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International Journal of Engineering, Science and Technology
Vol. 15, No. 2, 2023, pp. 48-57

INTERNATIONAL JOURNAL OF ENGINEERING, SCIENCE AND TECHNOLOGY

www.ijest1-ng.com
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Comparative bioactive compounds and antifungal potential evaluation of *Mentha longifolia* (L.) Hudson (Lamiaceae) extracts against plant pathogenic fungi

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Abstract

The present study was design to evaluate the *Mentha longifolia* stem, flower and leaves infusion, decoction and hydro-alcoholic extracts to determine the bioactive molecules and antifungal activities by zone of inhibition technique, Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were assay by broth dilution technique and spore germination assay using Light Compound Microscope against plant pathogenic fungi (*Fusarium oxysporum*, *Aspergillus parasiticus*, *Rhizoctonia solani* and *Alternaria alternata*). The *Mentha longifolia* phytochemicals were found in the range flavonoids 3.8 - 2.4%, saponins were 5.6-3.4%, alkaloids 0.7-0.1% and total phenolic were 40-15 mg Gallic acid/g. The maximum zone of inhibition 23±1 mm and 22±1 mm were found by hydroalcoholic leaves extract against *Fusarium oxysporum* and *Aspergillus parasiticus* respectively. The MIC range of hydroalcoholic were 12.5-100 mg/mL, infusion were 25-100 mg/mL and decoction were 25-200 mg/mL. While MFC range of hydroalcoholic were 25-200 mg/mL, infusion were 50- 200 mg/mL and decoction were 50-200 mg/mL. Spore germination assay reaveled that increse in the extract concenetration decrese the spore germination. The results revealed that *Mentha longifolia* chemical composition might be build up as latent bio-fungicides against plant pathogenic fungi.

Keywords: Wild mint, extraction techniques, phytochemicals, zone of inhibition, spore germination.

DOI: http://dx.doi.org/10.4314/ijest.v15i2.5

Cite this article as:
Ali J., Abbas J. 2023. Comparative bioactive compounds and antifungal potential evaluation of *Mentha longifolia* (L.) Hudson (Lamiaceae) extracts against plant pathogenic fungi. *International Journal of Engineering, Science and Technology*, Vol. 15, No. 2, pp. 48-57. doi: 10.4314/ijest.v15i2.5

Received: January 21, 2023; Accepted: March 29, 2023; Final acceptance in revised form: April 6, 2023

1. Introduction

Plants are the core foundation of crops, fiber, food and numerous other products that are beneficial to human beings. Plants are assaulted at numerous phases of their development with various pathogens like viruses, fungi, bacteria, insects and pests. This minimizes crops yields cause's huge financial losses to the farmers. Due to plant diseases, approximately 10% of crops products are vanished (Reem *et al*., 2017). The main infectious agents of plants/crops are pathogenic fungi, leading changes during growth phases together with post-harvest. As vegetables and fruits are concerned, there is a broad diversity of fungi genera leading to quality issues connected to characteristic, organoleptic properties, dietary value and minimum shelf life (Paola *et al*., 2011). In numerous circumstances, toxic fungi are as well ultimately accountable for the poisoning and allergic disorders among customers (Kursa et al., 2022).

For many decades, a number of numerous synthetic substances and chemicals has been applied as antifungal compounds to diminish the pathogenic fungi. Nevertheless, the need for sustainability and green chemistry has show the way to a renewal research attention in the application of substitute techniques for disease and pest control that create least harm human, animal and ecosystem, counting the utilization of bio antifungal substances (Loran et al., 2022). Usually, chemical fungicides manage plants pathogenic fungi; though, its applications are progressively constrained because of poisonous effects on ecosystem and human health (Harris *et al*., 2001). The rising order of regulations and production application of pesticides and the appearance of pests opposed to the manufactured goods in use, support the exploration for new control strategies and novel active molecules (Paola *et al*., 2011). Currently it is the moment to explore and utilized alternate, environment friendly and secure pesticides to control plant diseases. Therefore, in this connection we select the wild mint for our study.

The herbs' wonderful biosynthetic capabilities create it probable to utilize them to develop natural preparation and use them as a substitute for man-made chemicals. Natural fungicides called natural bioactive compounds are non-specific and its outcome on pests is comprehensive. The result of natural bioactive agents comprised on herbs extracts depends mostly on the composition of alkaloids, terpenes and phenols (Kursa et al., 2022). *Mentha longifolia* (L.) Hudson is commonly known as a wild mint. It is native to Africa, Europe and Asia. It is a herb extensively utilized in cosmetic, confectionary, food, perfumery and pharmaceutical industries (Saeedeh and Parissa 2016). It is employed to provide distillate, infusion, additives, food, decoction and household (Mir *et al*., 2018). Wild mint is extensively used for menstrual pain, cough, headache, sunstroke, stomachache, shortness of breath, hemorrhoids and sore throat (Ozer, 2018). The bioactive compounds analysis exposed that the wild mint posses numerous phytochemicals like glycosides, flavonoids, nicotinic acid, tannins, terpenes derivatives, terpenes, menthone and menthol (50%) by which the mint plant have credited biological activities (Walaa et al., 2021). The phytochemicals synthesized by herbs under the pressure of foreign molecules (elicitors) also have a defense position in connection to pests (Kursa et al., 2022). Therefore, to assess the antifungal potential of bioactive agents, it is crucial to find out their ability on the fungal growth inhibition. The current study objectives was to quantified the selected phytochemicals and antifungal potential of *Mentha longifolia* stem, leaves and flower hydroalcoholic, infusion and decoction extracts.

2. Material and Methods

2.1. Plant Collection and Preparation

Mentha longifolia L. healthy plants were collected from the main campus lawns of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar, Khyber Pakhtunkhwa-Pakistan. The plants parts (flower, leaves and stem) were separated and washed thoroughly in tap water and dried under forced circulation of heated air at 40 °C to minimize deterioration of the herbs active molecules and ground to powder using Standard Model No.3 Wiley Mill USA in the Pilot Plant of PCSIR Peshawar-Pakistan. The powder samples shifted aseptically into sterilized brown bottles and kept at -10 °C in cold incubator (Gallenkamp- England) for further experimental work.

2.2. Hydroalcoholic (HA) Extract

The hydroalcoholic solvent comprised 50% ethanol and 50% H_2O (100 mL) were added into each designated flask for each powder samples (20g). The aluminum foil was used to close the Erlenmeyer flasks and store at dark room for ten days. Subsequently, the flasks were putted for 24 hours on a shaker working at 90 rpm. The mixer filtration was carried out using 45 µm membrane filter. The Vacuum Rotary Evaporator was used to dry the filtrate. The evaporation was stopped when the sample materials in the rotary flask remain $1/5^{th}$. The viscous materials were poured into a beaker and kept on water bath at 45°C, until a constant weight of the beaker obtained. The dried extract was precisely weighed, labeled and refrigerated.

2.3. Preparation of Infusion and Decoction

The procedure of Ozer, 2018 with slight amendment was applied for infusion and decoction extraction. For infusion; the plant material 4 g were added into 200 mL of boiling distilled H_2O and permitted to stand for fifteen minutes. For decoction; the plant sample 4 g were added into 200 mL of distilled H_2O and warm together in a beaker and permitted to keep for fifteen minutes following it boiled. The 45 µm membrane filter paper were used to filtered the mixture. The mixture were concentrated at 45 °C under pressure using the Vacuum Rotary Evaporator and the viscous concentrate were decanted in a beaker and kept on hot plate to obtained a constant weight of the dry plant extract.

2.4. Quantitative Screening of Phytochemical Compounds

The alkaloids concentration (%) was determined using the alkaline precipitation gravimetric technique, flavonoids (%) was quantified according to the gravimetric procedure and saponins (%) was analyzed by double extraction gravimetric protocol (Harborne, 1998). Phenolic compounds were analyzed by Folin–Ciocalteu method using spectrophotometer (Mir *et al*., 2018). The content of phenol were quantified in expressions of Gallic acid milligrams of equivalent per gram of plant dry weight (mg GAE/g DW).

Aqueous Extract

2.5. Antifungal Potential Assays

The study fungi were procured from Environmental Research Laboratory at Food Technology Center (FTC) of PCSIR Laboratories Complex Peshawar-Pakistan. These cultured were maintained on Potato Dextrose Agar (PDA, Oxoid, Hampshire, United Kingdom) slants.

2.5.1. Agar well diffusion Method

Sterile and solidified PDA plates were aseptically swab with fungal inoculums. Subsequently, in the cultured plates seven (07) millimeter diameter wells were aseptically hollow out and the extracts of *Mentha longifolia* different parts in a quantity of 100.0 mg/mL of 10% dimethyl sulfoxide (DMSO), negative control blank (10% DMSO) and positive control Mancozeb (20 mg/mL) were shifted into the particular tag wells individually. The inoculated plates were kept in an incubator for 72 hours at 35°C in an upright situation and around the well the inhibition zone appearance was documented (Narasimharaju *et al*., 2015).

2.5.2. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) Determination

The MIC and MFC assayed by the broth macrodilution technique in 10 mL test tubes. Experiments were performed in potato dextrose broth (PDB, Oxoid, Hampshire, United Kingdom) to determine MIC. The plant extracts were added at different concentrations (200, 100, 50, 25 and 12.5 mg/mL) dissolved with 10% DMSO in the PDB. Control treatment Mancozeb (20, 15, 10 and 5 mg/mL) were prepared in the same manner but without adding the tested extracts. The spore suspension (10^6 spores/mL) 100 µL were inoculated in all the test tubes, mixed thoroughly, and incubated for 10 days at 25 °C. MIC was quantified as the minimum dosage of the added extracts, which was capable to inhibit totally the observable growth of the plant pathogenic fungi. To assess MFC, 100 µL of the medium of each trial in which fungal growth was not observed was sub-cultured on PDA plates and incubated at 25 °C for 7 days. The MFC was considered as the minimum concentration at which no growth was observed on the plates (Loran et al., 2022).

2.5.3. Spore Germination Assays

The tested fungi spore suspension having 30 spores per microscopic filed was arrange from seven days old cultures of individually experimented plant pathogenic fungi. Put on a glass slides one drop of spore suspension along with a drop of selected extracts of *Mentha longifolia*. Consequently, slides were reserved in humid compartment arranged by placing of two-fold filter paper in the Petri plates in both side were kept in an incubator (Memmert-Germany) for 24 hours at 28 °C. The light microscope were used to examine the slides at X400 magnification to observed the sporangia germination rates. The germ tubes generated spores were counted and spore germination percentage were recorded. The germination percentage was observed applying bellow formula (Ibrahim *et al*., 2014).

Spore Germination % = Spore Germination Number/ Spores Total Number X 100

3. Statistical analysis

Each experiment were run in triplicate, average were determined with standards deviation (±SD) for phytochemicals quantification and zone of inhibition. Averages of spore germination percentages (standard error of the mean (SEM) for each treatment were used.

4. Results and discussion

4.1. Phytochemicals Compounds and Extraction System

The phytochemicals analysis of *Mentha longifolia* leaves, stem and flower using infusion, hydroalcoholic and decoction extraction system shown in Table 1. The results showed that the phytochemicals compounds were assay in high concentration in leaves, followed by stem and flower. The hydroalcoholic extraction system was the found to exhibit the maximum quantity of bioactive compounds, followed by infusion and decoction. The flavonoids were found in the range 3.8-2.4 %, alkaloids range 0.1-0.7 %, saponins range 5.6-3.4% and total phenolic content 40-15 mg Gallic acid/g.

Table 1. Phytochemicals Analysis of *Mentha longifolia*

Plant Parts	Extraction system	Flavonoids (%)	TPC (mg Gallic acid/g)	Saponins (%)	Alkaloids (%)
Leaves	Infusion	3.7±01	33±01	5.5±0.2	0.6±00
	Hydro-Alcoholic	3.8±0.6	40±02	5.6±0.5	0.7±00
	Decoction	3.4±0.3	21±0.4	5.3±0.4	0.4±00
Stem	Infusion	3.3±1.5	25±01	4.4±01	0.4±00
	Hydro-Alcoholic	3.5±0.1	37±01	5.4±01	0.5±00
	Decoction	3.1±00	18±1.5	4.2±0.5	0.2±00
Flower	Infusion	3.1±00	20±01	3.9±0.6	0.3±00
	Hydro-Alcoholic	3.3±00	26±01	4.3±01	0.4±00
	Decoction	2.4±00	15±01	3.4±01	0.1±00

Each values showed as average ± SD of triplicate analysis (n = 3), TPC=Total Phenolic Content

The phytochemicals analysis showed the occurrence of flavonoides and phenols in enormous quantity in wild mint extracts (Ghoulami *et al*., 2001; Akroum *et al*., 2009; Seyed *et al*., 2012; Mir *et al*., 2018). These reported phytochemicals quantification in *Mentha longifolia* as found to dissimilar and varied as compared to our current study. Many researchers as explained these; differences in herbs ingredients might be because of the variations in soil composition, interaction of various microorganisms with herbs, climatic conditions of each season, maturity stage, plants portions, altitude, plant species and geographic region of collection (Saeedeh and Parissa 2016; Javid *et al*., 2017; Demo and Oliva, 2008; Webster *et al.*, 2008). Our study showed that hydroalcoholic extracts were the most efficient to extracts the bioactive compounds. These findings were close agreements to the previous studies (Leila *et al*., 2015; Murtada and Abdelkarim 2013; Tatiya *et al*. 2011). However, dissimilarities from our studies observed by previous findings of Mir *et al*., 2018 were concluded that infusion were more actives to extracts phenolic compounds as compared to ethanol and Ozer 2018 observed that decoction were found more phenolic compounds as compared to infusion. The variation might be concerned local, climatic and seasonal differences.

The bioactive molecules studies reported the occurrence of numerous flavonoids (Ghoulami *et al*., 2001), saponins, tannins and ketones in herbs of Mentha genus (Seyed *et al*., 2012). The phytochemicals in the cold water extracts of wild mint leaves possessed the occurrence of resins, phenols, alkaloids, tannins and saponins, although the glycosides were absent (Walaa et al., 2021). These substances are accredited for various biological and pharmacological activities of the mentha species (Seyed *et al*., 2012). The specific kind of phenolic substances, like tannins, flavonoids and phenolic acids have an extra straight effect on pathogenic fungi (Kursa et al., 2022). The ethyl acetate and n-hexane of wild mint were abundant in menthone with 15.28% and 18.37% respectively (Mohamed et al., 2020). The menthone a common phenolic monoterpene that was blamed to hold hydrophobic properties leading distraction of microorganism discriminating porosity in the course of its absorption into microorganisms cell membrane (Saharkhiz et al., 2012).

The solvent chemical properties, extraction process method and different compositional and structural feature of the bio products come out from each substances solvent system observing distinctive actions (Pinelo *et al*. (2004). Polarity distinctions among numerous solvents have been documented to account for the variations in solubility of active herbal bioactive characteristics, therefore differences in the level of potential (Paola *et al*., 2011).

4.2. *In-Vitro* Antifungal Activities

4.2.1. Agar well diffusion, MIC and MFC

Antifungal activities of *Mentha longifolia* leaves, stem and flower infusion, decoction and hydroalcoholic extracts were tested against four phytopathogenic fungi such as *Fusarium oxysporum*, *Aspergillus parasiticus*, *Rhizoctonia solani* and *Alternaria alternata*. Agar well diffusion technique was employed to determine the antifungal activity by quantifying the zone of inhibition diameter. The antifungal potential with zone of inhibition of *Mentha longifolia* extracts of leaves, stem and flower are shown in Table 2. The maximum zone of inhibition 23±1 mm was calculated against *Fusarium oxysporum* by hydroalcoholic extract of leaves. The leaves extracts have found the most high zone of inhibition, stem observed the moderate zone of inhibition and flower shoed the least zone of inhibition. Similarly, the hydroalcoholic extracts calculated the maximum zone of inhibition, infusion the moderate and decoction have found the least zone of inhibition.

Table 2. Antifungal Activity (Zone of Inhibition) of *Mentha longifolia*.

Fungi	Plant Parts	Extracts/Control				
		Zone of Inhibition in millimeter (mm)				
		Decoction	Hydro-Alcoholic	Infusion	10% DMSO	Mancozeb
Fusarium oxysporum	Leaves	18±1	23±1	19±1	--	25±01
	Stem	16±1	21±0	17±1		
	Flower	13±0	13±0	12±1		
Aspergillus parasiticus	Leaves	18±1	22±1	17±1	--	27±0.5
	Stem	14±1	19±0	15±0		
	Flower	10±0	11±0	11±0		
Rhizoctonia solani	Leaves	11±0	20±1	12±0	--	24±1.5
	Stem	10±0	16±0	11±0		
	Flower	--	14±1	10±0		
Alternaria alternata	Leaves	17±1	21±1	18±2	--	26±01
	Stem	12±0	14±0	13±0		
	Flower	10±0	12±0	11±0		

Each values expressed as average ± SD (n = 3), -- = No zone of inhibition.

The MIC and MFC of decoction, hydroalcoholic and infusion extracts of leaves, stem and flower of *Mentha longifolia* were calculated and shown in Figure 1. The hydroalcoholic extracts of leaves and stem showed maximum potency (low MIC=12.5 mg/mL and MFC=25 mg/mL) against *Fusarium oxysporum*. Leaves hydroalcoholic extracts calculated 25 mg/mL MIC against

Aspergillus parasiticus and *Alternaria alternata*. The infusion leaves extracts observed 25 mg/mL MIC against *Fusarium oxysporum* and *Alternaria alternata*. While stem and flower infusion extracts MIC were found in the range of 50-100 mg/mL. The decoction MIC range was 25-200 mg/mL. The flower MFC range 100-200 mg/mL, stem MFC range were 25- 200 mg/mL and leaves MFC range were 25-100 mg/mL for three extracts system. The decoction flower extract showed relatively less activity (high MIC and MFC values) when judge against with the leaves and stem hydroalcoholic and infusion extracts against all examined plant pathogenic fungi.

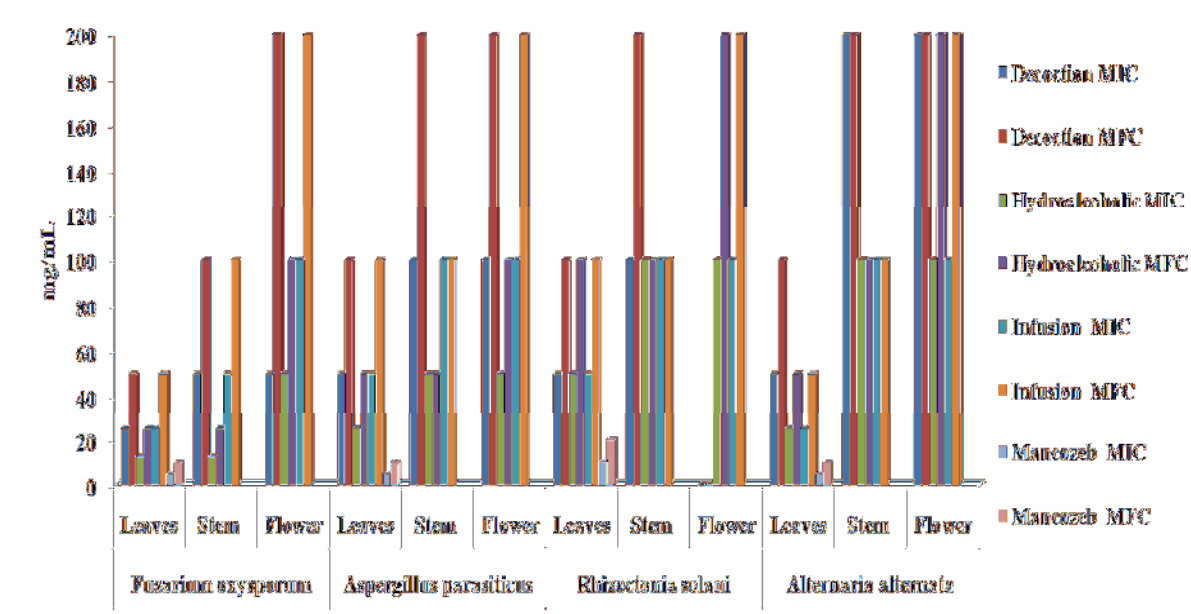

Fig.1. Antifungal Activity (MIC and MFC) of *Mentha longifolia*.

The antifungal activities of *Mentha longifolia* against various phyto pathogenic fungi were well documented (Maisa *et al*., 2015; Seyed *et al*., 2012; Khattak *et al*., 2004). The leaves extracts of *Mentha piperita* exhibited antifungal potential against plant pathogenic fungi (*Aspergillus oryzae*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus niger*) (Javid et al., 2017). Alkaloids possess antifungal activities, which are commonly present in toxic herbs. Alkaloids are effectual against fungi, parasites and bacteria. These compounds enter into the fungus cell wall and DNA (Kursa et al., 2022). The dissimilarities and variation from our study may be due to the solvent extraction system, solvents, plants parts, methods, geographical variation, seasonal and plants maturity.

The phenolic acids could destroyed the porosity of the microorganisms membrane through interrelate with lipid bilayers, resultant in homeostasis disturbance by means seepage of cell ingredients and growth destruction (Loran et al., 2022). The phenolics compounds antifungal activities are accredited to the occurrence of the hydroxyl groups in their shape and/ or their lipophilicity properties**.** Because of its binding characteristics with protein and adhesions, they are capable to disturb complex metal ions, inactivate enzymes and membrane, thus exerting deadly properties upon fungi. In exacting, the hydroxyl groups are occupied in the disconnection of oxidative phosphorylation, while phenolics lipophilicity properties make easy diffusion of the cytoplasmic membrane **(**Kursa et al., 202). Generally the plants leaves were found many flavonoids such as kaempferol glycosylated derivatives, apigenin, luteolin and quercetin. It was reported that these compounds are accountable for elevated antimicrobial potential of the herbs water, ethanol and methanol extracts. It was reported that a maximum antimicrobial activities of the methanol extract than hexane or dichloromethane might be because of the presence of flavonoids as polar substances. Numerous glycosylated flavonoides hold a synergism outcome in the antifungal and antibacterial activities (Seyed *et al*., 2012). Additionally, non-polar volatile substances such as menthol possessed in the essential oil of the wild mint were claim to hold a high antifungal and antibacterial activities (Seyed *et al*., 2012). The plant bioactive phenolic molecules alone or in mixture hold up the fungi life process by fastening their protein compounds, behave as chelating substances, changing shape constituent synthesis, destroying or weakening the porosity wall of the cell membrane and altering the cells physiological condition (Domenico *et al*., 2015). The mechanisms engaged behind the antifungal activities of phenols substances are; fungi membrane alterations have an effect on functions and permeability, inhibition of oxidases, oxidative stress reduction and mycotoxin involved key genes synthesis downregulation expression (Loran et al., 2022).

The water extracts were found least bio-fungicide characteristics aligned with the investigated fungi might be proved by the reality that when herbal samples are soaked in aqueous, various hydrolases and phenolases are extracted and might exerted modulating effects on the efficiency of the molecules in the extracts. Additionally it might also be blamed to unfinished extraction of the bioactive molecules (El-Mahmood *et al*., 2008). The phytochemicals of the plants and antimicrobial activities have a strong

correlation (Leila *et al*., 2015). The wild mint antimicrobial activities could be accredited to the occurrence of oxygenated monoterpenes in their phytochemicals substances (Mimica-Dukić et al., 2003, Şahin et al., 2003).

The prominent bioactive constituents in the essential oil of Mentha sp. are carvone and menthone, and they hold antifungal activities because they acts on the ergosterol of cell membrane biosynthesis causing of the death of the fungal cells (Samber et al. (2015). Usually, it observed that the plants extract antifungal effect varies because of the distinct solvents, plants and tested fungi with no precise tendency related to the solvents polarity (Thembo *et al*., 2010). All used fungi insensitivity in the experiment against the extracts could be due to their distinctive cell wall (Murtada and Abdelkarim 2013). Contrasting the bacteria, fungi are eukaryotic and composed hard cell walls holding polysaccharides as well as chitin and a cell membrane comprised of ergosterol (Harvey and Champe, 2000). The sensitivity level variations of the fungal species could be due to the inherent tolerance of the fungi species and to the mixtures of bioactive molecules occurred in the extracts (Leila *et al*., 2015). The fungi illustrated different level of tolerance and sensitivity to the herbal extracts. The findings observed that there was no identical reaction between or within the experimented fungi of the similar strains in conditions of vulnerability to antifungal activities in the extracts (Thembo *et al*., 2010). Sensitivity differences between the microorganisms to antibiotic compounds in the extracts of plants could be sum up by the differences in the cell wall composition, genes inheritance on plasmids might be merely altered among bacterial and fungal strains (Sandrock and van Etten 1998). It was reported that both non-polar and polar substances exerted antifungal activities of the plants extracts (Thembo *et al*., 2010). The response of particular fungi to a particular extracts is well significance consider. This would mean that the different genetics of fungus and the choice of particular plants ingredients are significant when plant fungicides development (Thembo *et al*., 2010). An immune system is not present in plants and therefore definitely depends on additional systems to protect themselves from attacked by a diversity of pathogens. As fungal infections are concern, these principles comprise synthesis of peptides (Broekaert *et al*., 1997), antifungal proteins (Selitrennikoff, 2001) and bioactive organic compounds (Morrisey and Osbourn, 1999). In response to the microbial infection the plants are synthesized the phenolic compounds. As a result, it is probable that they can perform as effectual antimicrobial agents against a broad range of pathogens (Leila *et al*., 2015). The antimicrobial activities variation might be due to the diverse protocol adopted or a dissimilar geological environment, method of substances extraction, physiological age of the plant, seasonality and cultivar type (Rajinder *et al*., 2015).

4.2.2. Percent Spore Germination

The *Mentha longifolia* leaves, stem and flower extracts (infusion, decoction and hydroalcoholic) at different concentration 12.5, 25, 50, 100 and 200 mg/mL for the spore germination assay. Germination percentage of tested fungi conidia decreased as the concentration increase in all *Mentha longifolia* extracts treatments (Table 3, 4, 5 and 6). The *Fusarium oxysporum* spore germination (%) is shown in Table 3. The result shows that at 200 mg/mL concentration of leaves extraction system observed no spore germination. The stem extracts are found spore germination (%) in the range 20 - 46.7 at 200 mg/mL. While the flower extracts observe the spore germination (%) range 36.7 - 46.7 at 200 mg/mL. The spore germination rates are reducing as the concentration increases. The conidia of *Aspergillus parasiticus* did not germinate in leaves (hydroalcoholic, infusion and decoction) and stem hydroalcoholic extracts at 200 mg/mL (Table 4). The *Rhizoctonia solani* spores germination is shown in Table 5. The results showed that the no spore's germination was found at 100 mg/mL and 200 mg/mL of leaves infusion and hydroalcoholic extracts against *Rhizoctonia solani*. The stem and flower extracts system of *Mentha longifolia* at 200 mg/mL spore germination percentage range were 23.3±01 - 66.7±01.

Table 3. Percentage Conidial Germination of *Fusarium oxysporum*.

Plant Parts	Extraction System	Percent Germination of Conidia				
		Concentration (mg/ mL)				
		12.5	25.0	50.0	100.0	200.0
Leaves	Hydro-Alcoholic	90.0±01	16.7±01	13.3±01	3.3±00	0.0±00
	Infusion	93.3±01	23.3±01	20.0±00	6.7±00	0.0±00
	Decoction	96.7±01	50.0±01	36.7±00	10.0±00	0.0±00
Stem	Hydro-Alcoholic	90.0±01	60.0±1.5	53.3±01	43.3±01	20.00±00
	Infusion	96.7±01	66.7±01	56.7±1.5	46.7±01	23.3±00
	Decoction	96.7±1.5	90.0±02	73.3±01	66.7±01	46.7±01
Flower	Hydro-Alcoholic	90.0±02	70.0±01	50.0±00	40.0±01	36.7±00
	Infusion	96.7±0.5	86.7±1.5	73.3±01	66.7±00	43.3±00
	Decoction	96.7±1.5	80.0±02	76.7±01	66.7±02	46.7±01
Positive Control = Mancozeb (20 mg/mL) = 0.0±00						
Negative Control = Absence of extract (Blank/ Distilled H_2O) = 100±00						

Each values are an average ± standard error of the mean (SEM), n = 3.

Table 4. Percentage Conidial Germination of *Aspergillus parasiticus*.

Plant Parts	Extraction System	Percent Germination of Conidia				
		Concentration (mg/ mL)				
		12.5	25.0	50.0	100.0	200.0
Leaves	Hydro-Alcoholic	90.0±03	26.7±01	23.3±01	10±0	0.0±00
	Infusion	90.0±02	60.0±02	36.7±01	16.7±0	0.0±00
	Decoction	93.3±01	63.3±01	40.0±01	23.3±0	0.0±00
Stem	Hydro-Alcoholic	90.0±02	50.0±01	46.7±01	43.3±01	0.0±00
	Infusion	93.3±01	63.3±01	60.0±00	56.7±01	36.7±01
	Decoction	93.3±01	60.0±1.5	53.3±01	50.0±01	20.0±00
Flower	Hydro-Alcoholic	90.0±01	83.3±01	76.7±01	63.3±01	30.0±01
	Infusion	93.3±01	86.7±01	80.0±02	70.0±02	36.3±00
	Decoction	93.3±01	90.0±03	86.0±03	73.3±01	43.3±01
Positive Control = Mancozeb (20 mg/mL) =		0.0±00				
Negative Control = Absence of extract (Blank/ Distilled H_2O) =		100±00				

Each values are an average ± standard error of the mean (SEM), n = 3.

Table 5. Percentage Conidial Germination of *Rhizoctonia solani*.

Plant Parts	Extraction System	Percent Germination of Conidia				
		Concentration (mg/ mL)				
		12.5	25.0	50.0	100.0	200.0
Leaves	Hydro-Alcoholic	90.0±02	33.3±01	30.0±00	0.0±00	0.0±00
	Infusion	90.0±01	46.7±01	50.0±00	0.0±00	0.0±00
	Decoction	90.0±03	66.7±01	63.3±00	40.0±01	20.0±00
Stem	Hydro-Alcoholic	90.0±01	46.7±01	43.3±01	40.0±02	26.7±01
	Infusion	90.0±04	50.0±00	46.7±01	43.3±03	30.0±01
	Decoction	90.0±02	53.3±01	50.0±00	46.7±01	26.7±00
Flower	Hydro-Alcoholic	90.0±02	63.0±02	60.0±01	56.7±01	23.3±01
	Infusion	90.0±02	66.7±01	63.3±01	60.0±03	26.7±01
	Decoction	90.0±01	86.7±01	83.3±02	73.3±01	66.7±01
Positive Control = Mancozeb (20 mg/mL) =		0.0±00				
Negative Control = Absence of extract (Blank/ Distilled H_2O) =		100±00				

Each values are an average ± standard error of the mean (SEM), n = 3.

Table 6. Percentage Conidial Germination of *Alternaria alternata*.

Plant Parts	Extraction System	Percent Germination of Conidia				
		Concentration (mg/ mL)				
		12.5	25.0	50.0	100.0	200.0
Leaves	Hydro-Alcoholic	90.0±03	40.0±01	36.7±01	0.0±00	0.0±00
	Infusion	93.3±01	56.7±01	53.3±00	0.0±00	0.0±00
	Decoction	96.7±02	70.0±01	66.7±00	0.0±00	0.0±00
Stem	Hydro-Alcoholic	90.0±02	60.0±00	50.0±01	33.0±00	0.0±00
	Infusion	93.3±01	66.7±01	53.3±00	36.00±00	13.3±00
	Decoction	96.7±03	70.0±02	63.3±01	40.00±01	16.7±01
Flower	Hydro-Alcoholic	90.0±02	73.3±01	70.0±02	66.7±01	20.0±01
	Infusion	96.7±01	80.0±03	76.7±01	73.3±00	33.3±01
	Decoction	96.7±01	83.3±01	80.0±1.5	70.0±02	36.7±01
Positive Control = Mancozeb (20 mg/mL) =		0.0±00				
Negative Control = Absence of extract (Blank/ Distilled H_2O) =		100±00				

Each values are an average ± standard error of the mean (SEM), n = 3.

The *Mentha longifolia* parts extracts % spore germination of *Alternaria alternata* is shown in Table 6. The results showed that at 100 mg/mL and 200 mg/mL concentration of leaves extracts (hydroalcoholic, infusion and decoction) were found no germination.

The stem and flower extracts at 200 mg/mL showed spore germination % in the range of 13.3 - 36.7. The augmented concentration showed an involvement of an increase in the phytochemicals in the solutions that take action on phyto-pathogenic fungi. Germination of conidia of the tested fungi in the current study reduces with raise plant extract concentration and generally, all the investigated concentration observed maximum inhibitory activities at high dosage. It was observed in our study that increase in extracts concentration reduce the germination percentage of spores in all tested fungi. The positive control also showed that germination of conidia did not observe on tested concentration. Conidia germination was reduce when *Mentha longifolia* extracts increases. All the wild mint extracts concentration had an inhibitory potential comparatively high as compared with negative control.

The phytochemicals compound utilize in herbs protection not merely fungistatic effect however also involve in protection system of the plant (Kursa *et al*., 2022). Fungal spore germination was inhibited by flavonoids and have been reported to manage toxic fungal strains**.** *Mentha piperita* have possessed flavonoid diglycosides and have the potential to inhibit the growth of the mycelium of *Phoma sorghina* and *Fusarium moniliforme* (Saul *et al*., 2020). Direct effect of the phytochemicals is based on the reduction of hyphae growth, inhibition of spores germination and fungal sporulation (Kursa *et al*., 2022). The water extract (200g/200mL) of Mentha arvensis against *Rhizopus stolonifer* and *Alternaria alternata* spore germinations were 22.57% and 20.44% respectively (Taskeen *et al*., 2010). Different plant extracts such as garlic, safeda, mehendi, mint and neem were applied for their antifungal potential to inhibit and control the spore germination of Alternaria brassicae a causative agent of Alternaria blight in mustard and rapeseed. These plants extract showed spore germination inbition (Khurana et al., 2005).

It was obvious from our research findings that *Mentha longifolia* extracts showed the antibiotic potential against under study plant pathogenic fungi at numerous degree because of differences in fungi, plant parts and solvent extracts. Commonly all *Mentha longifolia* extracts revealed antifungal potential at diverse dosages as depicted in all figures and tables. However the current study limitation includes; low samples quantity, tested fungi, extraction techniques.

5. Conclusions and way forward

Wild mint was investigated for its phytochemicals and antifungal properties. Findings indicated that wild mint has great antifungal value and thus, it could be considered as a source of natural antifungal agents for combating of plant pathogenic fungi. The outcomes of the study are the first round stage for additional field trials to investigate the antifungal potential of wild mint extracts in the field tests, their biological stability and allelopathic effects, while further prepared a bio-fungicide to safeguard plants against phyto-pathogenic fungi. Though, the findings acquired verification of the fungistatic ability of the wild mint under *In-vivo* circumstances and appropriately synthesized a formula of wild mint extracts (Bio-fungicide) might maximize the power of fungistatic potential and give the guideline for the formulation of plant origin new biofungicide. As a result it will give a secure substitute for organic and sustainable crop production.

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