PHYTOCHEMICAL ANALYSIS OF *MORINGA OLEIFERA, PETROSELINUM CRISPUM, ACACIA NILOTICA*

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Roll No. 051631001

Session 2016-2020

DEPARTMENT OF BOTANY

GOVT. POST GRADUATE COLLEGE FOR WOMEN, SAMNABAD LAHORE

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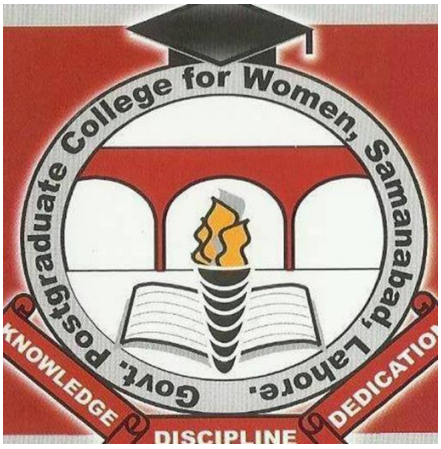

A THESIS SUBMITTED TO LAHORE COLLEGE FOR WOMEN UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELORS OF SCIENCE (HONS)

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BY

AIZA AMIR

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DEPARTMENT OF BOTANY

GOVT. POSTGRADUATE COLLEGE FOR WOMEN

SAMNABAD, LAHORE

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CERTIFICATE

This is to certify that the research work described in this thesis submitted by **Ms.** Aiza Amir to Department of Botany, post graduate college for women has been carried out under my/our direct supervision. I have personally gone through the raw data and certify the correctness and authenticity of all results reported herein. I further certify that thesis data have not been used in part or full, in a manuscript already submitted or in the process of submission in Partial/complete fulfillment of the award of any other degree from any other institution or home or abroad. I/We also certify that the enclosed manuscript has been prepared under my/our supervision and I/We endorse its evaluation for the award of BS/MS /Ph. D degree through the official procedure of University.

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DEDICATION

I DEDICATE THIS EFFORT TO MY BELOVED PARENTS AND TEACHER

ACKNOWLEDGMENTS

First and foremost I would like to express my gratitude to Allah SWT, who is the most beneficent and the most merciful, on whom ultimately we depend for sustenance and guidance, for his blessing and mercy and for granting me with the patience and perseverance required for the completion of my thesis. He has been bestowed upon me during this research project and indeed throughout my life. Salawat and Salam are always delivered to Muhammad (S.A.W) and may peace and salutation be given to him (S.A.W) who has taken all human being from the darkness to the lightness being blessed by the allah almighty.

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AIZA AMIR

CONTENTS

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List of abbreviations

AAS	Atomic Absorption Spectrometry
Biotech	Biotechnology
DPPH	1- diphenyl-2- picryl hydrazyl radical
DPPH	Diphenyl-2-picrylhydrazyl
EQ	Ethoxyquin
H_2SO_4	Sulfuric Acid
HCL	Hydrochloric Acid
HIV	Human Immune Deficiency
HPLC	High Potential Liquid Chromatography
IRS	Infra Rays Spectrum
ITK	Internal Transcribed Spacer
Mat	Maturase K
MIC	Minimum Inhibitory Concentration
MTCC	Mutual Type Culture Collection
NMR	Nuclear Magnetic Resonance
NPAA	Non-Protein Amino Acid
Phyto	Phytochemical
PKM1	Pyruvate Kinase Muscle Isozyme
RF	Retardation Factor
S.D	Standard deviation
Spp	Specie
TLC	Thin Layer Chromatography

UV Ultra violet

WHO World Health Organization

WP Western parsley

Abstract

Three plants (*Moringa oleifera*, *Petroselinum crispum* and *Acacia nilotica*) were tested for the qualitative phytochemical analysis. Extracts were prepared in two different solvents (acetone and distilled water). Extracts were tested for the presence of proteins, carbohydrates, phlobatannins, steroids, alkaloid, flavonoids, saponins and tannin. Aqueous and acetone extract of *M. oleifera* gave positive result for protein, carbohydrates and tannin. Aqueous extract of *M. oleifera* extract also tested positive for steroid, and saponins while acetone extract showed the presence of terpenoids, and alkaloid. The phytochemical analysis of *Petroselinum crispum* aqueous extract gave the positive result for carbohydrates, flavonoids, steroid, terpenoids and alkaloids while gave negative results for proteins, tannins, phlobatannins test and saponins. The acetone extract of *P. crispum* showed positive result for proteins, carbohydrates, tannins, terpenoids and alkaloids while phlobatannins, steroids and saponins were negative. *Acacia nilotica* resulted positive for protein, carbohydrates, tannins, steroid and saponins with aqueous extract while aqueous extract tested negative for terpenoids and alkaloids. Flavonoids, steroids and saponins were tested negative in acetone extract.

Chapter 1

Introduction

Plants are one of six big groups (kingdoms) of living things. They are autotrophic eukaryotes, which means they have complex cells, and make their own food. Usually they cannot move (not counting growth). Plants include familiar types such as trees, herbs, bushes, grasses, vines, ferns, mosses, and green algae (Asimov and Isaac, 1968). The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. Important bioactive constituents of plants include steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facet of life have served a valuable starting material for drug development (Ajayi *et al*., 2011).

Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Davidson *et al*., 2000). Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals (Hayat and Ahmad, 2007). Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. These phytochemicals have potential for use as drugs, and the content and known pharmacological activity of these substances in medicinal plants is the scientific basis for their use in modern medicine, if scientifically confirmed. (Ahn *et al*., 2017). Alkaloids are bitter-tasting chemicals, very widespread in nature, and

often toxic, found in many medicinal plants (Aniszewski, 2007). Glycosides are found in medicinal plants such as rhubarb, cascara, and Alexandrian senna (Wang *et al*., 2013). The cardiac glycosides are powerful drugs from medicinal plants including foxglove and lily of the valley. They include digoxin and digitoxin which support the beating of the heart, and act as diuretics. Terpenes and terpenoids of many kinds are found in a variety of medicinal plants (Wiart and Christopher, 2014). They are strongly aromatic and serve to repel herbivores. Their scent makes them useful in essential oils, whether for perfumes such as rose and lavender, or for aromatherapy (Singsaas and Eric , 2000).

The bark of willow trees contains salicylic acid, the active metabolite of aspirin, and has been used for millennia to relieve pain and reduce fever (Lichterman, 2004). The Thorn apple *Datura stramonium* has been used for asthma, because it contains the alkaloid atropine, but it is also a powerful and potentially fatal hallucinogen (Freyo and Eno, 2010). *Achillea millefolium* had antibacterial effects; *Capparis spinosa* is used for headache, renal complaints and stimulating tonic; *Echium amoenum* is used for common cold and had sedative effects; *Smyrnium cordifolium* is edible and used as tonic; Viola odorata is used for fever and migraine; *Ziziphora clinopodioides* is used for cold, infections and stomachache (Khaleghi-sigaroodi *et al*., 2010).

Moringa oleifera belongs to the Moringaceae family and is considered to have its origin in the north-west region of India, south of the Himalayan Mountains. It is now widely cultivated and has become naturalized in many locations in the tropics (Fahey *et al*., 2001). Different parts of the *MO* tree have been established as being good sources of unique glycosylates, flavonoids and phenolic acids (Amaglo *et al*., 2010; Coppin *et al*., 2013), carotenoids, tocopherols (Saini e*t* al. 2014e), polyunsaturated fatty acids (PUFAs) (Saini *et al.*, 2014d), highly bioavailable

minerals (Saini *et al*., 2014a), and folate (Saini *et al*., 2016) The varying concentrations of phenolics with the antioxidant capacity of the tested foliage established 'Pakistan Black' and 'Techiman' as the most nutritive cultivars, compared to the other major cultivars of *MO* from Pakistan (Nouman *et al*., 2016) *M. oleifera* is a fast-growing, deciduous tree that can reach a height of 10–12 m (32–40 ft) and trunk diameter of 45 cm (1.5 ft) (Parotta and John, 1993) The bark has a whitish-grey color and is surrounded by thick cork. Young shoots have purplish or greenish-white, hairy bark. The tree has an open crown reported that *Moringa* foliage's are a potential inexpensive protein source for livestock feeding. The flowers are fragrant and hermaphroditic, surrounded by five unequal, thinly veined, yellowish-white petals. The flowers are about 1.0–1.5 cm (1/2") long and 2.0 cm (3/4") broad. They grow on slender, hairy stalks in spreading or drooping flower clusters which have a length of 10–25 cm (Parotta and John, 1993).

Acacia nilotica (commonly known as gum Arabic tree, babul, thorn mimosa, Egyptian acacia or thorny acacia is a tree in the family Fabaceae. It is native to Africa, the Middle East and the Indian subcontinent. This tree was originally the type species of the genus Acacia. Small tree, 2.5–14 m tall, quite variable in many aspects; bark of twigs not flaking off, gray to brown; branches spreading, with flat or rounded crown; bark thin, rough, fissured, deep red-brown; branchlets purple-brown, shortly or densely gray-pubescent, with lenticels; spines gray-pubescent, slightly recurved, up to 3 cm long; leaves often with 1–2 petiolar glands and other glands between all or only the uppermost pinnae; pinnae 2–11 (-17) pairs; leaflets 7–25 (-30) pairs, 1.5–7 mm long, 0.5–1.5 mm wide, glabrous or pubescent, apex obtuse; peduncles clustered at nodes of leafy and leafless branchlets; flowers bright yellow, in axillary heads 6–15 mm in diam.; involucel from near the base to about half-way up the peduncle, rarely somewhat higher; calyx 1–2 mm long,

subglabrous to pubescent; corolla 2.5–3.5 mm long, glabrous or pubescent outside; pods especially variable, linear, indehiscent, 8–17 (-24) cm long, 1.3–2.2 cm broad, straight or curved, glabrous or gray-velvety, turgid, blackish, about 12-seeded; seeds deep blackish-brown, smooth, subcircular, compressed, areole 6–7 mm long, 4.5–5 mm wide (Duke, 1981a).

Parsley or garden parsley (*Petroselinum crispum*) is a species of flowering plant in the family Apiaceae that is native to the central Mediterranean region (Cyprus, southern Italy, Greece, Portugal, Spain, Malta, Morocco, Algeria, and Tunisia), but has naturalized elsewhere in Europe, and is widely cultivated as a herb, and a vegetable). Parsley (*Petroselinum crispum*) originates from the Mediterranean region; however, today it is grown throughout the world. It is a biennial plant growing up to 60–100 cm; the roots are thin, thick, and vertical. The fruit is rounded with a greenish gray color, and a length of 2.5 mm. Root and leaves are used, and sometimes even seeds. It is used for purposes of traditional medicine, contains essential oils, flavonoids, sugar, and starch. Leaves are rich in vitamin C, iron, iodine, and magnesium (Farzaei *et al*., 2013). Parsley is therefore, a medicinal herb with proven pharmacological properties, as well as antioxidant, analgesic, and antibacterial qualities (Farzaei *et al*., 2013).

Chapter 2

Review of literature

Ali *et al*. (2019) discussed plants generally contain chemical compounds both nutritional and non-nutritional which may be used and applied in nutritional and pharmaceutical industries. The study was aimed to evaluate phytochemical constituents and to determine the nutritional components of *Moringa oleifera* in Kano, Northern Nigeria. The leaves were air dried for two weeks, then grounded into a fine powder and extracted using water and acetone by maceration. The extract was subjected to phytochemical screening, proximate and mineral analysis using conventional laboratory methods. The result of phytochemical screening showed that the Moringa leaf extract contained alkaloid, saponins and flavonoids, reducing sugar, glycoside, anthraquinones, tannin and terpenoids. The proximate composition of the leaf presented as carbohydrate with 59.86%, protein 15.23%, fats 2.38%, crude fiber 9.26%, moisture content 4.42% and ash content 8.85%. The result of mineral analysis showed that the leaf of Moringa oleifera contain calcium (1.93%), potassium (0.95%), magnesium (0.39%), zinc (59.37ppm), phosphorous (28.85ppm), iron (105.20ppm) and copper (7.05ppm). The presence of the phytochemicals and nutrients in Moringa leaf has authenticated its usefulness by traditional herbalists in ethno medicine and potentials in drug formulation and as well used as food supplement.

Zengin *et al*. (2019) studied that Legumes produce a high diversity of secondary metabolites which serve as defense compounds against herbivores and microbes, but also as signal compounds to attract pollinating and fruit-dispersing animals. As nitrogen-fixing organisms, legumes produce more nitrogen containing secondary metabolites than other plant families. Compounds with nitrogen include alkaloids and amines (quinolizidine, pyrrolizidine, indolizidine, piperidine,

pyridine, pyrrolidine, simple indole, *Erythrina*, simple isoquinoline, and imidazole alkaloids; polyamines, phenylethylamine, tyramine, and tryptamine derivatives), non-protein amino acids (NPAA), cyanogenic glucosides, and peptides (lectins, trypsin inhibitors, antimicrobial peptides, cyclotides). Secondary metabolites without nitrogen are phenolics (phenylpropanoids, flavonoids, isoflavones, catechins, anthocyanins, tannins, lignans, coumarins and furanocoumarins), polyketides (anthraquinones), and terpenoids (especially triterpenoid, steroidal saponins, tetraterpenes). While some secondary metabolites have a wide distribution (flavonoids, triterpenes, pinitol), however, others occur in a limited number of taxa. The distributions of secondary metabolites with an irregular occurrence are mapped on a molecular phylogeny of the Fabaceae, reconstructed from a combined data set of nucleotide sequences from *rbc*L, matK and ITS genes. In most cases, the distribution patterns of secondary metabolites do not agree with the phylogeny of the plants producing them. In contrary, the distribution of many secondary metabolites is patchy and irregular. Thus, the use of phytochemical data to reconstruct a phylogeny of plants is often not informative and can be misleading. The patchy distribution may be due to convergent evolution, a contribution of endophytic fungi or more likely, to an early acquisition of the key genes of secondary metabolism in the evolution of land plants among others by horizontal gene transfer from bacteria. Thus, it would be a matter of gene regulation whether these genes are active in some but not all taxa.

El Sayed *et al*. (2018) demonstrated that background: *Petroselinum crispum* is a common vegetable or spice in Egypt and worldwide. It possesses many pharmacological and medicinal properties. Aims: In the current research, the total phenolic and flavonoid contents as well as the antioxidant activities of *P. crispum* macetoneic extract and its fractions were evaluated. Methodology: The total

phenolic content was estimated by Folin-Ciocalteus method and total flavonoid content was tested by aluminum chloride assay. The antioxidant activity was evaluated by 1, 1- diphenyl-2- picryl hydrazyl radical (DPPH) assay, 2, 2′-azino-bis (3-ethylbenzthiazoline-6- sulphonyl acid) assay (ABTS), and total antioxidant capacity assay. Results: The ethyl acetate fraction derived from the macetoneic extract exhibited the highest total phenolic content (121.95±2.15, mg GAE/ g extract) and total flavonoids content (106.45±2.18 mg rutin equivalent / g extract). Furthermore, the ethyl acetate fraction demonstrated the more potent.

Ancuceanu *et al*. (2018) studied that Petroselinum crispum (Mill.) Fuss., parsley, Apiaceae, is often used in the folk therapy as an anti-anemic product and is claimed to be rich in iron. Previous reports have stated iron contents in leaves varying between 126.2 and 1100 mg/kg, but no report on plants cultivated in Romania are available. We have measured iron contents in the vegetative organs (roots, stems and leaves) of plants cultivated in Romania on three different soils, at three development stages, using AAS. Since iron absorption may be influenced by the polyphenols and flavones, we have also measured these phytochemicals spectrophotometrically, using the Folin-Ciocalteau and aluminum chloride chelation methods, respectively. Correlations with the soil chemical composition were also explored. The iron contents in all vegetative organs of the plant varied between 21.6 (s.d. 10.8) in stems and 645.2 (21.9) mg/kg in leaves. The iron contents tended to increase with the development stage and the soil type had very limited influence on the iron contents. Polyphenol and flavone contents varied by soil type, vegetative organ and development stage, with interactions among these variables.

Reddy *et al*. (2018) demonstrated that Babbula is an important traditional medicinal plant in Ayurveda and Unani systems of medicine. All parts of the plant are useful as medicine/therapeutic agents to cure various ailments. Its bark is useful in cough, bronchitis, diarrhea, dysentery, biliousness, burning sensation, piles, leucoderma, urinary discharges, ascites etc. Leaves are useful in bronchitis, piles, liver tonic, healing of fractures, eye diseases and the fruit is astringent to the bowls, cures biliousness. The gum cures biliousness, leprosy, liver tonic, urinary discharges, vaginal and uterine discharges, healing of fractures, sour throat, lung troubles etc. The flowers are a powerful tonic and good for insanity. Fruits are useful dysentery and in ophthalmia. Though all parts of the plant are used for curing in different ailments many works have carried out on gum, bark, seeds and leaves. But Pharmacogenetic work has not been carried out on pods/fruits. Hence, we have carried out microscopical, powder studies, physio-chemical and preliminary phytochemical studies on the fruits of *Acacia nilotica*. The microscopic studies revealed the presence of elongated microsclerotia, abundant small starch grains and oil globules, uni seriate short tuffted trichomes, small rounded stone cells, lumen filled with brown content of tannin and thick walled parenchymatous cells of mesocarp region. The preliminary phytochemical analysis showed the presence of carbohydrates, proteins, saponins, phenols, steroids and tannins.

Ahmed *et al*. (2018) reported that the phenolic and antioxidant constituents in *Acacia nilotica* fruits have become an important source of medicinal and therapeutic benefit with powerful biological properties. This study investigated the phenolic content and antioxidant capacity of powdered Acacia fruits with seeds and without seeds. The phenolic content and antioxidant capacities in them were determined using Folin–Ciocalteus and DPPH free radical-scavenging assays. The

total phenolic and antioxidants of *A. nilotica* with seeds were spectrophotometrically determined to be 47.61 and 6.18% greater than when the seeds were removed from the dried fruits, respectively. The LC–MS/QTOF analysis shows the presence of 282 and 214 phenolic compounds in the macetone extracts of *A. nilotica* with seeds and without seeds, respectively. The present study, therefore, revealed that dried *A. nilotica* fruits with seeds have higher total phenolic content, antioxidant capacity, and bioactive constituents, which indicated that they have more medicinal value than fruits without seeds.

Muhammad *et al*. (2018) studied that Background: Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer. Studies have shown that extracts from natural products, such as fruits, have positive effects against cancer, compared with chemotherapy or recent hormonal therapy. The *A. nilotica* can provide nutrients and therapeutic ingredients to preclude, mitigate or treat many diseases or conditions. The Aim: This study aimed to analyze phytochemicals of the extracts of fruits of *A. nilotica* collected from majdool town. Material and Methods: These fruits were powdered and extracted with different solvents as macetone, chloroform, petroleum ether and water. Then, all the fruits extracts were analyzed quantitively looking for their phytochemical's constituents whether primary or secondary metabolites. Results: These experiment's results showed that the *A. Nilotica* fruits extracts have several different phytochemicals among which phenols, Alkaloids, Flavonoids, Tannins, Terpenoids', Cardiac glycosides, saponins and resins, and secondary metabolites and other primary phytochemicals that may have bioactivities. The macetone and the aqueous medium are more efficient in extracting secondary phytochemicals than the other solvents.

Conclusion: Overall, these results reveal that the *A. Nilotica* fruits may have medical therapeutic values due to the types of phytochemicals detected.

The antimicrobial activity of extracts was evaluated against four standard bacteria species (gram positive bacteria; Staphylococcus aureus, Bacillus subtilis) and (gram negative bacteria; *Pseudomonas aeruginosa*, *Escherichia coli*). The plates were inoculated for sensitivity testing, minimum inhibitory concentration (MIC) was measured.

The results of antimicrobial investigation show that the distilled water and macetoneic extracts inhibited the growth of all microorganisms (Specified by the zone of inhibition).

The results provide promising baseline information for potential use of these crude extracts in drug development programs in the pharmaceutical industries.

Sahay *et al*. (2017) demonstrated that Nowadays, with continuously changing socio-economic status, people have become more concerned about their health. Utilization of natural products of plant origin having lesser side effects has gained popularity over the years. There is immense scope for foods that can impart health benefits beyond traditional nutrients. *Moringa oleifera* (Drumstick tree) is one such tree having enormous nutritional and medicinal benefits. It is rich in macro and micro nutrients like protein, carbohydrate, calcium, phosphorus, potassium, iron, vitamins, beta carotene and other bioactive compounds which are important for normal functioning of the body and prevention of certain diseases. Most of the parts of *Moringa oleifera* including leaves, flowers and seeds are edible and other parts like bark, pods have use in biodiesel production and water purification. *Moringa oleifera* has tremendous therapeutic properties including anticancer, antiulcer, antimicrobial, antioxidant. Various researches have concluded that

Moringa should be used as functional ingredient in food products. This review aims to highlight the use of *Moringa oleifera* as a potential ingredient in food products as well as in other industries to serve as a background for future research works.

Agyare *et al*. (2017) reported that *Petroselinum crispum* belongs to the family Apiaceae is a bright green plant, which is cultivated widely in the tropic, subtropics, and temperate regions. It is a biennial plant which is widely cultivated as an annual plant. Traditionally, roots of *P. crispum* has been used as a powerful diuretic, seeds have been used as antimicrobial, antiseptic, antispasmodic, and in the treatment of gastrointestinal disorders, inflammation, halitosis, kidney stones, and amenorrhea. Leaves of *P. crispum* have been employed in the treatment of hemorrhoids, gastrointestinal disorders, diuretic, and as a food-flavoring agent in addition to its common usage as vegetable. *P. crispum* has been found to possess many pharmacological effects including, antioxidant, antibacterial, antifungal, hepatoprotective, antidiabetic, analgesic, spasmolytic, immunosuppressant, and gastroprotective properties. Hence this section reviews the phytochemical constituents and pharmacological activities of *P. crispum*.

Yadav *et al*. (2016) stated that the phytochemicals are the most important sources for the treatment of common diseases. The present investigation deals with the qualitative phytochemical analysis of leaves of ten medicinal plants. These are *Bauhinia variegata Linn*. (Caesalpiniaceae), *Calotropis procera* (Ait.) R.Br. (Asclepiadaceae), *Catharanthus roseus* (Linn.) Don. (Apocynaceae), *Lantana camara* (Linn.) Var. (Verbenaceae), *Mangifera indica* Linn. (Anacardiaceae), *Moringa oleifera* Lamk. (Moringaceae), *Ocimum sanctum* Linn. (Lamiaceae), *Pithecellobium dulce* (Roxb) Benth. (Mimosaceae), *Solanum nigrum* Linn. (Solanaceae), *Tinospora cordifolia* (Willd.) Mier. ex Hook. f. and Th. (Menispermaceae). Methonolic extracts of powder of leaves were screened for

qualitative determination of different phytochemicals like alkaloids, carbohydrates, glycosides, phytosterols, flavonoids, protein and amino acid, diterpenes, phenols and tannin.

Rajput *et al*. (2017) demonstrated that the aim of the present study was to evaluate the chemical and phytochemical properties of fresh and dried *M. oleifera* (PKM-1) leaf powder. Dried moringa leaves powder was prepared using cabinet dryer at 60-70 0C for 6-8 hrs. and grind into fine powder. Proximate, mineral, phytochemical properties were carried out on the leaf samples. Fresh and dried moringa leaf powder exhibited moisture levels varying from 72.83 to 7.43%, ether extract from 4.59 to 9.53%, crude fiber from 5.75 to 22.03%, total minerals from 4.59 to 9.53%, crude protein from 5.29 to 20.42%, and carbohydrates from 10.57 to 50.16%. The predominant mineral elements in the fresh and dried moringa leaf powder were Ca, Mg, K, Fe, Cu, 475.33, 40.33, 328.33, 7.63, 0.15 and 20.32, 387.83, 1545.33, 26.69, 0.83 mg/100g respectably. The phytochemical properties revealed that the ascorbic acid and total phenols of fresh and dried moringa leaves ranged from (231.28 to 106.28) and (62.32 to 253.00) mg/100 g, respectively. Antioxidant activities were found to be best in dried moringa leaves 9489.80 % activity. We concluded that *M. oleifera* samples could be employed in edible and commercial applications.

Moringa oleifera could be used in curing many diseases like typhoid fever, diarrhea, high blood sugar, hypertension, gastro intestinal disorder. It is advised that this plant be utilized in cooking and making other formulations that are edible.

Abdulkadir *et al*. (2016) demonstrated lack of proper nutrition is one of the most serious problem across the globe, affecting majorly African countries, where many children died not only due to inadequate food but also as a result of insufficient nutrient in their diet. This research was aimed to study the proximate composition

and screening of chemical components in different part of *Moringa oleifera* Lam. Proximate analysis was adapted from the standard methods developed by Association of Official Analytical Chemists (AOAC). We have determined percentage of moisture content, ash content, crude protein, crude fiber, crude fat and carbohydrate which were ranges from 8.1–4%, 5.59–9.28%, 11.70–26.57%, 21.03–44.27, 11.82–20.19 and 8.16–31.12% respectively. Different bioactive compounds of polyphenol, flavonoids, tannins and saponin were determined. Different parts of plants accumulate different levels of polyphenol, flavonoids, tannins and saponin. Our study suggest that *Moringa oleifera* can served as a good supplement to our nutrition, due to high content of fiber, protein, fat. However intake of Moringa plant might reduce the risk of many oxidative related problems due to the present of some polyphenols and flavonoid in almost all part of the plant used.

Santhi and Sengottuvel (2016) reported that Moringa concanensis Nimmo (Moringaceae) is an important medicinal plant. The present study deals with the analysis of Phytochemical constituents by qualitative and quantitative analysis of *Moringa concanensis* leaves, flowers and seeds were done using macetone extract. Alkaloids, flavonoids, terpenoids, carbohydrates, protein and amino acids were analyzed. Phenol and saponin were present in only macetone extracts of leaves and flowers. Steroids, anthraquinone, tannin, oils and resins were absent in the extract. Quantitative analysis was also conducted to determine the number of alkaloids, flavonoids, phenol and carbohydrate.

Sankhalker and vernekar (2016) discussed that background: Number of secondary compounds is produced by plants as natural antioxidants. *Moringa oleifera* Lam. and *Ocimum tenuiflorum L*. are known for their wide applications in food and

pharmaceutical industry. Objective: To compare phenolic and flavonoid content in *M. oleifera Lam* and *O. tenuiflorum L*. by quantitative and qualitative analysis. Materials and Methods: Phenolic and flavonoid content were studied spectrophotometrically and by paper chromatography in *M. oleifera Lam*. and *O. tenuiflorum* Results: Higher phenolic and flavonoid content were observed in Moringa leaf and flower. Ocimum flower showed higher phenolic content and low flavonoid in comparison to Moringa. Flavonoids such as biflavonyl, flavones, glycosyl flavones, and kaempferol were identified by paper chromatography. Phytochemical analysis for flavonoid, tannins, saponins, alkaloids, reducing sugars, and anthraquinones were tested positive for Moringa and Ocimum leaf as well as flower. Conclusions: In the present study higher phenolic and flavonoid content, indicated the natural antioxidant nature of Moringa and Ocimum signifying their medicinal importance. SUMMARY: *Moringa oleifera Lam.* and *Ocimum tenuiflorum L*. are widely grown in India and are known for their medicinal properties. Number of secondary metabolites like phenolics and flavonoids are known to be present in both the plants. The present study was conducted with an objective to qualitatively and quantitatively compare the phenolics and flavonoids in these two medicinally important plants.

Quantitation of total phenolics and flavonoids was done by spectrophotometrically while qualitative analysis was performed by paper chromatography and by phytochemical tests. Our results have shown higher phenolics and flavonoid content in Moringa leaf and flower. However, higher phenolic content was absent in Ocimum flower compared to that of Moringa. Phytochemical analysis of various metabolites such as flavonoids, tannins, saponins, alkaloids, anthraquinones revealed that both the plant extracts were rich sources of secondary metabolites and thus tested positive for the above tests. Various flavonoids and Phenolics were

identified by paper chromatography based on their Rf values and significant colors. From the above study we conclude that Moringa and Ocimum are rich in natural antioxidants hence are potent source in pharmaceutical industry.

Udosen *et al*. (2016) studied that the choice of medicinal plant by traditional medical practitioners was not based on knowledge of the plant constituents or phytochemicals, but the search for safer and cheaper remedies. The effect of Moringa oleifera leaf tea bag extracts on *Salmonella typhi* and *Escherichia coli* from stool sample was investigated using the agar well diffusion method. Five different extracts were obtained from the *Moringa oleifera* leaf tea bag. The result shows that the first extraction has the widest zone of inhibition on the two test organisms of 8mm and 3mm respectively. Only the first extraction has effect on Escherichia coli with the zone of inhibition of 3mm. The phytochemical analysis of the tea bag extract showed that it contains saponins, tannins, phenols, glycosides, alkaloids, flavonoids and the absence of reducing sugar. Thus, extract of the plant can be used in treating infections caused by these test organisms.

Bwai *et al*. (2015) reported that objective: *Acacia nilotica* fruit has been used to treat different diseases. The significance of the plant in traditional medicine and the importance of the distribution of these chemical constituents were discussed with respect to the role of this plant in ethno-medicinal usage in Nigeria. In the present study, the fruit of *A. nilotica* was subjected to elemental, phytochemical and antifungal analysis. Methods: The extraction was done with acetone and hexane using soxhlet apparatus. The elemental analysis was done using an Atomic Absorption Spectrophone-eastern India has been known for its rich biological diversity. For this study, seven medicinal plants such as *Bryophyllum pinnatum, Ipomea aquatica, Oldenlandia corymbosa, Ricinus communis, Terminalia bellerica, Tinospora cordifolia, and Xanthium strumarium*, were selected. The aim

of the present study was to investigate the presence of phytochemicals and to determine the total phenolic and flavonoid contents of the selected medicinal plants. Soxhlet apparatus was used for the organic solvent extraction. Solvents used were water, macetone, acetone, and acetone. Total phenolic contents of the aqueous extracts of the plants were determined by the Folin-Ciocalteus reagent method whereas total flavonoid contents of the aqueous extracts were determined by the Aluminum Chloride method. Proteins, carbohydrates, phenols, tannins, flavonoids, saponins, were detected in all of the plants tested. Total phenolic contents obtained were 18.4mg/gm, 18.8mg/gm, 11.6mg/gm, 29.2mg/gm, 29.6mg/gm, 40.8mg/gm, 12.8mg/gm, 71.6mg/gm of the extract and total flavonoid contents obtained were 8.4mg/gm, 37.6mg/gm, 4.4mg/gm, 6mg/gm, 42.8mg/gm, 18mg/gm, 6mg/gm, 28.8mg/gm of the extract for the plants *Bryophyllum pinnatum* (Leaves), *Ipomea aquatica* (Leaves), *Oldenlandia corymbosa* (Whole plant), *Ricinus communis* (Roots), *Terminalia bellerica* (Leaves), *Tinospora cordifolia* (Leaves), *Tinospora cordifolia* (Stem), and *Xanthium strumarium* (Leaves)Â respectively. Our findings provided evidence that crude aqueous and organic solvent extracts of these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases. The phytochemical investigation of the acetone and hexane extract of *A. nilotica* fruit was carried out. The extracts were evaluated for their antifungal activity. Results: The proximate analysis showed that moisture content was 12.6±0.02 %, crude fiber 11.1±0.03 %, crude lipid 15.8±0.01 %, ash content 5.0±0.01 %, crude protein 1.3±0.02 % and carbohydrate 54.2±0.02 %. The elemental analysis showed various concentrations of Ca, Zn, Mg, Mn, Ni, Cr, Fe, Cd while Co was absent. The phytochemical investigation revealed the presence of tannins, steroids, saponins, phenols, alkaloids, cardenolides, terpenoids, carbohydrates, cardiac glycosides, resins and balsams. The antifungal analysis of

acetone extract on *Aspergillus niger, Aspergillus flavon, Fusarium oxyfurum and Penicillium Spp* showed an increase in the zone of inhibition and an increase in the concentration of the *A. nilotica* fruit extracts when measured in mm. Conclusion: *Acacia nilotica* has both nutritional and medicinal values based on the presence of numerous secondary metabolites and essential metals. The plant studied here can be seen as a potential source of useful drugs and further studies are going on in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

Banu and Catherine (2015) discussed that plants are recognized in the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological activities. The biologically active compounds present in plants are called phytochemicals. These phytochemicals are derived from various parts of plants such as leaves, flowers, seeds, barks, roots and pulps. These phytochemicals are used as sources of direct medicinal agents. They serve as a raw material base for elaboration of more complex semi-synthetic chemical compounds. This paper mainly deals with the collection of plants, the extraction of active compounds from the various parts of plants, qualitative and quantitative analysis of the phytochemicals.

Rather et al.(2015) stated Acacia nilotica (L.) is an important ornamental and medicinal plant of tropical and sub-tropical regions belongs to family Fabaceae of genus *Acacia* commonly known as babul, is a source of many active secondary metabolites which may serve as potential candidates for drug development with greatest possibility of success in near future. The present review aims at providing an up-to-date summary of the traditional usage, phytochemistry, and pharmacological profile of *A. nilotica* (L). An exhaustive survey of literature has revealed that tannins, flavonoids, alkaloids, fatty acids

and polysaccharides (gums) constitute major classes of phytoconstituents of this plant. Pharmacological data base reports have revealed significant anti-inflammatory, antioxidant, antidiarrheal, antihypertensive and antispasmodic, antibacterial, anthelmintic, antiplatelet aggregatory, anticancer and acetyl cholinesterase (AChE) inhibitory activities. Through this review authors have tried to explore the therapeutic potential of *A. nilotica* and thus may be a promising rout for new, safe, biodegradable and renewable source of drugs with high therapeutic index.

Mohammad (2015) demonstrated that Sudan is rich in medicinal plants which synthesize substances that are useful to the maintenance of health in humans and animals. kikkar and Rumman were reported to have some antimicrobial activity. The aim of the present study is to investigate and analyze the phytochemical compounds and the antimicrobial activities of the two plants. The extracts of the different parts of these plants were tested against mycelial growth and spore germination, using selective media and the cup plate Inhibition zone method was used for the solvents tested. The results showed that some parts of the two plants were containing flavonoids, tannins, saponin, sterol and alkaloids. The microbial tests indicated that the Kikkar seeds extract was highly effective against the radial growth of *P. italicum.* Leaves and bark extracts were effective against *A. niger*. Rumman Flower and peel extracts were better against P. italicum. However, they were not effective against *A. nige*r. All Rumman part extracts were effective against *P. italicum*. and only the flower extract was effective against the fresh and dry weights of A. niger. The spore germination tests showed that all the extracts of Kikkar plant parts were effective against *P. italicum.* Only the Rumman peels extracts were effective against *P. italicum* and *A. niger*. The inhibition zone tests showed that the Kikkar leaves and pods extracts were highly effective against C.

albicans, and bark extract was highly effective against *C. valida.* The bark and the seeds extracts were effective against *C. tropicalis*, while, the pods extract was more effective against *Pichia sp.*, *E. coli* and *S. paratyphi*. However, the pods, bark and leaves extracts were highly effective against *Staphylococcus sp*. Rumman peels extracts were more effective against *C. tropicalis* and all of it' s parts were effective against the three bacteria (*E. coli, S. paratyphi and Staphylococcus sp*.). The solvent tests showed that the macetoneic pod extracts of Kikkar were effective against the tested fungi, as well as *Pichia sp*. and *C. tropicalis*. The leaves extracts were effective against *C. albicans,* and *Staphylococcus sp*. while, the bark extracts were effective against *C. tropicalis* and *C. valida*. However, the seeds extracts were effective against *C. albicans, C. valida,* and *C. tropicalis*. The macetoneic Rumman peels extracts were effective against the two fungi, and *C. valida.* The acetoneic bark, seeds and pods extracts of Kikkar were effective against *E. coli* and *S. paratyphi. C. valida* was the only sensitive yeast against the bark and the peel acetoneic extracts of Rumman. Peels extracts were more effective against the three tested bacteria. The bark and flower effective against *E. coli.* All the petroleum ether and hexane extracts of the two plants parts were not effective against all the tested organisms. Both Kikkar and Rumman plants were containing inhibitory compounds against different microorganisms. It could be suggested that Kikkar and Rumman extracts could be used for treating some skin diseases.

Solana *et al*. (2015) reported that *Moringa oleifera* is a tree distributed in Mexican semiarid and coastal regions. *M. oleifera* is used in practice in the treatment of various diseases and is available without a medical prescription, often in the form of an herbal infusion for everyday use. The aim of the present study was to evaluate the chemical composition and nutritional values of dried *M. oleifera* leaf powder collected from two different regions in Mexico. All samples of *M.*

oleifera exhibited moisture levels varying from 3.06 to 3.34%, lipids from 10.21 to 10.31%, fiber from 7.29 to 9.46%, ashes from 10.71 to 11.18%, crude protein from 10.74 to 11.48%, and carbohydrates from 54.61 to 57.61%. The predominant mineral elements in the leaf powder according to ICP-MS were Ca (2016.5–2620.5 mg/100 g), K (1817–1845 mg/100 g), and Mg (322.5–340.6 mg/100 g). The HPLC analysis indicated the presence of phenolic acids (gallic and chlorogenic acids) and flavonoids (rutin, luteolin, quercetin, apigenin, and kaempferol). We concluded that Lombardia *M. oleifera* samples could be employed in edible and commercial applications. Our results showed that the highest mean value of As from the San Pedro samples exceeds the recommended level and may constitute a health hazard to consumers.

Nawel et al. (2014) stated that in this paper, we extracted, analyzed and studied the antimicrobial activity of Algerian parsley essential oil on several microbes that cause infectious diseases and its effects on kinetics of lactic acid production by *Lactobacillus casei* subsp. rhamnosus. The essential oil of parsley (*Petroselinum crispum* Hoffm) obtained by hydro distillation was characterized by its physicochemical properties and by its chromatographic profiles. Myristicin and dillapiole were identified by gas chromatography spectrometry mass (GC/MS). The essential oil showed a high antimicrobial spectrum towards *Bacillus cereus and Candida albicans*, average effectiveness against *Clostridium perfringens*, *Staphylococcus aureus* and *Enterococcus faecalis* and no influence on *Escherichia coli.* The key odorant effects of parsley in the growth of *Lactobacillus casei subsp. rhamnosus* was studied. The results showed that *L. rhamnosus* can produce up to 10.96 and 13.78 g. L -1 of lactic acid on the control fermentation and on the second fermentation, respectively, characterized by the addition of 20 µL of the essential oil in the growth exponential phase.

Ojiako and E.N (2014) studied *Moringa oleifera* having been used as medicinal plant was subjected to soxhlet extraction and the extract was used to find the antimicrobial analyses. The result showed that acetone extract inhibits the action of staphylococcus aureus 9mm, *Escherichia coli* 4mm, *salmonella tiphy* 6mm, mucor 3mm, and candida albican 3mm.N-hexane extract inhibits *salmonella tiphy* 4mm, mucor 2mm, *candida albican* 2mm, while it had no action on *staphylococcus aureus* and *Escherichia coli*. Ethyl acetate possess the highest zone of 10mm in *staphylococcus aureus* and *salmonella tiphy* followed by E. coli 8mm, *candida albican* 4mm, and Mucor 2mm. Phytochemical analysis showed the presence of tannins and alkaloids in all the three extracts, phlobatannins were found to be present only in n-hexane extract, while they were absent in both acetone and ethyl acetate extracts, also saponins and phenols were present in both acetone and ethyl acetate extracts and absent in hexane extract. The quantitative analysis was found to be: tannins (8.22%), saponins (1.75%), alkaloids (0.42%) and phenols (0.19%).

Menpara and Chandra (2014) stated that Objective: The objective of this study was to carry out phytochemical and pharmacogenetic evaluation of leaves of *Pongamia pinnata L*. (Fabacea). Method: The present study provides pharmacognostic, physiochemical and phytochemical details of the leaves of *P. pinnata*. Macro and microscopic evaluation and WHO recommended parameters were followed in the study. Results: The macroscopic study showed that the leaf was ovate or elliptic with smooth margins, short petiole, alternate impar pinnate, hairless, acuminate at apex, rounded to cuneate at base and slightly thickened. Microscopic study revealed collateral, closed vascular bundles, trichomes, paracytic stomata, xylem vessels and prismatic calcium oxalate crystals. Physiochemical analysis of leaf showed total ash, water soluble ash and acid insoluble ash as 8.33±0.31, 2.33±0.36 and 0.5±0.0 %w/w respectively. Preliminary phytochemical screening showed the

presence of alkaloids, flavonoids, tannins, triterpenes, cardiac glycosides, steroids and saponins. Conclusions: The results of this study can serve as valuable source of information for identification of this plant for future investigation and applications.

Sharma and paliwal (2014) demonstrated that objective *Moringa oleifera* Lam. is the most widely cultivated species of the monogenetic family Moringaceae and has an impressive range of medicinal uses with high nutritional value. In this study saponin was isolated from *Moringa oleifera* pods. Methods: Thin layer chromatography (TLC) was performed using a mobile phase of Chloroform: macetone: H2O (7:3:1) on silica gel glass plates. High performance liquid chromatography (HPLC) of isolated compound from benzene extract obtained by Successive extraction method was carried out to confirm its nature by analyzing HPLC chromatograms. Results: Characterization of isolated saponin was done using IR and 1H NMR. Saponin from *M. oleifera* pods was isolated having Rf 0.90. The IR spectrum of isolated compound exhibited the presence for hydroxyl group (-OH), carboxylic acids, alkynes, presence of –C=O (esters) and >C-O (ethers) and the ring involvement or aromatic structure of the compound. 1NMR spectrum of isolated compound revealed presence of protons in the compound. Conclusion: The isolated compound was then nomenclature as SM (saponin from *Moringa* pods) and was further used to determine its biological and pharmacological properties. Ravichandran *et l.* (2009) studied that the plant *Moringa concanensis* Nimmo is a tree belongs to family Moringaceae locally known as Kattumurungai by tribal peoples of Nilgiris hill region in Tamilnadu. In view of its medicinal importance and taxonomic confusion, pharmacognostic studies, microscopical structure, morphological characters, chemical analysis and numerical values in epidermal study were carried out to supplement the necessary information for the systematic

identification and authentication of this plant, as per WHO guidelines. Pharmacognostic and preliminary phytochemical investigations of this plant were carried out and reported.

Nweze *et al*. (2014) reported that the phytochemical constituents aqueous and acetoneic leaf extracts of *Moringa oleifera Lam*. were assessed and compared. The mineral and proximate analyses were done on the whole leaf extract. The result of the qualitative phytochemical constituents of both leaf extracts of *Moringa oleifera* showed presence of all the tested phytochemicals (flavonoids, anthraquinone, alkaloids, saponins, steroids, terpenoids, cardiac glycosides, anthocyanin, tannins and carotenoids) with water extracting more of the phytochemicals. Results showed the presence of iron (0.03g/100g), calcium (2.09g/100g), magnesium (0.48g/100g), potassium (1.62g/100g), phosphorus (0.04g/100g), zinc (0.005g/100g), copper (0.01g/100g) and Sulphur (0.85g/100g). Proximate analysis revealed the presence of carbohydrate (57.01%), protein (18.92%), fats (2.74%), fiber (9.31%), moisture (4.09%) and ash (7.95%). The results from this study prove the extensive use of the leaves of this plant in ethnomedicine and its potentials in drug formulation.

Auwal et al. (2014) stated that Acacia nilotica (Thorn mimosa) is used locally for various medicinal purposes by traditionalists and herbalists in northeastern Nigeria. Plants products have been used since ancient times in the management of various conditions. The bark of *A. nilotica* has been reported to be used traditionally to manage diabetes, dysentery, leprosy, ulcers, cancers, tumor of the eye, ear and testicles, induration of liver and spleen and also in treatment of various condylomas. The objective of this study is to determine the phytochemical and elemental constituents of the extracts of *A. nilotica* pods. Flame emission and atomic absorption spectrometry were also used to determine the presence or

absence of micro- and macro-elements in the extracts. Phytochemical analysis of the aqueous, ethyl acetate and N-butanol fractionated portions of the pod extracts of *A. nilotica* revealed the presence of tannins, saponins, flavonoids, carbohydrate, whereas carbohydrates and tannins were the only constituent in the residue portion. Anthraquinones, alkaloids, terpene and steroids were not present in the extracts. The elemental screening revealed the presence of iron, potassium, manganese, zinc, calcium, phosphorous, magnesium, sodium, cadmium and copper. Lead, arsenic and molybdenum were not detected in the pod.

Chandra *et al*. (2014) studied that in the present investigation the antimicrobial activity of acetone, chloroform, water and macetone extracts of different parts (stem, leaf, seed) of *Acacia nilotica* (L.) Del. was observed. only macetoneic extract showed good activity against all the tested bacteria and fungi except *Aspergillus niger*. The determination of MIC value was found at the conc. of 5µl on *Escherichia. coli*. Phytochemical analysis revealed presence of alkaloids, saponin, flavonoid, tannin and glycosides in the leaf extract.

Patel *et al*. (2014) demonstrated that objective: The aim of the present study was to carried out phytochemical analysis of aqueous and acetoneic extract of *Moringa oleifera* and to find out antifungal property of *Moringa oleifera*. Methods: *Moringa oleifera* leaf extracts was used for plant component analysis and for determination of antifungal activity. *Saccharomyces cerevisiae* (MTCC No.170), *Candida albicans* (MTCC No.183), *Candida tropicalis* (MTCC No.1000) strain were used for experimental purpose. Well diffusion method was used to assess the antifungal effect of the extracts on micro-organisms. Results: The phytochemical screening indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins etc., in the both extracts. Antifungal activity of acetonic and aqueous extract of *Moringa oleifera* leaf was highly active against Saccharomyces cerevisiae inductive against

Candida tropicalis and not showing activity against *Candida albicans*. Conclusion: The present study conclusively demonstrates that *Moringa oleifera* is a good source of various phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, Terpenoids. The antifungal activity *Moringa Oleifera* was clearly shown by the present study against various fungi like *Saccharomyces cerevisiae, Candida albicans and Candida tropicalis*. All these preliminary reports warrant an in-depth analysis of the usefulness of *Moringa oleifera* as miracle drug against various ailments.

Dubey *et al*. (2013) stated that *Moringa oleifera Lam.* (Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics. Moringa is best known as excellent source of nutrition and a natural energy booster. Different parts of this plant are being employed for the treatment of different ailments in the indigenous system of medicine. The Moringa plant provides a rich and rare combination of zeatin, quercetin, sitosterol, caffeoylquinic acid and kaempferol. In addition to its compelling water purifying powers and high nutritional value, *M. oleifera* is very important for its medicinal value. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, antipyretic, antitumor, anti-inflammatory, antiepileptic, diuretic, antiulcer, antispasmodic antihypertensive, cholesterol lowering, antidiabetic, antioxidant, antibacterial, hepatoprotective, and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia. This review focuses on the detailed phytochemical composition, medicinal uses, along with pharmacological properties of different parts of this multipurpose tree

Al Hadi *et al*. (2013) studied that Phytochemical Screening, Antibacterial and Cytotoxic Activities of *Petroselinum crispum* Leaves Grown in Oman.

Petroselinum crispum belongs to the Apiaceae family, is a well-known spice and vegetable. The essential oil obtained from the fruit has also strong action on the central nervous system. Present study is designed to evaluate the phytochemical screening, antibacterial and cytotoxic activity of the leaves of *Petroselinum crispum* collected from Oman. Results of phytochemical screening indicate the presence of primary and secondary metabolites. Antimicrobial activity was measured using disc diffusion method against *S. aureus, E. coli, P. aeruginosa* and *K. pneumonia*. Brine shrimp test was used to estimate cytotoxic activity. In antibacterial assay, *Petroselinum crispum* leaves extracts showed very strong results, inhibition zones ranged from 7 – 20 mm. So this plant can be used as a good source of potential antimicrobial agent. Hydro alcoholic and Petroleum ether extracts showed the highest antibacterial activity. Furthermore, Ethyl acetate and Hydro alcoholic extracts have almost killed 90% of the shrimp larvae at higher concentration of 1000 µg/ml. LC50 values for the two extracts was 51.95 and 88.15 µg/ml, respectively. Non polar fractions like chloroform extract have displayed low cytotoxic activities as compare to polar extracts.

Wadood *et al*. (2013) described that Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. The present study involves ten different medicinal plants *Acacia nilotica, Psidium gujauva, Luffa cylindrical, Morus alba, Morus nigra, Momordica charantia, Fagonia cretica, Punica granatum, Ficus palmate* and *Prunus persica* locally available in Mardan region of Pakistan. The leaves of the selected medicinal plants were washed, air

dried and then powdered. The aqueous extract of leaf samples was used for the phytochemical analysis to find out the phytochemical constituents in the plants. The main objective of the research work was to check the presence or absence of the phytochemical constituents in all the selected medicinal plants. The results of the phytochemical analysis of these medicinal plants showed that the terpenoids, phlobatannins, reducing sugar, flavonoids and alkaloids were found to be present in afore mentioned medicinal plants. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases. It is expected that the important phytochemical properties recognized by our study in the indigenous medicinal plants of Mardan will be very useful in the curing of various diseases of this region.

Kamel (2013) reported that the aim of the present study was to evaluate the effect of microwave heating, (for one, two and three minutes) on some bioactive compounds of parsley (*Petroselinum crispum*) and dill (*Anethum graveolent*) leaves blended in water. The effect of microwave drying process of parsley and dill leaves was also studied. Total phenols, antioxidant activity, chlorophyll, carotenoids and color indices were determined in this study. According to the obtained results, dill leaves contain higher total phenols, antioxidant activity and carotenoids (1287.00 mg as gallic acid / 100g, 48.14% and 45.98mg/kg, respectively) than parsley leaves (1031.39 mg as gallic acid/100g, 40.10% and 40.00mg/ kg, respectively). Total phenols, antioxidants activity and carotenoid contents of parsley and dill heated samples significantly ($p<<0.05$) increased after one minute of heating. Then gradual decrease was observed in the same parameters after 2 and 3 minutes in both parsley and dill. Significant decrease in all tested parameters was observed in microwave dried samples compared to the fresh state.

Antioxidant activity decreased 20% in dried parsley and30.3% in dried dill compared to fresh samples. This work indicated that the tested bioactive compounds of parsley and dill was stable only after one minute of microwave heating, however, after 3min of heating 32.3 to 80% decrease was observed in these parameters.

U C and Nair (2013) conducted preliminary phytochemical analysis of extracts of the leaves of *M. oleifera* using petroleum ether, chloroform, and acetone and water solvents in succession Methods: The extracts of the dried powder of the leaves of *M. oleifera* Lam obtained using petroleum ether, chloroform, ethyl alcohol and water in succession were tested along with positive control and blank for the presence of tannins, alkaloids steroids, triterpenoids, flavonoids, hydroxyanthraquinones, cardiac glycosides, saponins, and for carbohydrates (glucose and fructose), proteins, amino acids, and fixed oils & fatty acid Result: The results suggest the presence of tannins, alkaloids steroids, triterpenoids, flavonoids, hydroxy-anthraquinones, cardiac glycosides, saponins, and carbohydrates (glucose and fructose) having selective solubility in selected solvents of varying polarities used in succession However, the tests for proteins and fixed oils and fats were negative in all the extracts. Conclusion: The selective solubility of the phytochemicals is probably responsible in conferring a wide spectrum of biological activities attributed to the leaves of *M oleifera* suggesting the importance of the solvent as a decisive factor and also suggest the importance of the employed test also as a decisive factor for confirming the presence of a phytoconstituent. The data in addition indicates that the leaves of *M. oleifera* are highly nutritious and could be a source of dietary supplement being rich in carbohydrates, proteins and amino acids. In addition, the present study suggests that the successive reextractions using solvents of varying polarities would

maximize the exploitation of the diverse bioactive compounds. The present information would be of help to isolate and characterize the diverse pharmacologically active principles of importance supporting their varied biological activities and the medicinal values.

Charles (2012) stated that Parsley is an annual culinary herb widely grown in Europe and Western Asia. This chapter describes the cultivation, parts of the parsley plant, its general uses and functional properties. The active constituents of the seed, root and leaves of parsley are discussed in detail together with the composition and physicochemical properties, and quality standards of essential oils. The chapter highlights the use of parsley in different recipes around the world. Finally, the functional properties and adverse effects of parsley are discussed in great detail. It has extensive information on the diuretic, carminative, antipyretic, antiviral, antibacterial, anti-inflammatory, anticancer and antioxidant properties of parsley.

Ali *et al*. (2012) published that *Acacia nilotica* Lam (Mimosaceae) indigenously known as 'Babul' or 'Kikkar' is a proverbial, medium sized tree and is broadly scattered in tropical and subtropical countries. It has an inspiring range of medicinal uses with potential anti-oxidant activity. This plant contributes a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes. *A. nilotica* is a medicinal plant acknowledged to be rich in phenolics, consisting of condensed tannin and phlobatannins, gallic acid, protocatechuic acid, pyrocatechol, (+) - catechin, (-) epi- gallocatechin-7-gallate and (-) epigallocatechin-5, 7-digallate. Different parts of this plant such as the leaves, roots, seeds, bark, fruits, flowers, gum and immature pods act as anti-cancer, antimutagenic, spasmogenic, vasoconstrictor, anti-pyretic, anti-asthmatic, cytotoxic, anti-diabetic, anti-platelet

aggregatory, anti-plasmodial, molluscicide, anti-fungal, inhibitory activity against Hepatitis C virus (HCV) and human immunodeficiency virus (HIV)-I and antioxidant activities, anti-bacterial, anti-hypertensive and anti-spasmodic activities, and are also engaged for the treatment of different ailments in the indigenous system of medicine. This review spotlights on the detailed phytochemical composition, medicinal uses, along with pharmacological properties of different parts of this multipurpose plant.

Deshpande and Kadam (2012) reported that *Acacia nilotica* commonly known in India as Babul has wide range of medicinal uses. In the present study, preliminary phytochemical screening of acetone and petroleum ether extract of stem bark of Acacia nilotica was carried out. It revealed the presence of alkaloids, carbohydrates, saponins, Tannins, Flavonoids, cardiac glycosides and anthraquinone in both acetone and ether extracts while fixed oils and fats, proteins and amino acids were absent. The antimicrobial activity of the extracts was determined by agar diffusion method. The acetone extract showed more significant activity against *Streptococcus mutants* as compared to petroleum ether extract. The minimum inhibitory concentration of acetone extract was 5 mg/ml while 10 mg/ml for ether extract. The results indicate that *Acacia nilotica* could be used as a source of antimicrobial agents to treat dental caries.

Savithramma *et al*. (2011) described that the traditional medicine involves the use of different plant extracts or the bioactive constituents. This type of study provides the health application at affordable cost. Secondary metabolites are responsible for medicinal activity of plants. Hence in the present study phytochemical screening of some important medicinal plants was carried out. Qualitative phytochemical analysis of these plants confirms the presence of various phytochemicals like saponins, terpenoids, steroids, anthocyanins, coumarins, fatty acids, tannins,

leucoanthocyanins and emodin's. The results suggest that the phytochemical properties for curing various ailments and possess potential antioxidant and leads to the isolation of new and novel compounds.

Raj *et al*. (2011) discussed that *Moringa oleifera* (Lam.) – (Moringaceae) is commonly known as horseradish tree or drumstick used as phytomedicine such as antioxidant, antimicrobial, anti-inflammatory, antipyretic, antidiabetic, antifertility, antiulcer and antitumor. *Moringa oleifera* (Lam.) root was successively extracted with petroleum ether, ethyl acetate, chloroform, acetone and aqueous extract were tested for antimicrobial activities against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Penicillium sp., Mucor sp., Aspergillus niger* and *Candida albicans* by disc diffusion method. Ethyl acetate extract showed high antibacterial activity against Pseudomonas aeruginosa (18.2 ± 0.2 mm). Chloroform extract were ineffective against *Escherichia coli* and *Proteus mirabilis*. Aqueous extract showed maximum number of inhibitions against *Penicillium sp*., (13.1 ± 0.2 mm) than other extracts and *Aspergillus niger* were ineffective in all the extracts except aqueous extract. The phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, tannins, cardio glycosides, amino acids and proteins.

Huijuan *et al*. (2011) demonstrated that Western parsley (WP), a culinary herb, has been reported to display significant antioxidative properties and to contribute to the elimination of heavy metals, but little information is available regarding its effect over food storage stability. In this study, the effect of this plant on the oxidative stabilities of food was investigated as compared with the synthetic antioxidant ethoxyquin (EQ) under accelerated oxidative stress. Headspace oxygen consumption, formation of primary and secondary oxidation products, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity, were used as the

parameters. Oxidative stabilities of food increased significantly ($P \leq 0.05$) with addition of WP, revealing potent antioxidant activities of WP for stabilization of food over a long storage period. Our results suggested that the synergistic action of the phytochemicals may be contributing to the improved oxidative stabilities. According to these results, WP, a commonly consumed herb, can be used as an alternative to synthetic antioxidants in the longterm storage of food.

Solomon and shittu (2010) stated that in vitro antimicrobial and phytochemical activities of the crude acetoneic leaf extract of *Acacia nilotica* on *Campylobacter coli* isolated from goats in Gwagwalada Abattoir was investigated. Hydrolysable tannins, saponin, saponin glycosides, volatile oils, phenols, triterpenes, flavonoids and alkaloid were present in the extract. Minimum inhibitory concentration was 70 mg/ml of the extract related to standardized bacteria colony of 3 x 108 organisms per ml. The highest zone of inhibition was observed with the 70 mg/ml concentration, following isolation and inoculation of test organisms on Muller Hinton Agar incubated at 37oC for 24 h. The basis of this plant extract in the traditional treatment of diarrhea in human is highlighted.

Shinwari (2010) reported that out of about 258,650 species of higher plants reported from the world; more than 10% are used to cure ailing communities. Beside many known drugs (e.g. Tubocurarine, reserpine, aspirin and morphine etc.) are discovered based on traditional knowledge. Majority of the people in Pakistan rely on medicinal plants to find treatment for their minor, even in some cases major diseases. Some wild plants are now being commonly used e.g. Ephedra, Artemisia, St. John's wort, Hippophage beside some that have been domesticated e.g. Garlic, Ginseng and Cumin etc. There is a local market system (Pansare) specifically dealing with medicinal plants business in Pakistan and several plants are exported. Plants having active constituents are used to treat

various ailments in both human and animal. In most instances, certain plant species are considered specific for a particular illness but occasionally they have mixed usage. Women, followed by children, are identified as the principal collectors of medicinal plants. Due to over-collection, several species have gone extinct in the Hindukush-Himalayan regions. Local collectors, vendors, herbal drug dealers and others are the ones who threaten the flora of Pakistan contribute (though unknowingly) to the extinction of some and bringing others to the brink of extinction. Though medicinal plants from wild are important source of income for local communities, but if not properly managed, this may lead to the destruction of habitat and in return extinction of species. There is therefore, a need to find ways to harvest medicinal plants sustainably from the wild, train local collectors (in proper collection techniques, train the people in growing medicinal plants, and remove some of the middlemen from the trading chain. In the present article, an effort was made to review the status of medicinal plants research in Pakistan.

Kasolo *et al*. (2010) discussed *Moringa oleifera* grown and used in many countries around the world is a multi-purpose tree with medicinal, nutritional and socio-economic values. In Senegal and Benin, *M. oleifera* leaves are dispensed as powder at health facilities to treat moderate malnutrition in children. It established the medicinal uses of *M. oleifera* leaves by local communities in Uganda and identified phytochemicals present in *M. oleifera* leaves extracts. It used quantitative and experimental methods that established the uses, and identified phytochemicals in *M. oleifera* leaves. Employed serial extractions, using ether, acetone and water as solvents. The phytochemicals were qualitatively identified using standard chemicals and standard outcomes. Twenty-four medicinal uses of *M. oleifera* leaves were established. Phytochemicals present included: tannins, steroids and triterpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars.

The local communities in Uganda use *M. oleifera* leaves to treat common ailments. Presence of phytochemicals in the extracts, indicate possible preventive and curative property of *M. oleifera* leaves. There is need to standardize *M. oleifera* leaves use for nutrition and herbal medicine.

Banso A. (2009) described that *Acacia nilotica* was assessed for active principles. The results showed that the stem bark extract of the plant possessed the active principles e.g. terpenoids, tannins, alkaloids, saponins and glycosides. The antimicrobial activity of the extracts was assayed against *Streptococcus viridians, Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Shigella sonnei* using the agar diffusion method. The plant extract exhibited antimicrobial activity against all the test microorganisms. *B. subtilis* was the most susceptible to the plant extract while *Candida albicans* was the most resistant. The minimum inhibitory concentration of the stem bark extract of the plant ranged between 35 and 50 mg/ml while the minimum bactericidal concentration ranged between 35 and 60 mg/ml. *A. nilotica* could be a potential source of antimicrobial agents.

Dahiru *et al*. (2006) stated that the effect of aqueous extract of *Moringa oleifera* leaf was studied on experimentally induced gastric ulceration in rats. Pretreatment with extract 200, 300 and 400 mg/kg bw reduced the characteristic lesions induced by indomethacin compared to untreated control group in a dose dependent manner. The effects observed could be due to the action of one or more of the phytochemicals present in the leaf extract.

Marine *et al*. (2006) determined that antioxidant and antibacterial activities of freeze-dried and irradiated parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum)* leaves and stems in macetone and water extracts. The total phenolic content was quantified with the Folin–Ciocalteau reagent. Several mechanisms of potential antioxidant activity of all extracts, including determining

relative free radical-scavenging and ferrous ion-chelating activities, as well as reducing power, were examined. Assessment of the total antioxidant activity of all extracts was done using an iron-induced linoleic acid oxidation model system. Antimicrobial activity towards *Bacillus subtilis* and *Escherichia coli* by different extracts was assessed by determining cell damage. Total phenolic content varied between parsley and cilantro, leaf and stem, as well as macetone and water extracts. Macetone-derived leaf extracts exhibited significantly ($p < 0.05$) greater radical-scavenging activity towards both lipid- and water-soluble radicals, which was attributed to the total phenolic content. Ferrous ion-chelating activity was significantly ($p < 0.05$) greater in the stem macetone extracts, and corresponded to antioxidant activity. Prooxidant activity was a feature of all aqueous extracts and corresponded to the reducing activity of both leaf and stem parts of parsley and cilantro. Bacterial cell damage, resulting in significant ($p < 0.05$) greater growth inhibition of *B. subtilis* and *E. coli*, corresponded to ferrous sequestering activity of macetone-derived stem extracts.

Hashim and Farhat (1999) demonstrated that in response to certain stress agents, infection with Cercospora and treatment of this infection with Cuprosan, changes in the quantity and quality of the volatile oils produced from *Mentha piperita L*. and *Petroselinum Crispum Mill* become apparent. The treated samples showed increase in the concentration of Cu ions in the leaves. Some components were produced under the effect of these stress agents (infection and treatment). Psoralen was isolated from samples treated with Cu salts. Corylidin, Angladin and Pereflorin B were isolated from Parsley herb under the influence of *Cercospora petroselini* infection. These compounds were examined for antimicrobial activity. Corylidin, Angladin and Psoralen, inhibited growth of *Pseudomonas putida , E.*

Coli and *Rhizobium* meloloti (gm−ve bacteria) , while Pereflorin B inhibited *Streptococcus lactis and Bacillus subtilis* (gm+ ve bacteria).

Bennett and Wallsgrove (1994) reported that many secondary metabolites found in plants ha\ e a role in defense against herbivores, pests and pathogens. In this review, a few examples art- described and discussed, and some often problems determining the precise role(s)of such metabolites highlighted. The role of secondary metabolites in defense may involve deterrent'/anti-feredetate\'it, toxicity or acting as precursors to physical defense s> stems. Many specialist herbivores and pathogens do not merely circumvent the deterrent or toxic effects of secondary metabolites but actually utilize these compounds as either host recognition cues or nutrients (or both). This is true of both cyanogenic glucosides and glycosylates, which are discussed in detail as examples of defensi\e compounds. Their biochemistry is compared and contrasted. An enormous \'ariety of secondary metabolites are derived from shikimic acid or aromatic amino acids, many of which have important roles in defense mechanisms. Several classes of secondary' products are " induced\ infection, wounding or herbivory, and examples of these are given. Genetic variation in the speed and extent of such induction may account, at least in part, for the difference between resistant and susceptible varieties. Botallackites and jasmonates have been implicated as signals in such responses and in many other physiological processes, though their precise roles and interactions in signalling and development are not fully understood.

Smitt *et al*. (1995) studied that *Thapsia garganica L*. and *T. transtagana Brot*. are classified as synonymous in Flora European. In the present investigation significant differences between the two taxa with regard to fruit anatomy and phytochemistry are demonstrated and they are considered as two separate species. Microscopic studies revealed a distinct difference in number and position of

secretory spaces in the pericarp of fruits from the two species and in addition pronounced differences were found in the presence of thapsigargins, the bioactive constituents of the two species. Quantitative HPLC analyses of thapsigargins were carried out on different plant organs from *T. garganica and T. transtagana* collected from various locations. Thapsigargin, thapsigargicin, nortrilobolid and thapsivillosin I and J were dominant compounds in all organs of *T. garganica* whereas none of these compounds could be detected in any organ of *T. transtagana*. On the contrary, thapsitranstagin and trilobolid were main thapsigargins of *T. transtagana. T. garganica* may include two chemotypes, as trilobolid was detected in some specimens of *T. garganica* only. As this is the first time trilobolid has been detected in these two species, it was isolated from both and identified by 1H NMR-spectroscopy.

Wassel G.M (1992) demonstrated that phytochemical screening of the different parts of the two Acacia species revealed the presence of tannins, saponins, coumarins, carbohydrate and/ or glycosides, unsaturated sterols and / or triterpenes, alkaloids and / or nitrogenous bases, flavonoids and cyanogenic compounds. Biological studies of the different extracts of the organs of the two Acacia species revealed that the acetoneic extracts of *Acacia nilotica* stem and *Acacia farnesiana* pod exhibit a remarkable hypoglycemic activity. Most of the acetoneic extracts of the two species were found to be stimulatory to the rat's uterus at the three stages of sex cycle. Antimicrobial testing revealed that most of the acetoneic extracts are inhibitory to gram positive bacteria, those of the leaf of the two species are inhibitory to gram negative bacteria while, most of them, being with no effect on fungi.

Chapter 3

Materials and Methods

3.1 Materials

All the chemicals used were of analytical grade and were purchased from Sigma, Merck, Acros and Fisher scientific chemical companies while the plants were collected from the local area of Lahore in April 2020 (Table 3.1)

Table 3.1:Sample collection

Scientific name	**Local name**	**Location**	**Part used**
Moringa oleifera	Sohanjanna	Lahore	Leaves
Acacia nilotica	Kikkar	Lahore	Leaves
Petroselinum crispum	Parsley	Lahore	Leaves

3.2 Preparation of Reagents

3.2.1 Ninhydrin Solution

One gram of Ninhydrin was mixed in 40 ml of acetone.

3.2.2 Ferric Chloride ($FeCl_3$) solution

1.25 gram of Ferric chloride was mixed in 25 ml of distilled water.

3.2.3 Sodium carbonate solution

Seven gram of Sodium carbonate was mixed in 20 ml of distilled water.

3.2.4 Sodium hydroxide solution (2%)

NaOH (0.5g) was mixed in distilled water (25 ml).

3.2.5 Hager's reagent

Picric acid (1 g) was dissolved in (100 ml) of distilled water.

3.2.6 Wagner reagent

Potassium iodide (6 g) and Iodine (2 g) were dissolved in (100 ml) of distilled water.

3.2.7 Mayer's reagent

1.35 gram of mercuric chloride was mixed in distilled water (20 ml). Then five gram of potassium iodide was mixed in the same volume of distilled water (20 ml). These solutions were joined in a specific ratio and the final volume obtained through distilled water.

3.2.8 Tannic acid stock solution

Tannic acid (2 mg) was dissolved in (2 ml) of distilled water.

3.2.9 Phenol

Phenol (5 g) was added in (100 ml) of distilled water.

3.2.10 Glucose solution

Glucose (1 g) was dissolved in 100 ml of distilled water.

3.2.11 Acetic acid

Acetic acid (20 ml) was added to 80 ml of distilled water.

3.3 Methods

3.3.1: Sample Collection

Leaves of different plants *(Moringa oleifera, Acacia nilotica, Petroselinum crispum)* were collected from the local area of Lahore in April 2020. The leaves were cleaned, air dried under shade, grounded with pestle and mortar and stored in air tight container.

3.3.2 Preparation of Extract

Powdered dried material (1 g) was macerated in (40 ml) distilled water in a 250 ml conical flask at stirrer with heating ability and stirring process was continued for 20 minutes. Then the mixture was filtered through Whatman No. 42 paper. This extract was used for Phytochemical screening tests.

3.4 Qualitative Phytochemical Analysis

3.4.1: Test for proteins

(a) Ninhydrin test

Crude extract (1 ml) was boiled with 1 ml of solution of Ninhydrin. Violet color indicated the presence of amino acids and proteins.

3.4.2: Test for Carbohydrates

(a) Molisch test

Five drops of Molisch reagent were added to crude plant extract (1 ml) and the mixture was mixed. After that, 1 ml of concentrated H_2SO_4 was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate

(b) Benedict's test

Crude plant extract (1ml) was mixed with (1ml) of Benedict's reagent and boiled for 5 minutes. Reddish brown precipitate indicated the presence of the reducing carbohydrates.

(c) Iodine test

Crude plant extract (1 ml) was mixed with few drops of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate (polysaccharides).

Test for tannins

Crude plant extract (1 ml) was mixed with 1 ml $FeCl_3$ solution. Blackish-blue or blackish-green color indicated the presence of tannins.

3.4.4: flavonoid's test

(a) Test for alkaline reagent

About 1 ml crude extract was dissolved in 2 ml solution of sodium hydroxide to make its 2% solution. Appearance of deep yellow color that converted into a colorless solution by the drop wise addition of diluted acetic acid indicated the flavonoids presence.

3.4.5: Phlobatannins test

1.0 ml of crude extract was dissolved in 1% aqueous hydrochloric acid and boiled on the stirrer having hot plat and appearance of precipitates of red color confirmed that the reaction is positive.

3.4.6: Steroid test

About 1.0 ml of crude extract was dissolved in 2.0 ml of chloroform and in addition, concentrated sulfuric acid was mixed through the side of test tubes. Red color produced with lower chloroform confirming the presence of steroids.

3.4.7: Terpenoids test

About 1.0 ml of crude extract was mixed in 2.0 ml chloroform and then dehydrated the solution. About 2.0 ml concentrated H_2SO_4 was mixed carefully and then boiled this solution for 2.0 minutes. Appearance of greyish color indicated presence of terpenoids.

3.4.8: Alkaloids test

About 1.0 ml of crude extract was mixed with 1.0 ml HCl and heated in a gentle way. Then few drops of Hager's reagents were mixed to this solution. Turbidity of resulting precipitate was taken as evidence for the presence of alkaloids.

3.4.9: Test for saponins

One gram of each of the powdered material was put within test tube and about 10 ml of water was mixed. The test tube was stoppered and shacked for thirty seconds and then allowed to stand undisturbed for 15 minutes. The appearance of foam that persists for 30 minutes confirmed presence of saponins.

Chapter 4

Results

4.1 Physical properties of extracts

The extracts of different plants (*Moringa oleifera, Acacia nilotica, Petroselinum crispum*) were made in two different solvents i.e., distilled water and acetone. Aqueous extracts of *Moringa oleifera was* brownish yellow while blackish green color was observed for acetone extract. Aqueous extract of *Acacia nilotica was* mustard yellow color while green color was observed for acetone extract and *Petroselinum crispum* acetone extract was light yellowish green and aqueous extract was green. Acetone extracts of *Moringa oleifera, Acacia nilotica, Petroselinum crispum* were free flowing while in case of aqueous extracts , extract of Kikkar was viscous while *Petroselinum crispum* and *Moringa oleifera* aqueous extracts were free flowing (Table 4.1).

Table 4.1: Physical properties of extracts

Plant	Extract	Physical properties	
		Color	**Consistency**
Acacia nilotica	Aqueous	Mustard yellow	Viscous
	Acetone	Green	Free flowing
Moringa oleifera	Aqueous	Brownish yellow	Free flowing
	Acetone	Blackish green	Free flowing
Petroselinum crispum	Aqueous	Light green	Free flowing
	Acetone	Yellowish green	Free flowing

4.2: Qualitative phytochemical analysis of ***Moringa oleifera***

Moringa oleifera aqueous extract gave positive result for proteins. It had some amount of carbohydrates because it gave positive results with Molisch test and benedict test but gave negative result with iodine. Tannins result was positive while phlobatannins, terpenoids, alkaloids in aqueous extract of *Moringa oleifera* leaves were absent. Aqueous extract gave positive result for saponins, steroid and alkaloids (Table 4.2). Negative result was observed for protein with *Moringa oleifera* acetone extract. Molisch test was positive for acetone extract while benedict test and iodine tests were negative. Tannin, alkaloid and terpenoid test were positive for acetone extract of *Moringa oleifera* extract while flavonoid, phlobatannins, saponins and steroids were negative (Table 4.2).

Table 4.3: Qualitative phytochemical analysis of *Moringa oleifera*

Sr. No.	**Tests**		**Aqueous Extract**	**Acetone Extract**
1	Proteins	Ninhydrin	+	-
2	Carbohydrates	Iodine Test	-	-
		Benedict Test	+	-
		Molisch Test	+	+
3	Tannin		+	+
4	Flavonoids		+	-
5	Phlobatannins		-	-
6	Steroid		+	-
7	Terpenoid		-	+
8	Alkaloids		-	+
9	Saponins		+	_

+, Positive: -, Negative

4.3: Qualitative phytochemical analysis of *Petroselinum crispum*

Petroselinum crispum aqueous extract gave negative result for protein. The carbohydrate test was positive because the benedict test and iodine test were positive but in case of Molisch test it was negative. Phlobatannins, tannins and saponins were negative in aqueous extract while flavonoids, alkaloids, steroids and terpenoids were positive (Table 4.4). Acetone extract gave positive result for protein. Carbohydrates were also present in *P. crispum* acetone extract because Molisch test was positive while iodine test and benedict test were negative. Tannins, flavonoids, terpenoids and alkaloids were tested positive in acetone extract while phlobatannins, steroids and saponin were tested negative in acetone extract of *Petroselinum crispum* (Table 4.4)

Table 4.4: Qualitative phytochemical analysis of *Petroselinum crispum*

Sr. No.	**Tests**		**Aqueous Extract**	**Acetone Extract**
1	Proteins	Ninhydrin	-	+
2	Carbohydrates	Iodine Analysis	+	-
		Benedict Testing	+	-
		Molisch Analysis	-	+
3	Tannin		-	+
4	Flavonoids		+	+
5	Phlobatannins		-	-
6	Steroid		+	_
7	Terpenoid		+	+
8	Alkaloids		+	+
9	Saponins		-	_

+, Positive : - , Negative

4.4: Qualitative Phytochemical analysis of *Acacia nilotica*

Aqueous extract of *Acacia nilotica* gave positive result for protein by showing violet color with Ninhydrin that indicated the presence of amino acid and proteins. Test of terpenoids and alkaloids were negative. *Acacia nilotica* aqueous extract gave positive result with Molisch and benedict test for carbohydrates while was negative for iodine test. Blackish green color appeared for tannins that showed that *Acacia nilotica* aqueous extract tested positive for phenols and tannins. Flavonoids, steroids and saponins were also tested positive (Table 4.5).

In acetone extract, protein test was negative. Molisch test was positive while benedict test and iodine tests gave negative result. Flavonoids, phlobatannins and saponins were tested negative while the tannins, alkaloid, terpenoids and steroid results were positive (Table 4.5)

Table 4.5: Qualitative phytochemical analysis of *Acacia nilotica*

Sr. No.	Tests		Aqueous Extract	Acetone Extract
1	Proteins	Ninhydrin	+	+
2	Carbohydrates	Iodine Analysis	-	_
		Benedict Testing	+	_
		Molisch Analysis	+	+
3	Tannin		+	+
4	Flavonoid		+	-
5	Phlobatannins		_	-
6	Steroid		+	_
7	Terpenoid		–	+
8	Alkaloids		_	+
9	Saponins		+	_

+, Positive: -, Negative

Chapter 5

Discussion

Medicinal plants have biological compounds that are used for treatment of various human diseases and also play an important role in healing. The traditional medicine involves the use of different plant extracts or the bioactive constituents. Phytochemicals have two categories i.e. primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain flavonoids, terpenoids, phlobatannins, phenols and alkaloid etc. Medicinal plants have antifungal, antibacterial and anti-inflammation activities.

The phytochemical test of *Moringa oleifera* showed that protein was present in aqueous solution but not in acetone solution, while checking the amount of carbohydrates iodine, benedict and Molisch test were performed in which iodine result was negative for both extracts but Molisch test was positive for both extracts, benedict showed positive test with aqueous extract but was tested negative in acetone extract. In aqueous extract, alkaloid, steroid and saponins were positive while in acetone extract terpenoids and alkaloids were positive. The phytochemical screening of *Moringa oleifera* exhibited that *Moringa oleifera* plant showed the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins and terpenoids in different solvent extracts (Patel *et al*., 2014). Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs (Rhoads and David, 1997). Flavonoids in *Moringa,* enhance the effects of Vitamin C and function as antioxidants. They

are also known to be biologically active against liver toxins, tumors, viruses and other microbes (Korkina and Afanase, 1997). Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal medicines and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical applications (Devi *et al*., 2011). Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects. Saponins cause hemolysis of red blood cells (Winter *et al*., 1993).

The phytochemical analysis of *Petroselinum crispum* showed that proteins were absent in aqueous extract while carbohydrates were slightly positive i.e., Molisch test and iodine test were positive while benedict test. Test for tannins, test for alkaloids and saponin test were all positive while test for flavonoid was positive but phlobatannins and terpenoids were tested negative with aqueous extract. Acetone extract of *Petroselinum crispum* showed positive result of protein with ninhydrin test. Test of carbohydrates, test for phenols and tannins were tested positive while saponins steroids, phlobatannins showed negative result. Phytochemical screening indicated the presence of primary and secondary metabolites in petroleum ether, chloroform, alcohol and water-soluble parts in the drug. Alkaloid, carbohydrate, phenolic compound, tannins, flavonoids, Proteins and amino acids and saponins were detected (Hadi *et al*., 2013)

Phenolic compounds are the most abundant secondary metabolites in plants, playing an important role for growth and protection from the harmful effects of pathogens, parasites and UV visible rays (Al-Qassabi and Hossain, 2018). The result of the ethyl acetate fraction derived from the macetoneic extract exhibited the highest total phenolic content (121.95±2.15, mg GAE/ g extract) and total flavonoids content (106.45±2.18 mg rutin equivalent / g extract). Furthermore, the ethyl acetate fraction demonstrated the more potent (Al Sayed *et al*., 2018).

Aqueous extract of *Acacia nilotica* gave positive results of protein. Test of terpenoids and alkaloids showed negative result. *Acacia nilotica* resulted positive with Molisch test and benedict test for carbohydrates while came negative in iodine test. Aqueous extract tested positive for tannins. Flavonoids, steroids and saponins were also tested positive.

The extract of *A. nilotica* contained the active components terpenoids, tannins, alkaloids, saponins and glycosides. This study showed that *A. nilotica* has useful antimicrobial properties (Banso *et al*., 2009). It suggested that medicinal plants used in the present study might have a general antimicrobial activity. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Fluck, 1973).

Acacia nilotica acetone extract tested negative for protein. Carbohydrates were detected positive only in Molisch test while were negative in iodine test and benedict tests. Tannins, steroid and terpenoid were detected positive. Alkaloids were also tested positive by showing precipitates in test tube while saponins and flavonoids tested negative with acetone extract.

Phytochemical analysis of different solvent extracts revealed the presence of carbohydrates and glycosides, phytosterols, phenolic compounds, saponins, flavonoids, proteins and amino acids, gums and mucilage's in both mace tone and acetone extract (Raghavendra *et al*.,2006). The phytochemical analysis revealed that the antibacterial activity of mace tone and acetone extract is due to the presence of acidic compounds. Antibacterial activity was not observed in isolated compounds indicating the loss of antibacterial activity on further separation of the active fraction (Roberts *et al*., 1981). The phytochemical screening of the *A. nilotica* leaf extracts has shown that the leaf contains saponins, saponin glycosides,

volatile oil, hydrolysable tannin, triterpenoid, tannins, flavonoids, phenol, alkaloids which are very important constituent when looking for pharmacologically active phytochemicals in the plants (Solomon *et al*., 2010).

Conclusion

The phytochemical analysis of *Moringa oleifera* showed the presence of Protein, carbohydrates, flavonoid and saponins in both extracts (aqueous and acetone). Phytochemical analysis of *Petroselinum crispum* indicated the presence of protein, small quantity of carbohydrates and tannins, terpenoids, steroid and alkaloid while in *Acacia nilotica* proteins, minor amount of carbohydrates, alkaline, steroids, terpenoids, alkaloids, and saponins were present.

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