International Journal of Secondary Metabolite
2022, Vol. 9, No. 4, 387–396
https://dx.doi.org/10.21448/ijsm.1079952
Published at https://dergipark.org.tr/en/pub/ijsm
Research Article

Composition analysis and antibacterial activity evaluation of different crude extracts of *Mentha piperita* (Lamiaceae)

Zahra Mohammed Al-Hajri[1], Mohammad Amzad Hossain[1*], Salim Said Al-Touby[2]

[1]Nizwa University, School of Pharmacy, College of Pharmacy and Nursing, Nizwa, Sultanate of Oman
[2]Nizwa University, School of Nursing, College of Pharmacy and Nursing, Nizwa, Sultanate of Oman

Abstract: Globally, the main target of scientists is to examine new medicinally beneficial plants for the preparation of herbal drugs as well as to explore possible uses in the food industry. In this regard, the target of our current study is to evaluate the antibacterial activity and biochemical analyses of the leaf extracts of Omani *Mentha piperita* L (*M. piperita*). The selected plant leaves coarse powder samples were extracted by using the Soxhlet extraction process with methanol at 65°C for a period of 72 hours. The evaporation of methanol was done by a rotary evaporator under reduced pressure and temperature. The hydro alcoholic extract was fractionated with the various polarities of solvents with an increasing pattern. The biochemical evaluation and pharmacological activity of the prepared local plant extracts were completed by using established methods. The different polarities of leaves extracts showed positive biochemical tests of alkaloids, flavonoids, glycosides, saponins, steroids, tannins and triterpenoids. The microbial activity of the fractioned plant extracts was tested with improved agar gel method. The different polarity extracts at different concentrations did not display any activity against the tested Gram (+) *Staphylococcus aureus* (*S. aureus*) and Gram (-) *Escherichia coli* (*E. coli*) and *Pseudomonus aeruginosa* (*P. aeruginosa*) bacterial strains. Based on the biochemical and pharmacological evaluation results, the selected whole plant and its potential extracts might be used traditionally as natural antibiotics to treat infectious diseases.

ARTICLE HISTORY
Received: Feb. 27, 2022
Revised: Aug. 19, 2022
Accepted: Oct. 28, 2022

KEYWORDS
Mentha piperita,
Organic crude extracts,
Biochemical evaluation,
Antibacterial activity.

1. INTRODUCTION

Mentha piperita L is known as a hybrid of two species watermint and spearmint belonging to the Lamiaceae family. This plant is native to Oman including European countries, but it is cultivated globally due to its medicinal values (Georgiev & Stoyanova, 2006). Several other varieties/species like apple mint, water mint, horsemint, pennyroyal, and spearmint belonging to the same family are also available in Oman. The height of this plant is about 30–90 cm. The stems are smooth and square. The cross-section and its rhizomes are wide spreading. The size of the leaves is about 1.5-3.5 inches long and 2.5 inches wide with an oval shape. They are dark green with reddish veins (Figure 1).

*CONTACT: Md. Amzad Hossain ✉ dramzadh@gmail.com School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, OMAN

ISSN-e: 2148-6905 / © IJSM 2022

Figure 1. Appearance of extracted organs (leaves) of *M. piperita*

The selected plant is used as a traditional folk remedy including complementary and alternative herbal therapy for the treatment of different disorders. In addition, it is widely used in Chinese traditional healing medicine for different ailments. Since ancient times, the Egyptian people have been using the powder of this plant in pyramids to kill bacteria (Georgiev & Stoyanova, 2006). Also, the leaf crude extracts of the selected plant are used to prepare various modern medicines. Due to their aromatic qualities, nowadays, the essential oil and leaves crude extracts are commercially used to produce foods, and medicines (Georgiev & Topalova 1989). In addition, the plant oil is used to prepare commercial products like toothpaste, chewing gum, mouthwash, soaps, sweets, balms or creams, and cough medicine (Georgiev & Topalova 1989; Stanev & Jelyazkov, 2004; Nedkov *et al.,* 2005; McKay & Blumberg, 2006). People use this plant mainly for its oil. The main ingredients in the oil are composed of menthol and menthone along with a high percentage of other biologically active minor compounds like pulegone, menthofuran and limonene (Georgiev & Topalova 1989; Sivropoulou *et al.,* 1995). Several scientific data are available in the literature that the selected plant species showed different pharmacological activities including scavenging activity, anticancer, antispasmodic, antiviral and antimicrobial activities (Spirling & Daniels, 2001; Aswatha *et al.,* 2008; Hossain *et al.,* 2008; Sun *et al.,* 2014). The pharmacological activity of the plant extracts and oil are directly linked to the bioactive compounds. Most researchers have detected anti-inflammatory, anti-bacterial and anti-cancer properties of this plant (Hills *et al.,* 2005; Tabari *et al.,* 2012; Sun *et al.,* 2014). Previously, several researchers have worked on leaves (Tabari *et al.,* 2012; Sun *et al.,* 2014) and roots of this plant (Hills *et al.,* 2005) and studies have exposed significant biological activity against several Gram (+ and -) bacteria strains (Hills *et al.,* 2005; Stepan *et al.,* 2016**)**. However, so far, no detailed information related to chemical analyses and the antibacterial activity of different polarity crude extracts has been published on the Omani *M. piperita* species. Keeping this in mind, the current study design is to determine the antimicrobial activity and chemical analyses of different extracts of locally collected *M. piperita leaves*.

2. MATERIAL and METHODS

2.1. Materials and Chemicals

Solvents with different polarities such as hexane, methanol, chloroform, and butanol (analytical grade, BDH, Germany) were used in this present study. Most of the reagents for biochemical analyses were of analytical grade. Antibiotic levofloxacin (positive control) and dimethyl sulphoxide (DMSO, negative control) were obtained from BDH, Germany. A blender machine (Jaipan, Super Deluxe, India) was used for the grinding of plant samples. The rotary evaporator (Model Yamato, R.E801, Japan) and Soxhlet extractor (MEDIZLAB) were from Borosil, India.

2.2. Microbial Strains

Both Gram (+) and Gram (-) bacterial strains were obtained from the Department of Microbiology, of Nizwa Hospital in December 2012. The bacterial strains were maintained on nutrient agar at 4°C. Then, the bacterial strains were cultured into a nutrient broth by an established technique at the Department of Biology, University of Nizwa, the Sultanate of Oman.

2.3. Plant Materials

The selected Omani *M. piperita* species were obtained from Hay Al Thurat, Nizwa, Oman. The sample was collected in February 2012 and after collecting the leaves samples, the morphological identification was accomplished by https://en.wikipedia.org/wiki/ peppermint. The leaves were separated from the plant, packed in a bag, and kept at 4°C until the preparation of crude extracts.

2.4. Preparation of the Samples

The leaf samples were processed and dried at normal temperature. The dried leaves (100 gm) were crushed into coarse powder and kept in an amber colour bottle for extraction.

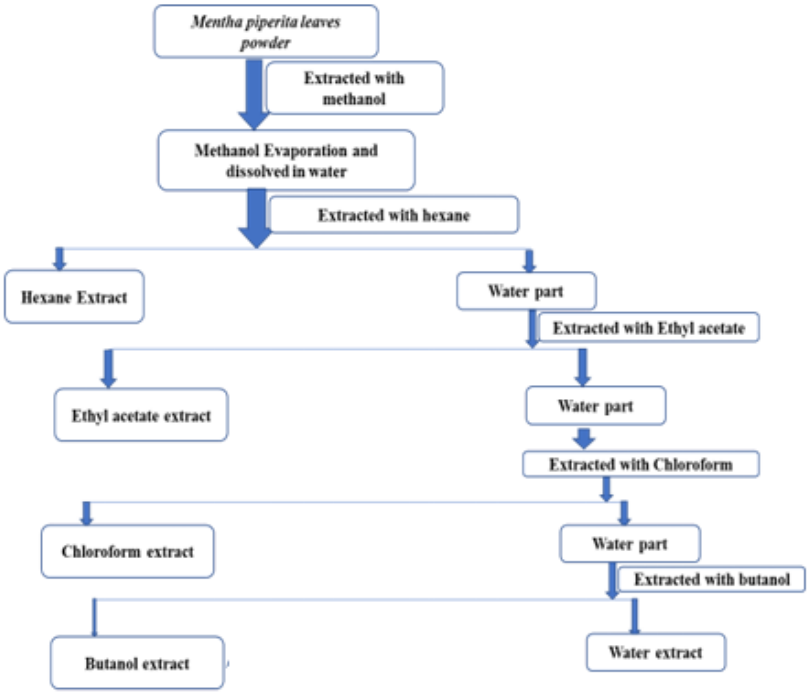
2.5. Extraction Procedure

About 80.33 gm of the powdered leaves samples were used for extraction by using a Soxhlet extractor with methanol (360 ml) at 68°C for a period of 72 hours (Asma *et al*., 2017; Raqiya *et al.,* 2017). After complete extraction and filtration, a rotary evaporator was used to remove methanol and give viscous semi-solid masses (7.99 gm, yield 9.94 %). The semi-solid extract (6.68 gm) was liquefied in water (75 ml) and then the sample was fractionated by various polarities of solvents: hexane (1.88 gm, yield 28.14%), ethyl acetate (1.65 gm, yield 24.7%), chloroform (0.12 gm, yield 1.79%) and butanol (1.51 gm, yield 22.60%) and the residual fraction was (0.90 gm, 1.34%) (Asma *et al.,* 2017; Raqiya *et al.,* 2017). The extraction process was repeated twice and then the solvent was evaporated under reduced pressure and temperature (Figure 2).

2.6. Biochemical Analyses

The plant powder materials, crude extracts and stock solutions were used for chemical analyses by the methods described by Tahiya *et al.,* 2014; and; Ali and Hossain, 2014). Each polarity plant leaf extracts (1 mg) was liquefied in it mother solvent (100 ml) to give the stock solution (1%, v/v).

Figure 2. Flow chart for extraction



2.6.1. *Identification of alkaloids*

The plant coarse powder (1 gm) was taken into a small beaker and dil NH_3 solution (3 ml) was added and it was kept for a few minutes with constant stirring. Chloroform (10 ml) was added to the same beaker and the mixture was shaken and filtered. The filtrate was evaporated to 50% by using heat and then Mayer's reagent was added drop by drop. A creamy colour precipitate appeared indicating the existence of alkaloids.

2.6.2. *Identification of flavonoids*

The stock solution of each plant extract (1 ml) was taken into an appropriate test tube. A few drops of dilute NaOH were added to each test tube. A deep yellow colour was found with the addition of dilute HCl that gradually becomes colourless. It showed the existence of flavonoids.

2.6.3. *Identification of saponins*

The stock solution of each plant extract (1 ml) was taken into six separate test tubes. The stock solution was diluted with the addition of H_2O and the whole solution was shaken for a few minutes. A foam layer appeared in the upper part of the test tube that showed the existence of saponins.

2.6.4. *Identification of steroids*

The stock solution of each plant extract (1 ml) was taken into a 20 ml beaker and was dissolved in $CHCl_3$ solvent (10 ml). Concentrated H_2SO_4 (1 ml) was added to the beaker dropwise. In the beaker, two layers were formed. The top layer turned red and the bottom layer turned deep yellow which indicated the existence of steroids.

2.6.5. *Identification of tannins*

The stock solution of each plant extract (3 ml) was taken into a beaker and was diluted with $CHCl_3$ solvent. Then, one millilitre $(CH_3CO)_2O$ was added to the beaker and finally Conc. H_2SO_4 was added carefully drop by drop. A green colour was obtained that showed the existence of tannins.

2.6.6. *Identification of triterpenoids*

The stock solution of each plant extract (5 mg) was taken in a beaker and dissolved in $CHCl_3$ solvent (2 ml) by shaking. One milliliter of $(CH_3CO)_2O$ and concentrate H_2SO_4 (1 ml) were added to the beaker drop by drop. It gave a violet colour that showed the existence of triterpenoids.

2.7. Antibacterial Activity Assay

The prepared leaf crude extracts of *M. piperita* were assessed for their *in-vitro* antibacterial activity by the agar diffusion method described by Fatma and Hossain (2016). Four different concentrations of each crude extract (2, 1, 0.5 and 0.25 µg/ml) were prepared by using a DMSO solution followed by a dilution method. The activity of the crude extracts at each concentration was determined against *S. aureus, E. coli* and *P. aeruginosa* bacterial strains. Approximately 5 mm diameter filter paper was used as a disc in the present experiment. Levofloxacin antibiotic and DMSO solvent were used as positive and negative controls. All discs were soaked in different concentrations of each plant crude extract along with the positive and negative controls. All the socked discs were placed on an inoculated agar plate and kept in an incubator at 37°C for 24 hours. The diameter of the inhibition zone of each incubated disc was manually measured. All the data were replicated three times and averaged.

3. RESULTS

Since ancient times, plant-based medicine has been used for the treatment of the majority of diseases. About 1200 plants have been considered medicinal plants all over the world due to their pharmacological activity. Specific plant species originate and grow well in certain regions with suitable environmental conditions and neighboring fauna and flora (Adamu *et al.,* 2004). The viscous semi-solid masses were obtained from the powdered leaves by the extraction of methanol and evaporation. The solvent-free methanol plant extract was liquefied by water and then extracted sequentially with various polarity of solvents to give hexane, ethyl acetate, chloroform, butanol and residual water fractions, respectively (Ali & Hossain, 2014; Tahiya *et al.,* 2014) (Figure 2).

3.1. Biochemical Analyses

The biochemical analysis of all prepared extracts and powders of *M. piperita* indicated the presence of alkaloids, flavonoids, saponins, steroids, tannins, and triterpenoids. However, chloroform leave extracts did not show any positive tests for tannin and steroids. Similarly, hexane plant crude extracts also did not show any positive tests for steroids (Table 1).

Table 1. Biochemical analysis of the hexane, ethyl acetate, chloroform, butanol and methanol crude extracts of *M. piperita*

Biochemicals	Inference				
	Hexane	Ethyl acetate	Chloroform	Butanol	Methanol
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	+
Saponins	+	+	+	+	+
Tannins	+	+	-	+	+
Triterpenoids	+	+	+	+	+
Steroids	-	+	-	+	+

+ = presence; - = absence

3.2. Antibacterial Activity

The antibacterial activity of different polarity crude extracts at different concentrations was qualitatively assessed against the employed bacteria strains in the existence or nonexistence of inhibition zones (Al-Rashdi *et al.*, 2021). The various polarity extracts at different concentrations (2, 1, 0.5 and 0.25 µg/ml) gave no significant activity against one gram (+) (*S. aureus)* and two gram (-) bacteria strains *(E. coli* and *P. aeruginosa)* at the range of 0-10 mm. Most of the crude extract of *M. piperita* did not show any impressive activity against *E. coli, P. aeruginosa* and *S. aureus* bacteria at the concentrations of 2, 1, 0.5 and 0.25 µg/ml is as presented in Table 2. The maximum antibacterial activity against *E. coli* was found in chloroform extract at the concentration of 2 mg/ml.

Table 2. Antibacterial activity of different crude extracts of *M. piperita* against *E. coli, P. aeruginosa* and *S. aureus*

Extract	Concentration (mg/ml)	*E. coli* (mm)	*P. aeruginosa* (mm)	*S. aureus* (mm)
Hexane	2	8±0.67	9±0.67	nd
	1	8±0.67	nd	nd
	0.5	8±0.67	nd	nd
	0.25	nd	nd	nd
	Control	nd	nd	nd
Ethyl acetate	2	8±0.67	nd	nd
	1	7±0.67	nd	nd
	0.5	7±0.67	nd	nd
	0.25	nd	nd	nd
	Control	nd	nd	nd
Chloroform	2	10±0.67	8±0.67	7±0.67
	1	8±0.67	7±0.67	nd
	0.5	nd	nd	nd
	0.25	nd	nd	Nd
	Control	nd	nd	Nd
Butanol	2	7±0.67	nd	7±0.67
	1	7±0.67	nd	6±0.67
	0.5	nd	nd	6±0.67
	0.25	nd	nd	6±0.67
	Control	nd	nd	Nd
Methanol	2	7±0.67	7±0.67	Nd
	1	6±0.67	7±0.67	Nd
	0.5	nd	7±0.67	Nd
	0.25	nd	nd	Nd
	Control	nd	nd	Nd

nd=not detectable

4. DISCUSSION

Nature has been an important source of medicines for a long time. The plant materials and their derivative products have been used as medicine in different traditional medicine systems to treat various diseases since old times. WHO estimated that approximately 50-60% of the world's population depend on plant derived medicines for their basic health care system. Based on their medical use in the traditional medicine system a good number of therapeutic agents have been extracted/isolated from natural resources (Frombi, 2003).

Treating various bacterial infection is growing difficult due to the development and spread of microbial resistance and the deficiency of the expansion of new antibacterial principles (Aishwarya, 2015). The natural product is one of the main sources that have provided the

pharmaceutical, agricultural and cosmetics industry with some of its natural sources of principal products in the search for new antibacterial drugs (Basheer & Abdullah, 2019). A huge number of local medicinal plants with a significant activity can represent a source of new antibacterial agents for the treatment of various infectious diseases (Aishwarya, 2015; Basheer & Abdullah, 2019). An active plant oils and extracts are used for food preservation, in finished pharmaceuticals products, and in therapies of alternative and complementary medicine systems (Bupesh *et al*., 2007).

M. piperita is a plant that grows well in all climates and exists all over the world including Oman. The oil from the selected plant is used to prepare toothpaste, chewing gum, mouthwash, soaps, sweets, balms or creams, and cough medicine (Spirling & Daniels, 2001; Aswatha *et al*., 2008; Hossain *et al.,* 2008; Sun *et al.,* 2014). A significant percentage of menthol and menthone are present in the plant. Menthol is a chemical ingredient used to treat a variety of gastrointestinal problems. In addition, it is also used for gastrointestinal problems, cramps, dyspepsia, nausea, the common cold, sore throat, colic, itching, inflammation and headaches (Hills *et al.,* 2005; Tabari *et al.,* 2012; Sun *et al.,* 2014).

4.1. Biochemical Analyses

The qualitative biochemical analysis of different polarity leaves extracts of *M. piperita* proved the presence of saponins, flavonoids, tannins, alkaloids, triterpenoids, and steroids. However, the hexane and ethyl acetate crude extracts did not show the presence of steroids. Similar chemical analysis results were reported on various extracts of the selected plant by other authors (Sujana *et al.*, 2013).

4.2. Antibacterial Activity

The antibacterial activity of different extracts of *M. piperita* was determined against the available three bacteria strains. Most leaf extracts from *M. piperita* showed no potentially significant activity against the selected Gram (+) and Gram (-) bacteria strains at the concentrations of 2, 1, 0.5 and 0.25 µg/ml (Abdulsattar & Hossain, 2020). Their calculated diameter zones of inhibition were within the range of 0-10 mm (Table 2). Among the extracts, the methanol extract only gave a low activity against *E. coli* and *P. aeruginosa* bacteria strains at the concentration of 2 and 1 mg/ml (Table 2). However, the methanol extract did not display activity against *S. aureus* at any applied concentrations. The same extract also failed to display any activity against *E. coli* and *P. aeruginosa* bacteria strains at low concentrations of 0.5 and 0.25 mg/ml. The butanol extract exhibited moderate antibacterial activity against *S. aureus* at all concentrations. But, less activity was obtained against *E. coli* at the concentration of 2 µg/ml. However, the other concentrations (1, 0.5 and 0.25 mg/ml) of butanol leaves extract did not give any activity against *P. aeruginosa*. In addition, hexane and ethyl acetate extracts from these leaves did not show any antibacterial activities against most of the tested bacteria strains. However, hexane and ethyl acetate extract crude extracts showed moderate activities at the concentrations of 2, 1 and 0.5µg/ml against *E. coli*. The chloroform extract of the selected plant also showed some activities at the high concentrations against all applied bacterial strains but at low concentrations did not show any activity. In our present study, the water extract also did not show any activity against all the tested bacterial strains (data not shown). It is very interesting that the negative and positive controls also did not inhibit the growth of the tested bacterial strains. Previous results of other authors showed that most of the polarities extracts give significant activities against the Gram (+ and -) bacterial strains (Hills *et al.,* 2005; Bupesh *et al.,* 2007; Tabari *et al*., 2012; Sujana *et al.,* 2013; Sun *et al.,* 2014; Aishwarya, 2015; Basheer & Abdullah, 2019)**.** Our experimental results are different from the reported values may be due to the chemical composition, concentration of ingredients, plant maturity, geographical region and processing conditions (Zainab & Hossain, 2016; Doha *et al.,* 2020). Therefore, further studies will be needed for the antibacterial activity of the Omani species by other standard

screening methods against *S. aureus* and *E. coli* and *S. aeruginosa*. In addition, further studies will be needed to separate the bioactive ingredients from the leaves of *M. piperita* species.

5. CONCLUSION

The antibacterial and chemical composition of the selected plant's various extracts were determined by well recognized methods. Our current report related to chemical analyses and antibacterial activity will help the isolation of new products/drugs with significant activity from the selected plant species. Based on our results, it could be concluded that the leaves extract of *M. piperita* has been moderate against the applied Gram (+) and Gram (-) bacterial strains. However, the experiment could be repeated to check the antibacterial activity of different polarity extracts at different concentrations against a large number of bacterial strains that will be useful for the preparation of antibiotics.

Acknowledgments

One of the authors is indebted to the University of Nizwa, Nizwa, Sultanate of Oman, for providing all required facilities to carry out this graduation research. The authors are thankful to Microbiology Department, Nizwa Hospital for providing microbial strains. Thanks to Lab Technician, Natural Products Lab for their continuous help to finish this study. Also thanks to Mr. Erno Muzamel, TOFEL Coordinator and SSS Instructor, Writing Center, University of Nizwa, Sultanate of Oman for his professional help to edit the present manuscript.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors

Authorship contribution statement

Zahra Mohammed Al-Hajri: Investigation, Data curation, Analysis, and Draft writing. **Mohammad Amzad Hossain**: Study design, Supervision, Data analysis, Edit manuscript. **Salim Said Al-Touby:** Logistic support, Data analysis, Final edit.

Orcid

Zahra Mohammed Al-Hajri https://orcid.org/0000-0003-2795-2442
Mohammad Amzad Hossain https://orcid.org/0000-0002-8970-0702
Salim Said Al-Touby https://orcid.org/0000-0002-3116-9023

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