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Original Research Article

GC-MS Characterization and Therapeutic Potential of Phytochemical Constituents of the Unani Medicine Uterotibb's Aqueous Alkaline Extract

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Abstract

Uterotibb is one of the potent Unani medicines that offers a natural solution to various human health issues. The present investigation explores the active phytochemical constituents of Unani medicine Uterotibb using GC-MS analysis. This study further evaluated the antioxidant potential and antimicrobial activity against a panel of human pathogens such as Staphylococcus aureus, Bacillus Subtilis, E. coli, and Klebsiella pneumonia. GC-MS studies revealed the presence of 388 phytochemical compounds in the alkali aqueous extract of Uterotibb. The main active biomolecules were stigmast-5– en-3-0l, and oleate (2.84%). Cyclotrisiloxane, hexamethyl (1.74%). 1,2,5-oxadiazole-3-carboxamide4-amino-N-(2-methoxyethyl)-(1.75%). Arsenous acid (1.48%). Pentane,1,1-thiobis (1.46%). 7,7,9,9,11,11-hexamethyl-3,6,8,10,12,15hexaoxa-7,9,11-trisilaheptadecane (1.46%). 4-(1,1dimethylpropyl-phenol, trimethylsilyl ether (1.43%). 1,2-bis (trimethyl silyl) benzene (1.36%). Adamantane methyl amine (1.32%). 1,4-benzenediole,2,5-bis(1,1 dimethyl ethyl)-(1.29%). 2-chloro aniline-5-sulphonic acid (1.28%); Acetic Acid, nitro-, methyl ester (1.24%) and Caprolactone oxime, (NB)-O-[(diethylboryloxy) (ethyl)boryl] (1.21%). Notably, Uterotibb extract exhibited antimicrobial activity in a concentration-dependent manner, where the highest concentration of 5 mg/mL showed a maximum zone of growth inhibition of 14.95±0.35 mm against *Staphylococcus aureus*, and the lowest zone of inhibition was 13.26±0.88 mm against *Bacillus subtilis* at the same concentration. The lowest MIC value was exhibited at 2 mg/mL concentration against *Staphylococcus aureus* where the highest MIC value of 4 mg/mL was observed against *K. Pneumoniae*. Further, the antioxidant potential of the extract exhibited scavenging activity of 67.115 ± 0.05 and 73.67 ± 0.09 using DPPH and ABTS assays, respectively. Finally, GC-MS profiling and bioactivity studies of the present work validate Uterotibb's potential as a natural Unani medicine for human care.

Keywords: Uterotibb, Aqueous Alkaline Extract, Phytochemicals, GC-MS, Antimicrobial Activity, Antioxidant Potential.

INTRODUCTION

Medicinal plants and its phytochemicals have been crucial to human health and wellness for centuries, offering a natural approach to disease prevention and treatment. Medicinal plants often contain a wide variety of secondary bioactive metabolites that forms the backbone of natural medicine, blending traditional knowledge with modern science. They serve as an integral part of Unani, Ayurveda, and Homeopathy medicines. This system emphasizes the utilization of natural bioactive substances to prevent and treat a myriad of diseases and disorders. The Unani medicine phytochemical constituents approach is grounded in the holistic principles of maintaining balance in bodily humors like blood, phlegm, yellow bile, and black bile (Ministry of AYUSH, 2024). In this regard, Gas Chromatography-Mass Spectrometry (GC-MS) is an invaluable tool for analyzing phytochemicals in medicinal plants, offering high precision and detailed insights. GC-MS excels in separating, identifying, and quantifying these complex mixtures even detecting trace amounts of phytochemicals that might be crucial for medicinal properties. This analytical approach determines the chemical composition of various secondary metabolites, thereby ensuring their quality, safety, and efficacy (Parveen *et al*., 2016). In the case of

Uterotibb, GC-MS plays a crucial in identifying its diverse secondary metabolites and phytochemical constituents. GC-MS analysis of Uterotibb, a Unani formulation, is crucial for identifying its chemical composition and ensuring the presence of secondary bioactive compounds responsible for its therapeutic effects. It helps maintain quality and safety by detecting contaminants or harmful substances. Moreover, it supports the standardization and scientific validation of the formulation, bridging traditional knowledge with modern science (Zahiruddin *et al*., 2020). On the other hand, Antimicrobial studies focus on evaluating the effectiveness of herbal medicine against microorganisms such as bacteria, fungi, and viruses. These studies are essential for determining if herbal medicine has potential applications in treating infections or preventing microbial growth (Parveen *et al*., 2021). The current study evaluated the antimicrobial potential of Uterotibb extract against a spectrum of pathogenic microorganisms. The formulation's efficacy was assessed to explore its potential as a natural antimicrobial agent to overcome the hurdles of the available commercial antibiotics. They target a wide range of pathogens, often acting on multiple pathways, which reduces the likelihood of resistance. Being natural and biodegradable, and their synergistic effects with antibiotics can enhance efficacy. Additionally, they are cost-effective, and hold great potential for novel drug discovery.

Further, the antioxidant studies of Unani medicine will contribute to the advancement of evidence-based traditional medicine and its integration into modern healthcare (Chaudhary and Kalia, 2014, Raza and Tabassum, 2024). Unani medicines are also used in the treatment of several Gynecological disorders (Aqeel and Qaiser, 2019). Recognizing the significance of Unani medicine in women's health care we have selected Uterotibb, a traditional formulation that is recommended for the treatment of several Gynecological disorders such as uterine weakness, Leucorrhea, Menorrhagia, Uterion tumors, and Uterion fibroids. The Uterotibb formulation consists of Ocimum Sanctum (Rehan seeds), commonly known as holy basil. Ocimum Sanctum has been recognized for its anti-inflammatory and antimicrobial properties, making it valuable in treating infections and reducing inflammation (Zhakipbekov *et al*., 2024; Borah and Biswas, 2018). *Lawsonia inermis Linn*, commonly known as henna, is traditionally used in Unani medicine for its therapeutic properties. Research has demonstrated that *Lawsonia inermis* exhibits significant antimicrobial effects, offering potential protection against a variety of infections. These antimicrobial properties are attributed to the presence of bioactive compounds such as saponins, tannins, steroids, and anthraquinones. The plant's extracts have shown efficacy against both gram-positive and gram-negative bacteria, making it a valuable natural remedy in combating microbial infections (Dev *et al*., 2016; Das *et al*., 2020). *Crocus sativus* (Zaffron) contains potent antioxidants and has been used for centuries to improve reproductive health (Anaeigoudari *et al*., 2023; El Faridi *et al*., 2024; Rahmani *et al*., 2022). *Nigella Sativa* (kalonji) is an herb recognized for its broad spectrum of antimicrobial and anti-inflammatory effects, making it useful in treating infections and other health conditions (Shafodino *et al*., 2022: Hanafy, 1991; Yesuff, 2015). *Trigonella foenum graecum* (Methi Dana), commonly known as fenugreek, has been studied for its hormone-regulating properties, contributing to its use in addressing gynecological issues (Goyal *et al*., 2016; Walli, 2015; Kumaravel, 2016). *Quercus infectiria* (Mazo Phal), often used for its astringent properties. *Quercus infectiria* can help manage conditions such as excessive menstrual bleeding (Banc *et al*., 2023; Nair *et al*., 2020; Hussein *et al*., 2016). *Santalum album L* (Sandal surq), Known for its calming and anti-inflammatory effects, sandalwood is a valuable addition to the Uterotibb's formulation (Srisopan *et al*., 2012; Misra *et al*., 2012). The present study aims to perform a detailed phytochemical characterization of Uterotibb secondary metabolites using GC-MS analysis and its bio-active components.

Additionally, the present study evaluated anti-microbial and anti-oxidant potential of Uterotibb, contributing to a better understanding of its therapeutic potential. This study validates the therapeutic properties of Uterotibb secondary metabolites (phytochemicals) constituents through scientific approach so that healthcare providers can offer patients a broader range of treatment options.

MATERIALS AND METHODS

Sample Collection

The Unani medication dried powder sample was collected from the Halal Herbal Remedies, Milath nagar colony, Hyderabad – 500005, Telangana state, India. The medication carry license and registration number T584/U, Hyderabad, T. S., India.

Extraction Procedure

Dried powdered of Unani medicine Uterotibb's (10 g each) were taken and extracted with 100 mL of 0.1N NaOH (aqueous alkali extraction) in two 250 ml conical flasks for 24 h at room temperature. The flasks were plugged and placed on an orbital shaker at 200 rpm/min at room temperature for 24 h. The aqueous alkali extracts were filtered using sterile Whatman No. 1 filter paper. After the filtration the aqueous alkali extracts were concentrated in a Rota Vapor at 60-70 °C temperature. The final extracts obtained were stored in a refrigerator at 4°C for further analysis.

DPPH Free Radical Scavenging Activity

Total free radical scavenging activity of the aqueous alkali extracts of Unani medicine were carried out using DPPH assay according to the protocol prescribed earlier (Nejhad *et al*., 2023). The stock solution of ethanolic extract was prepared at

concentration of 1 mg/mL. The solution was diluted to arrange of 10-500 µg/mL. Working solution containing 0.2 mM DPPH in 1 mL ethanol was added. The reaction mixture was shaken well and incubated in the dark for 30 min at room temperature. Then the absorbance was taken at 517 nm. The experiment was performed in triplicate. The antioxidant activity was estimated based on the percentage of DPPH radical scavenged as per the following equation:

Antioxidant activity % = ($A_{blank} - A_{sample}$)/A_{blank}) X100

Where, A_{sample} and A_{blank} indicate the absorption of extract sample and blank sample, respectively. The antioxidant activity of the extract was compared to that of natural antioxidants vitamin C.

ABTS Free Radical Cation Scavenging Assay

The ability the aqueous alkali extract to inhibit ABTS radicals was determined as previously prescribed by the standard procedure (Labiad *et al*., 2017; Saeed *et al*., 2012). A free radical solution (7 mM ABTS, 2.4 mM potassium per sulfate was prepared and left in the dark for 14h at 24°C. 200 µL of the aqueous alkali extract samples used to prepare different concentrations ranging from 10–500 µg/mL were added to the free radical solution (2 mL) and mixed completely. After 30 min incubation, the absorbance of the samples was measured at 734 nm and the ABTS free radical scavenging activity was reported. Vitamin C and TBHQ were used as controls.

GC-MS Analysis

The phytochemical investigation of ethanolic and alkali extracts were carried out on a GC-MS equipment (GCMS-QP2020; SHIMADZU) comprising an AOC-20s auto-sampler, an AOC- 20i auto-injector and a Gas Chromatograph (GC-2010 Plus) interfaced to a Mass Spectrometer. Experimental conditions of GC-MS system were as follows: SH-Rxi-5Sil-MS capillary standard non-polar column, dimension: 30 m, ID: 0.25 mm, film thickness: 0.25 µM. Flow rate of mobile phase (He-99.999% as carrier gas) was set at 1 mL/min and an injection volume of 1 µL was used with an injector at temperature of 80 °C, interface at 280 °C and ion source temperature of 200 °C. In gas chromatography, initial temperature was maintained at 80°C (isothermal holding time 5.0 min) then rose to 150°C at 5°C/min (hold time 5.0 min) and finally the temperature was increased to 280°C at 10°C (hold time 5.0 min). The injection volume was 1 µL with a split ratio of 10:1. Mass spectra were taken at 70 eV; a Mass spectra of metabolites were detected at two scans per second with a scanning interval of 50–600 m/z. The solvent delay was 0 to 2 min, and the total GC/MS running time was 30 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The software adopted to handle mass spectra and chromatograms was a TurboMass ver-5.2.

Identification of Phytochemicals

Interpretation of GC-MS mass-spectrum was conducted using the database of the Wiley and National Institute Standard and Technology (NIST) Librarie 11 that having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST08, NIST08s and NIST14 Library Search Programs.

Antimicrobial Studies

Bacterial strains

Four bacterial strains were used, two Gram-positive, *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6051) and two Gram-negative, *E. coli* (ATCC 25922) and *Klebsiella pneumonia* (ATCC 700603). These bacteria's strains were procured from American type culture collection (ATCC), USA. All the strains were grown in trypticase soy broth (TSB) at 37 °C with shaking at 210 rpm for 18 h then sub cultured on blood agar, nutrient agar and MacConkey, slants were prepared and preserved.

Inoculum Preparation

The bacterial strains were grown in trypticase soy broth to an optical density at 600 nm of 0.5 which is equivalent to that of 0.5 McFarland standard (1–2 × 10^8 CFU/mL). 1 mL of the bacterial suspension was centrifuged at 3400 g for 10 min at 4 °C. The pellets were washed and suspended in cold phosphate buffer saline (pH 7.4). The bacterial suspensions were further diluted to give a final bacterial concentration of approximately 5 × 10^6 CFU/mL (Al Saiqali *et al*., 2018).

Antibacterial activity of the Unani medicine Uterotibb's alkali extracts were determined by agar well diffusion assay at four concentrations (1, 2, 3, 4, 5 mg/mL). Muller Hinton agar was prepared according to the manufacturer's instructions and the plates were seeded with appropriate bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia and Escherichia coli*). Six wells were made using sterile Borer in each agar plate seeded with the test organisms.100 µL of each sample concentration were loaded into each well. The plates were incubated at 37 C for 18-24 h. The plates were checked for the appearance of zone of growth inhibition Standard antibiotic discs were used as a positive control to validate the antibacterial activity. After incubation the plates were observed for the appearance of zone of growth inhibition around the wells. The inhibition zones were measured and recorded, the experiment was carried out in triplicates (Al Saiqali *et al*., 2024).

Statistical Analysis

One way ANOVA and student's test revealed significant differences in antioxidant activity among different concentrations of Uterotibb's extract ($p < 0.05$).

RESULT AND DISCUSSION

GC-MS Analysis

The GC-MS chromatogram of the basic aqueous extract of Uterotibb revealed the presence of 388 diverse bioactive phytochemical compounds (Fig 1). Of these, 13 compounds are found to be predominant. These thirteen compounds have a significant medicinal value (Table 1, Fig 2)(Parihar and Balekar., 2017; Alshatwi and Alkaltham., 2021; Glomb and Swiatek., 2021; Genchi *et al*., 2022; Zhao *et al*., 2019; Pubchem compound summary for CID 6643., 2025; National institute of Health., 1992; Spilovska *et al*., 2016; Zumaidar *et al*., 2024) . However, our study revealed the presence of a small quantity of arsenious acid, which is within permissible limits (Ministry of AYUSH., 2018). However, our study also proved the presence of other bioactive compounds in small proportions. These compounds are also known for their therapeutic value. The present study also includes the evaluation of the antimicrobial activity of Uterotibb.

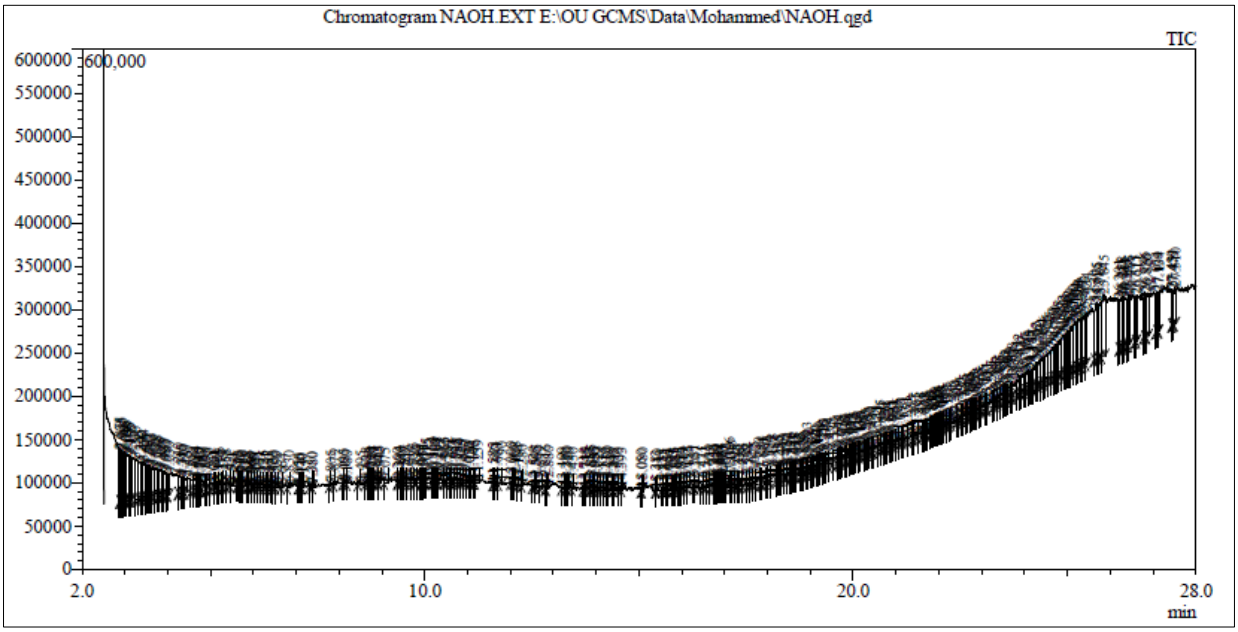

Fig. 1: GC-MS analysis of Uterotibb Aqueous Alkali extract

Table 1: Selected predominant compounds detected by GC-MS analysis of Uterotibb Alkali aqueous extract

S. No	Name of the compound	Peak area %	Medicinal Activity	References
1.	stigmast-5-en-3-0l, oleate	2.84%	Antibacterial, antifungal, antioxidant and anti-inflammatory	[32]
2.	Cyclotrisiloxane, hexamethyl	1.74%	Anticancer, antioxidant, Antidiabetic.	[33]
3.	1,2,5-oxadiazole-3-carboxamide 4-amino-N-(2-methoxyethyl)	1.75%	antibacterial, antifungal and useful for the treatment of cancer	[34]
4	Arsenous acid	1.48%	treatment of cancer and hematological malignancies	[35]
5.	pentane,1,1-thiobis	1.46%	not known	
6.	7,7,9,9,11,11-hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane	1.46%	Antimicrobial	[36]
7.	4-(1,1dimethyl propyl-phenol, trimethylsilyl ether	1.43%	Antimicrobial	[37]
8.	1, 2-bis (trimethyl silyl) benzene	1.36%	Antibacterial and anticancer	[38]
9.	Adamentane methyl amine	1.32%	Antibacterial, antiviral and anti-Parkinson	[39]
10.	1,4-benzenediole, 2,5-bis (1,1 dimethyl ethyl)-	1.29%	Not known	
11.	2-chloro aniline -5-sulphonic acid	1.28%	Antimicrobial	[40]
12.	Acetic Acid,nitro-methyl ester	1.24%	Not known	
13.	Caprolactone oxime,(NB)-O-[(diethylboryloxy)(ethyl) boryl]	1.21%	Not known	

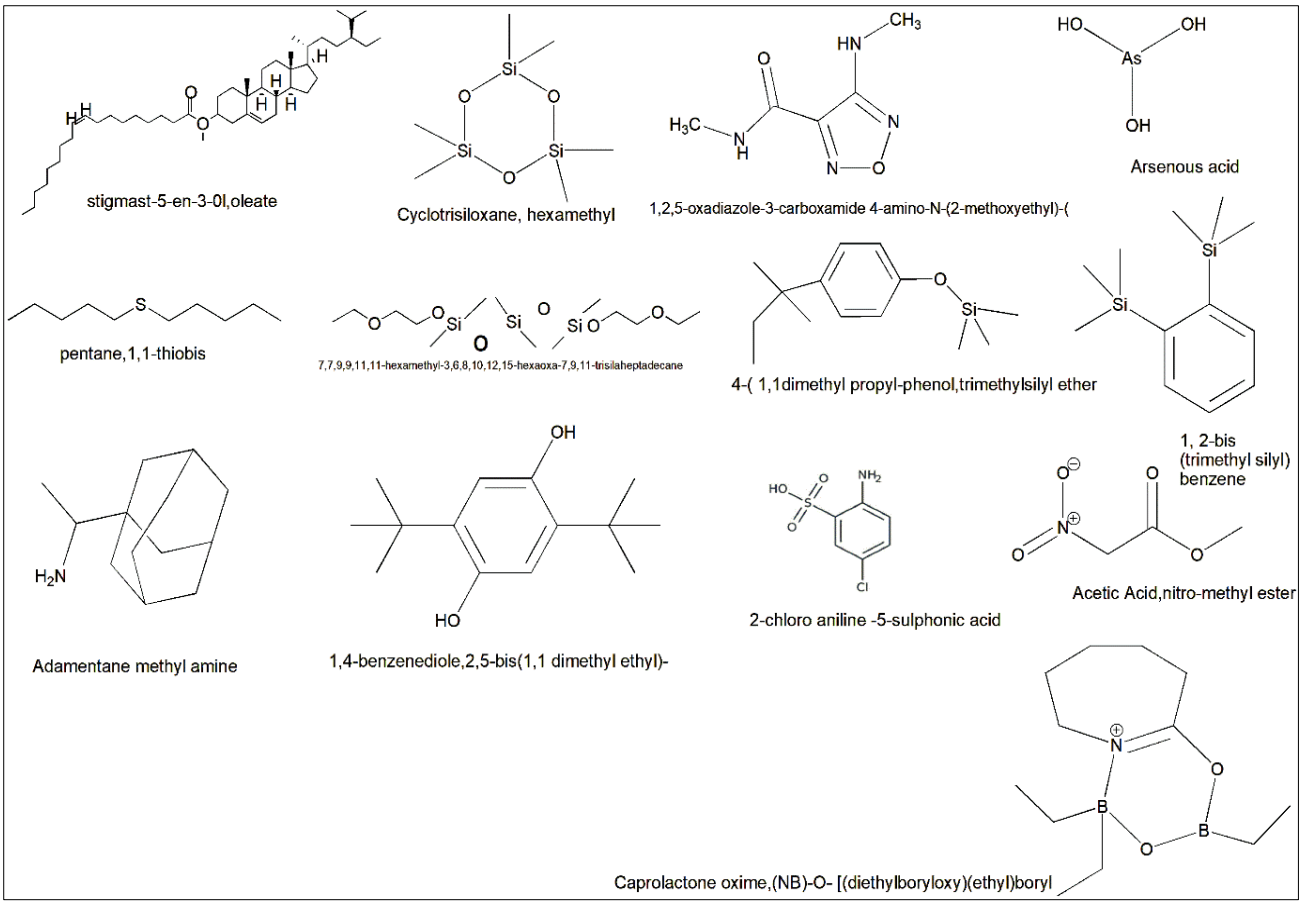

Fig. 2: Structure of key phytochemical compounds detected from Uterotibb aqueous alkali extract

Antimicrobial Activity

The alkali aqueous extract of Uterotibb exhibited remarkable antimicrobial activity against some pathogens such as *Staphylococcus aureus*, *Bacillus subtilis*, Klebsiella pneumonia, and Escherichia coli with MIC values ranging from 1 to 5mg/ml. The results of the antimicrobial investigation presented in (Table 2, Fig. 3).

The Uterotibb exhibited antimicrobial activity at a concentration-dependent manner, showcasing its potential as a promising solution in combating infections. This makes Uterotibb a noteworthy choice for those seeking effective antimicrobial treatments. Two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*E. coli* and *K. pneumoniae*) were used in this study. The aqueous NaOH extract of Unani medicine Uterotibb's exhibited the highest antimicrobial activity against *Staphylococcus aureus* with a zone of growth inhibition 14.95±0.35 mm at 5 mg/mL concentration. The lowest antimicrobial activity was observed against *Bacillus subtilius* with a 13.26±0.88 mm zone of inhibition at the sane concentration (Table 2). The extract showed considerable antimicrobial activity against *E. coli at* 4 mg/mL and 5 mg/mL with 12.97±0.45 mm and 13.98±0.87 mm zone of growth inhibition respectively. On the other hand, at 1 mg/mL concentration the aqueous alkali extract possessed highest antimicrobial activity against *Staphylococcus aureus* with 8.93±0.67 mm zone of growth inhibition while the lowest zone of inhibition of 7.32±0.91 mm was observed against *Bacillus subtilius*. At 3 mg/mL concentration, the aqueous alkali extract showed good antimicrobial activity against *Staphylococcus aureus* and *K. Pneumoniae* with 11.27±0.76 mm and 11.13±0.81 mm zone of inhibition respectively. In contrast, the minimum inhibitory concentration (MIC) of NaOH aqueous extract was 2 mg/mL and 3 mg/mL against *Staphylococcus aureus* and *Bacillus subtilis* respectively. For *E.coli* the MIC was determined to be 3 mg/mL where in case of *K. Pneumoniae* the MIC was observed at 4 mg/mL. Streptomycin (5 µg/mL) was used as a control, as shown in (Table 2, Fig. 3).

Table 2: Antimicrobial susceptibility potential of alkali aqueous extract of Uterotibb's against selected human pathogens

Bacterial species	Zone of growth inhibition in (mm)					
	Antibiotic discs	Different concentrations Uterotibb aqueous NaOH extract				
	Streptomycin 5 µg/mL	1 mg/mL	2 mg/mL	3 mg/mL	4 mg/mL	5 mg/mL
K. Pneumoniae	16.81±0.98	7.73±0.46	8.00±0.50	11.13±0.81	12.03±0.90	13.81±1.62
Staphylococcus aureus	16.33±0.63	8.93±0.67	10.68±0.28	11.27±0.76	12.33±1.04	14.95±0.35
Bacillus subtilius	24.17±0.12	7.32±0.91	9.92±0.68	10.17±0.72	11.30±0.92	13.26±0.88
E. coli	12.00±0.88	7.41±0.38	8.49±0.47	10.98±0.71	12.97±0.45	13.98±0.87

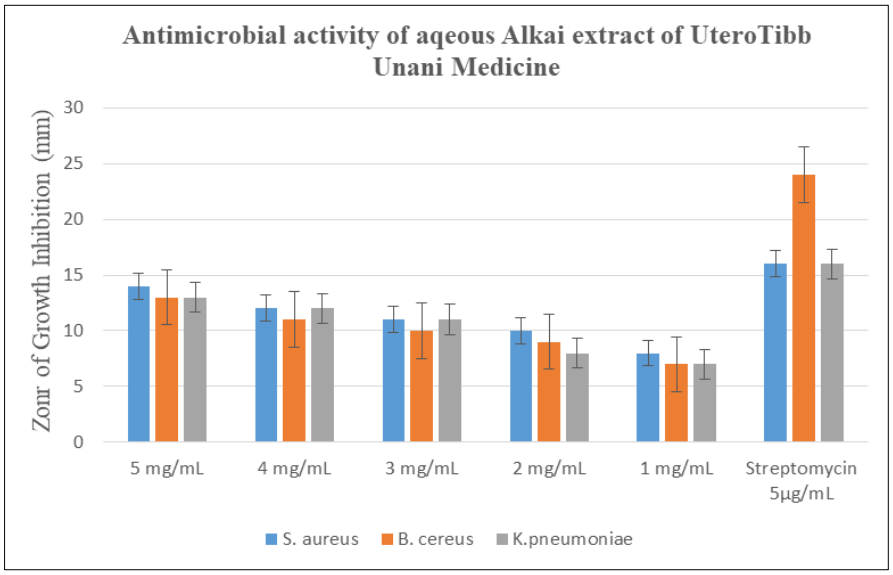

Fig. 3: Showing antimicrobial activity of the Uterotibb NaOH aqueous extract

The present study investigates a Unani medicinal formulation comprising more than five distinct medicinal plants. To the best of our knowledge, no prior studies have been conducted on this specific formulation. Consequently, the findings of this study are correlated with previously published data on individual constituent plants.

The antibacterial efficacy of the alkali extract demonstrated broad-spectrum antimicrobial activity against all tested bacterial strains. Notably, Moutawalli *et al*., (2024) reported that Henna seed extract exhibited no inhibitory effect against Escherichia coli. However, in contrast to these findings, the aqueous alkali extract in the present study displayed significant antimicrobial activity against E. coli at concentrations of 4 mg/mL and 5 mg/mL. Furthermore, our results are consistent with those of the same study, which reported that the ethanolic fraction exhibited antibacterial activity against Staphylococcus aureus and Bacillus cereus, with respective inhibition zone diameters of 15.5 ± 0.7 mm and 14.0 ± 0.0 mm. In terms of the minimum inhibitory concentration (MIC), the findings of the present study closely align with those obtained for the ethanolic fraction reported in the referenced study, where a MIC value of 1.563 mg/mL was observed against both Staphylococcus aureus and Bacillus cereus strains. Notably, regarding Escherichia coli, the current study demonstrated superior antimicrobial efficacy compared to the previously reported results. The referenced study did not report an MIC value for E. coli, whereas our findings established an MIC of 2 mg/mL against this bacterial strain, highlighting the enhanced inhibitory potential of the tested extract.

A study conducted by (Al-Timimi, *et al*., 2019) reported that aqueous and ethanolic extracts of fenugreek seeds exhibited no antimicrobial activity against Escherichia coli. In contrast, the findings of the present study demonstrated significant antibacterial activity against E. coli, as illustrated in (Table 2; Fig. 3). Conversely, Al-Timimi's study observed that both aqueous and ethanolic fenugreek seed extracts displayed antimicrobial efficacy against Staphylococcus aureus, with inhibition zones of 12 mm and 22 mm, respectively. These findings are consistent with the results obtained in the current study. Additionally, the MIC values reported by Al-Timimi (2019) for S. aureus and E. coli align with the MIC values determined in this study for the same bacterial strains, further supporting the reliability and reproducibility of our findings.

In another previous study conducted by Bag *et al*., (2012), aqueous and ethanolic extracts of *Terminalia chebula* exhibited varying degrees of antimicrobial activity against drug-resistant clinical isolates, with inhibition zones ranging from 8.67 ± 0.92 mm to 24.51 ± 1.28 mm. The ethanol and aqueous extracts

demonstrated antibacterial effects against Staphylococcus aureus, including both multidrug-resistant (MDR) and non-MDR strains, with inhibition zones ranging from 9 mm to 13 mm for MDR strains and 11 mm to 22 mm for non-MDR strains. These findings are in agreement with the results obtained in the present study.

Several classes of phytochemicals exhibit antimicrobial activity such as polyphenols, including phenolic acids and flavonoids, are particularly potent. Other significant classes include alkaloids, tannins, saponins, Flavonoid and terpenoids. Additionally, specific compounds like eugenol and coumarins also possess antimicrobial properties (Bag, *et al*., 012).

ABTS Assay

The antioxidant activity of the alkali aqueous extract of Uterotibb's was performed using ABT Assay and DPPH Assay. The antioxidant potential of the alkaline extract from Uterotibb was assessed through the ABTS Assay, using concentrations between 0.2 and 1 mg/L. The highest tested concentration of 1 mg/L exhibited a notable scavenging activity of 73.67 ± 0.09%. These findings are represented in (Fig. 3), highlighting the effectiveness of the extract in neutralizing free radicals. The antioxidant potential of the alkaline extract of Uterotibb was confirmed using the ABTS Assay, conducted at concentrations ranging from 0.2 to 1 mg/L. The highest scavenging activity recorded was 73.67 ± 0.09% at 1 mg/L. The results of this study are shown in (Fig. 4).

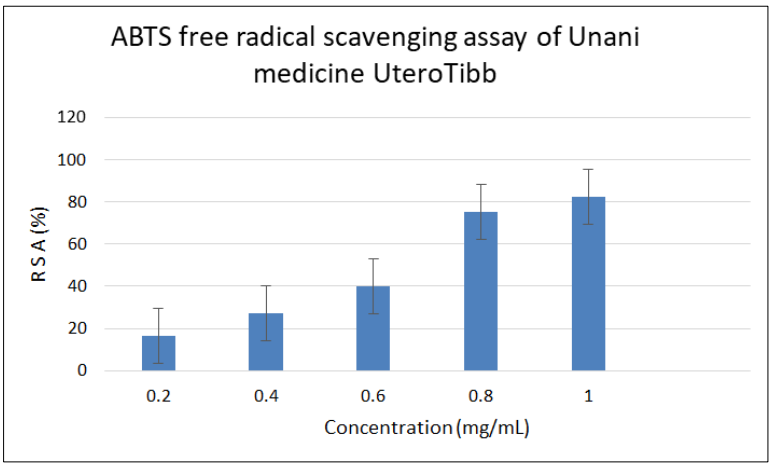

Fig. 4: ABTS free radical scavenging property of alkaline extract of Uterotibb

DPPH Assay

The antioxidant potential of the alkaline extract of Uterotibb was further evaluated using DPPH Assay at various concentrations (0.2 – 1mg/mL) and the results of this study are shown in (fig. 4.) Uterotibb's exhibited a concentration dependent increase in antioxidant activity. At the highest concentration (1mg/L), Uterotibb's showed the maximum scavenging activity (67.15 ± 0.05%) against DPPH radicals (Fig. 5). The free radical theory (Wickens, 2001) has prompted the search for compounds with antioxidant properties to enhance the body's physiological conditions. The DPPH and ABTS assays are among the most commonly used methods for evaluating antioxidant potential, as they are simple, rapid, and cost-effective (Alam *et al*., 2013; Ilyasov *et al*., 2020).

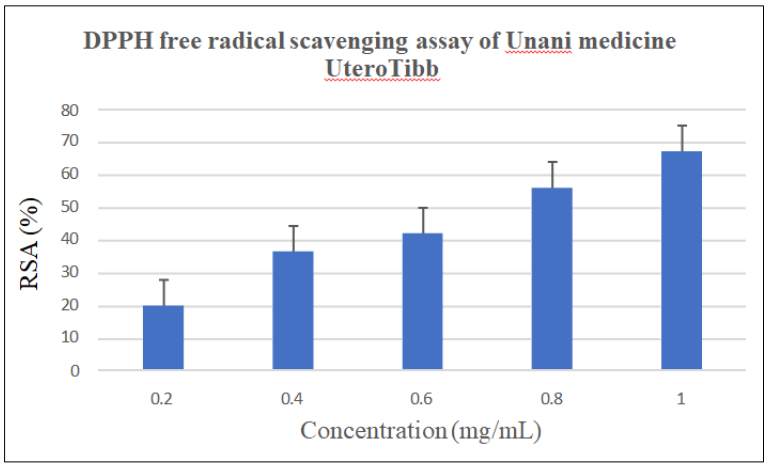

Fig. 5: DPPH free radical scavenging property of Uterotibb NaOH aqueous extract

Antioxidants play a crucial role in protecting the body from the damage caused by free radicals by donating hydrogen atoms or electrons to neutralize these harmful molecules. The aqueous extract of Uterotibb showed significant electron-donating activity in a concentration-dependent manner by reducing ABTS and DPPH. The notable antioxidant property of Uterotibb highlights its potential as a natural oxidant. The Uterotibb extract showed a dose-dependent antioxidant activity with a maximum scavenging activity of 80.0% against ABTS radical and 76.19% against DPPH radical. Linear regression analysis confirmed a significant relationship between antioxidant activity and Uterotibb aqueous extract ($p<0.05$). The results of the current study showed significant antioxidant activities using ABT and DPPH assays compared to the results obtained by (Jcob, *et al*., 2011) the henna ethanolic extract that exhibited lower scavenging of free radicals activities with IC50 value of 3.06±0.01 µg/M. Thus, the high free radical scavenging capacity observed in the aqueous alkali extract may be attributed to the elevated levels of polyphenolic compounds, flavonoids and tannins present in the extract. These results align with the earlier findings were the ethanolic extraction exhibited the reducing power of 14.40±0.01 µg/mL and 406.70±0.01 respectively.

Previous research has indicated that phenolic compounds, particularly those containing hydroxyl-phenolic structural groups, exhibit potent antimicrobial properties by enhancing microbial inhibition. Subsequent studies have further validated the antimicrobial efficacy of polyphenols and flavonoids. Polyphenols exert their antimicrobial activity through multiple mechanisms, including structural modification of the microbial cell membrane, regulation of membrane permeability, modulation of cellular interactions via hydrogen bonding, reduction of lipid content, and ultimately, suppression of microbial growth. Considering these established findings, the results of the present study provide further support for these conclusions, reinforcing the antimicrobial potential of phenolic compounds (Habbal, *et al*., 2005; Elansary, *et al*., 2020).

Several phytochemicals exhibit potent antioxidant activity, effectively neutralizing free radicals and mitigating oxidative stress to support overall health. Among the most significant are flavonoids, abundant which safeguard cells from oxidative damage. Polyphenols, enhance cellular defence mechanisms, while carotenoids contribute to eye health and immune function. Tannins, possess strong free radical-scavenging properties. Additionally, phenolic acids help reduce inflammation and oxidative stress. Terpenoids exhibit both antioxidant and anti-inflammatory effects. Lastly, alkaloids play a critical role in protecting cells against oxidative damage. Collectively, these phytochemicals contribute significantly to the prevention of oxidative stress-related diseases and the maintenance of overall health (Muscolo, *et al*., 2024). Phytochemicals exert antioxidant activity through various mechanisms that effectively neutralize free radicals and mitigate oxidative stress. It prevent oxidative stress by scavenging free radicals, chelating metal ions, regulating antioxidant enzymes, and inhibiting oxidases that generate reactive oxygen species. They also stabilize cell membranes and reduce lipid peroxidation, preserving cellular integrity and function. These combined mechanisms highlight their role in combating oxidative damage and related diseases (Zhang, *et al*., 2024; Sundaram, *et al*., 2021).

CONCLUSION

Uterotibb in Unani medicine refers to treatments and remedies specifically aimed at addressing issues related to the uterus. Unani medicine, a traditional system of healing, uses herbal formulations and natural substances to restore balance in the body.

In the present work, we have investigated the phytochemical composition and antimicrobial and antioxidant potential of Uterotibb. The GC-MS analysis revealed the presence of 388 bioactive secondary metabolites (phytochemicals), out of which compounds were predominant with their significant medicinal properties. To the best of our knowledge this is the first study to provide the details GC-MS analysis of the aqueous NaOH extract of the Unani medicine Uterotibb. However, the other detected compounds were in small proportions and exhibited good biological activity. The antimicrobial studies showed that Uterotibb traditional Unani herbal medicine possesses significant antimicrobial action against selected human pathogens where the lowest MIC was observed against Staphylococcus aureus at 2 mg/mL concentration but in case of *E. coli* the MIC was observed at 3 mg/mL. The extract demonstrated excellent antioxidant activity. The present study underscores the potential application of Unani herbal medicine in pharmaceutical and medical contexts. Furthermore, our research highlights the crucial role of the Uterotibb formulation for treating different human disorders and diseases.

Conflict of Interest: Authors declare no conflict of interest.

Authors Contributions

MOHD, SSA, and MN designed the concept, carried out and monitored the experiments. All authors. NB, manuscript writing and proof reading. All the authors read and approved the final manuscript.

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