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RESEARCH ARTICLE

PRELIMINARY PHYSICO-PHYTOCHEMICAL & PHYTOGNOSTICAL EVALUATION OF THE LEAVES PARTS AND EVALUATION OF HERBAL OINTMENT USING LEAVES OF *MORINGA OLEIFERA* LAM LEAF EXTRACT

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INTRODUCTION

Herbal drugs and their constituents play important role in the in different medicinal system like Unani, Siddha, homeopathy, Naturopathy and Ayurveda. More than 70% populations are uses other than allopathic system of medicine. Herbal plants are wonderful origin of traditional & modern medicine, useful for primary health care system worldwide. Herbal plants have ability for the formation of secondary metabolites such as steroids, phenolic substances, flavonoids, alkaloids, etc. These secondary metabolites are used to treatment of many diseases. In the last few decades due to exponential improvement in the herbal medicine. *Moringa oleifera* is popular in develop countries due to its medicinal importance. Also it is obtained from the natural source and less adverse effects. *Moringa oleifera* Lam belonging to the family Moringaceae brought its importance for its different traditional uses throughout India.

In present scenario some herbal medicines are used as dietary supplement. They are found different dosage form as tablets, capsules, powders, teas, extracts, and fresh or dried plants. Now a day's people use herbal medicines to prevention, treatment and improve their health. *Moringa oleifera* is fast growing, tall, soft wood tree, evergreen or deciduous tree. It normally grows up to 10 to 12 m in height (Basu, 2005). *Moringa oleifera* have been used traditional medicine system associated for centuries, in the ayurvedic system of medicine associated with the prevention or cure of the disease because of its water compelling, water purification capacity and nutritional importance. It is traditionally used as *infections disease*, gastric cancer and gastric ulcer. *Moringa oleifera* also known as horse radish tree and drum stick tree. *Moringa oleifera* Lam is a species of flowering plant within the Moringaceae family, native to plant of sub-Himalay an tracts of India, Pakistan, Bangladesh and Afghanistan.

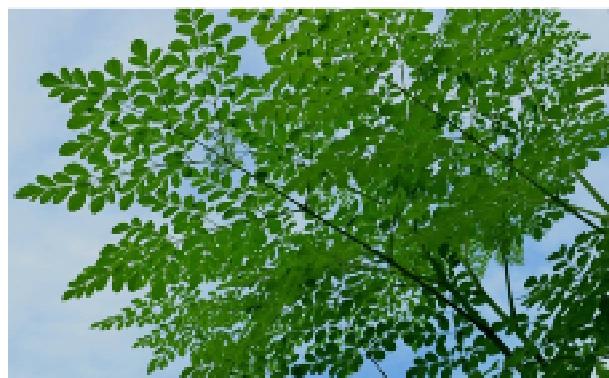
Other species are *M. oleifera*, *M. arborea*, *M. longituba*, *M. peregrine*, *M. pygmaea*, *M. carcanesis*. In India a relatively large percentage of these is used as food by humans. Different parts of the plant is used as medicine such as bark, leaves, seeds, flowers, roots and immature pods. The plant contains important phytoconstituents like alkaloids, tannins, steroidal glycones and reducing sugars. Plant leaves also contains essential amino acids. The whole plant, leaves, roots, flower and bark have huge medicinally importance. *Moringa oleifera* leaves contain vitamin A, vitamin C, iron. It have a rich nutritional profile of leaves which contains vitamins, minerals, and essential amino acids. It contains rich source of antioxidants including beta carotene, vitamin c, quercetin and chlorogenic acid. Chlorogenic acid has been found to lower blood sugar levels (Vaidya, 2007). From a digestive point of view the plant *Moringa oleifera* Lam. good for digestion health. It is highly rich in fibers that as the epoch times put it works like a mop in your intestine, to clean up any of that extra grunge left over from a greasy diet. Also found iso-thiocyanates, which have antibacterial activity that may help to rid our body of *H. pylori*, bacteria implicated in gastric ulcer and gastric cancer. The leaves and seeds of *Moringa oleifera* Lam. may protect against some effects of the arsenic toxicity. Contamination of ground water by arsenic are cause of global health concern. *Moringa oleifera* seeds have been found to work better for water purification function (Gupta, 2010). Taxonomic classification of plant are Kingdom-Plantae, Division-Magnoliophyta, Class- Magnoliopsida, Order- Capprales, Family-Moringaceae, Genus- Moringa, Species- oleifera. Common name of plant are Latin-Moringa oleifera, Sanskrit- Subhajnana, Hindi-Saguna, Sainjna, Unani-Sahajan, Arabian- Rawag, French-Morungue, Spanish-Angela/ Ben, Chinese-La ken, English: Drumstick tree, Hoiseradish tree. Leaves are compound leaves, alternate and non-stipulous, bi-tripinate leaves. Stem are dirty white color with thin skin and rough surface.

The flowers are cream or white, bisexual flowers arranged in axillary panicles with 5 thin spatulated petals, 5 linear lanceolate sepa and 5 yellow stamens. The stamens are inserted at the edge of the disc, with free filaments and unilocular anthers, bent downwards and oblong. The ovary is stipitate and lanceolate. The fruits are in long capsule, trivalved and some with oilseeds. They are dehiscent and 20 to 40 cm in length and contain 12 to 35 seeds per fruit. During vegetative growth they are white and change their color to brown at maturity. Seeds are fleshy and winged 2.5 to 3 mm long. Roots are pivoting with abundant branching reaching 0.4 to 3 m in length (https://www.efloraofindia.org/plant_details.php?cateUrl=tree&plantUrl=moringa-oleifera). Flowering and fruiting time are March-August. *Moringa Oleifera* plants contain zeatin, quercetin, beta-sitosterol, kaempferol and caffeoylguinic acid and minerals- iron, potassium, calcium, copper, zinc, magnesium, manganese etc. Standardization of herbal drugs are difficult because generally mixture of constituents and the active constituent in most cases is unknown. The aim of the current study deal the standardize leaves parts of *Moringa oleifera* L. Secondary metabolites are used to treatment of many diseases. The secondary metabolites provide a rich biogenic source for novel drug discovery. The metabolites produced by different plants vary from each other. In recent scenario herbal ointment is more popular formulation use for external application. The conveying of drugs through the skin are encouraging concept because easy to access, large surface area, vast exposure to the circulatory and lymphatic networks and protective nature of the treatment (Sukanya, 2013). Instead of the alternative formulation like herbal medicine may also be prepared in the form of ointment. These ointment mention a viscous semisolid preparation applied externally on body surfaces area such as the skin, mucus membranes of the eye, vagina, anus, and nose etc. These ointments have specific medicinal values. The medicated ointments contain a medicinal ingredient mixed, suspended or emulsified in the ointment base. Herbal ointment applied externally such as antipuritic, keratolytics, protectants, antiseptics, emollients and astringents. Ointment bases are mainly free from water and generally contain one or more chemical in suspension or solution or dispersion form. Hence Ointment bases may be different types like absorption bases, dehydrating hydrocarbon water soluble type (Bhandari, 2012).

Today in the modern era, the pathogenic bacteria have developed resistance against existing antibiotics because of the extensive use of antimicrobial drugs against the infectious diseases. So some of the active compounds prohibit growth of the disease causing microbes either singly or in combinations. For a long period of time plants have been a precious source of natural products which are used for maintain the human health, especially in last decades with more extensive studies for natural treatments. There is a continuous and immediate need to invent the new antimicrobials compounds with the varied chemical structure and innovative mechanisms of action for new and re-appearing infectious diseases. So scientists are increasingly turning their attention to community medicines, looking for new leads to develop better drugs against microbial infections. Considering that extracts of *Moringa oleifera* show broad spectrum antimicrobial activity. *Moringa oleifera* Lam. is antibacterial activity and also has high potential as antibacterial agent when synthesized as ointment for topical use methanolic extract of *Moringa oleifera* Lam. No proper report was found regarding and preliminary physico-phytochemical phyto-cognostical evaluation of *Moringa oleifera* Lam. till the date. Standardization of herbal drugs are difficult because generally mixture of constituents and the active constituent in most cases is unknown. Now the present study deal the standardize leaves of *Moringa oleifera* Lam. Keeping this view the aim of the current study deal the a Preliminary physico-phytochemical phyto-cognostical evaluation of the leaves parts and formulation and evaluation of herbal ointment using *Moringa oleifera* Lam. leaves extract.

MATERIALS AND METHODS

Fresh leaves parts of *Moringa oleifera* Lam were collected from field of Itaura, Chandeshwar Azamgarh, UP, India in the month of January 2021 and authenticated by Dr. Arti Garg, Scientist-E & Head of Office, Botanical Survey of India, Allahabad, Uttar Pradesh, India. A voucher specimen has been preserved in Department of Natural Product Pharmacy College, Itaura, Chandeshwar, Azamgarh 276128, Uttar Pradesh, India for future reference (Vouchers specimen no. No.PCA /2020-2021/453). The leaves parts were dried under shade and powdered (40 mesh size) and stored in airtight containers. The macroscopic characters were studied as per given procedure in WHO guidelines on quality control methods for medicinal plants materials (World Health Organization, 2002). Fluorescence analysis of powdered leaves carried out according to these method Kokoski et al. (Kokoski, 1958) and Pratt & Chase, (1949).



MACROSCOPICAL STUDIES: The leaves of the plant were studied for their organoleptic evaluation of drug refers to the evaluation of drugs by color, odour, size, shape, taste, size of the leaf and special features including touch and texture etc. Organoleptic evaluation can be done by means of organs of special sense which includes the above parameters and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity and degree of purity (WHO, 2002).

MICROSCOPICAL EVALUATION: Microscopical parameters carried out by using compound microscope attached with a camera (WHO, 2002).

Table 1. Macroscopical evaluation of *Moringa oleifera* Lam leaves

S. No.	Feature	Observation
1.	Color	greenish
2.	Odour	Strong aromatic
3.	Taste	Characteristic
5.	arrangement	Alternate, bi-tripinate

Table 2. Physiochemical Analysis of *Moringa oleifera* Lam Leaves

S. No.	Solvent	Wt. of Plant material (gm)	% age of yield	Color of extract
1.	Pet. Ether	4	1.95%	Yellowish green
2.	Chloroform	4	4.11%	Greenish brown
3.	Methanol	4	6.8%	Dark green
4.	Aqueous	4	4.74%	Blackish green

Table 3. Phytochemical screening of *Moringa oleifera* Lam Leaves

S. No.	Test	Methanolic extract
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Carbohydrates	+
5.	Saponins	+
6.	Triterpenoids	-
7.	Proteins	+

(+)- present, (-)-absent

Table 4. Data showing the Physio- chemical standard values of *Moringa oleifera* Lam Leaves

S. No.	Parameters	Values
1.	Total ash(mg/gm)	3.65%,
2.	Acid insoluble ash(mg/gm)	1.61%,
3.	Water soluble ash(mg/gm)	1.81%
4.	Loss on drying(mg/gm)	5.45%,

Table 5. Composition of the methanolic extract of *Moringa oleifera* Lam leaves ointment

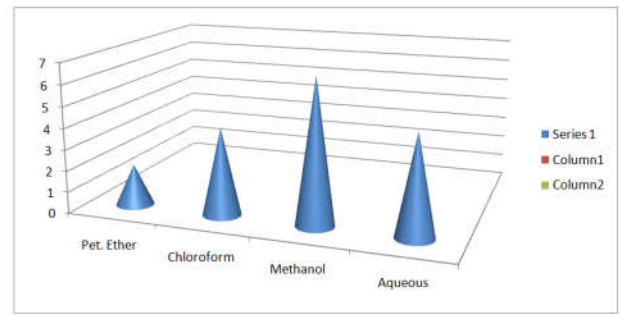
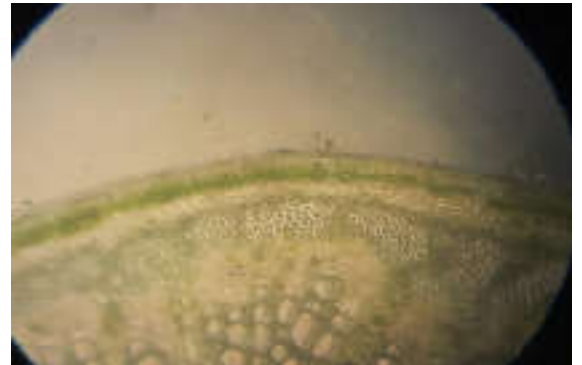
S. No.	Components	Amount(gm)
1.	methanolic extract of leaves Plant <i>Moringa oleifera</i> Lam	2
2.	Emulsifying wax	28
3.	White soft paraffin	50
4.	Liquid paraffin	20

Table 6. Physicochemical parameters of *Moringa oleifera* Lam. herbal ointment formulation

S. No	Physicochemical parameters	Observation
1.	Colour	Greenish
2.	Odour and taste	Characteristic
3.	Loss of drying	0.28%
4.	pH	6.11
5.	Diffusion study	2.11 cm in 1 min
6.	Stability study	Stable with pH 6.7

Table 7. Zone of inhibition of the methanolic extract of *Moringa oleifera* Lam (MEMO) leaves

S. No	Concentration (mg/ml) of MEMO	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
1.	100	18.64 ± 3.07	17.55 ± 2.03
2.	250	20.66 ± 1.55	18.65 ± 3.08
3.	500	23.05 ± 3.03	21.33 ± 3.07
4.	Ciprofloxacin (5 µg)	28.33 ± 3.05	26.00 ± 2.01

**Fig. 1. Extractive value (%) different extract of *Moringa oleifera* Lam Leaves****Fig 2. T.S. of stem of *Moringa oleifera* Lam**

POWDER MICROSCOPY: The dried Leaves of plant *Lantana camara* Linn were powdered and sieved to obtained fine powder. A small quantity of powder was kept on a slide and after mounting on glycerine, after 10 min it was spared. Finally, powder microscopy study was done with the powdered leaves (WHO, 2002; Asoka, 2006).

PHYSICOCHEMICAL STUDIES: The ash values (total ash, acid insoluble ash, water soluble ash), the loss of drying (Bhatia, 2008; Anonymous, 2010), extractive values (petroleum ether 60-80 °C, chloroform, methanol, aqueous) were determined according to the official methods of ayurvedic pharmacopoeia of India (. The Ayurvedic Pharmacopoeia of India; Pharmacopoeia of India, 1996;

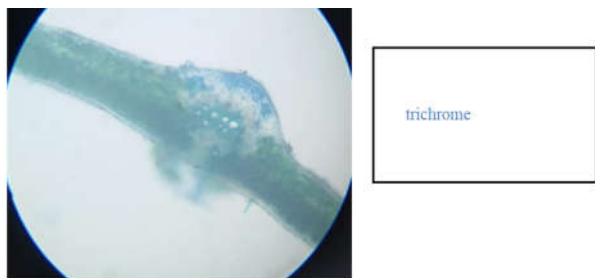


Fig. 3. T.S. of leave of *Moringa oleifera* Lam



Fig. 4. power of leave of *Moringa oleifera* Lam

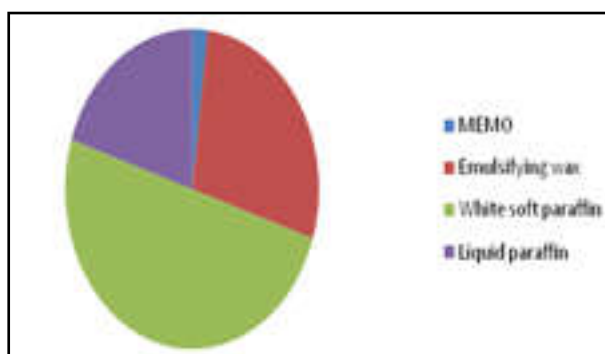


Fig. 5. Composition of the methanolic extract of *Moringa oleifera* Lam leaves ointment

World Health Organization, 1998) were performed according to the official methods prescribed in Indian Herbal Pharmacopeia (Anonymous, 1998). and the WHO guidelines (World Health Organization, 2002).

Total ash: Weigh accurately previously weighed and tarred crucible. Add 2 gm of ground material. Ignite the material by increasing the heat to 500-600 °C. Cool in desiccators and weigh. If carbon free ash cannot be obtained in this manner, cool the crucible and moisten the residue with 2ml. of water or a saturated solution of ammonium nitrate. Dry on a waterbath or hot plate and ignite. Allow the residue to cool for 30 min and weigh. Calculate content of total ash (Khandelwal, 2006).

Acid-insoluble ash: To the crucible containing total ash, add 25 ml of hydrochloric acid, cover with a watch glass and boil gently for 5 minutes. Rinse the watch glass with 5ml of hot water and add this liquid to crucible. Collect the insoluble matter on an ash less filter paper and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, and then ignite at 450-500°C to constant weight. Cool in desiccator for 30 min and weigh without delay. Calculate the content of acid-insoluble ash in mg per gram of air-dried material (Khandelwal, 2006).

Water-soluble ash: To crucible containing total ash, add 25 ml. of water and boil for 5 min. collect water on ash less filter paper. Wash with hot water and ignite for 15 min. at temperature not exceeding 450°C. Subtract the weight of this residue in mg obtained from total weight of total ash (Khandelwal, 2006).

Loss on drying: This test determines both water and volatile matter. Drying can be carried out either by heating to 100-150 °C or by drying in a desiccators over phosphorus pentoxide R under atmospheric or reduced pressure at room temperature for a specified period of time (Khandelwal, 2006).

EXTRACTION METHOD AND PRELIMINARY PHYTOCHEMICAL SCREENING

Moringa oleifera Lam leaves washed with water gently, and then the washed leaves were kept on the tissue paper and shade dried at room temperature for two weeks. The dried plant leaves was powdered and sieved to get fine powder using an electric blender. For the phytochemical screening, the powdered leaves were extracted with petroleum ether (40 °C), chloroform, methanol, aqueous respectively in a series using cold maceration technique. All extract were concentrated in a rotary vacuum evaporator below 40°C and subsequently dried in high vacuum to get solid crude petroleum ether extract (PEMO), chloroform (CEMO), methanol (MEMO), aqueous (AEMO) respectively. Phytochemical screening of the methanolic extract of *Moringa oleifera* Lam leaves was performed for the detection of various phytoconstituents such as alkaloids, steroids, saponins, proteins, flavanoids, tannins, carbohydrates as per standard procedure (Khandelwal, 2006; Khandelwal, 2006; Trease and Evans, 2005; Mukherjee, 2002).

Tests for Alkaloid:

- Extract was treated with 1 ml of Dragendorff's reagent. An orange-red precipitate indicates the presence of alkaloid.
- Extract was treated with 1 ml of Mayer's reagent. Whitish yellow or cream-colored precipitate indicates the presence of alkaloids

Tests for Carbohydrates:

- To 1 ml of the extract, add equal quantities of Fehling A and B, upon heating formation of brick red precipitate indicate the presence of sugar.
- To 1 ml of Benedict reagent, add 1 ml of extract solution and boil.

Test for Tannin

To 1 ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.

Test for Flavanoids: Extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappear on addition of an avid indicate the presence of flavanoids.

Test for Saponins: Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1 cm layer of foam indicates the presence of saponins.

Test for Triterpenoids: Extract (300 mg) was mixed with 5 ml chloroform and warmed for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well. The appearance of red color indicates the presence of triterpenes.

Test for Proteins: The plant powder was extracted in different solvent and solvent free plant extract was mixed with few ml of diluted HCl and filtered. Two drops of ninhydrin solution was added to the filtrate. The mixture was mixed properly. Purple color not seen that indicates absence of proteins.

Test microorganisms: The microorganisms used for the study were *Staphylococcus aureus* and *Escherichia coli*. In this study, multi drug resistant wound separates bacteria from pathology, Civil Line, Azamgarh were used. The bacterial strains were raised and managed on Mueller Hinton agar at 37 °C.

Microbiological media: Chemicals and standard drugs Mueller Hinton Agar and Nutrient broth are collect from the Chemical store of the Pharmacy College, Azamgarh. Gentamicin ointment (1mg of Gentamicin in the form of Gentamicin Sulphate), obtain by medical store of MahaMriyunjai Hospital, Azamgarh.

Evaluation of antibacterial activity of methanolic Extract (MEMO): The antibacterial activity of the methanolic extract of the leaves of *Moringa oleifera Lam* (MEMO) at concentrations of 100mg/ml, 250mg/ml and 500mg/ml were determined using the cup plate method. A molten Mueller Hinton agar stabilized at 45 °C was seeded with 0.1 ml of a 24 h broth culture of the test organism (*E. coli* and *S. aureus*) containing approximately 10⁸ cfu / ml in a sterile Petri dish and allowed to set. Wells of 6mm diameter were created with a sterile cork borer and filled to about three-quarters full with solutions of the methanolic extract of the leaves of *Moringa oleifera Lam*. (MEMO).The plates were pre-incubated for 1 h at room temperature to allow for diffusion of the solution and then incubated for 24 h. The zones of inhibition were measured (mean, n=2). Streptomycin and Gentamycin were used as positive and negative controls respectively. The in vitro bacterial response to the extract are evaluate using the diameter of the zones of inhibition as follows; resistant: 10mm and below, intermediate: 11-15mm and susceptible: 16mm and above (Singh, 2019).

Preparation of Ointments: Three topical ointment bases of varying degrees of aqueous/anhydrous character namely simple ointment BP, emulsifying ointment BP and aqueous cream BP were prepare by fusion method. In this method the constituents of the base were placed together in a melting pan and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for about 5 minutes and then cooled with continuous stirring. Formulation of ointment done by incorporating 10 g of the semisolid methanolic extract of *Moringa oleifera Lam*. into the various bases by triturating in a ceramic mortar with a pestle to obtain 100 g of herbal ointments containing 10 % w/w of *Moringa oleifera Lam*. extract (Guideline, 2012). The prepared herbal ointments were put in ointment tube, labeled and were stored at room temperature.

Evaluation of ointment: The evaluations were carried out on the ointment by using the following parameters

Color and odour: Color and odour of ointment, examine by visual examination.

Loss on drying: 1 g of ointment was placed in the Petridis and heated in the water bath at 105 °C every 30 min until it get constant weight.

pH: The pH of ointment was determined by digital pH meter. 1 g of ointment was dissolved in 50 ml of distilled water and the pH was measured.

Diffusion study: The diffusion study was carried out by preparing agar nutrient medium of any concentration. It was poured into Petridis. A hole bored at the centre and ointment was placed in it. The time taken for the ointment to get diffused was noted.

Stability study: The stability studies are carried out for the prepared ointment at temperature of 37 °C for 2 months.

RESULT AND DISCUSSION

In literature survey it was found that the plant possesses several traditional and pharmacological uses. The macroscopical study of the leaves of *Moringa oleifera Lam* was done. The leaves were evergreen in colour with compound leaves, alternate and non-stypulous, bi-tripinate leaves, in older pale yellow colour (Table-1). Pharmacognostical standardization was essential tool for proper utilization of the plant for pharmaceutical uses. The values of the physical constant like ash values, loss on drying, extractive value were determined. Extractive value and color of extract was investigated (Table-2). Preliminary qualitative phytochemical screening (methanolic extract) shown that presence of alkaloids,

tannins and flavonoids showed the leaves are rich sources of secondary metabolites responsible for different pharmacological activities. (Table-3). *Moringa oleifera Lam* Leaves powder microscopy showed trichome. The preliminary in vitro antimicrobial activity of the methanolic extract of *Moringa oleifera Lam*. (MEMO) presented showed excellent activity against *Staphylococcus aureus*. The in vitro antimicrobial activity of the methanolic extract of *Moringa oleifera Lam*. (MEMO) based herbal ointments. In various case history show that most of the infections are caused by the gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes*. Less common cause by the gram-negative bacteria such as *Escherichia coli*. The methanolic extract of *Moringa oleifera Lam*. (MEMO) leaves showed significant antibacterial activity against all the tested microorganisms. This observation indicates that the activity due to the presence of large varieties of phytoconstituents present in the extract. Hence, the observed antibacterial activities of the ointment are due to the presence of active constituents of the extract and the activity also possess as ointment. This was good sign to do further studies on that to make it as one of the commercial ointment for the treatment of bacterial infections. In literature survey it was found that the plant possesses several traditional and pharmacological uses. The formulation and evaluation of herbal ointment study of the leaves extract of *Moringa oleifera Lam*. were done.

CONCLUSION

Preliminary physico-phytochemical study of the *Moringa oleifera* (L.) Leaves study concluded to macroscopic, other physical values and parameters will help to identify the species of plant, phytochemical screening will help the presence of secondary metabolites, Microscopy is an important tool in the evaluation of crude drugs which is applicable at various levels such as the authentication of the crude drugs, study of powdered drugs, which is responsible for the medicinal & pharmacological importance of the plant. *Moringa oleifera Lam* Leaves is known as wide range of medicinal value, it helps to identification, authentication and standardization. It also require to research on phytochemical and pharmacological aspect. However research going on it would be easier to develop new drugs.

Preliminary physico-phytochemical study of the *Moringa oleifera* (L.) study concluded to macroscopic, other physical values and parameters will help to identify the species of plant, phytochemical screening will help the presence of compounds, Microscopy is an important tool in the evaluation of crude drugs which is applicable at various levels such as the authentication of the crude drugs, study of powdered drugs, study of T.S. *Moringa oleifera* (L.) is known as wide range of medicinal value, it helps to identification, authentication and standardization. In concluded that the present investigation comes out with the fact that *Moringa oleifera* (L.) are required so that better, safe and cost effective drugs for treating *S. aureus* causing diseases. This study shows that *Moringa oleifera* (L.) are antibacterial activity and have high potential as antibacterial agent. When formulated as ointment for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions. The potency of the *Moringa oleifera* (L.) herbal ointment against *Staphylococcus aureus* could be harnessed in the containment of the organism implicated as the commonest etiologic agent of boils, carbuncles, infantile- impetigo and wound. The final product readily spread on skin surface, showed no irritant effect, diffused well and stable at different temperature. It also require to research on phytochemical and pharmacological aspect. However research going on it would be easier to develop new formula.

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