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Review on Lemon Balm Herb and its Evaluation

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Abstract: *The development of drugs from medicinal herbs may be good to find novel therapeutic agents in the treatment of anxiety. Melissa officinalis L. (lemon balm) belongs to the Lamiaceae family and grows widely in the Mediterranean region, western Asia, southwestern Siberia, and northern Africa. Melissa officinalis L. has been used in traditional medicine to treat headaches, indigestion, colic, nervousness, cardiac failure and depression. In addition, it has been reported in several researches that lemon balm had many beneficial effects such as anti-inflammatory and ant nociceptive, Antioxidant. leaves contain several classes of constituents including polyphenolic compounds (rosmarinic acid, caffeic acid and protocatechuic acid), essential oils (geranial, neral, citronellal, geraniol, beta-pinene, alpha-pinene, beta-caryophyllene, germacrene D, and ocimene), monotherpenoid aldehydes, sesquiterpenes, flavonoids (luteolin) and tannins. The aims of this study were to evaluate the phytochemical screening of ethanolic extract of Melissa officinalis L. One study examined a chemically-validated essential oil derived from Melissa and found that Melissa inhibited binding of GABAA to receptor channel in the rat forebrain, but had no effect on or nicotinic acetylcholine receptors (Abuhamdah et al., 2008). They also found that Melissa elicited a significant dose-dependent reduction in both inhibitory and excitatory transmission.. The aromatic balm leaves are often used in beverages and as a seasoning in salads, dressings and sauces, as well as in cooked foods, e.g., in soups and stews. Some vernacular names are balm, common balm, blue balm, dropsy plant, honey plant, Herzkraut, citronelle, cytria, cedronella.*

Keywords: Melissa officinalis L, Lemon balm, Essential oils, Phenolic compounds, Headaches, Anti-inflammatory.

I. INTRODUCTION

Plants are always key source of drugs or treatment strategy in different additional medicinal systems. In recent years, many people are choosing to plant based medicine or product to improve their health condition or as curative substance either alone or in combination with others. Herbal medicine includes herbs, herbal materials (like plant parts) or preparation, processed and finished Herbal products and active ingredients. In India approximately 70% of modern drug are discovered from natural resources and number of other synthetic analogue have been prepared from photo type compounds isolated from plants.

Ayurveda - Three classical Ayurvedic literature, charaka Samhita,sushruta samhita, and astanga hridaya mention about 526,573 and 902 number of plants. About 25% prescription drug found globally are derived from plant sources and nearly 121 such drugs entity in use. 13 drugs of natural origin are approved in US between 2005 to 2007 and clinical trial are going on more than 100 natural product based drugs. Herbs and plants can be processed and can be taken in different ways and forms, and they include the whole herb, teas, syrup, essential oils, ointments, salves, rubs, capsules, and tablets that contain a ground or powdered form of a raw herb or its dried extract. *Melissa officinalis L.* (lemon balm) belongs to the Lamiaceae family and grows widely in the Mediterranean region, western Asia, southwestern Siberia, and northern Africa. *Melissa officinalis L.* has been used in traditional medicine to treat headaches, indigestion, colic, nervousness, cardiac failure and depression. In addition, it has been reported in several researches that lemon balm had many beneficial effects such as anti-inflammatory and antinociceptive, Antioxidant. anti-tumoral, anti-microbial, anti- bacterial, Anxiolytic and antidepressant, anti-herpes, anti-HIV, anti-cardiovascular diseases, Antidysrhythmic,sedative, hypolipidemic and spasmolytic. Phytochemical studies showed that *Melissa officinalis L.* leaves contain several classes of constituents including polyphenolic compounds (rosmarinic acid, caffeic acid and protocatechuic acid), essential oils (geranial, neral, citronellal, geraniol, beta-pinene, alpha-pinene, beta-caryophyllene, germacrene D, and ocimene), monotherpenoid aldehydes, sesquiterpenes, flavonoids (luteolin) and tannins.

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II. MATERIAL AND METHODS

2.1 Plant Profile

Melissa officinalis L.

Melissa officialis (lemon balm) Plants grows in bushy and upright to Maximum height of 100 cm (39 inch) belonging to family lamiaceae. The heart shaped leaves are 2- 8cm (0.79 to 3.50) inch long and have a rough vein surface. They are soft and hairy with scalloped edges and have a mild lemon scent. During summer small white or pale pink flowers are appear. It gives zesty scent that make it perfect for aromatic teas. Lemon balm (Melissa *officinalis L)* is a perennial herbaceous plant in the mint family and native to south central Europe, The Mediterranean Basin, Iran and Central Asia, but now naturalized elsewhere. The second name, "officinalis" originates from the use of the herb by Apothecaries, who sold herbal remedies directly to their customers. *Melissa Officinalis* also known by different names, Bee Balm, Cure All, Dropsy Plant, Honey Plant, Sweet Balm, sweet Mary, Toronjil. Romans introduced lemon balm to Great Britain where it became a favourite cottage garden herb.

Chemistry

Lemon Balm contains high flavonoids – which can have antioxidant effect. also contains Eugenol, Tannins and Terpenes, (+) – Citronellal, 3- octanol, Caffeic Acid, Caryophyllene, Catechin, Citral A, Citral B, Geranial, Geraniol, iso – geranial, Rosmarinic Acid. VITAMIN – Vit B and Vit C, MINERALS – Potassium, sodium, magnesium, calcium and iron potassium. (Potassium is useful for heart). Rosmarinic acid appears to be the most important active component leaf contains, 36.5 + /- 0.8 mg Rosmarinic acid per gram.

III. TAXONOMIC HIERARCHY AND NOMENCLATURE

Kingdom	Plantae
Subkingdom	Viridiplatae
Infrakingdom	Streptophyta
Super division	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophyta
Class	Magnoliopsida
Superorder	Asteranae
Order	Lamiales
Family	Lamiaceae
Genus	Melissa L.
Geographic division	North America
Origin	Continental US, Canada.

Table 1: Taxonomical hierarchy and nomenclature

3.1 Collection of Plant Material

The *Melissa officinalis L*. was collected from Vishwa High-tech Nursery, Virgoan, Akole.plant was harvested in October 2020. The samples were packed instantly in polyethylene bags to avoid decomposition of some bioactive compounds**.**

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Figure 1: Lemon balm leaves Fig no.2. Dried powder of lemon balm

3.2 Preparation of Dried Powder

The leaves were dried under shade and some are also in hot air oven by spreading stems and leaves on the drying trays of a dehydrator. Setting the temperature at its lowest setting (95°F or 35°C) and dry for 12 to 18 hours and then made into powdered form using mortar and pestle and then sieved.

Figure 3: Dried powder of lemon balm

3.3 Herbarium of Plant

Fig. 4: Herbarium of plant

For preparation of herbarium, Whole specimen is used. Specimens are properly dried in the paper and taking a change of the plant, press and mounted on sheet and filling of herbarium table.

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IV. EVALUATION SCREENING

4.1 Morphological Evaluation

Lemon balm is a perennial plant that reaches a height of 1 m with hairy, deeply veined, heart-shaped leaves which are 2–8 cm long. Flowers are white or pale pink in small clusters. Lemon balm is a cross-pollinating species and its ovate seeds are black or dark brown. Lemon balm has a hairy root system with many lateral roots which better adapt to different environmental conditions. The number of lateral shoots ranged from 16.6 to 38.5. The length of the leaf was from 3.68 cm to 5.71 cm and its width oscillated from 2.82 cm to 4.42 cm. The internode length varied from 2.18 cm to 4.03 cm. The dry weight of the herb ranged from 157.0 g to 339.3g. The seed yield oscillated from 22 g to 182 g and the weight of 1000 seeds w compounds of lemon balm essential oil. New shoots start to appear early in spring, so they can also suffer from spring frosts. It gives zesty scent that's makes it perfect for aromatic teas. as from 0.356 g to 0.632 g. Essential oil content oscillated from 0.05% to 0.44%. Citral, citronellal and caryophyllene were the main compounds of lemon balm essential oil. New shoots start to appear early in spring, so they can also suffer from spring frosts. It gives zesty scent that's makes it perfect for aromatic teas.

4.2 Microscopic Evaluation

Safranin, light green, phloroglucinol, concentrated hydrochloric acid and iodine were used for preparation the plant sections and the powder. Surface preparations, transverse sections (T.S.) as well as the powder of the leaf, stem and root were used for observation of various microscopic features. All microscopical investigations were done by using microscope.

4.3 Extraction

Powdered leaves (40 g) were exhaustively extracted in a Soxhlet apparatus at a temperature of 60 °C with 250 ml of 96% ethanol. Extract was filtered, evaporated using rotary evaporator at 40 °C, and stored at 4°C. The yield of the extract was 9.814% based on dry weight.

V. PHYSICO-CHEMICAL EVALUATION

- **pH:**Lemon balm will grow in a relatively wide pH range between 5.6 (acidic) and 9.0 (strongly alkaline) with a preferred range of 6.0 to 7.5.
- **Loss on drying :** Leaves are dried under the shade and dried powder of *Melissa Officinalis* was taken and weighed. The weight is taken repeatedly until the constant weight was not obtained. The most important factor of drying conditions is the drying air temperature profile. Five different drying air temperature profiles was used to maintain the quality parameters of lemon balm samples while reducing the drying time as much as possible.
- **Ash value : a.** An accurately weighed quantity (2 g) of the shade dried powder of *Melissa Officinalis L* was incinerated in a crucible at a temperature of 450°C in a muffle furnace until carbon free ash was obtained and then cooled, weight **b.** Determination of acid insoluble ash **:** The ash obtained was boiled with 2M HCL (25ml) for five minutes and it was filtered using an ash less filter paper. insoluble matters retained on the filter paper and it was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace.

VI. PHYTOCHEMICAL SCREENING

The qualitative assay of the major metabolite of families focused on the crude extract, all was based on the staining reactions and precipitation.

6.1 Detection of Sterols and Polyterpenes

These families of compounds were tested using **Liebermann-Burchard's test.** A blue-green ring between layers indicates the presence of steroids and pink- purple ring is an indication of the presence of terpenes.

6.2 Detection of Redusing Sugar

Reducing sugars were identified in crude extract using the Fehling reagent, and then Sugars presence was confirmed by **Tollens's test**. To 5 ml of crude extract, we added 5 ml of Fehling's solution. The appearance of a red brick precipitate

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after 2-3 min of heating bath at 70 °C indicates the presence of reducing sugars. The detection of reducing sugars by the Tollens test consisted of the addition of 5 ml of crude extract to 5 ml of the Tollens reagent. After a few minutes, a silver mirror is formed which is an indication about a positive reaction.

6.3 Detection of Alkaloids

Alkaloids were characterized utilizing **Boucharde's reagent** (iodo-iodized solution) and **Dragendorff's reagent** (potassium bismuth iodide solution). 6 ml of extract were evaporated to dryness. The residue was taken up in 6 ml alcohol at 60°. Two drops of Dragendorff's reagent were added to the alcoholic solution; occurrence of reddish-brown p**9.4**

Detection of Protein

The proteins were estimated in the extracts using the biuret method. An aliquot of extract was dissolved in 2 ml of 20% aqueous NaOH in a test tube, followed by the addition of 2 -3 drops of an aqueous solution of 2% CuSO4. The appearance of a purple colour indicates the presence of proteins in the solution.

6.4 Detection of Coumarins

The coumarins have been identified in the extract using the reaction with the lactone ring. In two test tubes, we introduced 2 ml of ethanolic solution obtained from the residue. We added 0.5 ml of 10% NaOH to the contents of one of the tubes. Next, we held the test tubes in a water bath to boiling. After cooling the mixture, 4 ml of distilled water were added to each test tube. In the case of a positive reaction, the liquid from the test tube where the alkaline solution was added is transparent or more transparent compared to the control test tube liquid (without alkaline solution). Acidifying the clear solution with a few drops of concentrated HCl, it loses its yellow colour, is troubled or it forms a precipitate.

6.5 Detection of Tannins

The tannin contents were determined using Stiasny's reagent. A volume of 5 ml of extract was evaporated to dryness. After adding 15 ml of reagent Stiasny to the residue, the mixture was heated in a water bath at 80°C for 30 min. The formation of a precipitate characterizes catechic tannins. For gallic tannins, we filtered the previous solution. The filtrate was saturated with sodium acetate and 3 drops of FeCl3. The appearance of an intense blue-black coloration indicates the presence of gallic tannins.

6.6 Detection of Saponosides

To find saponosides, contributed in a test tube, 10 ml of total extract. After a vigorous shake for 15s, the tube was left to stand for 15 min. A height of persistent foam greater than 1cm showed the presence of saponosides.recipitate was taken as a positive reaction.

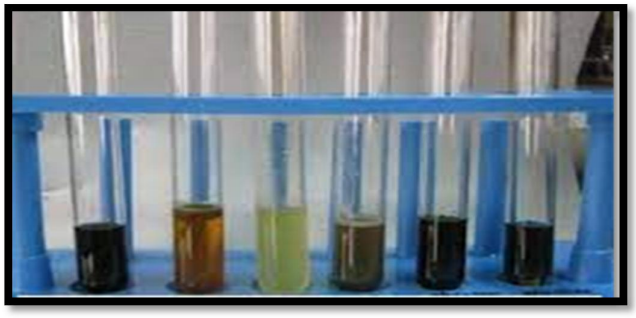

Fig 5: Phytochemical screening tests

VII. DETERMINATION OF FLAVONOID CONTENT

The total flavonoid contents were determined based on a colorimetric method. Extract solution (0.25 ml, 1mg/ml) and 1.25 ml of distilled water were pipetted into a test tube and then mixed with Sodium nitrite solution (5%, 0.075ml). After 5 min of incubation for of the mixture, 0.15 ml of 10% aluminium chloride was added. The mixture was kept for 6 min

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and 0.5 ml of 1 M sodium hydroxide was finally added. The mixture was diluted with 0.275 ml of distilled water. The absorbance of the mixture was read against blank at 510 nm and compared to a standard calibration curve. The flavonoid contents were expressed as mg quercetin equivalent (QE)/g of plant dry wt.

VIII. DETERMINATION OF PHENOLIC CONTENT

Total phenolic contents were estimated according to the Folin-Ciocalteu essay. Briefly, 0.5 ml of sample solution was mixed with 2.5 ml of Folin-Ciocalteu reagent diluted with distilled water 1:10, to which was subsequently added 4 ml of Na2CO3 (7.5 %, w/v). After a 30 min incubation of the mixture in a water bath at 45°C, the absorbance was measured at 765 nm in a UV-Vis spectrophotometer against a blank sample. The standard curve of Gallic acid was obtained under the same conditions as above with serial concentrations (0-200 mg/l) and was used for calibration. The results were expressed as Gallic acid equivalents (mg GAE/g of dried extract). Data were presented as the average of triplicate analyses.

IX. RESULT AND DISCUSSION

9.1 Morphological Evaluation

Sr. no	Parameter	Observation
1.	Size	100 cm (39 inch)
2.	Shape	Heart shaped leaves
3.	Surface	Long and rough hair surface, scalloped edges

Table 2. Morphological Evaluation

9.2 Microscopic Evaluation:

Fig. 8 Under Projection microscope

Fig. 9 Stock Image

A transverse section in the leaf blade is biconvex in outline showing a prominent midrib on the lower surface and a raised rounded ridge on the upper one (Fig. 9). Both the upper and lower epidermises carry glandular and non-glandular trichomes that are more distributed on the midrib. The transverse section also reveals a dorsiventral mesophyll interrupted by the cortical and vascular tissues at the midrib region. In addition, a mass of sub-epidermal collenchyma is found under both the upper and lower epidermises of the midrib region. The vascular system of the midrib is formed of a large collateral vascular bundle surrounded by non-lignified pericyclic fibres.

Extraction

The extract yield of Melissa Officinalis was 9.814% based on dry weight.

Physico-Chemical Evaluation

pH – The pH range was found to be between 5.6 (acidic) and 9.0 (strongly alkaline).

Moisture content –Drying trials with the constant low temperature (35 °C) and the increasing temperature profile gave the closest colour values of dried samples to the ones of fresh samples. The maximum colour changes occurred with the constant high temperature (60 °C) and the triangle temperature profile. The drying trials with the constant low temperature gave the lowest essential oil loss (24.28% loss) while the drying trials with the constant high temperature gave the highest essential oil loss (63.95% loss). The increasing temperature profile in which the drying air temperature increased from 35 °C to 60 °C in 8 hours became the most convenient drying air temperature profile for drying lemon balm.

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Ash Value – Ash content was about 6%, superior to the average value corresponding to woods, which makes pulping difficult.

Phytochemical Screening

In this study, preliminary phytochemical analysis of the leaf extract of *Melissa Officinalis* L. has been shows (table no 2), the presence of many constituents including tannins, saponins, alkaloids, terpenoids, steroid, flavonoids, followed by other accessions. Also note the absence of other compounds such as coumarins and proteins.

Medicinal plants have historically proven their value as a source of various secondary metabolites with curative properties such as alkaloids, flavonoids, glycosides, saponins, steroid, etc. thus the screening tests may be useful in the detection of the bioactive principles and subsequently presents an important tool for the identification of novel drug leads.

The biological function of alkaloids and their derivatives is very important because of their antispasmodic, analgesic, and bactericidal activities. Saponins have properties of precipitating and coagulating red blood cells and were reported to exhibit cholesterols binding properties, appearance of foams in aqueous solutions and haemolytic activity.

Traditionally, saponins are also used as detergents and molluscicides. Plant steroids are known to be important for their cardiotonic activities and also their use in nutrition and cosmetics.

Phytochemical constituents	Result
Sterols and polyterpenes	+
Catechic tannins	+
Hydrolysable tannins	+
Flavonoids	+
Saponins	+
Reducing sugar	_
Coumarines	_
Alkaloids	+
Proteins	_

Table 3: Preliminary phytochemical screening of the ethanolic extract from *Melissa Officinalis L*

Total Phenolic, Flavonoids and Tannin Contents

The total phenolic content (TPC), flavonoid content (TFC) and catechic tannins (TTC) are shown in Table 2. Analysis of these results indicated that the ethanolic extract of Melissa officinalis L. was rich in those compounds. Phenolic Plant compounds are documented in the literature as antioxidants or free radical scavengers. Plant polyphenols are known to be effective as singlet oxygen scavengers, reducing agents and hydrogen atom donators. In the present study, after the reaction of the active extract with Folin-Ciocalteau reagent in an alkaline medium, a blue-coloured solution due to the presence of phosphor-molybdic- phosphotungstic-phenol complex- was produced. The content of phenolic was calculated using the regression equation of the calibration curve (R2 =0.989, y = 0.009x + 0.0464), and expressed in GAE as milligrams per gram of the extract or fraction (mg GAE/g of dried extract). The total phenolic content. ISSN: 01173375 Volume 10, Issue 07, December, 2020.1039 established in our ethanolic extract was (46.90±0.72 mg GAE/g DM). In comparison, reported that TPC was 14.91 mg GAE/g DM for infusion of Melissa officinalis L., while established 48.86 mg GAE/100 g DM for methanolic extract. demonstrated that the extracting solvent significantly affects the polyphenol compound content and the measured antioxidant activity. Flavonoids are one class of secondary plant metabolites that also represent a large group of polyphenol compounds. Many plants contain flavonoids such as methanol extract of Datura metal L. methanol extract of Lepidium sativum, and water extract of Limnophilia aromatic. Polar extracts including water, methanol and ethanol showed more flavonoids compounds than a polar extracts. Flavonoids seem to display antioxidant and antimicrobial activities. The total flavonoid values indicated in this study (15.068±0,711 mg EQ/g DM). These results are similar to those reported, Tannins were reported to exhibit many biological activities such as antitumor, anti-plasmin inhibitors, antioxidants and can bind to the protein. Tannins can be found in both leaves and stem bark, and they are used to help the regulation of tissue growth and also as a pesticide. We found that tannins content of ethanolic extract of Melissa officinalis L. was (14.958±0,487 mg ECa/g DM).

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X. CONCLUSION

Examination of both the macroscopical and microscopical features of M. officinalis leaves represents a good method in the identification of this plant species. In addition, from a pharmacognostical point of view, these botanical characters will be helpful in future phytopharmacological investigations of this plant. Modern pharmacological studies have now validated many traditional uses of M. officinalis. The data reviewed here revealed that M. officinalis is a potential source for the treatment of a wide range of diseases especially anxiety and some other CNS disorders, though confirmatory trials are warranted to substantiate these effects in the clinical setting. Data regarding many aspects of this plant such as mechanisms of actions, pharmacokinetics, adverse effects of the extracts, potential interactions with standard-of-care medications and active compounds is still limited which call for additional studies particularly in humans. The present study allowed us to highlight the presence for the first time of bioactive molecules in the leaves of Lemon balm of Moroccan origin. These are actually polyphenols, flavonoids, flavones and flavanols known to have remarkable antioxidant properties.Many studies confirmed the antioxidative effects of *Melissa officinalis*; thus, its effect in preventing and treating oxidative stress-related diseases might be reliable.

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