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EVALUATION OF ANTIBACTERIAL ACTIVITY OF MORINGA OLEIFERA

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ABSTRACT

Each part of the *Moringa tr*ee (fruits, seeds, leaves, flowers, bark and roots) is associated with the presence of at least one, or in most number of benefits. *Moringa oleifera* is one of the world's mostly used plants. All parts of the species are traditionally used for different purposes, but leaves are generally the most used all over the world. Fodder scarcity and Low quality of available fodder are considered to be the major constraints hampering the productivity of farm animals. The available feed particularly in a dry season when natural pastures are mature is highly fibrous and inadequate with low nutritive value due to low crude protein content.[1] *Moringa* species are all native to Asia, from where they have been propagated across many parts of the world especially more seen in warm countries, such as Malaysia and other tropical countries. This tree can tolerate temperatures from 19^{o}C to 28^{o}C, and has height from 5 to 10 m and can be cultured throughout the plains. *Moringa* leaves are being used as a medicine because it is said to contain many phyto-chemicals, hence it is used as preventive and curative purposes.

KEYWORDS: *Moringa*, Subabul, Morus, Glyricidia and Sesbania.

INTRODUCTION

INTRODUCTION OF *MORINGA OLEIFERA*

Each part of the *Moringa tr*ee (fruits, seeds, leaves, flowers, bark and roots) is associated with the presence of at least one, or in most number of benefits. *Moringa oleifera* is one of

the world's mostly used plants. All parts of the species are traditionally used for different purposes, but leaves are generally the most used all over the world. Fodder scarcity and Low quality of available fodder are considered to be the major constraints hampering the productivity of farm animals. The available feed particularly in a dry season when natural pastures are mature is highly fibrous and inadequate with low nutritive value due to low crude protein content.[1] *Moringa* species are all native to Asia, from where they have been propagated across many parts of the world especially more seen in warm countries, such as Malaysia and other tropical countries. This tree can tolerate temperatures from 19^{o}C to 28^{o}C, and has height from 5 to 10 m and can be cultured throughout the plains. *Moringa* leaves are being used as a medicine because it is said to contain many phyto-chemicals, hence it is used as preventive and curative purposes.[2] Tree, seeds, fruits as shown in the below Figure.

***MORINGA OLEIFERA* (TREE, FRUITE, SEEDS).**

Many underutilized crops and trees are the main target for the studies by plant scientists, nutritionists, and growers. Of the many plant and tree verities, *Moringa oleifera* is one of these, which has been neglected since several years, but of late the *moringa* is being investigated for its fast growth, higher nutritional attributes, and utilization as a livestock fodder crop. *Moringa* can be grown in areas where growing of other crops is difficult. It can also be grown as a crop on marginal lands with high temperatures and low water availability.[3-6] *M. oleifera* are originated in sub-Himalayan tracts of the Indian sub-

continent. This is one of the fast growing, evergreen, deciduous medium sized perennial tree of about 10 m to 12 m height. The bark has whitish-grey colour and is surrounded by thick cork. Young shoots have purplish or greenish-white bark. Flowers are yellowish creamy white and sweet smelling. The matured fruit is a hanging capsule of 20-45 cm size having 15 to 20 dark brown globular seeds of 1 to 1.2 cm diameter.[7] At present country facing the deficit of green, dry and concentrate at the level of 63.5%, 23.5%, and 64% respectively as a result the CP and TDN availability are not meeting the requirement causing deficit of about 26.5% and 23.70% respectively.[8] Further due to ever-increasing population pressure of human beings, arable land is mainly used for food and cash crops, thus there is little chance of having good quality arable land available for fodder production unless milk production becomes remunerative to the farmer as compared to other crops. The unconventional fodder resources such as Azola, *moringa*, sesbania, cactuses, etc are emergency fodders with high nutritive values.[9] To meet the current level of livestock production and its annual growth in population, the deficit in all components of fodder, dry crop residues, and feed has to be met through increasing productivity, utilizing untapped feed resources, increasing cultivable land area or through imports. Trees and browse species like Subabul, Morus, Glyricidia and Sesbania have been used as livestock fodder for centuries in India and many other countries. Most trees and shrubs are easily propagated and not require high management inputs (fertilizers and pesticides) or advanced technology.[10]

Growing condition of *Moringa oleifera*

Moringa oleifera is a widespread multipurpose tree reported to have nutritional, therapeutic and prophylactic properties with several industrial applications. It is well known to the ancient world, but only recently it has been rediscovered due to the tremendous variety of its potential uses. It is a fast growing, a perennial tree which can reach a maximum height of 7 to 12 m up to the crown[11] and found growing naturally at elevations of up to 1000 m above sea level. It can grow well on hillsides, but is more frequently found growing on pasture land or in river basins as a non-cultivated plant. *Moringa oleifera* belongs to the monogeneric family of shrubs and tree *Moringaceae*, considered to have its origin in Agra and Oudh, in the northwest region of India and south of the Himalayan Mountains. It is now cultivated throughout the Middle East, almost the whole tropical belt and it was introduced in Eastern Africa from India at the beginning of 20th century. About 33 species have been reported in the family Moringaceae.[12] Among those, thirteen species namely, *Moringa arborea, Moringa borziana, Moringa concanensis, Moringa drouhardi, Moringa hildebrandtii,*

Moringa longituba, *Moringa oleifera*, *Moringa ovalifolia*, *Moringa peregrina*, *Moringa pygmaea*, *Moringa rivae*, *Moringa ruspoliana*, *Moringa stenopetala* are well known and found worldwide.

1.2 Taxonomical Classification

1.2.1 Kingdom – Plantae

1.2.2 Sub kingdom – Tracheobionta

1.2.3 Super Division – Spermatophyta

1.2.5 Class – Magnoliopsida

1.2.6 Sub class – Dilleniidae

1.2.7 Order – Capparales

1.2.8 Family – *Moringceae*

1.2.9 Genus – *Moringa*

1.2.10 Species – oleifera

1.3 Vernacular names

The plant *Moringa oleifera* is known by several names throughout the world. The synonyms are given below.

1.3.1 Latin – *Moringa oleifera*

1.3.2 Sanskrit – Subhanjana,

1.3.3 Hindi – Saguna, Sainjna

1.3.4 Unani – Sahajan

1.3.5 Ayurvedic – Haritashaaka,

1.3.6 English - Drumstick tree, Horseradish tree

1.3.7 Telugu - Munagakaya[13]

1.4 Morphology

Moringa oleifera is a small fast – growing evergreen or deciduous tree usually grows up to 10 or 12 m in height. It has spreading, fragile branches, feathery foliage of tripinnate leaves, and whitish gray bark.

1.4.1 Leaves

The leaves are bipinnate or commonly tripinnate up to 45 cm long the leaflets are hairy, green and almost hairless on the upper surface. The twigs are hairy and green, these are compound leaves with leaflets of 1–2 cm long. Leaves as shown in the below Figure.

***MORINGA OLEIFERA* LEAVES**

1.4.2 Flowers

The fragrant, bisexual, yellowish white flowers are hairy stalks in spreading or drooping axillary panicles 10 – 25 cm long. Individual flowers are approximately 0.7 to 1 cm long and 2 cm broad and five unequal yellowish – white, thinly veined, spathulate petals, five stamens with five smaller sterile stamens and pistil composed of a 1-celled ovary and slender style. Flowers as shown in the below Figure.

***MORINGA OLEIFERA* FLOWERS**

1.4.3 Fruits

Fruits are trilobed capsules and are referred to pods it is pendulous, brown triangular, and splits into three parts lengthwise when dry 30 – 120 cm long, 1.8 cm wide fruits production mostly occurs in march and April. Fruits contain around 26 seeds during their development stage. Immature pods are green in color they turn brown on maturity. Fruits as shown in the Figure No.1.4.

***MORINGA OLEIFERA* FRUITS**

1.4.4 SEEDS

Seeds are round 1cm in diameter with brownish semi – permeable seed hull with 3 papery wings hulls of seed are brown to black but can be white if kernels are of low viability. Viable seed germinate within 2 weeks, each tree can produce around 15,000 to 25,000 seeds/year. Average weight is 0.3 gm/seed. Seeds are as shown in the below Figure.[14]

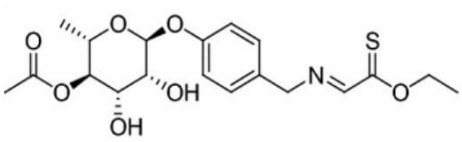
***MORINGA OLEIFERA* SEEDS**

1.5 Phytochemical constituents[15]

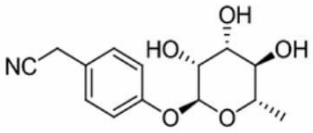
Some phytochemical constituents of *Moringa oleifera* plant are found in various parts of the plant. According to the literature; these constituents are listed in tabular form in table-1 and their chemical structures are shown in figure.

Table-1.1 Chemicals and phytoconstituents.

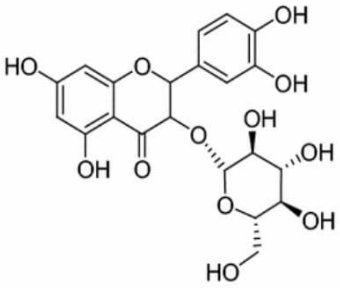
Sr. No	Plant part	Extract	Phytoconstituents
1	Leaves	Aqueous and alcoholic	Niazirin and Niazirinin – nitrile glycosides, Niaziminin A, and Niaziminin B, alpha and gammatocopherol.2
2	Seeds	Aqueous and Hydro-alcoholic	Methionine, cysteine, benzyl glucosinolate, niazimicin niazirin.
3	Flowers	Hydro-alcoholic	D-glucose, quercetin, iso-quercetin, kaempferol, kaempferitrin and ascorbic acid, protein, D-mannose.
4	Root	Alcoholic	Moringine, moringinine, spirachin, 1,3-dibenzyl urea, alpha- phellandrene, p-cymene,



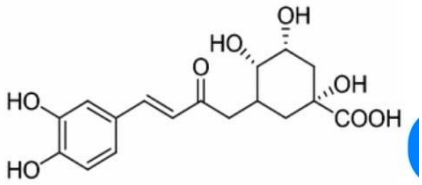
Niaziminin A



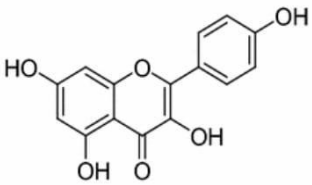
Niazirin



Quercetin-3-glucoside



Chlorogenic acid



Kaempferol

1.6 GEOGRAPHICAL DISTRIBUTION

Moringa (*Moringa oleifera*) is a plant native to the sub-Himalayan regions of northern India and Pakistan. It has been introduced to all tropical and subtropical regions and has naturalized in many African countries (Bosch, 2004; Vélez-Gavilán, 2017). It is widely cultivated in Ethiopia, the Pacific Islands, Florida, Sudan, the Caribbean, the Philippines, West Africa,

South Africa, Asia and Latin America. It is therefore very popular and has several names around the world. The generic name comes from the Tamil name morunga (Olson, 2010).

Global *moringa* production was estimated at 46,000,000 tons in 2017, up from 32,000,000 tons in the previous year or an increase of 43.75% (Nils-Gerrit, 2020). India is the world's largest producer of *moringa* with an annual production of 1.1 to 1.3 million tons of tender fruit on an area of 38,000 hectares (Rachana *et al*., 2020).[16]

India is the largest producer of *Moringa* with an area of 43,600 hectares and an annual production of 2.6 million tonnes of fruits, producing approximately 63 tonnes per hectare in the year of (2023). Tamilnadu leads in area and production, followed by Andhra Pradesh and Karnataka. Tamilnadu covers an area of 20,684 hectares. Among these areas, Dindigul has an area of 5538 hectares followed by Theni (2951 hectares). The specific objectives of this study include the production and marketing of *Moringa*. A list of *Moringa* farmers was selected, including 120 farmers from Dindigul and Theni districts. Farmers were interviewed through a previously developed and tested survey. Likewise, 30 traders were selected from the study area and interviews were conducted. Farmers reported an average annual return of Rs. 1, 95,000 per acre/year, net return was Rs. 1,31,000 per acre per year for cultivation of perennial *Moringa* and Total gross income per acre/year was Rs 2 Lakh and the annual net income per acre was Rs 1.19 million from annual *moringa* cultivation. Of the two marketing channels identified, the producer's share in consumer rupee was highest in channel II (61%) and lowest in channel I (53%). Channel II has the best marketing efficiency followed by Channel I.

Moringa oleifera is a shrub that can reach 10 m high with opposite, tripinnate and deciduous leaves. This means that the old leaves turn yellow and fall off at the foot of the tree. Each leaf is up to 50 cm long, often up to about 90 cm long.[17]

1.7 INTRODUCTION OF ANTIBIOTICS

1.7.1.1 DEFINITION: An antibiotic is a product produced by a microorganism or a similar substance produced wholly or partially by chemical synthesis, Which in low concentrations, inhibits the growth of other microorganisms.

1.7.1.2 HISTROY

Antibiotics use is found since ancient times. Topical application of mouldy bread, with references to its beneficial effects arising from ancient Egypt, China, Serbia, Greece and

Roman was used by many civilizations. John parkinson (1567-1650) was the first person to directly document the use of moulds to treat infection.

Antibiotics truly revolutionized medicine in the 20th centuary by Alexander Fleming (1881-1955). The first commercially available antibacterial was Prontosil, a sulphonamide developed by the German biochemist Gerhard Domagk in the 1930s.

Before this in 1928, Alexander Fleming had discovered the first antibiotic, penicillin, but it took over a decade before penicillin was introduced as a treatment for bacterial infections. The inroduction of penicillin marked the beginning of the so- called “golden era” of antibiotics. Most of the antibiotic classes used at present were discovered between 1940 and 1962. Each class typically contains several antibiotics that have been discovered over time or are modified versions.[18]

1.7.1.3 CLASSIFICATION

Antibiotics can be classified in several ways, including by mechanism of action, chemical structure, or spectrum of activity. Here's a breakdown by each method.

1.7.1.3.1 By Mechanism of Action

1.7.1.3.1.1 Inhibitors of Cell Wall Synthesis

1.7.1.3.1.1.1 Beta-lactams (Penicillins, Cephalosporins, Carbapenems, Monobactams)

1.7.1.3.1.1.2 Glycopeptides (Vancomycin, Teicoplanin)

1.7.1.3.1.2 Inhibitors of Protein Synthesis

1.7.1.3.1.2.1 30S ribosomal subunit inhibitors: Aminoglycosides, Tetracyclines.

1.7.1.3.1.2.2 50S ribosomal subunit inhibitors: Macrolides, Chloramphenicol, Clindamycin, Linezolid.

1.7.1.3.1.3 Inhibitors of Nucleic Acid Synthesis

1.7.1.3.1.3.1 DNA gyrase inhibitors: Fluoroquinolones (Ciprofloxacin, Levofloxacin)

1.7.1.3.1.3 RNA polymerase inhibitors: Rifampicin.

1.7.1.3.1.4 Antimetabolites (Inhibit folate synthesis)

1.7.1.3.1.4.1 Sulfonamides (e.g., sulfamethoxol, sulfadiazine),

1.7.1.3.1.4.2 Trimethoprime

1.7.1.3.1.5 Disruptors of Cell Membrane Function

1.7.1.3.1.5.1 Polymyxins (e.g., Polymyxin B, Colistin)

1.7.1.3.1.5.2 Lipopeptides (e.g., Daptomycin)

1.7.1.3.2 By Chemical Structure

1.7.1.3.2.1 Beta-lactams: Penicillins, cephalosporins, carbapenems, monobactams

1.7.1.3.2.2 Macrolides: Erythromycin, azithromycin, clarithromycin

1.7.1.3.2.3 Fluoroquinolones: Ciprofloxacin, levofloxacin, moxifloxacin

1.7.1.3.2.4. Aminoglycosides: Gentamicin, tobramycin, amikacin

1.7.1.3.2.5 Tetracyclines: Tetracycline, doxycycline, minocycline

1.7.1.3.3 By Spectrum of Activity

1.7.1.3.3.1 Broad-spectrum antibiotics

Examples: Tetracyclines, Chloramphenicol, Carbapenems

Amoxycillin

1.7.1.3.3.2 Narrow-spectrum antibiotics

Examples: Penicillin G (Gram-positive), Aztreonam (Gram-negative)[19]

1.7.1.4 AMOXYCILLIN (STANDARD DRUG)

Amoxicillin (also spelled amoxicillin) is a semi-synthetic antibiotic derived from penicillin, developed to overcome some of the limitations of earlier penicillin such as poor oral absorption and a narrower spectrum of activity. First introduced in the 1970s, amoxicillin has since become one of the most commonly prescribed antibiotics worldwide due to its efficacy, safety profile, and broad spectrum of activity.

Amoxicillin belongs to the β-lactam class of antibiotics, which are characterized by the presence of a β-lactam ring in their molecular structure. This ring is critical to their mechanism of action, as it allows the antibiotic to bind to and inhibit penicillin-binding proteins (PBPs) that are essential for bacterial cell wall synthesis.

1.7.1.4.1 Compared to ampicillin, another early aminopenicillin, amoxycillin has.

1.7.1.4.2 Improved oral bioavailability

1.7.1.4.3 Longer duration of action

1.7.1.4.4 Better absorption from the gastrointestinal tract, even in the presence of food

1.7.1.4.5 Because of these advantages, amoxycillin is suitable for both children and adults, and it is available in a variety of dosage forms, including tablets, capsules, oral suspensions, and pediatric drops.

1.7.1.4.6Amoxycillin is effective against a wide range of aerobic Gram-positive and certain Gram-negative organisms, including Streptococcus.

1.7.1.4.7 Enterococcus and non-β-lactamase-producing strains of Haemophilus influenzae and Escherichia coli.

1.7.1.4.8 However, its effectiveness can be limited by β-lactamase enzymes produced by resistant bacteria. To address this, amoxycillin is often combined with clavulanic acid, a β-lactamase inhibitor, in commercial preparations like Augmentin.

1.7.1.4.1 In clinical practice, amoxycillin is commonly used for.

1.7.1.4.1.1 Respiratory tract infections

1.7.1.4.1.2 Otitis media

1.7.1.4.1.3 Urinary tract infections

1.7.1.4.1.4 Sinusitis

1.7.1.4.1.5 S kin infections

1.7.1.4.1.6 Part of combination therapy for Helicobacter pylori in peptic ulcer disease

Due to its extensive use, bacterial resistance to amoxycillin has become a growing concern, emphasizing the need for judicious use and antibiotic stewardship

1.7.1.4.1.7 Amoxicillin is included in the World Health Organization's List of Essential Medicines, highlighting its importance in global health.

1.7.1.4.2 Mechanism of Action of Amoxycillin

1.7.1.4.2.1. Targets Penicillin-Binding Proteins (PBPs)

1.7.1.4.2.1.1 Amoxicillin binds to PBPs on the inner membrane of the bacterial cell wall.

1.7.1.4.2.1.2 PBPs are enzymes that help build the peptidoglycan layer, a key structural component of the bacterial cell wall.

1.7.1.4.2.2. Inhibits Peptidoglycan Cross-Linking

1.7.1.4.2.2.1 By binding to PBPs, amoxicillin blocks the cross-linking of peptidoglycan chains, which are essential for cell wall strength and rigidity.

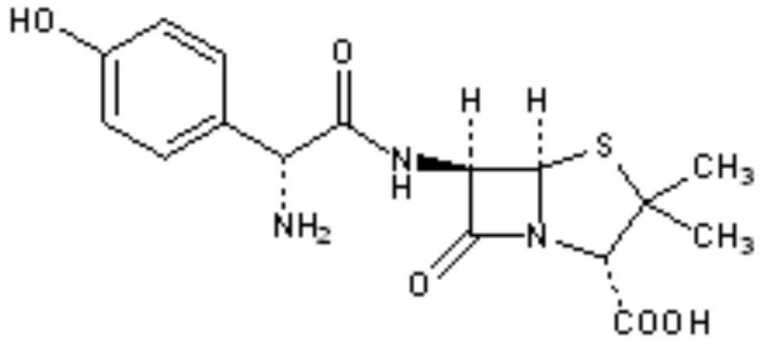
1.7.1.4.2.3. Weakens the Cell Wall

1.7.1.4.2.3.1 Without proper cross-linking, the cell wall becomes weak and unstable.

1.7.1.4.2.4. Causes Cell Lysis and Death

1.7.1.4.2.4.1 The weakened cell wall cannot withstand osmotic pressure, leading to bacterial lysis (bursting) and ultimately death of the bacteria.

Amoxycillin as shown in the below Figure.[20]



CHEMICAL STRUCTURE OF AMOXYCILLIN

CHAPTER-2

2. LITERATURE REVIEW

2.1 S Sukla, R Mathur.[1988]

An aqueous extract of *Moringa oleifera* roots was investigated for its estrogenic, antiestrogenic, pregestational and antiprogesterone activities. Oral administration of extract progressively increased the uterine wet weight of bilaterally ovariectomized rats. This estrogenic activity was supported by stimulation of uterine histo-architecture. When the extract was given conjointly with oestradiol dipropionate (EDP), there was a successive reduction in the uterine wet weight when compared to the gain with EDP alone and uterine histological structures were also inhibited. In the deciduoma test, the highest dose of 600 mg/kg interfered with the formation of deciduoma in 50% of the rats, showing some antiprogesterone activity. Doses up to 600 mg/kg of the extract orally failed to induce a decidual response in the traumatized uterus of ovariectomized rats. The antifertility effect of the extract appears to be due to multiple attributes.[20]

2.2 Jameel Fahad, Vijayalaxmi. [2010]

In the present study, aqueous extract of bark of *Moringa oleifera* administered orally, was evaluated for its antiurolithiatic potential in albino rats of Wistar strains. The stones were produced in this study by zinc disc foreign body insertion in the bladder supplemented with

1% ethylene glycol in drinking water. The reduction in weight of the stones was used as criteria for assessing the preventive or curative antiurolithiatic effect of the bark of this plant. Two doses of extract for prophylactic and curative groups were used. In both groups the oral administration of the extract of bark of *Moringa oleifera* has resulted in significant reduction in the weight of bladder stones compared to the control group.[21]

2.3 Amrutia Jay, Lala Minaxi. [2011]

Moringa oleifera which is commonly known as drumstick tree has been used for its nutrition value and extensively used as a CNS depressant traditionally. Present work has been carried out to evaluate the anticonvulsant activity of methanolic extract of M. oleifera leaves against pentylenetetrazole and maximal electroshock induced convulsions at different dose level (200 mg/kg and 400 mg/kg i.p.). Diazepam and phenytoin(5mg/kg i.p. and 25mg/kg i.p., respectively) were used as a reference standard. At both the doses it significantly (P< 0.0001)delayed the onset of clonic seizures in PTZ induced convulsions and significantly reduced (P< 0.0001) duration of hind limb extension in MES test. The phytochemical investigation of plant revealed the presence of alkaloids, flavonoids, tannins and saponins as major constituents. The data obtained indicates that methanolic extract of M. oleifera leaves may help to control grand mal and petit mal epilepsy.[22]

2.4 Rajnish Gupta, Manas Mathur. [2012]

Moringa oleifera, a widely cultivated species in India, is an exceptionally nutritious vegetable with a variety of potential uses in treating rheumatism, venomous bites, and microbial infections. In the present study, we investigated the antidiabetic and antioxidant effects of methanol extracts of M. oleifera pods in streptozotocin -induced diabetic albino rats.[23]

2.5 Jacob Olagbenro Popoola; {2013}

All parts of *Moringa oleifera* are medicinally valuable with overlapping uses in treating myriads of ailments and diseases including body pains and weakness, fever, asthma, cough, blood pressure, arthritis, diabetes, epilepsy, wound, and skin infection. *Moringa* also has robust ability to challenge terminal diseases such as HIV/AIDs infections, chronic anemia, cancer, malaria and hemorrhage. The present study was to obtain ethnobotanical information on the use and local knowledge variation, geographical distribution, and to collect different landraces of *Moringa oleifera* from the different agro-ecological regions in Nigeria, for further studies. Materials and methods: Ethnobotanical data were collected through face to face interviews, semi structured questionnaires and discussions with selected people who had

knowledge about the plant. The fidelity level (FL %) and use value for different use categories of *Moringa oleifera* and its parts were estimated. The variation in ethnobotanical knowledge was evaluated by comparing the mean use value among ethnic, gender and age groups using sample T test. Garmi GPS was used to determine the locations (latitude and longitude) and height in different areas to assess the geographical spread of the species. Results: Seven (7) categories of use (Food, medicine, fodder, fencing, firewood, gum and coagulant) were recorded for *Moringa oleifera*. Food and medicinal uses showed highest fidelity level while the leaves and the seeds were the plant parts most utilized for the same purposes. There were significant differences among the ethnic, gender and age groups regarding the ethno-botanical use value.[24]

2.6 NIVEDITA PATEL, PINAL PATEL et, al (2014)

The aim of the present study was to find out antibacterial property of *Moringa oleifera*, family *Moringaceae*. *Moringa oleifera* is a very useful tree in tropical countries. In ayurvedic all parts of the tree used in different healing procedures for different diseases. The plant leaves are very good nutrient supplement for malnutrition and also used as an antibiotic. To evaluate the antibacterial activity of *Moringa oleifera* leaf extracts, Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus, Proteus vulgaris, Streptococcus mutans, Bacillus subtilus, and Staphylococcus epidermidis bacteria were used. Phytochemical analysis of the leaf in solvents of varying polarity; viz., aqueous, ethanol were also carried out. The phytochemical screening indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins etc., in the both extracts. Well diffusion method was used to assess the antibacterial effect of the extracts on micro-organisms. The ethanolic and aqueous extract were active against all strains but the ethanol leaf extract showed maximumactivity against Streptococcus mutantandaqueous extract shows maximum activity against Proteus vulgaris.[25]

2.7 Dr. Monther Faisal Mahdi, Dr. Rafah Al-Smaism (2015)

Sulfamethoxazole (SMX) belong to the sulfonamide group of antibiotic. It was chosen to be the representative of this group due to its widespread use and detection frequency in the aquatic environment. The thiazolidinones ring has been integrated into a widespread range of known biologically energetic compounds, either as a substituent group or as a replacement of another ring inspired researchers to produce several compounds containing this moiety. Furthermore the chemistry of chalcones has produced serious scientific

readings during the world. Chiefly interest has been concentrated on the creation and biodynamic actions of chalcones, so that diversity of novel heterocycles with good pharmaceutical shape can be designed. Synthetic procedures have been successfully developed for the generation of the target compounds were used six different aromatic para benzaldehydes of (H,OH,OCH3,NO2,Cl & N(CH3)2), and following multi steps reaction procedure. The purity of the products was checked by using thin layer chromatography (TLC). The chemical structure of intermediate and final compounds were characterized and confirmed by measuring their melting points, FT-IR spectroscopy, elemental microanalysis (CHNS) and 1HNMR of final compounds. The preliminary study of antimicrobial activity were done on 3 different strains of bacteria and showed that the final compounds M3(a-f) have significant activity compared with standard drug (Sulfamethoxazole), have moderate to good activity.[26]

2.8 A Agunu, BA Lwal.[2016]

The knowledge of natural drugs has developed together with the evolution of scientific and social progress. In Nigeria, it is a common practice to see herbalist combining the leaves of *Moringa oleifera* Lam (*Moringaceae*) and Andrographis paniculata Burm. F. (Acantha*ceae*) in the treatment of hypertension, diabetes, arthritis, general pains, etc. This study determined the LD50 of this leaves combination, the diuretic property, the anti-inflammatory activity, and compared with each plant alone and standard drugs. The acute toxicity LD50 was carried out using modified method of Lorke. The diuretic activity was determined using the method of Lahlou. The anti-inflammatory activities were determined using the method of Sudipta. The LD50 was observed to be greater than 5000 mg/kg. The combination of leaves extract did not produce any significant activity (diuretic and anti-inflammatory) compared to each plant leaves extract alone at $p < 0.001$. This study suggests that there is no scientific rationale for combining the leaves of these two plants in diuretic and anti-inflammatory medication.[27]

2.9 Kantilata Thapa, Mousami Poudel (2019)

Moringa oleifera (M. oleifera) which is commonly known as Drumstick tree or Horse radish tree, is an angiospermic plant which belongs to family *Moringaceae*. This plant typically belongs to sub-himalayan Northen India, Pakistan, Bangladesh and Afghanistan. The Tropical and sub-tropical area of the world is suitable for the growth of this plant. The Terai, Siwalik, and Middle Mountain regions are the best-suited regions of Nepal for *Moringa*

cultivation due to their elevation and climate conditions. It is commonly referred as "The Miracle Tree" due to its extensive practical and nutritional benefits as well as a remedy to cure many fatal diseases. *Moringa* is also a great supplement to provide essential amino acids, micronutrients, and protein to the typically nutrient-lacking rural Nepalesediet. Especially, pregnant women and infant are benefitted more through high nutrient content of leaves. Every part of *Moringa* has useful properties which can serve mankind and it can be one of the promishing plant for further research activities. The main purpose of this review was to highlight the nutritional, medicinal and commercial properties of *Moringa*, to know the potentiality of *Moringa* cultivation in the context of Nepal, to suggest future directions for research, market and development strategies and to assess the published scientific journal, article on multipurpose use of *Moringa oleifera.*[28]

2.10 Dr Chinmoyee Rani Das (2020)[29]

Moringa oleifera Lam (*Moringa ceae*) is a highly desired plant that can be found in a variety of tropical and subtropical environments. The leaves, roots, seed, bark, and flower of this plant have anticancer, antipyretic, antiepileptic, anti-inflammatory, antiulcer, andantibacterial properties, and are used in indigenous medicine, especially in underdeveloped nations, to treat a number of maladies. The goal of this review is to give thorough information on the phytochemical compounds and pharmacological activities of.

3. AIM AND OBJECTIVES

3.1 AIM

3.2 OBJECTIVES

- To collect and authenticate *Moringa oleifera* leaf samples
- Collect fresh leaves from a reliable source.
- Use solvents (e.g., water,) for extraction.
- Perform hot extraction methods to obtain crude extracts.
- Use methods such as agar well diffusion,
- Test against common pathogenic bacteria like *Escherichia coli, Staphylococcus aureus.*
- Measure and compare zones of inhibition.
- Compare results with a standard antibiotic (positive control)
- Establish the lowest concentration of extract required to inhibit and kill bacterial growth.
- To analyze and interpret results statistically.

- To explore the potential of *Moringa oleifera* as a natural source of antibiotics

CHAPTER-4

PLAN OF WORK

INTRODUCTION

LITERATURE REVIEW AIM AND OBJECTIVES

METHODOLOGY

- ✓ COLLECTION OF MORINGA OLEIFERA LEAVES
- ✓ DRYING OF MORINGA OLEIFERA LEAES
- ✓ GRINDING
- ✓ EXTRACTION
- ✓ CRYSTALLIZATION
- ✓ EVALUATION OF ANTIBACTERIAL ACTIVITY
- ✓ TESTING ORGANISMS
- ✓ PREPARATION OF NUTRIENT AGAR MEDIA
- ✓ ANTIBACTERIAL ACTIVITY

RESULTS AND DUSCUSSION

CONCLUSION REFERENCES

CHAPTER-5

5. METHODOLOGY

5.1 COLLECTION OF *MORINGA OLEIFERA* LEAVES

The collection of *Moringa oleifera* leaves is a critical step in ensuring the quality of the final product, whether it is intended for fresh consumption, medicinal purposes, or processing into powder, capsules, or extracts. Proper methods of harvesting, handling, and post-harvest treatment are essential to maintain the nutritional and medicinal properties of the leaves.

5.1.1 Objectives of Leaf Collection

5.1.1.1 To obtain high-quality *moringa* leaves rich in nutrients.

5.1.1.2 To maintain the sustainability and regenerative capacity of the *moringa* trees.

5.1.1.3 To ensure that collected leaves are suitable for intended uses (fresh market, drying, extraction).

5.1.1.4 To minimize post-harvest losses and maintain leaf quality

5.1.2 Timing of Leaf Collection

The appropriate timing of leaf collection significantly influences the yield and quality.

5.1.2.1 First Harvest: Typically, *moringa* trees can be harvested for the first time 6–8 months after planting, depending on growth conditions.

5.1.2.2 Subsequent Harvests: After establishment, the tree can be harvested multiple times per year generally every 35–45 days in favorable conditions.

5.1.2.3 Ideal Time of Day: Early morning or late afternoon is preferred for harvesting to prevent heat stress and wilting of the leaves. Harvesting during cooler parts of the day also helps in retaining moisture and nutrients.

5.1.3 Maturity Stage for Harvest

5.1.3.1 Young Leaves: Tender and more suitable for fresh consumption.

5.2 Methods of Leaf Collection

5.2.1 Manual Harvesting

Manual harvesting involves hand-picking the leaves or cutting young branches. This method ensures minimal damage to the tree and allows selective picking of high-quality leaves. Steps.

5.2.1.1 Identify healthy, disease-free branches.

5.2.1.2 Pluck individual leaves or cut small branches (15–30 cm long).

5.2.1.3 Place harvested material into clean, ventilated baskets or cloth bags to avoid overheating. (Figure 5.1)

***MORINGA OLEIFERA* LEAVES**

5.3 Precautions During Collection

We avoid harvesting during rainy conditions to prevent microbial contamination. Use sanitized tools (scissors or pruning shears) to prevent the spread of disease.

Do not harvest heavily from a single tree: Leave sufficient foliage to allow for regrowth and maintain the health of the tree.

5.3.1 Select leaves from healthy plants: Plants showing signs of disease or pest infestation should be avoided.

5.3.2 Post-Harvest Handling

Immediately after collection, proper handling is essential to preserve the quality of the moringa leaves.

5.3.3 Cleaning

5.3.3.1 We were rinsed out leaves gently with clean water to remove dust, dirt, and any insects.

5.3.3.2 Allow leaves to drain and dry on clean surfaces or in perforated baskets.

5.3.4 Sorting

5.11.4.1 We removed yellow, diseased, or damaged leaves.

5.11.4.2 Only healthy, green leaves are selected for further processing or sale.

5.3.5 Preservation

5.3.5.1 For drying: We spread leaves thinly in a well-ventilated, shaded area. Avoid direct sunlight which can degrade nutrient content.

For powder production: Dried lea ves should be ground and stored in airtight containers away from moisture, light, and heat.[33]

5.4 Drying Process of *Moringa oleifera* Leaves

5.4.1 INTRODUCTION

Drying is a critical post-harvest operation for *Moringa oleifera* leaves, especially when the leaves are intended for longer storage, powder production, or medicinal use. Proper drying techniques are essential to preserve the nutritional content, color, aroma, and overall quality of the leaves. Due to the delicate nature of *moringa* leaves, special care must be taken to avoid nutrient loss, microbial contamination, and degradation of bioactive compounds.

5.4.1.1 Drying serves to

Reduce the moisture content of leaves to safe levels (< 10% moisture) for storage. Prevent microbial spoilage (fungi and bacteria).

Retain maximum vitamins (especially Vitamin A and Vitamin C), minerals, and antioxidants.

5.4.1.2 Objectives of Drying

5.4.1.2.1 To extend shelf life by lowering the moisture content.

5.4.1.2.2 To maintain the natural green color and prevent browning.

5.4.1.2.3 To preserve the nutritional and medicinal properties.

5.4.1.2.4 To prepare the leaves for further processing such as powdering, encapsulation,or extraction.

5.4.2 Pre-Drying Preparations

5.4.2.1. Selection of Leaves

We selected Only fresh, mature but tender, healthy, and pest-free leaves are selected. We avoid yellow, wilted, diseased, or damaged leaves.

5.4.2.2 Cleaning

Leaves are gently washed in clean, potable water to remove dust, dirt, insects, and other contaminants.

5.4.2.3 Draining

After washing, leaves are drained thoroughly to remove excess surface water. This prevents clumping and microbial growth during drying.[34]

5.5 Drying Methods

There are several techniques available for drying *Moringa oleifera* leaves, depending on the scale of operation, available resources, and intended final quality. Each method has specific pros and cons.

5.5.1 Shade Drying

5.5.1.1 Procedure

5.5.1.1.1 We spread cleaned leaves thinly (in a single layer) on cloth sheets.

5.5.1.1.2 We place the trays in a shaded, well-ventilated area away from direct sunlight.

5.5.1.1.3 We turn the leaves gently 1–2 times daily to ensure uniform drying.

Drying usually takes 4days. (depending on humidity and temperature) Figure as shown in the below.(5.2)

***MORINGA OLEIFERA* DRY LEAVES**

5.5.2 ADVANTAGES

5.5.2.1 Preservation of Nutrients

Moringa leaves are rich in vitamins (especially vitamin A and C) and antioxidants, which are sensitive to sunlight. Shaded drying helps retain more nutrients by protecting them from UV radiation and excessive heat.

5.5.2.2 Better Color Retention

Shade drying preserves the natural green color of *moringa* leaves, which is a sign of quality. Sun drying can bleach or yellow the leaves, making them look less appealing.

5.5.2.3 Improved Flavor and Aroma

Harsh sunlight can degrade delicate compounds responsible for *moringa's* flavor and aroma. Shaded drying maintains a fresher taste and smell.

5.5.2.4 Reduced Risk of Contamination

In shaded, controlled environments, leaves are less exposed to dust, insects, and animal droppings compared to open sun drying.

5.5.2.5 Slower, Controlled Drying

Shaded drying happens at a moderate pace, allowing moisture to escape gradually without "cooking" the plant tissue, which helps maintain texture and medicinal properties.

5.5.2.6 Minimized Loss of Bioactive Compounds

Important bioactive molecules like polyphenols and flavonoids are better preserved in shade drying, enhancing moringa's medicinal value.

5.5.2.7 Energy Efficient

Like sun drying, shaded drying uses no electricity or fuel, making it eco-friendly and cost-effective, especially in rural settings.

5.5.3 DISADVANTEGES

5.5.3.1 Longer Drying Time

Shaded drying is slower than direct sun drying, which can take several days depending on humidity and airflow.

5.5.3.2 Higher Risk of Mold and Spoilage

If the drying area has poor ventilation or high humidity, moisture may stay longer, leading to mold growth or fermentation.

5.5.3.3 Requires More Space

To avoid piling up the leaves (which can trap moisture), you need larger, well-ventilated areas for shaded drying.

5.5.3.4 Labor Intensive

We have to monitor the drying process closely — regularly turning or flipping the leaves to ensure even drying and prevent mold.

5.5.3.5 Not Suitable for Rainy or Very Humid Seasons

In regions or seasons with high humidity or frequent rains, shaded drying without proper control becomes very ineffective.

5.5.3.6 Potential for Contamination if Not Managed Well

If the shaded area is not clean, covered, or protected from insects, rodents, or dust, there's still a risk of contamination.

5.5.3.7 May Need Additional Structures

To create a proper shaded drying setup (like drying racks, mesh trays, or special shelters), there might be a small cost or effort involve.[35]

5.6 GRINDING PROCESS

To process *Moringa oleifera* leaves using an electric grinder, you'll need to first wash and dry the leaves thoroughly. Then, use an electric grinder to pulverize the dried leaves into a fine powder.

5.6.1 Grinding

Electric Grinder: Use a clean electric grinder (such as a small mill or blender) to grind the dried leaves into a fine powder. (Figure 5.3)

Pulverization: Ensure the leaves are completely dry before grinding to avoid clogging the grinder.

5.6.2 Sieving: For a finer powder, we sieve the ground powder through a fine mesh screen. (Figure 5.4)

Storage: We store the powdered leaves in an airtight container in a cool, dry place.[36]

ELECTRIC GRINDER

SEIVING PROCESS OF *MORINGA*

SEIVING PROCESS OF *MORINGA* POWDER

5.7 EXTRACTION PROCESS OF *MORINGA OLEFERA*

5.7.1 Principle

Soxhlet extraction is a continuous extraction technique in which a solvent repeatedly washes over a solid sample to extract desired compounds. The solvent is heated, evaporated, and condensed to drip onto the sample. The soluble components dissolve into the solvent and are collected after many cycles.

5.7.2 Requirements

5.7.2.1 Materials

5.7.2.1 *Moringa oleifera* leaves powder

5.7.2.2 Water

5.7.2.3 Filter paper, Cotton, Grinder.

5.7.3 Apparatus

5.7.3.1 Soxhlet extractor

5.7.3.2 Round-bottom flask (RBF)

5.7.3.3 Heating mantle or water bath

5.7.3.4 Condenser

5.7.3.5 Thimble

5.7.3.6 Stand and clamps

5.7.4 PROCEDURE

5.7.4.1 Setup Preparation

5.7.4.1.1 Place the powdered *Moringa leaves* inside a thimble (or wrap in filter paper) and place it in the Soxhlet extractor.

5.7.4.1.2 Fill the round-bottom flask with an appropriate volume of solvent (e.g., 250 mL water for 50 g powder).

5.7.4.1.3 Attach the Soxhlet extractor above the round-bottom flask and connect the condenser above the extractor.

5.7.4.1.4 Secure the setup with stands and clamps tightly.

5.7.5 Extraction

5.7.5.1 Start the heating mantle to gently heat the solvent.

5.7.4.2 The solvent will evaporate, rise into the condenser, condense into liquid, and drip onto the powdered leaves.

5.7.5.3 When the extractor fills up to the siphon level, it will automatically siphon the solvent (with dissolved extracts) back into the round- bottom flask.

5.7.5.5 This process is repeated continuously for 12 hours or until the solvent in the siphon becomes colorless (indicating complete extraction).

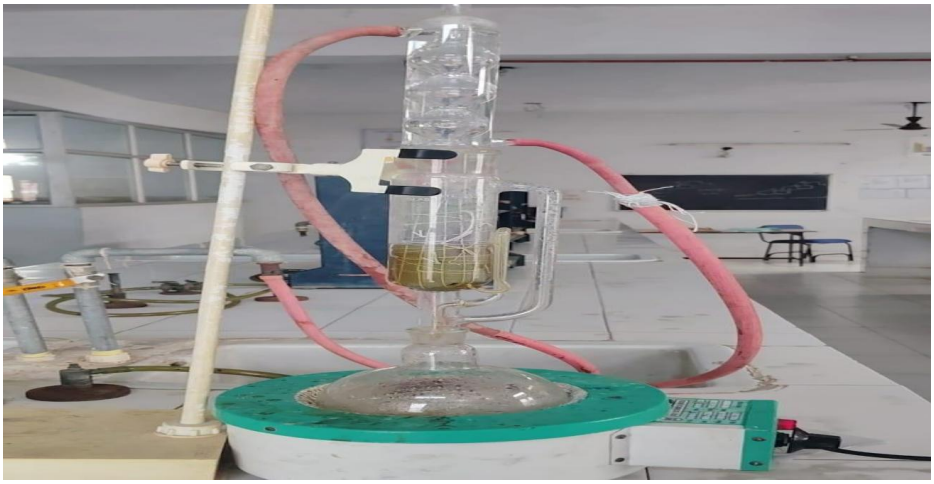
5.7.6 Post-Extraction

5.7.6.1 After extraction, allow the apparatus to cool down.

5.7.6.2 Disconnect the setup carefully.

5.7.5.3 Collect the extract-containing solvent from the round-bottom flask.

5.7.6.4 Concentrate the extract by evaporating the solvent under reduced pressure using by slow evaporation on a water bath.[37] (Figure 5.5)



SOXHLET APPARATUS

5.8 CRYSTALLIZATION

5.8.1 Transfer the concentrated extract to a clean conical flask.

5.8.2 Add a minimal amount of hot water to dissolve the syrupy mass completely (forming a saturated solution).

5.8.3 Allow the saturated solution to cool slowly at room temperature.

5.8.4 Once at room temperature, place the flask in an ice bath for 2–3 hours to promote crystallization.

5.9 ISOLATION OF CRYSTALS

5.9.1 Filter the formed crystals using filter paper.

5.9.2 Wash the crystals gently with a small amount of cold water to remove impurities.

5.10 DRYING OF CRYSTALS

5.10.1 Dry the isolated crystals in a desiccator. (Figure 5.6)

Collect the dried, purified crystals for further analysis.[3]

5.11 EVALUATION OF ANTIBACTERIAL ACTIVITY

The crude extracts of *Moringa oleifera* were screened for their antibacterial activity against Gram-positive and Gram-negative bacteria using Cup plate method.

5.11.1 TESTING ORGANISMS

[staphylococcus aureus (Gram+), E.coli *(Gram⁻)]*

5.11.2 PREPARATION OF NUTRIENT AGAR MEDIA

5.11.2.1 Ingredients (for 1 liter of Nutrient Agar):

5.11.2.2 Peptone: 5.0 g

5.11.2.3 Beef extract (or yeast extract): 3.0 g

5.11.2.4 Sodium chloride (NaCl): 5.0 g

5.11.2.5 Agar: 15.0 g

5.11.2.6 Distilled water: 1000 mL

5.11.3 PROCEDURE

We prepare nutrient agar media, by dissolving 5 g of peptone, 3 g of beef extract, and 5 g of sodium chloride in about 800 mL of distilled water. Add 15 g of agar and heat the mixture while stirring until the agar is completely dissolved. Adjust the final volume to 1000 mL with distilled water. Check the pH and adjust it to 7.0 using HCl. Dispense the medium into suitable containers and sterilize it by autoclaving at 121°C for 15–20 minutes. Once cooled to

around 45–50°C, the medium can be poured into sterile Petri dishes for solid culture used.[39] (Figure 5.7)

5.12 Antibacterial activity

The antibacterial activity of the extracts was determined by using the agar-well diffusion method (Cup plate method). Nutrient agar media was sterilized by autoclaving, cooled, cultures of each test organism was added to Nutrient agar media by spreading and each plate was properly labeled and allowed to set. Cups were made in each Petri plate using sterile cork borer (10mm diameter and about 2cm apart). About 100µl of different concentrations of plant extracts, water and standard drug were added into the wells and allowed to diffuse for 15mins. Then bacterial plates were incubated at 37C for 24 hours. Water serves as blank, Amoxicillin 500mg was taken as standard drug. After incubation measured the diameter of zone of inhibition by using zone reader and mean values were determined at the end of the period, inhibition zones formed on the medium were evaluated in mm.[40] (Figure 5.8)

CHAPTER-6

6. RESULTS AND DISCUSSION

Aqueous extract of *Moringa oleifera* was taken and evaluated for their antibacterial activity against gram positive and gram negative bacteria. The antibacterial activity of the aqueous extracts of *Moringa oleifera* (sample) was shown inhibitory activity against gram positive Staphylococcus aureus and the zone of inhibition is 10mm. Zone of inhibition of aqueous extract *Moringa oleifera* against gram negative Eschericia coli is 12mm. Zone of inhibition of Amoxycillin 500mg (standard) against Staphylococcus aureus is 15mm and E. coli is 15mm. Zone of inhibition of water which act as a negative control against two test organisms is 0mm.[41] (Table 6.1) (Graph6.1)

Table 6.1: Zone of Inhibition.

sample	Concentration (mg/ml)	Zone of Inhibition (mm)	Zone of Inhibition (mm)
		E. coli	Staphylococcus
Aqueous extr act	1mg/1ml	12mm	10mm
Amoxycillin	1mg/1ml	15mm	15mm
Water	1ml	0	0

CHAPTER-7

7. CONCLUSION

The present work shows good antibacterial activity of aqueous extract of *moringa oleifera* against gram positive and gram negative bacteria. It is prooved to be good antibacterial, immunity booster. It can be included in our diet inorder to boost our immunity in current scenario.

REFERENCES

1. Moyo B, Masika PJ, Muchenje V. Effect of supplementing crossbred Xhosa lop-eared goats castrates with *Moringa oleifera* leaves on growth performance, carcass and non-carcass characteristics. Trop. Anim. Health and Prod., 2012; 44(4): 801-09.
2. Udikala M, Verma Y, Sushma, Lal S. Phytonutrient and Pharmacological Significance of *Moringa oleifera*. Int. J. Life. Sci. Scienti. Res., 2017; 3(5): 1387-91.
3. Nouman W, Siddiqui MT, Basra SMA. *Moringa oleifera* leaf extract: An innovative priming tool for rangeland grasses. Turk. J. Agric. For., 2012; 36: 65-75
4. Nouman W, Siddiqui MT, Basra SMA, Afzal I, Rehman H. Enhancement of emergence potential and stand establishment of *Moringa oleifera* Lam. by seed priming. Turk. J. Agric. For., 2012; 36: 227-35.
5. Nouman W, Siddiqui MT, Basra SMA, Farooq H, Zubair M, et al. Biomass production and nutritional quality of *Moringa oleifera* as field crop. Turk. J. Agric. For., 2013; 37: 410-19.
6. Wasif N, Shahzad B, Muhammad TS, Azra Y, Tehseen G, et al. Potential of *Moringa oleifera L.* as livestock fodder crop: A review. Turk. J. Agric. For., 2014; 38: 1-14. 10.3906/tar-1211-66.
7. Singh D, *Moringa* Cultivation for Green Fodder by NDDB. Accessed on 22nd April, http://www.dairyknowledge.in/sites/default/files/moringa-oleifera-eng.pdf and also available on facebook.com/NationalDairyDevelopmentBoard, 2018.
8. Datta D. Indian Fodder management towards 2030: A Case of Vision or Myopia. Int. J. Manag. Soc. Sci. Res., 2013; 2(2): 33-41.
9. Gouri, Mahadevappa D, Sanganal JS, Gopinath CR, Kalibavi CM. Importance of azolla as a sustainable feed for livestock and poultry. Agric. Review, 2012; 33(2): 93- 103.

10. Mendieta-Araica B, Sporndly R, Sanchez NR, Sporndly E. *Moringa (Moringa oleifera)* leaf meal as a source of protein in locally produced concentrates for dairy cows fed low protein diets in tropical areas. Livestock Sci., 2011; 137: 10-17.
11. Nitin, G.S., Bonde, C.G., Patil, V.V., Narkhede, S.B., Patil, A.P., Kakade, R.T. (2008). Analgesic activity of seeds of *Moringa oleifera* Lam, Int. J. Green. Pharm, 2: 108–110.
12. Nadkarni. K.M. (2009). Indian Materia Medica. Bombay Popular Prakashan, I: 811-816.
13. Iswar Chandra, G., Shamim, Q. M.D., Safwan, A.K., Jitendra, P., Rohit Choudhary and Anoopsingh. (2010): Short Communication Evaluation of the Anthelmintic Activity of *Moringa oleifera* seeds. International Journal of Pharma Professional's Research, 1(88).
14. Rhoades, David F. (1979). Evolution of Plant Chemical Defense against Herbivores. In Rosenthal, Gerald A., Janzen, Daniel H. Herbivores: Their Interaction with Secondary Plant Metabolites. New York: Academic Press, p. 41.
15. Julia coppin. (2008). A study of nutritional and medicinal values of *Moringa oleifera* leaves from susaharan Africa: Ghana, Rwanda Senegal and Zambia.
16. Trapti Rastogi. (2009). Comparative Studies on Anthelmintic Activty of *Moringa oleifera* and Vitex Negundo.Asian J. Research Chem, 2(2).
17. Roloff, A., Weisgerber, H., Lang, U., Stimm, B. (2009). *Moringa oleifera* Lam, 1785; Weinheim ISBN: 978-3-. 527-32141-4.
18. Pal, S.K., Mukherjee, P.K., Saha, B.P. (1995). Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. Phytotherapy Research, 9: 463– 465.
19. Talhaliani, P. and Kar, A. (2000). Pharmacological Research, 41(3): 319–323.
20. Faizi, S., Siddiqui B.S., Saleem, R., Siddiqui, S., Aftab, K., Gilani, A.H. (1994). J Nat Prod, 57: 1256-1261.
21. Manguro, L.O., Lemmen, P. (2007). Nat. Prod. Res, 21: 56-68.