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Tasleem Ahmad

^(a)Regional Research Institute of Unani Medicine, Patna, Bihar, India-80008
^(b)Central Research Institute of Unani Medicine, Erragadda, Hyderabad, India

Ayesha Mateen

Central Research Institute of Unani Medicine, Erragadda, Hyderabad, India

Mohammad Abdul Waheed

Central Research Institute of Unani Medicine, Erragadda, Hyderabad, India

Mohammad Abdul Rasheed N

Central Research Institute of Unani Medicine, Erragadda, Hyderabad, India

Syed Gulnawaz Ahmad

Central Research Institute of Unani Medicine, Erragadda, Hyderabad, India

Mohammad Ishtiyaque Alam

Regional Research Institute of Unani Medicine, Patna, Bihar, India-80008.

Najmus Saher

Regional Research Institute of Unani Medicine, Patna, Bihar, India-80008.

Mohammad Wasim Ahmed

Regional Research Institute of Unani Medicine, Patna, Bihar, India-80008

Pawan kumar Yadav

Central Council for research in Unani medicine, Janakpuri, New Delhi, India

Zaki Ahmad Siddiqui

Central Council for research in Unani medicine, Janakpuri, New Delhi, India

Shakir Ali

Department of Biochemistry, Jamia Hamdard, New Delhi, India, India

Correspondence:

Dr. Tasleem Ahmad

Research Officer (Biochemistry) S-1
Regional Research Institute of Unani Medicine, Patna city, Patna India
Email: tasleem786@rediffmail.com

Antimicrobial activity of some herbal drugs used in unani system of medicine

Tasleem Ahmad, Ayesha Mateen, Mohammad Abdul Waheed, Mohammad Abdul Rasheed N, Syed Gulnawaz Ahmad, Mohammad Ishtiyaque Alam, Najmus Saher, Mohammad Wasim Ahmed, Pawan kumar Yadav, Zaki Ahmad Siddiqui, Shakir Ali

Abstract

Cyperus rotundus, *Operculina turpethum* and *Acorus calamus* are used in unani system of medicine for the treatment of various ailments. In this study, these three plants were selected to investigate their antimicrobial activity against *Escherichia coli* (ATCC 259220), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), Clinical isolates-*Salmonella paratyphi*, and *Klebsiella*. Methanolic extract of *C. rotundus* showed antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Methanolic extract of *O. turpethum* showed antimicrobial activity against *Staphylococcus aureus*. Interesting results were found in case of *Acorus calamus*. Methanolic extract of *Acorus calamus* showed antimicrobial activity against *S. paratyphi*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Ethanolic extract of this drug showed antimicrobial activity against *Pseudomonas aeruginosa*, *Klebsiella*, *E. coli* and *Staphylococcus aureus*. The presence of phytochemicals such as flavonoids, sesquiterpenoids, shyobunone, acolamone and β -asarone in the extracts of these plants supports their traditional uses as medicinal plants for the treatment of various ailments. The present study reveals potential use of these plants for developing new antibacterial compounds against pathogenic microorganisms.

Keywords: Antimicrobial, *Cyperus rotundus*, *Operculina turpethum*, *Acorus calamus*

1. Introduction

Natural products have been used for combating human diseases for thousands of years, since they exhibit a wide range of biological properties that can be exploited for medical application [1]. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases [2]. This resistance has increased due to indiscriminated use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. So that many scientist have search antimicrobial agents such as medicinal plants [3]. Medicinal plants represent a rich source of antimicrobial agents and they are used in different countries and are a source of many potent and powerful drugs [4]. In this study, three herbal drugs *Cyperus rotundus*, *Operculina turpethum* and *Acorus calamus* are used for antimicrobial activity. *Cyperus rotundus* (Family Cypreace), also known as nutt grass, is perennial sedge distributed throughout India. In Unani system of medicine, it is used as diuretic, emmenagogue, aphrodisiac, stone bladder, strengthens memory, palpitation, loss of appetite, scorpion bite and also possesses anti-inflammatory, antiestrogenic, antimicrobial, antihelminthics, antihistaminic activity[5-6]. Turbud safed or *Operculina turpethum* (Family Convolvulaceae) grows throughout India up to 900 m. The plant is a large perennial twiner with milky juice and fleshy branches. It cures brain diseases. Alcoholic extract of *O. turpethum* has potent anti-bacterial activity against *Micrococcus pyogenes* and *E. coli* [7]. Ethanolic extract, aqueous extract and ethereal extract of root of this plants showed anti-inflammatory activity [8]. Chemical constituents present in *O. turpethum* include resin, a fatty substance, volatile oil, albumin, starch, yellow colouring matter, lignin, salts and ferric oxide. Root of *Turbud safed* contains 10% resin, glucoside and turpethinic acid A and turpethenic acid B. It is used for the removal of dropsical effusion and in such cases it acts best in combination of ginger and bitartrate of potash [9]. *Acorus calamus* (Family Araceae), commonly known as Sweet Flag, is recognized by the name of Waj-e-turki in Unani System of Medicine. It is a perennial shrub growing in damp marshy places. It is also used in cough, bronchitis, gout, inflammation, diarrhea, convulsions, depression, tumors, haemorrhoids, skin

diseases, numbness and general debility and also possess insecticidal, antibacterial hypolipidemic, and antifungal, [10-13]. β -asarone Fraction of *Acorus calamus* possesses potent antimicrobial, antifungal activity [14].

2. Material and Method

2.1 Plant material

Rhizomes of *Cyperus rotundus*, *Operculina turpethum* and *Acorus calamus* were collected from local herbal dealer of Hyderabad, and were authenticated by Botanist at CRIUM Hyderabad, Andhra Pradesh, India. This study was done in the year of 2011-2012.

2.2 Bacteria Cultures

Microorganisms tested in this study were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), Clinical isolates - *Salmonella paratyphi*, and *Klebsiella*.

2.3 Extraction for bacterial culture

The collected herbal drugs were washed and air-dried for 48h at the room temperature, chopped into small pieces and then soaked with two types of solvent at room temperature. The extract was filtered and then entire extract was concentrated to dryness using rotary evaporator under reduced pressure.

2.4 Evaluation of antibacterial activity

Anti-Bacterial activity of the extract was determined by agar diffusion assay [15]. Bacterial strains were first grown in Mueller Hinton broth (MHB) under shaking condition for 24 h at 37 °C and after the incubation period 0.1ml of the test the inoculum was spread evenly with a sterile glass spreader on Mueller Hinton Agar (MHA) plates. In seeded plates, wells were made using sterile 6 mm cork borer in the inoculated MHA plate. The wells were filled with 150 μ l of the extracts. The concentration of stock extracts were 200 mg/ml. The inoculated plates were incubated at 37 °C for 24 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of the zones of inhibition was measured and the antibacterial activity was expressed in terms of average diameter of the zone of inhibition in millimeters. The test was conducted in triplicate the photograph was taken in UV-Visible documentation system.

3. Results and discussion

An alarming increase in bacterial strains resistant to a number of antimicrobial agents demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit growth of pathogenic bacteria. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross resistance with agents already in use may be minimal [16]. In this study, three herbal plants, *C. rotundus*, *O. turpethum*, *Acorus calamus* are used for antibacterial activity against *S. paratyphi*, *Pseudo aetrogenosa*, *Klebsiella*, *E. coli* and *Staphylococcus aureus*. Methanolic extract of *C. rotundus* and *O. turpethum* and both Methanolic and Ethanolic extract of *Acorus calamus* are used for this study. Present study report Methanolic extract of *O. turpethum* has antibacterial activity against *staphylococcus aureus*. Methanolic extract of *C.*

rotundus has zone of inhibition against *P. aeruginosa* and *staphylococcus aureus*. Interesting results were found in case of *Acorus calamus*. Methanolic extract of *Acorus calamus* has zone of inhibition against *S. paratyphi*, *Pseudo aetrogenosa*, and *staphylococcus aureus*. Ethanolic extract of this drug has zone of inhibition against *Pseudomonas aeruginosa*, *Klebsiella*, *E. coli* and *staphylococcus aureus*. Results are depicted in Table no-1. Clear zone of inhibition was also shown in plate 1-5.

A possible explanation for responses of the herbal medicine against different bacterial strain can be provided by a quick survey of the ingredients in the herbs such as *Cyperus rotundus* that include β -sitosterol, β -Cyperone, Cyperolone, Isocyperol, Isokobusone, Kobusone, Linolenic acid, Linoleic acid, Myristic acid, Oleic acid, Stearic acid, Suganol, Phenylpropanoid, cyperene, cyperol, flavonoids, sesquiterpenoids, vitamins, polyphenols, cyprotene, cypera-2, 4-diene, a-copaene, cyperene, aselinene, rotundene, valencene, trans-calamenene, d-cadinene epi-a-selinene, a-murolene, cadalene, nootkatene, cyperotundone, acyperone [17-18]. *O. turpethum* contains Turpethinic acids- A, B, C, D, E, soluble resin, volatile oil, albumin, starch, lignin salts, ferric oxide, Scopoleptin, Betulin, lupiol & β -sitosterol [19-20]. The rhizomes of *Acorus calamus* are empirically used in the treatment of a wide variety of human diseases. Shyobunone and acolamone, β -asarone are important chemical constituents of *Acorus calamus* [21].



Plate 1: Effect of herbal drugs on *E. coli*. WTM means methanolic extract of *Acorus calamus*, WTE means ethanolic extract of *Acorus calamus*, SKM means methanolic extract of *Cyperus rotundus*, and TSM means methanolic extract of *Operculina turpethum*

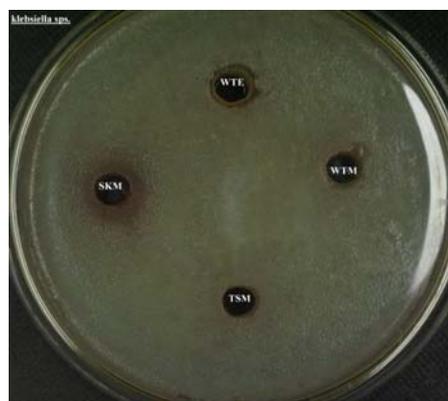


Plate 2: Effect of herbal drugs on *Klebsiella*. WTM means methanolic extract of *Acorus calamus*, WTE means ethanolic extract of *Acorus calamus*, SKM means methanolic extract of *Cyperus rotundus*, and TSM means methanolic extract of *Operculina turpethum*

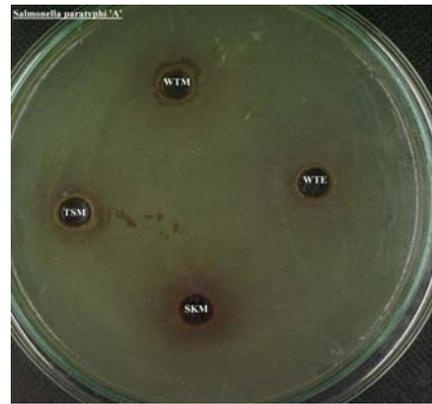
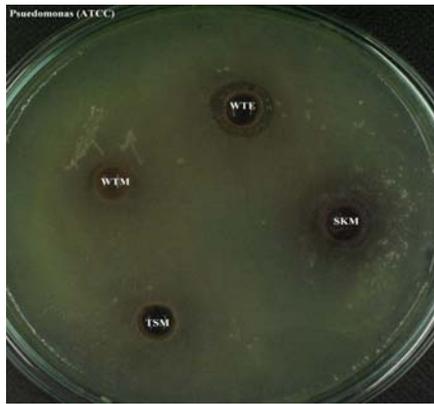


Plate 3: Effect of herbal drugs on *Pseudomonas aeruginosa*. WTM means methanolic extract of *Acorus calamus*, WTE means ethanolic extract of *Acorus calamus*, SKM means methanolic extract of *Cyperus rotundus*, and TSM means methanolic extract of *Operculina turpethum*.

Plate 4: Effect of herbal drugs on *Salmonella paratyphi A*. WTM means methanolic extract of *Acorus calamus*, WTE means ethanolic extract of *Acorus calamus*, SKM means methanolic extract of *Cyperus rotundus*, and TSM means methanolic extract of *Operculina turpethum*.



Plate 5: Effect of herbal drugs on *Staphylococcus aureus*. WTM means methanolic extract of *Acorus calamus*, WTE means ethanolic extract of *Acorus calamus*, SKM means methanolic extract of *Cyperus rotundus*, and TSM means methanolic extract of *Operculina turpethum*.

Table 1: Effect of some herbal drugs against bacterial strain tested by agar diffusion assay

Bacterial sps	ZONE OF INHIBITION (ZOI)			
	<i>Operculina turpethum</i>	<i>Cyperus rotundus</i>	<i>Acorus calamus</i>	
	M.Ext	M.Ext	M.Ext	E.Ext.
<i>S. paratyphi</i>	----	----	11 ± 1	---
<i>Pseudo. aeruginosa</i>	----	10 ± 1.7	09 ± 1	12 ± 2
<i>Klebsiella sps</i>	----	----	----	10 ± 2
<i>E. coli</i>	----	----	----	11 ± 1
<i>Staph. aureus</i>	11 ± 1.7	6 ± 2.6	2 ± 1	12 ± 1

Values are mean Zone of Inhibition (mm) ± SD of three replicates
M.Ext-Methanolic extract and E.ext- Ethanolic extract

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