma) into a sterile microcentrifuge tube using a pipette. The samples should be handled with a temperature between 2 and 8°C. If the plasma is not going to be investigated immediately, it must be transported and kept at –20°C or lower. It is important to avoid repeated freeze-thaw cycles. Samples that are lipemic, icteric, or hemolyzed can invalidate some assays. (Nurul Huda Mohd Nor *et al*., 2019)

Plate -3 Prepared the blood sample in five groups for Centrifugation

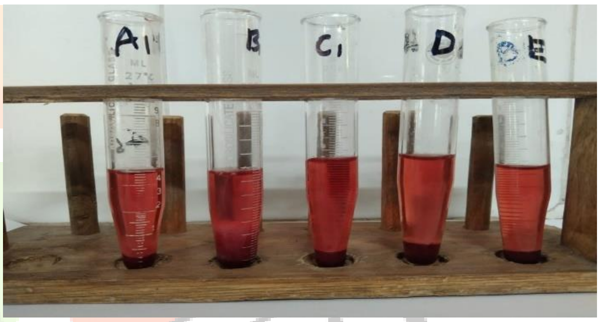


Plate- 4 Separation of Plasma and Serum Pure Platelet plasma(PPP)

Coagulation Test

Using the Prothrombin Time test, the length of the blood coagulation process was determined. Each subject's measurement was divided into five study groups for a total of two cycles. Five groups were created using 0.1 mL of PPP (Pure Platelet Plasma) in test tubes. After that, the tubes were incubated for 180 seconds at 37°C.

Group 1: 0.1 mL of PPP with addition of 0.3 mL CaCl2 (Positive control)

Group 2: 0.1 mL of PPP (Negative control)

Group 3: 0.1 mL of PPP with addition of 0.05 mL 25Percentage Concentrate of Moringa oleifera leaves extract + 0.3 mL CaC12

Group 4: 0.1 mL of PPP with addition of 0.05 mL 50Percentage Concentrate of Moringa oleifera leaves extract + 0,3 mL CaCl2

Group 5: 0.1 mL of PPP with addition of 0.05 mL 100Percentage Concentrate of Moringa oleifera leaves extract + 0.3 mL CaC12

Every ten seconds, each tube was gently shaking and assessed to see if a plasma clot had formed. Using a digital stopwatch, the length of the coagulation process was determined from the start of the shaking procedure to the development of a plasma clot.

RESULTS

Phytochemical Screening done in Aqueous Extract of *Moringa oleifera* Leaves:

The results obtained following the phytochemical screening of the leaves of *Moringa oleifera* are recorded in the above table. We thus note in the *Moringa oleifera* leaves has a very strong presence of flavonoids and tannin , a weak presence of reducing sugar, terpenoids carbohydrate and sterols , a strong presence of reducing compounds such as Amino acid, steroids, glycosides , saponins, alkaloids and phenol and no results found in anthraquinones compound in the aqueous extraction of *Moringa oleifera* leaves as shown in the Table 1

Table -1: Results of phytochemical screening of Moringa oleifera

S. No.	Phytochemical test	Aqueous
1	Alkaloids	++
2	Flavonoids	+++
3	Phenol	++
4	Tannins	+++
5	Saponins	++
6	Glycosides	++
7	Steroids	++
8	Sterols	+
9	Anthraquinones	-
10	Carbohydrate	+
11	Amino acid	++
12	terpenoids	+
13	Reducing sugar	+

Legend; *+: Low presence ,++:strong presence ,+++Very strong presence, -:Absence*

Quantitative Analysis: The total phenol content (µg/g GAE) in Moringa—expressed in GAE—was 52.08 ± 0.21 µg/g in aqueous extract (Fig 1). The total flavonoid content (µg/g QE) in Moringa—expressed in quercetin equivalents—was 116.93 ± 1.22 µg/g in aqueous extract (Fig 1). The Total Tannin content (µg/g) in Moringa was 86.91±0.42 µg/g in aqueous extract as shown in the Fig 1 respectively.

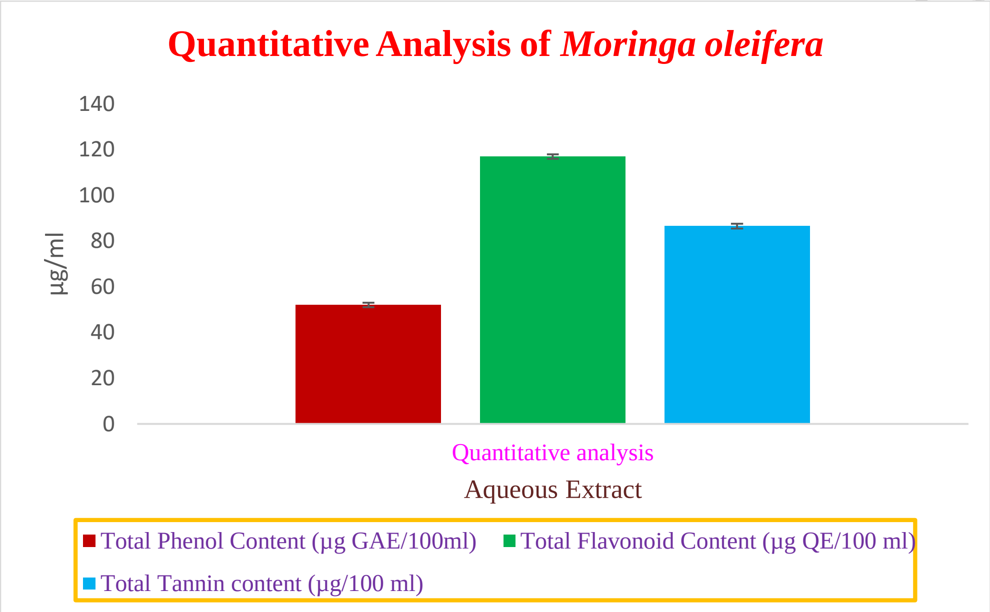


Figure 1: The total amount of Tannin, phenolic and flavonoid compounds of aqueous extracts of *Moringa oleifera* leaves..

Phytocomponent Identification by GC–MS analysis of Aqueous extract of *Moringa oleifera* Leaf

Analysis of the mass spectrum The National Institute of Standards and Technology (NIST) database, which contains more than 65,000 patterns, was used for GC-MS analysis. A comparison was made between the spectrum of the unknown component and the known components kept in the NIST collection. We determined the components of the test materials' names, molecular weights, and structures. GC-MS analysis was used to identify the twenty main chemicals found in the aqueous extract of *Moringa oleifera* leaves. The compounds applications, concentration (Percentage), retention period, molecular formula, and molecular weight of the active principles are displayed in (Table 2 and Fig 2). The extract of *Moringa oleifera* leaves contained 20 major compounds of which the maximum quantum was 2-(3,4 – dihydroxyphenyl)- trihydroxychromen-4-one, 3TMS derivative (38.9Percentage) has effective in anti-cancer, antioxidant and also gives anti-arthritis, antihistamine effects followed by 1H-Indene, 2-butyl-5-hexylocta hydro (15.29 Percentage) activate in antioxidant and anti-lung cancer properties, and may also protect skin cells from UV radiation next followed by 3-(2-aminoethyl)-1H-indol-5-ol (7.46Percentage). referred to as the "happy chemical" since it promotes happiness and well-being, its found in the brain, intestines, and blood platelet. It Assist with mood regulation and memory, sleep, sexual function, bone health and blood coagulation.

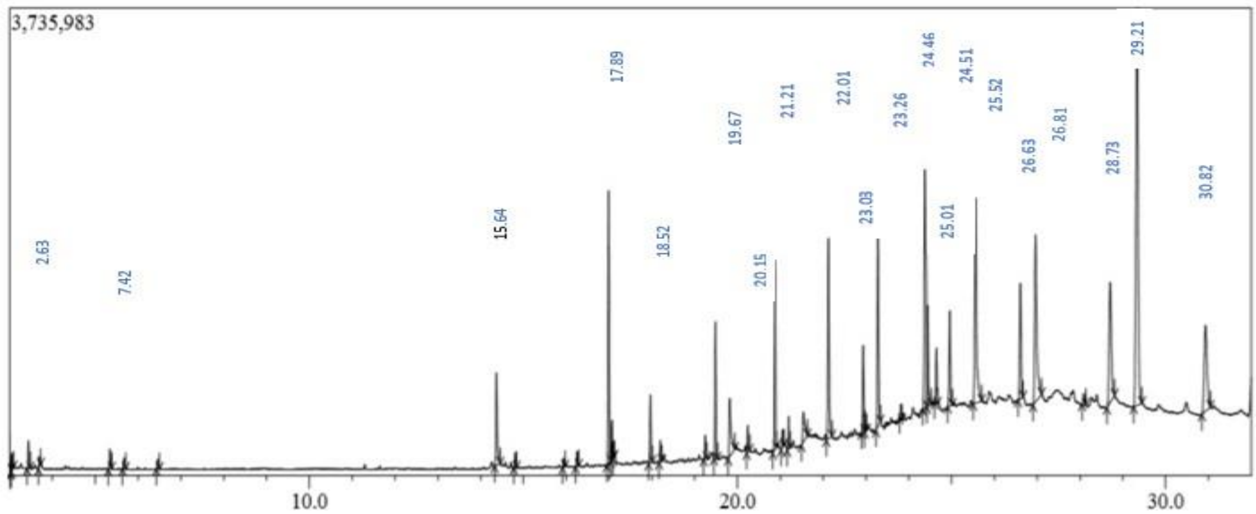


Figure 2: GCMS Analysis of *Moringa oleifera* Aqueous Extract

Table 2: Major Compounds found in the Aqueous Extract of Moringa oleifera Leaves

S. No.	Compound Name	R. Time	Peak Area %	Molecular Formula	Molecular Weight(g/mol)	Uses
1	5-Nonanol–dibutylcarbinol	2.63	0.65	$C9H20O$	144.25	It has a role as a pheromone and an animal metabolite. (PubChem)
2	Carbamic acid	7.42	0.36	$CH3NO2$	61.04	It is insecticides, herbicides, and fungicides are used to protect crops and gardens and to protect human and animal health from insect-borne diseases(Horacio Heinzen, María Verónica Cesio 2024)
3	n-Hexadecanoic acid	15.64	1.53	$C_{16}H_{32}O_2$	256.43	Anti- inflammatory, neurotrophic, Antibacterial, Antioxidant, Nematicides Hypocholesterolemia, and Pesticides (Vasudevan Aparna *et al.*, 2012)
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	17.89	2.97	$C6H8O4$	144.12	Strong Anti-oxidant (Yu *et al.*, 2013; Čechovská *et al.*, 2011
5	1, 2-epoxyhexadecane (oxirane)	18.52	1.55	$C16H32O$	240.42	It used as a diluent for UV-curable coatings. (Atul Kumar *et al.*, 2022)

6	Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-	19.67	1.89	C7H14N2O	142.20	Anti-inflammatory, analgesic and ulcerogenic activities (Siddiqui *et al.*, 2010)
7	1-Propanamine, 3-propoxy	20.15	0.51	C6H15NO	117.19	Textile resins, Drugs, Pesticides (Odochi O. Chukwu *et al.*, 2024)
8	3-(2-aminoethyl)-1H-indol-5-ol	21.21	7.46	C10H12N2O	176.22	Assist with mood regulation and memory, sleep, sexual function, bone health and blood coagulation (Shubhada Tayade *et al.*, 2022)
9	Dodecyl-	22.01	1.93	C12H26O	288.38	It is used in plant and marine-derived antimalarial agents (Marjan Talebi *et al* 2023)
10	Butanedioic acid, 2-hydroxy-2-methyl-, (S)-	23.07	2.01	C5H8O5	134.087	Mobilize the soil Phosphates for agricultural applications (Leila Rezakhani *et al.*, 2019)
11	L-Galactose, 6-deoxy-	23.26	3.24	C6H12O5.	164.16	It is a mediate cell-cell recognition and adhesion-signaling pathways. Blood transfusion reactions and also a tool to determine cancer diagnosis, prognosis. (Jiawei Meng *et al.*, 2023)
12	D-Mannoheptulose	24.46	4.71	C7H14O7	210.18	D-Mannoheptulose widely studied for its activity against breast cancer and to suppress the D-glucose induced insulin release. (Al-Ziaydi *et al.*, 2020)
13	Propenamide	24.51	0.84	C3H7NO	73.09	Propenamide derivatives studied for antimicrobial and antiviral efficacy (Nitesh Bhalla *et al.*, 2021)
14	Z-10-Tetradecen-1-ol acetate	25.01	1.56	C16H30O2	254.41	It is an insect pheromone used for therapeutics (PubChem)
15	2-Ethylacridine	25.52	2.14	C15H13N	207	Anti-inflammatory anticancer, antimicrobial, antiparasitic, antimalarial, antiviral and fungicidal activities (Upe Francisca *et al.*, 2017)
16	Pentadecanoic acid	26.73	2.44	C15H30O	242.3	Antimicrobial (Mujeeb *et al.* 2014)
17	Tetra acetyl-d-xylonic nitrile	26.85	3.93	CHNO	342.29	Anti-tumor and Anti-oxidant (Kanhar and Sahoo 2018)

18	Cis-Octadecenoic acid	28.73	5.99	$C_{18}H_{34}O_2$	282.46	Prevents the function of protein kinase C lymphocytes, releases myeloperoxidase and chemotaxis in human neutrophils(Igwe *et al*., 2015)
19	2-(3,4 – dihydroxyphenyl)-trihydroxychromen-4-one	29.21	38.9	$C_{15}H_{11}O$	302.238	It is an effective antioxidant and anti-arthritis effects. It has anticancer characteristics, which might help prevent malignant cells from tumor formation. It also helps in neurodegenerative illnesses and efficient antihistamine (Younis Khalaf *et al*. 2021)
20	1H-Indene, 2-butyl-5-hexyloctahydro	30.82	15.3	$C_{19}H_{36}$	264.48	Antioxidant and anti-lung cancer properties, and also protect skin cells from UV radiation (Pichnaree Kraokaew *et al*., 2022)

Blood Coagulation activity

Formation of blood clot was seen when the test tubes were inverted. In negative control, plasma dropped when the test tube was rearranged. In this gathering no plasma clump happened. The plasma in sure control, 25Percentage, half, and 100 percent concentrate bunch the plasma didn't drop at the point when the test tubes were upset which implies the plasma clumps were shaped. Each gathering had different length of plasma cluster arrangement the blood coagulation season of two subjects in two cycles.

Mean of blood coagulation time in short order and standard deviation (SD) in each gathering as follows: positive control was 215.66 plus/minus 4.19 seconds, negative control showed plasma coagulation in 373.33 plus/minus 2.41 seconds gathering of centralization of 25Percentage was 181.66 plus/minus 9.55 seconds, gathering of centralization of half was 125.33 plus/minus 7.23 seconds, and the gathering of centralization of 100 Percentage was 103.33 plus/minus 8.93 seconds. *Moringa oleifera* leaves showed the briefest length of plasma coagulation in 100 Percentage as shown in Table-3

Table 3: The mean difference of blood clotting time

Mean time of Blood Coagulation (seconds)					
	Positive Control	**Negative Control**	**25% Concentration**	**50% Concentration**	**100% Concentration**
Mean Time	215.66±4.19	373.33±2.41	181.66±9.55	125.33±7.23	103.33±8.93

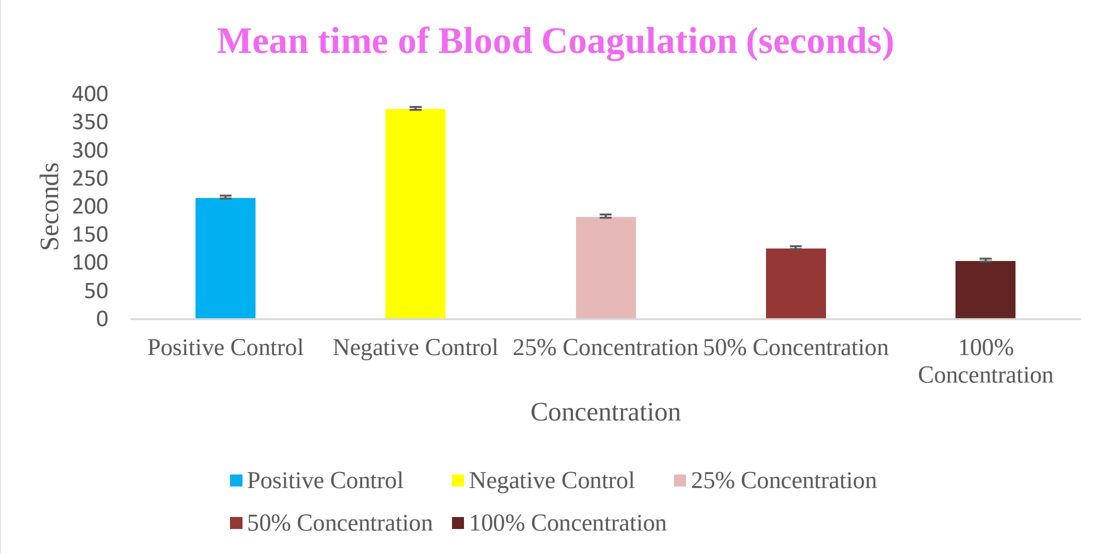


Figure 3: The mean difference of Blood Clotting Time

The average blood coagulation time of the positive control group was 155seconds longer compared to the group of 100Percentage concentrate; 1.96times increased the blood coagulation time, 140 seconds longer than the group of 50Percentage concentrate; 1.8 times increased the blood coagulation time. The positive control group and the group of 25Percentage concentrate showed no significant difference ($P>0.05$) with an average blood coagulation time 18 seconds longer than the positive control group; 1.06 times increased the blood coagulation time. (Plate -5 and 6)

Table 4: Mean Difference for Test Groups

Groups	Concentrations	Mean Difference
Positive Control	100%	112.33
	50%	90.33
	25%	34
100%	50%	-22
	25%	-78.33
50%	25%	-56.33

From the data analysis , the fastest blood coagulation time occurred in the group of concentration of 100Percentage *Moringa oleifera* leaves extract with significant differences compared to other treatment extract resulting shorter average blood coagulation time shown in Plate-7 and Table-4

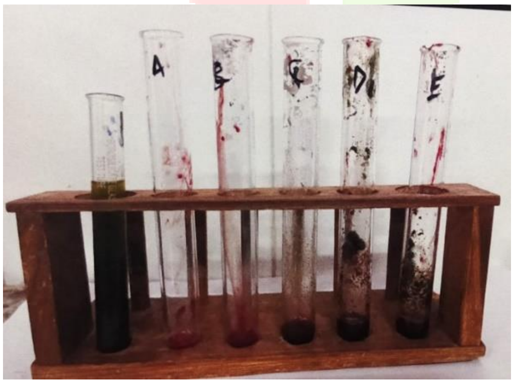


Plate -5 Coagulation Formation

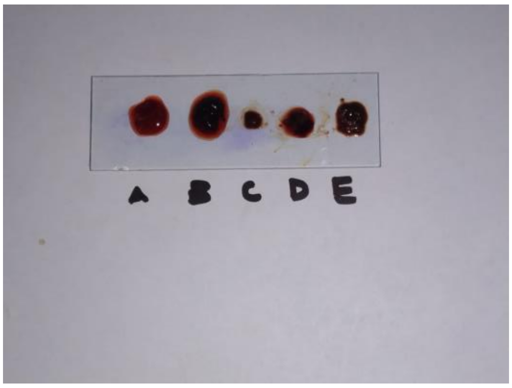


Plate -6 Blood clot formation Process

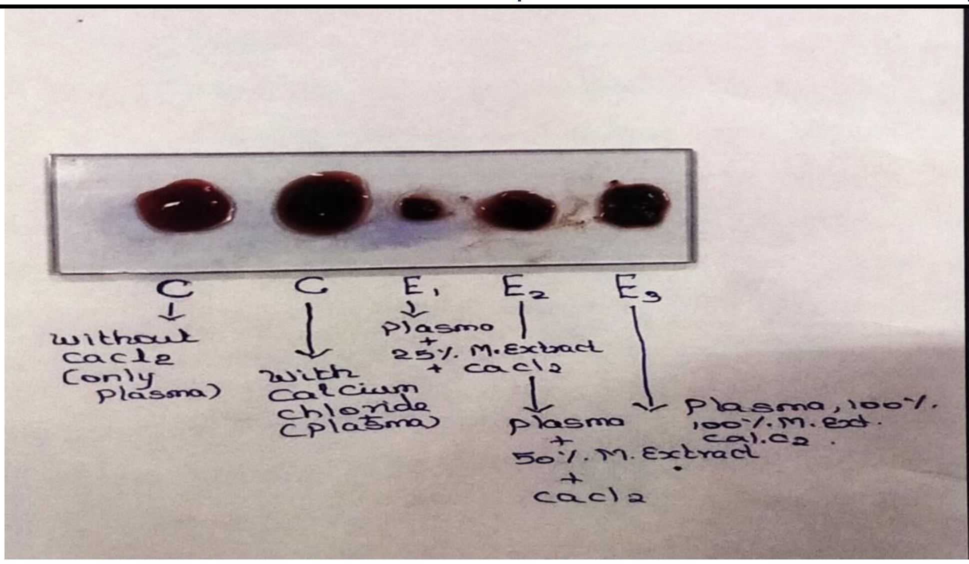


Plate -7 Blood clot after coagulation test with Aqueous *Morienga oleifera* Leaves Extract

DISCUSSION :

The majority of traditional healers' knowledge of medicinal plants comes from oral tradition, and many are unaware of the active elements in herbal treatments. Accurately identifying herbal material and active components is crucial for quality assurance, safety and effectiveness, public acceptance, and potential integration with the national healthcare system. (Abideen Adeyinka Adekanmi *et al*., 2020). The potential of plants to produce aromatic compounds is actually limitless. This includes phenolics and polyphenols such as quinine, phenolic acids, flavonoids, and flavanols, as well as tannins, coumarins, terpenoids, essential oils, alkaloids, lectins, and polypeptides, (Cowan MM. 1999). In regards, several experiments relying on the chemical analysis of secondary metabolite content of plants in general and the plant species being studied in particular were carried out.

Alkaloids are nitrogen containing organic compounds present in plants and derived from amino acids metabolism. (M. Cheraghi *et al*., 2017). Fresh Moringa leaves are also known as pro vitamin which are rich source of β-carotene. Moringa has been found to be a rich source of polyphenols (flavonoids, phenolic acids and tannins) that containing all phenolic molecules in a sample including those found in extractable proteins.(R.K. Saini *et al* ., 2016) . Glucosinolates are an extensive category of glycosidic chemicals prevalent in many plant families that include sulphur and nitrogen. The Moringa plant's roots, stem, leaves, and pods were all found to include a variety of glucosinolate chemicals. (Maldini *et al*., 2014). There are also plenty of tannins compounds in moringa leaves. Moringa leaves also contain an appreciable amount of tannins. These are complex polyphenol molecules that can bind to and precipitate protein, amino acids, alkaloids and other organic molecules in aqueous solutions. (E.M. Teixeira *et al*., 2014).

In recent years, gas chromatography and mass spectrography (GC–MS) has been applied unambiguously to identify the structures of different phytoconstituents from plant extracts and biological samples with great success (Pichnaree Kraokaew, *et al*., 2022). Gas chromatography and mass spectrum is a reliable technique to identify the phyto constituents of volatile matter, long chain branched hydrocarbons, alcohols, acids and esters (Shubhada Tayade , *et al*., 2022)

Still medicinal plants are used significantly in healthcare by different populations throughout the world. (Majeed R *et al*., 2012) The natural compounds extracted from medicinal plants have been used as conventional or complementary remedies for both treatable and untreatable diseases. (López D *et al*., 2009). Yet, these natural products are considered to possess a good source of several kinds of chemicals that exhibit various biological properties, that can help build complementary medicines. For instance, it is believed that herbal remedies made from the plant *Allium sativum L.* (garlic) are supposed to inhibit platelet activation (Zhang A, Sun H, and Wang X. 2013). Also, other herbs such as *Salix alba* L. are ethnomedicinally used as an anti-inflammatory agent. Later on, the extracted salicylic acid from the second plant species was transformed into a powerful anti-platelet drug acetylsalicylic acid that also called aspirin. (Rahman K and Billington D. 2000). Out of all the plants studied, *Strophanthus hispidus* proved to be the most effective. The dose-response profiles obtained showed that the increased clotting time was due to different processes for the different plant extracts. (Peter J. Houghton, Karl P. Skari 1994). *In vitro* activity of *Aizoon hispanicum* L. (Aizoaceae), *Centaurea hyalolepis* Boiss. (Asteraceae), *Heliotropium maris-mortui* Zohary. (Boraginaceae), *Parietaria judaica* L. (Urticaceae), *Polygonum arenarium* Waldst. & Kit. (Polygonaceae), and *Verbascum sinuatum* L. (Scrophulariaceae) on blood coagulation was estimated and it is apparent that these plants should be cautiously consumed with anticoagulant drugs (*e.g.* heparin) and stops their consumption before surgery. (Lubna Abdallah *et al*., 2022)

For that reason, the current research worked on *Moringa oleifera* plant species was selected for this research according to their medical histories in various fields. (Vane JR and Botting RM. 2003) The quality features, data, and therapeutic potential of these medicinal plants can be obtained from the phytochemical tests of *Moringa* leaves. However, there are no previous studies on the effect of these plant species on blood coagulation, which is closely related to thromboembolic diseases. This study is trial lab research utilizing *Moringa oleifera* leaves removed as a lab test. Estimation of Blood coagulation time relates to an extraneous fountain of coagulation to assess the coagulant impact estimated by Prothrombin Time (PT) testing technique. A positive benchmark group, 25Percentage, half, and 100 percent removed bunch, PPP, was added with $CaC1_2$ going about as a recalcification agent on Chicken Blood Plasma, hence, animating the typical development of a blood coagulation. The outcomes show that the treatment gathering of *Moringa oleifera* leaves concentrate of 25Percentage, half, and 100 percent has a more limited blood coagulation time than the positive benchmark group was 1.64 times, 1.27 times, and1.08 times separately. In this review, the higher the level of *Moringa oleifera* leaves separate, the more limited blood coagulation time was acquired than the positive control bunch. (Ahmed M Sabo *et al*., 2022)

The higher level of *Moringa oleifera* leaves separate had a more limited blood coagulation time than the lower level of *Moringa oleifera* leaves remove. It may very well be closed if an expansion in fixation leads to a Swiffer plasma cluster arrangement. *Moringa oleifera* leaves separate demonstrates that it assumes a part in speeding up human blood coagulation. There calcification process happens by changing coagulation time in human blood plasma tests, portrayed by the arrangement of blood clumps through platelet actuation and the coagulation overflow. The quickest blood coagulation time happened in the 100 percent concentrate of *Moringa oleifera* leaves remove contrasted with other treatment gatherings; 1.64 times increment the blood coagulation time contrasted with the positive control, going about as expected new human blood plasma *in vitro*. *Moringa oleifera* leaves extricate has a fantastic capacity to abbreviate the coagulation season of new human ordinary blood plasma. (Goldhaber SZ. 1992).

Coagulation factor testing, a kind of blood test, looks at one or more of your clotting factors to determine whether you: suffering clotting factor levels that are unusually high or low. Our blood contains chemicals released by the platelets that activate the clotting factors. A more robust blood clot that will adhere to its location is formed when the clotting elements combine in a cascade (Martin N. Raber. 1990). Plasma is produced when whole blood is collected in tubes that are treated with an anticoagulant. The blood does not clot in the plasma tube. The cells are removed by centrifugation. The supernatant, designated plasma is carefully removed from the cell pellet using a Pasteur pipette.(Thavasu, PW *et al*. (1992) .

Conclusion : *Moringa oleifera* has many properties such as Anti-Inflammatory Anticancer, Antimicrobial, Antiparasitic, Antimalarial, Antiviral, Fungicidal ,Neurotrophic, Antioxidant, Nematicides, Hypocholesterolemia, and Pesticides activities According to the present study results to a research reveals that *Moringa oleifera* leaves also contain anti-coagulating properties and more research is needed to ascertain its molecular characterization, substrate specificity, and activity. For brief experiment can be done in chronic study for future work.

ACKNOWLEDGEMENT: The authors thank to PG and Research Department of Zoology, Faculty of Science, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Teynampet, Chennai 600018, India for providing the facilities needed to perform the study.

References

1. Abideen Adeyinka Adekanmi , Sherifdeen Adeniyi Adekanmi , Opeyemi shafiu Adekanmi (2020). Qualitative and Quantitative Phytochemical Constituents of Moringa Leaf. International Journal of Engineering and Information Systems 4, (5,), Pages: 10-17
2. Adcock D. M., D.C. Kressin and R.A. Marlar (1997). Effect of 3.2Percentage vs 3.8Percentage sodium citrate concentration on routine coagulation testing. American Journal of Clinical Pathology.107(1):105–110.
3. Ahmed Ghdhban Al-Ziaydi , Ahmed Majeed Al-Shammari , Mohammed I. Hamzah , Haider Sabah kadhim and Majid Sakhi Jabir(2020) Hexokinase inhibition using D-Mannoheptulose enhances oncolytic newcastle disease virus-mediated killing of breast cancer cells . Cancer Cell International. 20(420): 1-10
4. Al-Saadi, N. (2011). In vitro study of the anticoagulant activity of some plant extracts. Indian Journal of Applied Research. 3(7): 120–122.

5. **Atchade S. Pascal, Hoteyi Ismael, Seibou Toleba Soumanou, Bello Kaokab, Sezan Alphonse*(2018). Effect of Tridaxprocumbens Extracts on Blood Clotting. International Journal of current Pharmaceutical Research, 11(1): 8-13.**
6. Atul Kumar, Javeed Rasool, Qazi Naveed Ahmed. (2022) Applications of 2-Oxoaldehydes Chemistry of 2-Oxoaldehydes and 2-Oxoacids Pages 63-171
7. Bohn, S.K., N.C. Ward, J.M. Hodgson and K.D. Croft (2012). Effects of tea and coffee on cardiovascular disease risk. Food and Function, 3 (1): 575-591
8. Chemical characteristics and fractionation of proteins from Moringa oleifera Lam leaves Food Chemistry, 147 , pp. 51-54.
9. Cowan MM (1999). Plant products as antimicrobial agents. Clinical Microbiology Review. 12:564–582.
10. E.M. Teixeira, M.R. Carvalho, V.A. Neves, M.A. Silva, L. Arantes-Pereira. (2014)
11. Goldhaber SZ (1992). Pulmonary embolism thrombolysis: a clarion call for international collaboration. Journal of the American College of Cardiology. 19:246–247.
12. Horacio Heinzen, María Verónica Cesio (2024). Carbamate pesticides Encyclopedia of Toxicology (Fourth Edition) Volume 2, Pages 485-491
13. Igwe KK, Nwankwo PO, Otuokere IE,Ijioma SN, Amaku FJ.(2015). GCMS analysis ofPhytocomponents in the methanolicExtract of Moringa oleifera Leave. Journal of Research in pharmaceutical Scieence. 2(11):1-6
14. Jiawei Meng , Yingying Zhu, Ningning Wang , Wenli Zhang , Wanmeng Mu.(2023). Recent advances in a functional deoxy hexose L-fucose: Occurrence, physiological effects, and preparation. Trends in Food Science & Technology . 138(1) 16-26
15. Kanhar S. and A.K. Sahoo (2018).Ameliorative effect of Homalium zeylanicum against carbon tetrachloride-induced oxidative stress and liver injury in rats. Journal of Biomedicine and Pharmacotherapy. 111(1):305–314.
16. Kumar AK, M. Chalamaiah , RR. Kumar and KN. Babu (2009). Preliminary studies on biotransformation of drumstick (Moringa oleifera) and watermelon (Citrullus lanatus) seed oils using Baker's Yeast. Asian Journal of Biological Science. 2(4) :118-123.
17. Kumar, S., L. Joseph, M. George and A. Sharma (2011). A review on anticoagulant or anti-thrombotic activity of natural plants used in traditional medicine . .International Journal of Pharmaceutical Sciences Review and Research 8(1) :70–74.
18. L. Čechovská, K. Cejpek, M. Konečný, J. Velíšek(2011). On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one in antioxidant capacity of prunes. European Food Research and Technology., 233 (3) :367-376
19. Leila Rezakhani , Babak Motesharezadeh , MohammadMehdi Tehrani , Hassan Etesami and Hossein Mirseyed Hosseini (2019). Phosphate–solubilizing bacteria and silicon synergistically augment phosphorus (P) uptake by wheat (Triticum aestivum L.) plant fertilized with soluble or insoluble P source. Ecotoxicology and Environmental Safety 173(2): 504-513
20. Longba B.D,O.A. Ushie, E. Ogah, A.C. Kendenson and J.T. Nyikyaa(2018). Phytochemical Screening and Quantitative Determination of Phytochemicals in Leaf Extracts of Hannoa undulata. International Journal of Medicinal Plants and Natural Products. 4(2) : 32-38
21. López D, Fischbach MA, Chu F, Losick R, Kolter R (2009). Structurally diverse natural products that cause potassium leakage trigger multicellularity in Bacillus subtilis. Proceedings of the National Academy of Sciences U S A. 106:280–285.
22. Lubna Abdallah,* Ibtihaj Surakji, Tarteel Qawasme, Dania Ayyash, Ruba Shhadeh, Ghadeer Omar and Ali Barakat.(2022). In Vitro Activity of Some Medicinal Plants on Blood Coagulation. Turk J Pharm Sci. 2022 Jun; 19(3): 330–335.
23. M. Cheraghi, M. Namdari, H. Daraee, B. Negahdari (2017). Cardioprotective effect of magnetic hydrogel nanocomposite loaded N,alpha-L-rhamnopyranosyl vincosamide isolated from Moringa oleifera leaves against doxorubicin-induced cardiac toxicity in rats: in vitro and in vivo studies. Journal of Microencapsulation, 34 (2017), pp. 335-341.
24. M. Maldini, S.A. Maksoud, F. Natella, P. Montoro, G.L. Petretto, M. Foddai, G.R. De Nicola, M. Chessa, G. Pintore (2014). Moringa oleifera: study of phenolics and glucosinolates by mass spectrometry. Journal of Mass Spectrometry, 49 (2014), pp. 900-910.
25. Majeed R, Reddy MV, Chinthakindi PK, Sangwan PL, Hamid A, Chashoo G, Saxena AK, Koul S. (2012). Bakuchiol derivatives as novel and potent cytotoxic agents: a report. European Journal of Medical Chemistry. 49(1):55–67

26. Mallick EP and MB Singh (1980). Plant Enzymology and Histoenzymology; 1st ed: pp. 280. Kalyan Publisher. . New Delhi, India.
27. Manicam C, JO. Abdullah, ER .Tohit, Z. Seman , SC. Chin and M. Hamid (2010). In vitro anticoagulant activities of Melastoma malabathricum Linn. aqueous leaf extract: a preliminary novel finding. Jounal of Medicinal Plants Research. 4(14):1464–1472.
28. Marjan Talebi, Saeed Samarghandian, Tahereh Farkhondeh and Mohsen Talebi.(2023). Plant and marine-derived antimalarial agents. Natural Products in Vector-Borne Disease Management Pages 201-221
29. Martin N. Raber.(1990) Chapter 157Coagulation Tests Walker HK, Hall WD, Hurst JW, editors. Boston: Butterworths
30. Mass Spectrometry (GC-MS) Biological Forum – An International Journal 14(4): 1209-1220
31. N. Siddiqui, B. Azad, M.S. Alam, R. Ali (2010).Indoles: role in diverse biological activities. International Journal of Pharmaceutical and Clinical Research.2 (4) : 121-127
32. Nitesh Bhalla , Nitin Ingle, Srilakshmi V. Patri and D. Haranath(2021). Phytochemical analysis of Moringa oleifera leaves extracts by GC-MS and free radical scavenging potency for industrial applications . Saudi Journal of Biological Sciences, 28 (12) 6915–6928
33. Novais J. (2004). Studies on pharmaceutical ethnobotany in Arrabida Natural Park (Portugal) . Journal of Ethnopharmacol, 93(2-3):183-95.
34. Nurul Huda Mohd Nor, Fauziah Othman, Eusni Rahayu Mohd Tohit, Sabariah Md Noor, Rosniza Razali, Hazlina Ahmad Hassali, and Hadijah Hassan (2019). In Vitro Antiatherothrombotic Effects of Extracts from Berberis Vulgaris L., Teucrium Polium L., and Orthosiphon Stamineus Benth Hindawi Evidence-Based Complementary and Alternative Medicine Volume 2019(1): 1-10
35. Odochi O. Chukwu , Cordilia O. Iyare , Chinedum U. Emelike , Anthony C.U. Ezimah , Nnaemeka T Asogwa and Nwaeze G. Konyefom(2024). GC–MS analysis of Moringa oleifera leaf extract and effects of administration on histology of reproductive organs and liver of female rats exposed to chronic unpredictable stress Food Chemistry Advances. 4(1): 1-12
36. Peter J. Houghton, Karl P. Skari (1994). The effect on blood clotting of some west African plants used against snakebite. Journal of Ethnopharmacology 44(2): 99-108
37. Pichnaree Kraokaew , Preeyanuch Manohong , Prachayaporn Prasertsuksri , Prapaporn Jattujan , Nakhon Niamnont , Montakan Tamtin , Prasert Sobhon and Krai Meemon .(2022). Ethyl Acetate Extract of Marine Algae, Halymenia durvillei, Provides Photoprotection against UV-Exposure in L929 and HaCaT Cells. Marine Drug.20(11): 707.-723
38. Pichnaree Kraokaew , Preeyanuch Manohong , Prachayaporn Prasertsuksri , Prapaporn Jattujan , Nakhon Niamnont , Montakan Tamtin , Prasert Sobhon and Krai Meemon (2022). Ethyl Acetate Extract of Marine Algae, Halymenia durvillei, Provides Photoprotection against UV-Exposure in L929 and HaCaT Cells. Drugs , 20, 7071-16
39. R. Ullah, S.A. Alqahtani, M.A.O. Noman, M.A. Alqahtani, S. Ibenmoussa, M. Bourhia. (2020). A review on ethno-medicinal plants used in traditional medicine in the Kindgdom of Saudi Arabia Saudi Journal of Biological Sciences , 27(10): 2706-2718
40. R.K. Saini, I. Sivanesan, Y.-S. Keum(2016). Phytochemicals of Moringa oleifera: a review of their nutritional, therapeutic and industrial significance. Biotechnology, 6(1):. 203-209.
41. Rahman Gul, Syed Umer Jan, Syed Faridullah, Samiullah Sherani, and Nusrat Jahan (2017). Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. Scientific World Journal. 5873648.pp 1-7.
42. Rahman K, Billington D (2000). Dietary supplementation with aged garlic extract inhibits ADP-induced platelet aggregation in humans. Journal of Nutrition. 130:2662–2665.
43. Riya Kadia, Smit Bhavsar, Palak Sapra, Himanshu Pandya, Archana Mankad and Nainesh Modi (2022). Phytochemical Investigation and Antioxidant Activity of Ethnomedicinal Plants from Ratanmahal and Udalmahuda Forest, Dahod. International Journal of Pharmacognosy and Phytochemical Research 14(1);24-31
44. Shubhada Tayade, G.J. Janavi , T. Arumugam , B. Rajagopal and P. Geetharani (2022). Phytochemical characterization of moringa (Moringa oleifera Lam.) using Gas Chromatography
45. Shubhada Tayade, G.J. Janavi , T. Arumugam, B. Rajagopal and P. Geetharani (2022). Phytochemical characterization of moringa (Moringa oleifera Lam.) using Gas Chromatography Mass Spectrometry (GC-MS) Biological Forum – An International Journal 14(4): 1209-1220

46. Shunmugapriya K, P. Vennila , S.Thirukkumar and M. Ilamaran (2017). Identification of bioactive components in Moringa oleifera fruit by GC-MS. Journal of Pharmacognosy and Phytochemistry 6(3): 748-751
47. Upe Francisca Babaiwa , Osayemwenre Erharuyi , Abiodun Falodun and John O Akerele.(2017). Antimicrobial activity of ethyl acetate extract of Citrullus lanatus seeds. Tropical Journal of Pharmaceutical Research 16 (7): 1631-1636
48. Vane JR, Botting RM (2003). The mechanism of action of aspirin. Thrombosis Research. 110:255–258.
49. Vasudevan Aparna [1], Kalarickal V Dileep, Pradeep K Mandal, Ponnuraj Karthe, Chittalakkottu Sadasivan, Madathilkovilakathu Haridas (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical biology drug design .80(3):434-9.
50. Younis Khalaf, H., A.A. Thaker, and M. Salih Shalal (2021). Study of the chemical composition, amino acid, Flavonoids and Vitamin C of Moringa oleifera Leaves Extract Grown in AL-Ramadi-Iraq. Indian Journal of Forensic Medicine & Toxicology, 15(3) : 1-6.
51. Yu, X., M. Zhao, F. Liu, S. Zeng, J. Hu(2013). Identification of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one as a strong antioxidant in glucose–histidine Maillard reaction products. Food Research International Journal., 51 (1) : 397-403
52. Zhang A, Sun H, Wang X (2013). Recent advances in natural products from plants for treatment of liver diseases. European Journal of Medical Chemistry. 63:570–577.