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International Journal of Engineering, Science and Technology
Vol. 16, No. 4, 2024 pp. 1-10

INTERNATIONAL JOURNAL OF ENGINEERING, SCIENCE AND TECHNOLOGY

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www.ajol.info/index.php/ijest

Nutrient evaluation, minerals quantification and antibacterial potential of *Mentha longifolia* (L.) flower, leaves and stem against foodborne bacterial pathogens

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Abstract

This study was conducted for the assessment of the nutrition, minerals and antibacterial activities of leaves, stem and flower of *Mentha longifolia* (wild mint). Proximate compositions were carried out using standards methods, mineral analysis were determined using Atomic Absorption Spectrophotometer and Flame Photometer, antibacterial activities were assessed using Zone of Inhibition Assay. Broth dilution method was used to determine the Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The proximate composition of *Mentha longifolia* observed that moisture was found in the range of 2.3-2.8%, ash was 2.5-7.5%, crude fat was 0.5-2.1%, crude fiber was 8.5-22.5%, protein was 2.1-4.5%, carbohydrate was 69.6-78.7% and energy was 291.3-347.7 Kcal/100 g dw. Sodium concentration was found in the range of 1250-1590 mg/kg, Potassium was 3240-4820 mg/kg, Calcium was 12400-24000 mg/kg, Magnesium was 4800-9600 mg/kg, Zinc was 32-64 mg/kg, Iron was 140-498 mg/kg, Manganese was 32-107 mg/kg and Chromium was 3-6 mg/kg in *Mentha longifolia* leaves, flower and stem. The highest zone of inhibition (23±0.2) of *Mentha longifolia* leaves extract (tincture) was calculated against *Bacillus cereus*. Similarly the highest zone of inhibition of *Mentha longifolia* flower and stem was noted against *Bacillus cereus* in tincture extract were 17±01 mm and 16±01 mm respectively. The *Mentha longifolia* leaves MIC range was 40–120 mg/mL and MBC 60–200 mg/mL. The flower extracts MIC range 70–220 mg/mL and MBC 110–300 mg/mL. Generally the antibacterial activities of wild mint leaves extracts were higher as compared to flower and stem extracts ($p<0.05$). The results commonly observed Gram-negative bacteria are typically high opposed to the *Mentha longifolia* extracts as compared to Gram-positive bacteria. The proximate and minerals analysis concludes that this herb is used to formulate nutraceutical products and their extracts can be used against multi drug resistance bacteria accomplished of causing foodborne diseases.

Keywords: Proximate composition, human health, minerals, decoction, infusion, tincture, Gram positive and Gram negative bacteria.

DOI: http://dx.doi.org/10.4314/ijest.v16i4.1

Cite this article as:
Ali J., Hussain A., Rahman Z.U., Rehman I.U. 2024. Nutrient evaluation, minerals quantification and antibacterial potential of *Mentha longifolia* (L.) flower, leaves and stem against foodborne bacterial pathogens. *International Journal of Engineering, Science and Technology*, Vol. 16, No. 4, pp. 1-10. doi: 10.4314/ijest.v16i4.1

Received: March 20, 2024 Accepted: April 7, 2024; Final acceptance in revised form: June 8, 2024

1. Introduction

The occurrence of Toxic microorganisms in the contaminated food, which are consumed by human being, is the prime grounds of food poisoning in human. Bacteria are the chief agent of the microbial contamination of foods. Food scientist, engineers and researchers are worried regarding the expansion of bacterial ailments originated by means of pathogens and microbes in food. In recent years, food borne diseases are augmented regardless of the hygiene improvement. As a result, it gives the impression needed to give high concentration to toxic bacteria to stop food borne diseases. Numerous synthetic chemicals are utilized to stop the pathogenic microbial growth. But these chemicals additives developed toxic effects in human beings such as produce resistance to microbes, toxicity and cancer, various customers are insisting the application of microbial, animal and herbal based agents to do dual function i.e. eliminate the toxic effects of synthetic chemicals and enhance the shelf life of foods (Mohammad *et al*., 2017). Various deadly ailments are recognized to be cured by using the medicinal plants proved form the human history. Nowadays, herbal medicine maintains to act as a prime role in basic health system as healing therapies in various budding countries of the world. Herbs at a standstill go on to the nearly the special origin of medicine for the greater part of the global inhabitants (Firas, 2009). Herbal medicine has been employed all over the past civilization of human for treating the ailments. Though majority of the present medicine are derived chemically, so far it has been calculated that approximately one-third medicine is herbal origin or have been derived after isolated from botanical extracts (Mohammad *et al*., 2017).

Due to severe starvation mankind is in a condition of anarchy. Various diseases and ailments are caused due to deficiency of various minerals such as zinc, calcium, iodine and iron. The pregnant woman and young children in preschool are the most exposed populations. Deprivation of micronutrient is a worldwide health issue. The micronutrient mediators, basic fortification and the mainly price effectual nutritional managements have been find out (Muhammad *et al*., 2023). Wild mint or horse mint botanically known as *Mentha longifolia L. (M. longifolia)* belongs to family Lamiacea*,* cultivated quickly and has creeps a long underground rootstock which should grow up to one to two meter long. Its stalk is sometimes sparsely hairy, grey-villous and white (Zahra *et al*., 2017). In conventional medicine, the leaves of mint are utilized for the cure of nasal decongestants, aches and minor sore throat, digestive system and possess antiseptic potential (Firas 2009). The mint leaves tea is conventionally drink for the healing of digestive discomfort, headaches and fever. Mint essential oil is act as antiviral, carminative, antispasmodic and antibiotic agents (Daferera *et al.,* 2003). Currently *Mentha longifolia* are widely used in pharmaceutical, aroma, food, confectionary and cosmetic industries all over the world (Javid and Javed, 2023). The pharmacological potential of mints is because of phenolic substances. Mentha different species are uses as traditional herbal medicine for the treatment of liver complaints, ulcerative colitis, flatulence, anorexia, bronchitis and nausea (Cowan, 1999; Iscan *et al.,* 2002; Moreno *et al.,* 2002).

Proximate and nutritional profiling of fruits and vegetables perform a key role in judging their dietary significance. Many medicinal plants are also used as diet beside with their therapeutic values, assessing their food capacity can help to recognize the magnetism of these plants (Javid *et al*., 2017). The interior basis of various health issues are more over minerals defiance. For men and animal plants are the main sources for minerals. The researchers are trying to find out the minerals composition of medicinal herbs and explain a correlation to treat the ailments (Muhammad *et al*., 2013). Minerals and vitamin scarcity are a global problem in both developing and rich countries. Presently, approximately fifty percent of the global population exposed to malnutrition or is at threat of shortage in minerals, vitamins or other micronutrients. Utilization sufficient minerals are vital for both mental and physical growth. Because minerals are crucial for various metabolic purposes, a scarcity in them could outcome in a variety of health issues (Yousuf *et al*., 2021). Herbs have been reported as varied origin of natural compositions for boosting health for the previous ten years. The World Health Organization (WHO) has authorized the support of plants derived products because conservative treatment is basically neither within reach nor quantitative (Borsellino *et al*., 2020). So, keeping in view the above facts a study was designed to evaluate the nutritional value, mineral contents and antibiotic potential of *Mentha longifolia* flower, leaves and stem extracts against foodborne pathogens.

2. Materials and Methods

2.1 Plant Collection

The aerial parts of the plant were obtained from Herbal Research Garden of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar, Khyber Pakhtunkhwa-Pakistan. The plant collected parts were carefully washed with tape water to eliminate the soil and dirt particles. The flower, stem and leaves were separated and dried in a shaded room for a period of 10 days. The dried samples were grinded into powder form with the help of Waring® Commercial Laboratory Blender, USA and kept in an air tight brown glass bottle for study uses.

2.2 Proximate Analysis

Moisture and ash were determined by weight difference. The fiber was estimated from the crucible weight loss during its content on explosion. Soxhlet apparatus (Quickfit, England) was used quantify crude lipid by direct solvent (petroleum ether) extraction methods. Micro Kjeldahl (Bloc Digest, Spain) method was applied to calculate the nitrogen value. Nitrogen value was multiplying with a factor of 6.25 to determine protein (Abdurehman *et al*., 2012). The formula of (Ulfat *et al*., 2016) was sued to calculate the carbohydrates contents.

Carbohydrate (%) = 100 – (Protein +fats +crude fiber +ash + moisture).

Total energy was estimated using below formula (Tania *et al*., 2017).
Energy (kcal/100 g) = 4 (CHO + Proteins) + 9 (Fat).

2.3 Mineral Analysis

The powdered material (5 g) was measured in a crucible (silica), burned on open flame until smoke is stop, and then kept in a muffle furnace (Witeg- Korea) at 550 °C to obtain a clear white ash. At ambient temperature the crucible was cooled in a desiccator and remaining white content was wetted with 0.5 mL concentrated sulfuric acid (Merck KGaA, Damsttadt-Germany). On hot plate (PCSIR-Pakistan) the Crucible was kept and heated until sulfuric acid (Merck KGaA, Damsttadt-Germany) fumes completely evolved. The sulfated ash along with crucible kept on 600 °C in a muffle furnace (Witeg- Korea) turn over the ash received a constants weight. The resulted ash was cooled, liquefied in five-millimeter 6 N hydrochloric acid (BDH Laboratory, England) and waited for thirty minutes. The solution was filtered through Whatman® 40 filter paper 125mm Ø Whatman International Ltd. Maidstone, England and make up (50 mL) with deionized water. This solution was applied to quantify the heavy metals analysis through Atomic Absorption Spectrophotometer Z-2000 (Hitachi- Japan) and whereas Sodium and Potassium was analyzed by Flame Photometer Jenway PFP7-UK (Ali *et al*., 2016).

2.4 Crude Extract Preparation

Decoction: This extraction was prepared by boiling finely five-gram (5) ground powder of flower, stem and leaves separately in 100 ml water for twenty minutes at 100 °C. **Infusion:** To prepare **Infusion** add boiling H_2O to the five gram herbal powder 5g/100 mL and left it standing for 20 min. **Tinctures**: To prepare Tinctures add (10g) grinded powder sample with 200 mL ethanol (96%) in sealed flasks, moving (40 cycles/ minute) for seven days at 37 °C. All the extracts were subsequent to cooling, filtered the material with the help of Buchner funnel and concentrated through Rotary Evaporator Equipment (R-200 Buchi Rotavapor, B-490 Heating Bath) Switzerland. The thick concentrated material was obtained and weighed. The extracted material was kept in a cool incubator (Memmert- Germany) at four degrees centigrade for further experimental work.

2.5 Maintenance of Bacterial Culture

All experimental bacteria cultures were procured from the Environmental Research Section of PCSIR Peshawar. Nutrient agar (Oxoid, Hampshire-England) was used to maintained pure bacterial cultures. Sub-culturing was carried out for each microbial culture repeatedly on the same medium and kept at 4°C in a cool incubator (Memmert- Germany) prior to exercise in tests.

2.6 Well-diffusion assay

Nutrient agar (Oxoid, Hampshire-England) 15 mL for bacterial cultures were mixed with hundred microliters of microbial culture (10^8 CFU/mL) and after that shifted into Petri dishes. The inoculated media was standing to solidify and cool for 30 minutes at room temperature. A disinfected cock's borer was employed to make 06 mm diameter wells into the culture medium. Next 100 μL with a 300 mg/mL concentration dissolved in five percent Dimethyl sulfoxide (DMSO) solution of every extract (tincture, infusion and decoction) were poured to the wells. For positive control (Ampicillin) was used (1 mg/mL in sterile physiological saline), while negative control 5% DMSO was used. The plates were kept for two hours to mix uniformly at 4 °C and then at 37°C incubated in an incubator (Memmert-Germany) for twenty-four hours. The antibacterial potential was visually assessed as zone of inhibition around the wells. The inhibition zones diameter was calculated in millimeters (Bashir and Javid 2013).

2.7 MIC and MBC Determination

The *Mentha longifolia* extracts were diluted to different quantification ranging from 20 to 300 mg/mL in nutrient broth (Oxoid, Hampshire-England). Each concentration of 500 µL was transfer to 2 ml autoclaved nutrient broth (Oxoid, Hampshire-England) in test tubes. Afterward, one millimeter (1×10^8 CFU/mL) of bacterial strains of the individual culture was transfer to the test tubes content and for eighteen hours incubation period at 37**°**C. Ampicillin was applied as Positive control with diverse strength range (0.01 to 1.0) mg/mL. The minimum quantification of the experimental substances that did not permit any observable turbidity against tested microbes was considered as MIC. The MBC was found out by taking 100 µL of microbes from every of the culture tubes having no turbidity and shifted into sterile plates of agar. The Petri dishes were incubated for forty-eight hours in an incubator (Memmert-Germany) and afterward growth observation was carried out. The quantity of the testing materials with no observable growth was considered as MBC (Bashir and Javid 2013).

3. Statistical Analysis

The results were stated as means ± standard deviations (SD) of three readings. The P <0.05 were set as confidence limits. For the majority of the values obtained Standard deviations did not exceed 5%

4. Results

The proximate composition results are presented in Table 1.

Table 1. Proximate Composition of *Mentha longifolia*.

Parameters	Leaves	Flower	Stem
Moisture %	2.70±0.5	2.3±0.1	2.8±0.2
Ash %	2.5±01	7.5±0.3	2.5±0.2
Crude Fat %	2.1±0.2	0.8±0.2	0.5±00
Crude Fiber %	10.5±0.4	8.5±0.7	22.5±02
Protein %	4.5±0.5	2.2±0.1	2.1±0.3
Carbohydrate %	77.7±00	78.7±00	69.6±00
Energy (Kcal/100 g dw)	347.7	330.8	291.3

Each value is a mean of 03± SD.

This finding revealed that Proximate composition of *M. longifolia* revealed that moisture (%), ash% and crude fiber% was increases in the order stem> leaves >flower. While crude fat and protein was increases in the order leaves>flower>stem. Carbohydrate and energy value are high in flower as compared to leaves and stem. Table 2 shows the minerals composition of *M. longifolia*. Generally Sodium and Potassium contents were increases in the order stem>leaves>flower. Calcium, Magnesium, Zinc, Manganese and Chromium were increases in the order leaves>stem>flower.

Table 2. Minerals Analysis (mg/kg) of *Mentha longifolia.*

Elements (mg/kg)	Leaves	Flower	Stem	**RDA** (Bill, 1995)	
				Men	**Women**
Na	1470±05	1250±10	1590±12	500 mg	500 mg
K	4800±10	3240±15	4820±10	2,000 mg	2,000 mg
Ca	24000±20	12400±13	13600±04	800 mg	800 mg, for pregnant/nursing 1200 mg
Mg	9600±04	4800±14	5280±06	350 mg	280 mg, 355 mg (nursing), 320 mg (pregnant)
Zn	64±01	32±01	39±04	15 mg	12 mg, pregnant (15 mg), nursing (19 mg)
Fe	498±04	140±03	149±05	10 mg	15 mg, for pregnant 30 mg
Mn	107±07	30±02	32±02	2.0-5.0 mg	2.0-5.0 mg
Cr	06±01	03±01	05±00	50-200 mcg	50-200 mcg
Cu	ND	ND	ND	1.5-3.0 mg	1.5-3.0 mg
Pb	ND	ND	ND	-	-
Ni	ND	ND	ND	-	-
Cd	ND	ND	ND	-	-

ND= Not detected, Each values is a mean of 3± SD, RDA= Recommended Dietary Allowances.

Table 3 shows the antibacterial activities of *M. longifolia* leaves. The results showed that highest zone of inhibition 17±0.5 mm and 19±0.5 mm was observed against *Staphylococcus aureus* by decoction and infusion extracts respectively and lowest zone of inhibition was calculated against *Pseudomonas aeruginosa* was 10±00 mm and *Pseudomonas aeruginosa* was 11±0.5 mm by infusion and tincture respectively. Generally, the tincture observed the highest zone of inhibition against *Staphylococcus aureus, Enterococcus faecalis* and *Bacillus cereus* were 20±0.2 mm, 21±0.2 mm and 23±0.2 mm respectively. The decoctions MBC (mg/mL) were in the range of 100-200 mg/ml and MIC (mg/mL) were in the range of 70-120. While MIC (mg/mL) of infusion were in the range of 50-120 and MBC (mg/mL) were in the range of 80-200. Similarly tincture MIC (mg/mL) was in the range of 40-100 and MBC (mg/mL) were in the range of 60-180.

Table 3. Antibacterial Activities of *Mentha longifolia* Leaves.

Bacteria	Decoction			Infusion			Tincture		
	mm	mg/mL		mm	mg/mL		mm	mg/mL	
Gram-positive	ZI	MIC	MBC	ZI	MIC	MBC	ZI	MIC	MBC
Staphylococcus aureus	17±0.5	70	100	19±0.5	50	80	20±0.2	50	80
Enterococcus faecalis	16±0.3	70	120	18±0.2	60	90	21±0.2	40	60
Bacillus cereus	15±0.1	80	140	17±0.4	70	100	23±0.2	40	60
Bacillus subtilis	14±0.2	90	140	16±0.5	80	100	19±0.2	50	80
Gram-negative									
Escherichia coli	11±0.2	110	160	13±0.1	90	130	14±0.2	90	120
Salmonella Typhimurium	10±0.1	120	170	11±0.1	110	200	12±01	90	120
Pseudomonas aeruginosa	09±0.2	120	200	10±00	120	200	11±0.5	100	150
Klebsiella pneumoniae	13±0.2	90	180	14±01	80	170	15±01	70	180

ZI= Zone of inhibition, Each value represents average of 3 ± Standard Deviation.

Table 4 shows the antibacterial activities of *Mentha longifolia* flower. The highest inhibitory effect (zone of inhibition) by Gram positive bacteria was observed against *Bacillus cereus* was 17±01 mm (tincture).

Table 4. Antibacterial Activities of *Mentha longifolia* Flower.

Bacteria	**Decoction**			**Infusion**			**Tincture**		
	mm	**mg/mL**		**mm**	**mg/mL**		**mm**	**mg/mL**	
Gram-positive	**ZI**	**MIC**	**MBC**	**ZI**	**MIC**	**MBC**	**ZI**	**MIC**	**MBC**
Staphylococcus aureus	14±0.1	120	160	15±0.5	100	140	16±0.1	80	110
Enterococcus faecalis	12±0.2	120	190	13±01	110	170	14±0.5	100	170
Bacillus cereus	15±0.1	120	190	16±01	90	180	17±01	70	110
Bacillus subtilis	13±0.5	130	200	14±02	120	190	15±01	90	130
Gram-negative									
Escherichia coli	09±0.1	200	300	10±0.4	150	250	11±0.10	130	230
Salmonella Typhimurium	08±0.2	200	300	07±00	170	270	10±00	140	140
Pseudomonas aeruginosa	NZI	NA	NA	07±00	200	290	09±00	150	230
Klebsiella pneumoniae	07±00	220	300	07±00	200	300	08±01	170	240

ZI= Zone of inhibition, Each value represent average of 3 ± Standard Deviation.

Similarly, the Gram-negative bacteria *Klebsiella pneumoniae* demonstrated the weakest activity (zone of inhibition) 06±00 mm and 04±00 mm by decoction and infusion respectively. *Mentha longifolia* stem extracts against tested microbes are shown in Table 5.

Table 5. Antibacterial Activities of *Mentha longifolia* Stem.

Bacteria	**Decoction**			**Infusion**			**Tincture**		
	mm	**mg/mL**		**mm**	**mg/mL**		**mm**	**mg/mL**	
Gram-positive	**ZI**	**MIC**	**MBC**	**ZI**	**MIC**	**MBC**	**ZI**	**MIC**	**MBC**
Staphylococcus aureus	13±0.1	200	300	14±0.5	200	300	15±0.1	200	300
Enterococcus faecalis	11±0.2	200	300	12±01	200	300	13±0.5	200	300
Bacillus cereus	14±0.1	200	300	15±01	200	300	16±01	200	300
Bacillus subtilis	12±0.5	200	300	13±02	200	300	14±01	200	300
Gram-negative									
Escherichia coli	08±0.1	300	300	09±0.4	300	300	10±0.10	300	300
Salmonella Typhimurium	07±0.2	300	300	07±00	300	300	10±00	300	300
Pseudomonas aeruginosa	NZI	NA	NA	07±00	300	300	08±00	300	300
Klebsiella pneumoniae	NZI	NA	NA	07±00	300	300	07±01	300	300

ZI= Zone of inhibition; Each value represent average of 3 ± Standard Deviation.

It was observed that stem extracts showed a very weak zone of inhibition, high MIC and MBC values. Table 6 illustrates the antibiotic potential of standard used in the current study showed that standard antibiotic has high zone of inhibition and low MIC and MBC as compared to the all extracts of *Mentha longifolia*.

Table 6. Antibacterial Activities of standards used in the study.

Bacteria	**Ampicillin**			**5% DMSO**		
Gram-positive	**mm**	**mg/mL**		**mm**	**mg/mL**	
	ZI	**MIC**	**MBC**	**ZI**	**MIC**	**MBC**
Staphylococcus aureus	28±0.30	0.12	0.24	NZI	NA	NA
Enterococcus faecalis	26±0.21	0.20	0.40	NZI	NA	NA
Bacillus cereus	24±01	0.28	0.56	NZI	NA	NA
Bacillus subtilis	22±00	0.35	0.70	NZI	NA	NA
Gram-negative						
Escherichia coli	20±0.6	0.40	0.80	NZI	NA	NA
Salmonella Typhimurium	21±0.5	0.38	0.76	NZI	NA	NA
Pseudomonas aeruginosa	23±01	0.31	0.62	NZI	NA	NA
Klebsiella pneumoniae	27±01	0.19	0.38	NZI	NA	NA

ZI= Zone of Inhibition, NZI=No inhibition zone, Each value represent average of 3 ± Standard Deviation, -=Not applied.

Generally, the *M. longifolia* is more potent against Gram +ve microbes as judge against to Gram –ve. The antibacterial activity increases by extraction techniques in the order tincture> infusion> decoction. While in parts point of view increases leaves>flower>stem.

5. Discussion

These days the utilization of medicinal plants has gain significant amount of magnitude. Globally it was applied by the marginal and indigenous communities for treatment of numerous ailments. Therapeutics herbs are generally utilized as its oral decoctions along with foodstuff supplement. But, more research has been required so far to authenticate the utilization in this respect. The current study is an attempt in this direction.

5.1 Proximate Analysis

The minimum content of moisture percentage of *M. longifolia* leaves, flower and stem found in this study mean that *M. longifolia* leaves possess low quantity of water and could not contaminate generally due microorganisms activities have not effortlessly passed out. But the moisture quantity in the food items are lofty it might require a few moment to properly dried for storage and preservation. It would as well develop fungus and decompose without difficulty, but not sound dried prior to storage and preservation, methods such as shade drying, air drying could be used to protect it dietary compositions. The elemental composition of the herbs represents ash content. The ash amount in any herbs is directly proportion to the presence of specific minerals in high quantity, therefore satisfying the requirement of the body elementals nutrition (Sajjad *et al*., 2014).

The fat content of *M. longifolia* leaves in this study was found to be 0.5-2.1%. This result, compared with earlier research carried out by Erawati *et al*., 2022; Ali *et al*., 2017; Anyaoku *et al*., 2023 which reported that pepper mint leaves possessed various quantity of oil, applied as a booster in nanostructured lipid carriers (NLC), which influence the stability and characteristics of NLC. The oil of Pepper mint is extensively recognized and utilized in the skin care and pharmaceutical industries for the reason that of its pharmacological and medicinal characteristics for the synthesis of cough medicines, creams, lotions and topical gels etc. It was also reported (Mainsara *et al*., 2018) that a high quantity of volatile oil occurred in the pepper mint leaves.

The nutritional analysis also observed that *M. longifolia* possess considerable quantity of fiber (8.5-22.5%). The human body needed a sufficient amount of fiber, because it helps in weight management, bowel movement and food digestion. The *M. longifolia* will be a fine foundation of fibre in the body. The greater fiber composition of the herb might be because of the presence of cellulose in the succulent branches or leaves. For good absorption of the food in the intestine the high fiber content is beneficial (Sajjad *et al*., 2014).

The *M. longifolia* protein quantification in the current study was found to be 4.5-2.1%. This means that *M. longifolia* possess various quantity of amino acids required in the tissue for development, growth and maintenance of damaged out cells.

In the current study the carbohydrate content of *M. longifolia* was found to be 69.6-78.7%. The adding of this herb to diet will not only act as a herbal remedy or spice, however will also provide to the tissues with carbohydrates for the energy synthesis to utilize in numerous body functions.

Mentha longifolia moisture, ash, protein, fat, carbohydrates and fibre were 2.60±0.02%, 22.39±0.02%, 7.491±0.02%, 2.34±0.02%, 55.12±0.04% and 11.29±0.03% respectively (Ghani *et al*., 2014). The protein content showed in small quantity which observed that the herb ingredient would be composed of the carbohydrates. The current study proximate composition is a close agreement to the previous study except moisture which was found in high quantity 92% (Sajjad *et al*., 2014). The Mentha piperita dried leaves have maximum proximate composition i.e. ash 10.86%, protein 9.80%, fat 15.99%, fiber 11.27% and carbohydrate 34.13% (Sabrina *et al*., 2022) as compared to the current study. On the other hand the results of the previous study Ali *et al*., 2017 showed a close agreement to the obtained results in the current study. The broad variation in soil, species, climate, topographical and geographical factors could be the reason for the differences in these reports. Approximately twenty four percent of Pakistanis are presently occurrence malnourishment because of the country's worryingly elevated starvation rate. It is reported that approximately 37.5 million Pakistanis are malnourished (Muhammad *et al*., 2023). Herbals plants which are a high source of protein, fiber, fats, carbohydrates and minerals like the wild mint may be used as food and minerals supplements as the current study revealed.

5.2 Minerals Compositions

From the overall data it was obvious that all the plant parts contained all minerals in appreciable amount. The higher Na, K, Ca, Mg and Fe contents showed that plants might be helpful in restoring these minerals deficiency in the body when consumed regularly. The appreciable amount of Zn, Mn and Cr showed that the curing effect of plants might be related to their mineral composition. The Cu, Pb, Ni and Cd were not found in mint, it is very appreciable because generally these metals are toxic to human health. The previous studies (Ali *et al*., 2017; Mainasara *et al*., 2018; Anyaoku *et al*., 2023) have reported the occurrence of elements were to be in dissimilar quantification. Dissimilarities in minerals occurred and its quantities might be account to topographical, geographical and soil nutritional state differentiation.

Approximately two billion human beings globally possessed minerals deficiency, which is linked to diseases and preventable that create a severe danger to their health (Muhammad *et al*., 2023). Yet even if they are now somewhat essential for man body, minerals have an extraordinary impact on it (milligrams or micrograms per day). But these medicines (synthetic minerals) are utilized in ridiculous quantities as minerals supplements, the energy metabolism, reproductive system, immune system and the

brain might all be ill with the consequence. The outcomes of these shortages comprising ill health, decrease employment potential, learning trouble and so far death (Moetamedipoor *et al*., 2022).

Potassium and Sodium normalize osmotic pressure and acid base balance of the body fluid (Bashir and Ali 2013). Calcium is very important for nerve impulses, cardiac muscle, teeth health, bones, milk and blood clotting (Muhammad *et al*., 2013). Manganese is enhancing various enzymes and also a part of some enzyme (Naseem *et al*., 2012). Magnesium is vital for human being, because it assists to normalize glucose level, insulin level, vitamin D and calcium regulation. It may also aid to maintain healthy muscles, strengthen heat muscles and lower anxiety levels (Anyaoku *et al*., 2023). Zinc is required for brain health, behavioral development, normal insulin secretion, normal growth and wound healing (Muhammad *et al*., 2013). Iron plays a function in the metabolism of protein, carbohydrate and lipids as well as a part of hemoglobin. Anemia is caused due to deficiency of iron (Naseem *et al*., 2012). These results observed that Na, K, Ca, Mg, Zn, Fe and Mn were calculated in high amount as recommend daily allowance (Bill, 1995). All these minerals might at a standstill contribute to the dietary value of *M. longifolia*.

5.3 Antibacterial Potential

The antibacterial activities of wild mint leaves, stem and flower decoction, infusion and tincture were assayed against *Staphylococcus aureus, Enterococcus faecalis Bacillus cereus Bacillus subtilis, Escherichia coli, Salmonella Typhimurium, Pseudomonas aeruginosa* and *Klebsiella pneumonia.*

The *Mentha arvensis* antimicrobial activities using its aerial parts ethanol extract applied disc diffusion technique (Zone of Inhibition=ZI) and microdilution technique (MIC and MBC) reported (Shah *et al*., 2023) that *Klebsiella aerogenes* (ZI=7.42mm, MIC= 2.8µg/mL and MBC= 5.1µg/mL), Staphylococcus (ZI=9.14mm, MIC= 4.5µg/mL and MBC=4.6µg/mL), *Klebsiella pneumonia* (ZI= 13.39mm, MIC= 5.7µg/mL and MBC= 7.8µg/mL) and *Escherichia coli* (ZI=5.69mm, MIC= 3.2µg/mL, MBC= 6.2µg/mL) .

The antimicrobial activities of *Moroccan M. longifolia* reported that acetone extract observed that *Bacillus cereus* was the most susceptible bacteria observed MBC (1.50 ± 0.05 mg/mL) and MIC (1.17 ± 0.05 mg/mL) and *Staphylococcus aureus* showed MBC (6.25±0.43 mg/mL) and MIC (.34±1.10 mg/mL). Furthermore, acetone extracts observed maximum activities against E. Coli having MIC was 6.25 ± 0.00 mg/mL (Meryem *et al*., 2023).

The *Mentha piperita* Leaves aqueous and methanolic extracts showed a changeable inhibition activities using agar disc diffusion and agar dilution technique against all Gram-positive microbes at 200 mg/mL with ZI (8.00– 23.5 mm), additionally, the extracts assayed specify no activity against tested fungi *Candida albicans* and Gram-negative (Sabrina *et al*., 2022).

The *M. longifolia* extracts demonstrated antimicrobial activities in the current study could be explained by its wealthy bioactive compounds sketch, which exhibited isocoumarins, coumarins, rutin, quercetin, apigenin, luteolin, gallic acid, cafeic acid, chlorogenic acid, m-coumaric acid, ρ-coumaric acid, cryptochlorogenic acid, rosmarinic acid, and cafaric acid (Meryem *et al*., 2023).

The dissimilarities in the antimicrobial potential of herbs from the similar resource after extraction with dissimilar solvents have showed that not the whole bioactive compounds that are accountable for antimicrobial potential are extracted in a lone solvent. Therefore, solvents of dissimilar polarity must be used (non-polar: petroleum ether, ethyl acetate; polar: ethanol, acetone, H_2O). Successive or sequential solvent extraction is a fine choice for enhanced solubility of numerous bioactive compounds. Though, it is also required to understand the active compounds, isolated by every individual solvent therefore to keep away from addition of needless solvent at extraction methods. It is also vital to know the efficiency of every solvent in the extraction of a class or individual bioactive compounds (Ali *et al*., 2016). So in the current study it was found that tincture is a good choice, because it showed a prominent antibacterial potential. The antibiotic potential of *Mentha piperita* leaves extracts was point out that ethyl acetate, chloroform and the pet. Ether was observed high potency as compared to aqueous and ethanol (Rajinder *et al*., 2015). In our study tincture is more active as compared to aqueous extract. The *Mentha piperita* leaves extract calculated highest zone of inhibition (17.24 mm) where as stem extract showed 15.82 mm minimum zone of inhibition (Sabahat and Perween 2005). The current study is a close agreement of these finding because leaves have high antibacterial potential as compared to stem extracts.

Environmental factors are playing a key role in the synthesis of secondary metabolites in the plants. Factors like altitude, light intensity, precipitation and temperature, which predict the weather of an area, influence of the accumulation of the phytochemicals (Davise, 1994). It has been reported repeatedly that Gram-positive microbes are highly at risk to essential oils as compared to Gram-negative microbes. The Gram-negative microbe's tolerance to essential oils was due to the hydrophilic outer membrane existence which prevents the diffusion of hydrophobic essential oils into mark cell membrane (Firas, 2009). Several antimicrobial resistance mechanisms are willingly adopted by various bacterial species. Firstly, the bacteria may obtain β-lactamases (genes encoding enzymes), that demolish the antibiotic substances prior to effect. Secondly, microbes may obtain efflux pumps that extrude the antibiotic compounds from the cell prior it could arrived its destination site and showed its power. Thirdly, for metabolic pathway microbes may obtain numerous genes which finally synthesis changed microbial cell walls that no longer exhibited the attachment location of the antibacterial compounds agent or microbes may obtain mutations that low the entrée of antibacterial compounds to the intracellular goal place via porin genes down-regulation (Tenover, 2006).

In reality, Gram-positive microbes are high susceptible to plant extracts as compared to Gram-negative microbes. Gram-positive microbes cell walls compared with Gram-negative microbes, are greater susceptible to numerous antimicrobial agents, anti-boutiques (Norajit *et al*., 2007) as well as numerous botanical drugs (Sharifa *et al*., 2008). Periplasmic space and Lipopolysaccharides layer of Gram-negative microbes are the causative phenomena of resistivity of Gram-negative microbes (Koohsari *et al*., 2015). The previous findings concluded that microbes (Gram-positive) are highly vulnerable to plants oil and

extract as contrast to Gram-negative microbes (Sandasi *et al*., 2010; Mahboubi and Haghi 2008; Mimica-Dukic *et al*., 1999; Cosentino *et al*., 1999; Karaman *et al*., 2003; Sahin *et al*., 2002).The antibiotic capacity of herbs extracts observed maximum activity against bacteria (Gram-positive) as compared to Gram-negative germs (Italo *et al*., 2017). The sensitivity differences of the microbial cultures may be due to compositions of the phytochemicals present in the herbs extracts, nature of the microbes and intrinsic tolerance of the microbes (Basheer and Abdullah 2013). It is not amazing that standards drugs have low MBC, MIC and high zone of inhibition as compared to the *M. longifolia* extracts. Because the drugs are prepared by standard of the art technology, while common plant origin decoction are prepared from crude sources which are mostly time exposed to contamination and degradation (Bashir and Javid 2013). The present study revealed that wild mint is potent source of protein, fiber, minerals and its extracts can be used against foodborne bacterial pathogens. However, the current study has some limitation i.e. optimum dosage for different purpose i.e. protein, minerals and antibacterial extraction, minerals extraction , low sample volume, only three extraction techniques used, types of effective extracts and straightforward bacterial pathogens antimicrobial assay.

6. Conclusion

Our current study revealed that *M. longifolia* is an excellent foundation of nutrients (Protein, fiber and carbohydrates), minerals and can be used as substrates deficit in either of these nutrients. While the antibacterial activity results specified that *M. longifolia* extracts can be utilized as non-synthetic preservatives in food stuff and food beside the recognized connecting candidate pathogenic microbes of man, food spoilage bacteria and foodborne diseases. At the end non-synthetic items with plant basis contribute a significant function in treating ailments and solve the antibiotic resistance problems and discovered the new antibacterial compounds. It is necessary to recommended and point out the way forward that wild mint extraction processes have to be optimized on large scale to produce minerals, fiber and protein based nutraceutical products. An ecofriendly, low cost and feasible engineering processes will be developed to isolate active constituents of this plant to use as potent herbal based antibiotic. Additional research on mechanism and metabolism of substances of this plant could guide to novel and high efficient medicines and products formulation for the indigenous industries.

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