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Pharmacology and Enzyme Inhibitory Potentials of *Myrtus communis* L. Fruits Grown in Oman

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ABSTRACT

Background and Objective: *Myrtus communis* L. is a plant used in traditional medicine worldwide. Since many ages ago, this herb's berries have all been widely utilized as a traditional medicine to treat conditions like peptic ulcers, diarrhea, haemorrhoids, inflammation and skin diseases. The purpose of the study is to evaluate the antiulcer, antidiabetic enzymatic inhibition, antibacterial and antioxidant actions of different polarity extracts of fruits of *Myrtus communis* L. **Materials and Methods:** Methanol (crude extract) and its fractions (n-hexane (MCFH), dichloromethane (MCFD), ethyl acetate (MCFE), butanol (MCFB) and aqueous (MCFA)) from fruits of *Myrtus communis* L. were produced and assessed for their antimicrobial, antioxidant, α-glucosidase and antiulcer *in vitro* bioassays. All of the fractions were tested using a brine shrimp lethality assay to determine their cytotoxic effects. **Results:** The examined samples demonstrated a sizable capacity to fend off the gram-negative and gram-positive bacteria. The MCFB determined the best antibacterial activity of the five extracts, with an average inhibitions zone of 9.3 mm against *Staphylococcus aureus* and 6.75 mm against *Escherichia coli*. The MCFA extract displayed the second-highest antibacterial activity against *Pseudomonas aeruginosa*, with an average inhibition zone of 9.5 mm, while MCFH had the least amount of activity against the bacteria that were tested. In the anti-alpha glucosidase activity, MCFE was the best with IC_{50} of 20.04±0.38 µg mL^{-1} and the unease inhibition was best seen with the MCFA and MCFB extracts with IC_{50} values of 146.87±1.39 and 194.97±1.68 µg mL^{-1}, respectively. All extracts showed no significant cytotoxic activity. **Conclusion:** Therefore, all tested samples, especially MCFE and MCFB extracts, were assumed to have significant capacities for the studied activities.

KEYWORDS

Myrtus communis L., Myrtaceae, urease inhibition, antidiabetic, antibacterial, cytotoxic, LC-MS analysis

INTRODUCTION

Type 2 diabetes is one of the main metabolic illnesses, which is brought on by insufficient insulin production, diminished insulin activity or both[1]. Leading long-term consequences of diabetes, obesity, metastatic cancer and cardiovascular disorders are all correlated with persistent hyperglycaemia[2]. Orally administered antidiabetic drugs have been used either alone or in conjunction with insulin to treat hyperglycaemic individuals[3]. To reach a normal sugar level, numerous anti-diabetic medications were

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produced in the previous 10 years. To prevent diabetes consequences such as polyuria, polydipsia, polyphagia, recurrent weight loss, poor vision, nausea and skin infections, such interventions are unable to lower plasma glucose levels and achieve normoglycemia[3,4]. To overcome the high level of blood glucose, one of the approved therapeutic targets for the development of medications to prevent problems brought on by hyperglycaemia is α-glucosidase inhibition[4]. It is one of the hydrolase enzymes that catalyzes the breakdown of polysaccharides into monosaccharides, such as glucose, for absorption in the bloodstream is α-glucosidase[5]. The AGIs have received a lot of attention recently due to their potential clinical applications in the treatment of hyperglycaemia and as antiviral medications. Because α-glucosidase control the conversion of polysaccharides to monosaccharides in the small intestine, that is why its inhibition slowdown glucose absorption[1,5,6].

Urease, a heteropolymer amidohydrolase that contains nickel, catalyzes the hydrolysis of urea into ammonia and carbon dioxide. It is widely distributed in plants, bacteria, fungi and mammals[7]. Two nickel ions coupled to four histidine residues, carbamylated lysine and an aspartate carboxylate make up the active site of urease[8,9]. The quick rise in ammonia levels caused by this well-known enzyme creates a favourable environment in the stomach for the survival of *Helicobacter pylori*. Due to urease hyperactivity, which raises the stomach's pH and causes issues including peptic and gastric ulcers, hepatic coma, pyelonephritis and kidney stones, high amounts of ammonia are created[9]. This clinical situation necessitates the use of inhibitors that can control urease activity[10]. This colonization of harmful microbes results in several infections of the digestive and urinary tracts[10,11]. Therefore, inhibition of urease is a promising therapeutic target for urinary tract complication including peptic cancer and stomach ulcer. Hence, in the current study, the plant extract sources were evaluated for insight into their medicinal use.

Myrtus communis L. is the Greek word for myrtle. It belongs to the Myrtaceae family and grows in clusters[12]. It is a common plant that develops as a small tree or shrub and grows widely in the Middle East, Mediterranean region countries (Algeria, Spain, Tunisia, Turkey) and the Himalayan Northwest[13]. It was indeed utilized for treating various common ailments in ancient medicine, including urinary, gastrointestinal and skin issues and it is now widely used in the cosmetic, food and pharmaceutical sectors[14]. *Myrtus communis* possess several medical, pharmacologic and biological activities such as antiviral, antibacterial, antifungal, analgesic, anti-inflammatory, antioxidant, anti-hemorrhagic, antimutagenic, hepatoprotective, wound healing and anti-hyperglycemic activities[15]. Leaves of this plant had an anti-diabetic effect in rats with streptozotocin-induced diabetes[15,16]. The phenolic chemicals in the plant were recently discovered to be responsible for this action. The phenols isolated from *M. communis* L. greeneries have an anti-diabetic effect. There were no conclusive data on the effectiveness of *M. communis* L. in diabetic rats[15,16]. In traditional, *Myrtus communis* L. was used to treat gastrointestinal disorders due to its protective effect. Furthermore, many studies have been carried out to illustrate the correlation between the traditional use and the mechanism of action of MC[17,18]. Therefore, the current study is being conducted for the first time on the fruits by screening them for conducting *in vitro* tests for *Myrtus communis* L. potential antimicrobial, cytotoxic, antiulcer and anti-diabetic properties.

MATERIALS AND METHODS

Study duration and location: The study was taken place in Oman from May, 2021 to August, 2023.

Materials: The chemicals and solvents didn't need to be further refined because they were all of analytical purity. Most of the reagents were bought from Sigma-Aldrich Chemical Company (St. 142 Louis, MO, USA). Organic solvents were acquired from Fisher Scientific (Loughborough, United Kingdom). Yamoto rotary evaporator model no. RE801 was used for distillation and evaporation of organic solvents. xMark Microplate Absorbance Spectrophotometer from BIO-RAD, Hercules, California, USA. Other equipment includes Whatman Grade 1 qualitative filter paper, TLC silica gel 60F254 aluminum sheet 20×20 cm and TLC silica Gel 60F254 glass plate 20×20 cm, obtained from Merck (KGaA, Darmstadt, Denver, Germany), Instruments P-114.1 Analytical balance and incubator (INCU-Line®IL115).

Table 1: Different plant extracts of *Myrtus communis* L. fruit

Name of extract	Amount (g)	Yield (%)
MCFH	3.21	04.39
MCFD	1.84	02.52
MCFE	2.34	03.20
MCFB	3.38	04.63
MCFA	22.1	30.20

MCFH: n-hexane, MCFD: Dichloromethane, MCFE: Ethyl acetate, MCFB: n-butanol and MCFA: Aqueous

Collection and identification: *Myrtus communis* fruits (120 g) were collected (May 2021) from different location of Jabal Al-Akhdar, Oman. The plant was photographed during collection and a voucher specimen (MCJ-03-21) was identified by the plant taxonomist (Dr. Syed Abdullah Gilani, at the Department of Biological Sciences and Chemistry, University of Nizwa, Sultanate of Oman). After identification, the sample was immediately moved to the laboratory and washed to eliminate all debris and dust before being dried in the shade for 10-15 days at room temperature. Finally, the berries were pulverized into a coarse powder that was uniform.

Extraction and fractionation: The crushed plant material (73 g) was immersed twice in a closed container containing 100% methanol as a solvent and allowed to stand at room temperature for 3 to 5 days with continuous stirring, where the crushed plant material was cold-extracted. At the end of each dip, the solvent was decanted and filtered to produce a clear solution. Finally, both methanol extracts were combined. Methanol was removed using a rotary evaporate or until a thick sticky mass formed (37.2 g). The resulting mass was suspended in 250 mL of a 1:1 ethanol-water combination and extracted with hexane, chloroform, ethyl acetate and butanol. Then all solvents were evaporated using a rotary evaporator to obtain five extracts with different polarities and yield (%) (Table 1).

Antibacterial activity using disc diffusion method: The antibacterial activity of all crude extracts of *M. communis* fruits was determined using the disc diffusion method[19]. The microbiology lab at NMSRC (Natural and Medical Sciences Research Center) at the University of Nizwa in Oman provided all the organisms for this investigation. Each extract was evaluated in Dimethyl Sulfoxide (DMSO) at four distinct concentrations: 125, 250, 500 and 1000 μg mL^{-1}. By immersing filter paper discs (5 mm) in vials holding the prepared solutions, extracts were impregnated. After this time, the dipped filter papers were tested with three types of Gram-negative bacteria (*E. coli*, *P. aeruginosa* and *K. pneumoniae*) and two types of Gram-positive bacteria (*S. aureus* and *S. pneumoniae*). As a positive control, levofloxacin (300 μg mL^{-1}, analytical grade, Sigma-Aldrich, Germany) was utilized. The plates were incubated at 37°C for 24 hrs and the antibacterial activity of each extract was determined by measuring the diameter of the inhibitory zone surrounding each disc.

Cytotoxic activity of *Myrtus communis* L. fruit: For the lethality of brine shrimp, a test was run using brine shrimps (10 nauplii) to ascertain the cytotoxic impact of each produced extract of *M. communis* L.[19], the lethality of each extract was assessed against brine shrimp (*Artemia salina* Leach) larvae. The larvae were produced by putting brine shrimp eggs in artificial sea water made from 38 g of sea salt dissolved in a liter of distilled water. The shrimp larvae were exposed to five different concentrations of each extract: 25, 50, 100, 200 and 400 μg mL^{-1}. After 24 hrs, the number of survivors was counted to establish the %mortality in each concentration.

Alpha-glucosidase inhibition assay: About 1 mg of enzyme was dissolved in 50 mL phosphate-buffered saline (PBS), resulting in 0.2 units of enzyme in each well. In a 96-well plate, 135 μL of 100 mM phosphate buffer was poured to each required well. The freshly prepared enzyme solution 20 μL was then added followed by 20 μL of 0.5 mg mL^{-1} crude extract solutions. The plate was then incubated at 37°C for 15 min after incubation, a 25 μL substrate solution was added to the wells and the absorbance was measured

using an ELISA plate reader for 30 readings at 400 nm wavelength at 1 min intervals. To evaluate the IC_{50} values (inhibitory concentration at 50%) of the crude extracts that showed good inhibitory activity, they were serially diluted (0.25, 0.125, 0.0625, 0.0312 and 0.0156 mg mL^{-1}) and inhibition (%) and IC_{50} value were calculated[20,21].

Urease enzyme inhibition assay: The 96-well plates were used to incubate the reaction mixture, which included 25 µL of Jack bean (*Canavalia enisiformis*) urease (1 unit/well), 55 µL of 100 mM urea dissolved in phosphate buffer with a pH of 6.80 and 5 µL of various doses of crude extract from (0.2 to 0.05 mg mL^{-1}). Following that, each well contained 45 µL of phenol reagents (0.005% w/v sodium nitroprusside and 1% w/v phenol) and 70 µL of alkali reagent (0.1% w/v NaOCl and 0.5% w/v NaOH). The production of ammonia was used to determine urease activity by applying the Weatherburn indophenols method[20,21]. After 50 min, increase in absorbance at 630 nm was observed in a microplate reader (Spectra Max M2, Molecular Devices, California, USA). All reactions were performed in triplicate in a final volume of 200 µL. Thiourea, as the standard inhibitor of urease, was used and IC_{50} value was calculated using the published protocol[8,10].

RESULTS AND DISCUSSION

Antibacterial activity: The antibacterial activities of *Myrtus communis* L. extracts were examined against several types of bacteria using disk diffusion method. All the extracts displayed moderate antibacterial activity against the tested bacterial strains at the higher concentration only. Among the five extracts, MCFB fractioned determined the highest antibacterial activity with an average inhibition zone of 11.5 mm against *Staphylococcus aureus* and 6.75 mm against *Escherichia coli* (Table 2). The MCFA extract displayed average inhibition zones of 9 mm, which is the second highest antibacterial activity against *Pseudomonas aeruginosa*. The n-Hexane fraction showed the weakest activity against tested bacteria. The outcomes validate the antibacterial properties of the *M. communis* extracts and it was in agreement with the results obtained by Amensour *et al.*[22] and El Hartiti *et al.*[23].

Cytotoxic activity of *Myrtus communis* L. fruit: All extracts of *Myrtus communis* L. fruit were subjected to brine shrimp lethality bioassay for possible cytotoxic action. The results are presented in Table 3. All the extracts showed no significant cytotoxic activity indicating that *M. communis* L. is highly edible. *Myrtus communis* L. fruit contains combination of organic compounds and nutrients that gives it a dietary benefit, it contains antioxidant like quercetin, tannins, myricetin which is effective against acne and improve cell appearance, moreover they contain flavonoids including, linalool, pinene, tannins and other sugar which can boost immunity[24]. A study conducted by Mert *et al.*[25] who determined that leaves extract of the study plant have good cytotoxic potential. A variation in the activity might be possible due to different factors such as the nature and age of the plants, harvest time, plant genotype, climatic conditions in the respective locality where the plants were grown, the differences in the parts of the plants used for analysis as well as the extraction technique.

Antidiabetic assay: The inhibitory activity of four extracts of *M. communis* fruit against yeast α-glucosidase was investigated and the results are shown in Table 4. In this assay, MCFH (64.83 µg mL^{-1}), MCFD (45.36 µg mL^{-1}), MCFE (20.04 µg mL^{-1}) and MCFB (425.95 µg mL^{-1}) showed more than 50% α-glucosidase inhibition activity at the 0.5 mg mL^{-1} concentration. The results indicate that MCFC exhibited the best anti-alpha glucosidase activity followed by MCFD and MCFH. However, MCFB showed the least inhibitory activity. These findings provide crucial information about the biologically active constituents present in *M. communis* fruit truly responsible for the inhibition of the α-glucosidase enzyme. This study displayed that extract of *M. communis* fruit could be helpful in the effective management of postprandial hyperglycemia[26].

Table 2: Antibacterial potentials of different polarities extract of *Myrtus communis* L. fruits (ZOI, mm)

Fractions	Concentration (µg mL^{-1})	*Streptococcus pneumoniae*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Escherichia coli*
MCFH	1000	10	8	7.5	6	7.5
	500	8	7	7	6	6
	250	7	0	6	0	6
	125	7	0	0	0	0
Levofloxacin	300	27	35	23	31	36
MCFD	1000	8	8	8	11	8
	500	7	7	7.5	9	6
	250	7	7	7	9	0
	125	6	6	7	8	0
Levofloxacin	300	26	36	27	31	36
MCFE	1000	8	7	10	10	6.5
	500	7.5	6	9	9	6
	250	7	6	8	7.5	6
	125	6	6	7	6	0
Levofloxacin	300	26	36	26	29	35
MCFB	1000	8.5	11.5	9	11	8
	500	8	10	8	9	7
	250	7	8	7.5	8	6
	125	6.5	8	7	8	6
Levofloxacin	300	28	35	28	31	36
MCFA	1000	7.5	8	11	10.5	7.5
	500	7	7.5	10	10	7
	250	7	7	9	8.5	6
	125	6	6	8	7	0
Levofloxacin	300	28	34	29	30	37

MCFH: n-hexane, MCFD: Dichloromethane, MCFE: Ethyl acetate, MCFB: n-butanol and MCFA: Aqueous

Table 3: Mean percent mortality of brine shrimp larvae for different polarity extracts of *M. communis* L.

Plant extract	Concentration (ug mL^{-1})	Number of surviving nauplii (after 24 hrs) T1	T2	Total number of nauplii survivors (average)	Mortality (%)
MCFH	400	7	8	8	20
	200	8	8	8	20
	100	8	9	9	10
	50	9	10	10	0
	25	9	9	9	10
MCFD	400	6	7	7	30
	200	7	7	7	30
	100	7	8	8	20
	50	8	9	9	10
	25	9	8	9	10
MCFE	400	7	6	7	30
	200	7	7	7	30
	100	8	8	8	20
	50	9	10	10	0
	25	9	10	10	0
MCFB	400	8	7	8	20
	200	8	7	8	20
	100	9	9	9	10
	50	9	9	9	10
	25	10	10	10	0
MCFA	400	7	7	7	30
	200	7	8	8	20
	100	8	8	8	20
	50	9	10	10	0
	25	10	10	10	0

MCFH: n-hexane, MCFD: Dichloromethane, MCFE: Ethyl acetate, MCFB: n-butanol and MCFA: Aqueous

Table 4: α-glucosidase inhibition activity of different extracts of *M. communis* fruit

Code	Inhibition (%)	IC_{50} µg mL^{-1} (±SEM)
MCFH	91.10	64.83±1.56
MCFD	91.70	45.36±0.87
MCFE	92.90	20.04±0.38
MCFB	61.30	425.95±1.91
MCFA	20.67	N/A
Acarbose	57.25	608.21±1.74

N/A: Not active, Concentration: 0.5 mg mL^{-1}, MCFH: n-hexane, MCFD: Dichloromethane, MCFE: Ethyl acetate, MCFB: n-butanol and MCFA: Aqueous

Table 5: Urease inhibition activity of different extracts of *M. communis* fruits

Extract	Inhibition (%)	IC_{50} µg mL^{-1} (±SEM)
MCFH	63.52	229.75±3.14
MCFD	68.36	372.44±2.60
MCFE	64.00	225.54±2.73
MCFB	74.18	194.97±1.68
MCFA	78.59	146.87±1.39
Standard	91.02	1.58±0.95

Concentration: 0.5 mg mL^{-1}, MCFH: n-hexane, MCFD: Dichloromethane, MCFE: Ethyl acetate, MCFB: n-butanol and MCFA: Aqueous

Urease inhibition activity: Urease is an enzyme involved in the hydrolysis of urea to ammonia and carbon dioxide. *Helicobacter pylori*, a Gram-negative bacterium that lives on this enzyme, causes stomach inflammation and increases the risk of developing duodenal and gastric ulcers. Because urease is of great medical importance, highly stable and low toxicity urease inhibitors may be an effective treatment for diseases caused by urease-dependent pathogenic microorganisms. The order of activity for the urease enzyme inhibition is MCFA>MCFB>MCFE>MCFH>MCFD as shown in Table 5. The MCFA and MCFB showed IC_{50} values of 146.87 and 194.97 µg mL^{-1}, respectively and may be an excellent source of compounds with good urease inhibitory activity. Preclinical research would be extremely beneficial to ethnobotanical antibiotic drug development efforts, in addition to advancing and improving bioprospecting and the *in vitro* studies mentioned above.

CONCLUSION

Myrtus communis L. includes bioactive ingredients with a variety of phytochemicals that have a wide range of biological properties that may be the cause of its many therapeutic benefits. The MCFE extract and all of the tested samples collectively had the highest alpha glucosidase inhibitory activity. All extracts demonstrated notable antibacterial activity, with an average inhibition zone of 9.5 mm against the investigated bacterial strains. It was found that the MCFE extract effectively combated diabetes. The MCFA and MCFB extracts showed the most urease inhibition. Thus, it was determined that *M. communis* could be used to treat diabetes and ulcers, as well as to combat microorganisms. Numerous bioactive chemicals are thought to be the cause of these characteristics. To screen and separate the probable chemical components for the evaluated issues, more research is nevertheless advised. Furthermore, additional *in vivo* studies of the extracts required to be conducted to establish the safety and protection before recommending their practical use in pharmaceutical industries.

SIGNIFICANCE STATEMENT

In this study, fruits of *Myrtus communis* L. were evaluated against antidiabetic, antiulcer, antibacterial and antioxidant potential for the first time. The ethyl acetate fraction could be a promising source of antidiabetic compounds due to promising α-glucosidase inhibition. Due to significant urease inhibition, the aqueous and n-butanol fractions might be used in medication against gastric ulcers. Similarly, all extracts attributed significant antibacterial activity and were assumed to be caused by a variety of bioactive compounds. The numerous therapeutic effects of *M. communis* may be attributed to its bioactive

components, which comprise a number of phytochemicals with a wide spectrum of biological activities. However, more research is recommended to screen and segregate the likely chemical components for the assessed concerns.

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