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IN VITRO EVALUATIONS OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE UNSAPONIFIED COMPONENTS OF *SCILLA INDICA* BAKER

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ABSTRACT

The present investigations was aimed to study the antimicrobial activity (antibacterial and antifungal) of the unsaponified matter β-sitosterol from the plant Scilla indica Baker bulb belongs to family Liliaceae. The dried plant compound was obtained successively extracted by using organic solvents viz. ethanol, methanol, petroleum ether and water extract was assessed for their antimicrobial activity against pathogens like bacteria: Bacillus spp, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and fungi: Aspergillus niger, Aspergillus flavus and, Rhizopus stolonifer. The potential antibacterial and antifungal activity against bacteria and fungi was examined by MIC and zone of inhibition and MBC and MFC analysis by using agar well diffusion and micro dilution method respectively. All the extract shows the significant activity against the above microorganisms. But the alcoholic extracts show the maximum zone of inhibition and minimum inhibitory concentration. The minimum zone of inhibition and comparatively maximum inhibitory concentration was determined in petroleum ether and water extracts of compound showing the least antimicrobial activity against all the experimental strains. Hence, this compound from the plant of Scilla indica Baker could be source to obtain new and effective antimicrobial medicine to treat infection and may be exploited as the future antimicrobial drugs.

Keywords: Antibacterial Activity, Antifungal Activity, MIC, MBC, MFC, Scilla Indica Baker, E. Coli, A. Niger, P. Aeruginosa And Zone of Inhibition.

I. INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, the resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen 1992). There are many approaches to search for new biologically active higher plants (Farnsworth & Loub 1983). One such approach is systematic screening, which may result in the discovery of novel effective compounds (Janovska *et al*. 2003). Many efforts have been done to discover new antimicrobial compounds from plants. While systematic screening of medicinal plants may result in the discovery of novel effective compounds (Janovska *et al*. 2003). Various

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medicinal plants have been used for years in daily life to treat infective diseases all over the world. The characteristics of the plants that inhibit microorganisms and are important for human health have been researched in laboratories since 1926 (Vonderbank 1949; Erdoğrul *et al.* 2001; Erdoğrul 2002). Traditional medical treatments in daily life are now being tested with the use of empiric methods (Sokmen *et al*. 2000). Since plants contain a variety of chemical compounds in their leaves, roots, and flowers, they have been used in the treatment of various human diseases for thousands of years all over the world (Larhsini *et al*. 2001. Similarly, a lot of plants have been used by rural people for the treatment of several diseases, including microbial infections, (Baytop 1984). Most of the plants used for medicinal purposes have been identified, and their uses are well documented and described by different authors (Nadkarni 1876; Dastur 1985; Saradamma 1990), but the efficacy of many of these plants is yet to be verified. Natural plant extracts have also been tested in laboratories against bacteria and fungi (Sokmen *et al*. 1999). The first compound with antimicrobial activity was found in the 1930s (Goodman *et al*. 1991). Since that period, the development and use of these substances has increased, especially with the appearance of resistant strains (Zimhener & Mear 1972).

The plant *Scilla indica* baker is well known medicinal plant belongs to family Liliaceae. The aqueous bulb extract of plant *S. indica* was earlier identified to posses antiviral activity and inhibited virus infection and replication (Khan and Zaim, 1992).

The plant *Scilla indica Baker* was dried, powered and extracted with petroleum ether in a soxhlet extract for about 70 hrs.

After extraction was concentrated to a get yellow viscous mass. Which was saponified by refluxing with alcoholic potassium hydroxide. The contents obtained were dissolved in water and the unsaponified matter was recovered by extraction with ether in separating funnel.

II. MATERIAL AND METHODS

1. Selection of Medicinal Plant: The medicinal plant *Scilla indica* Baker was collected from CSIR-CIMAP, Lucknow. Healthy and disease free plants were selected for the antimicrobial screening studies.

2. Extraction Procedure: Powered material of *S. indica* bulb was extracted with different solvents (Like Ethanol, Methanol, Petroleum Ether and Water) respectively for 8-10 hrs in the soxhlet apparatus at a temperature not exceeding the boiling point of the respective solvents. After extraction excess solvent was removed by distillation and the concentrated extract so obtained were further dried in incubator at 40 ^{0}C.The residual extract after drying were dissolved in 50% DMSO and stored in refrigerator at 4 ^{0}C in small and sterile glass tubes.

3. Nutrient Agar: 500 ml of nutrient agar was made by placing 14 g of powdered mixture in 1 L flask, stirred, boiled and then autoclaved for 15 minutes at 121 ^{0}C. The plates were poured in a sterile environment under aseptic condition and allowed to cool for 2 hours. The microorganisms were streaked onto separate plates and discs were applied with for ceps, then labeled and placed in an incubator for 24-48 hours at 37 ^{0}C, both for bacteria and fungi respectively (Jagessar et al., 2008).

4. Potato dextrose agar (PDA): The potato was peeled and 100g was measured, finely chopped and boiled to a mash in distilled water.The dextrose was measured (12.5g) and placed in a 1L measuring cylinder. Agar was

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measured (12.5 g) and added to the measuring cylinder (with the dextrose). The potato mash was stirred and strained into the cylinder. Hot distilled water was added to make up 500mL.The contents was continuously poured and stirred until consistency was achieved. The content was then poured into a conical flask, plugged with cotton wool, over which aluminium foil was tightly wrapped. The flask was then autoclaved at 121 0C for 24hrs (Jagessar et al., 2008).

5. Reference and Control: The references were antibiotic in nature, streptomycin was chosen as the reference for all bacterial strains and greisofluvin was used as the reference for all fungus species. The control experiment consists of a plate solidifying agar onto which was inoculated pure solvent with microorganisms mixed in a 1:1 ratio (Jagessar et al., 2008).

6. Mother plates: These were made by culturing microorganism on PDA. A sterilized 6mm cork borer was used to cut agar discs in the plate.

7. Colonies Counting: Colonies were estimated with the assistance of a colony counter. The number was estimated for $1cm^2$ and then calculated for the entire plate. The plate radius was determined.

8. Culture and Maintenance of microorganism: Pure culture of all experimental bacteria and fungi were obtained. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on Potato dextrose medium (PDA).

9. **Microorganism use for test:** The bacterial strains used for antibacterial screening were;-

a) *Bacillus cereus* b) *Staphylococcus aureus* c) *Escherichia coli* d) *Pseudomonas aeruginosa*

The fungal strains used for antifungal screening were;-

a) *Aspergillus niger* b) *Aspergillus flavus* and c) *Rhizopus stolonifer*.

10. Media preparation and its sterilization: For agar well diffusion method (Maurya et.al 1995). Antimicrobial susceptibility was tested on solid (agar-agar) media in petri plates for bacterial assay nutrient agar (NA) (40 gm/l) and fungus PDA (39gm/l) was used for developing surface colony growth. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) value were determined by serial micro dilution assay. The suspension culture, for bacterial cell growth 2.4% w/v and PDA for fungus cell growth was taken for evaluation. All media prepared was then sterilized by autoclaving the media at (125 ^{o}C) for 20 min (Ahmad and Beg, 2001).

11. Agar Well diffusion method: Agar Well diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed with 8 hrs old broth culture of respective bacteria and fungi. Wells (10 mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of each plant extract was prepared at a concentration of 1mg/ml in different plant extract viz. methanol, ethanol, Petroleum ether, water. About 100ul of different concentration of plant solvent extract was added by sterile syringe into the well and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37 ^{o}C for 18-24 hr for bacterial pathogen and 28 ^{o}C for 48 hr fungal pathogen. The diameter of the inhibition zone (mm) was measured and the activity index was calculated. Experiment in triplicate were maintained and the reading were taken and the average value was recorded.

12. Minimum Inhibitory concentration: The minimum inhibitory concentration is defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates. This was determined from

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reading on the culture plates after incubation. The most commonly used methods are tube dilution method and agar dilution methods. Serial dilution is made of the products in bacterial and fungal growth media. The test organism is then added to the dilution of the products, incubated and scored for growth. This procedure is a standard assay for antimicrobials. Clinically, the minimum inhibitory concentrations are used.

13. Test for antibacterial activity: The antibacterial assay was carried out by micro dilution method in order to determine the antibacterial activity of compound tested against the pathogen bacteria. The bacterial suspension was adjusted with sterile saline to a concentration of 10^8 CFU/ml. The inocula were prepared and store at 4 ^{0}C until use. Dilution of the inocula was cultured on solid medium to verify the absence of contamination and to check the validity of inoculum. All the experiments were performed in triplicate and repeated two times.

14. Test for antifungal activity: The antifungal assay was carried out by micro dilution method in order to determine. The fungal spores were washed from the surface of the agar plates with sterile .85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of 10^8 as final volume of 100 ul per well. The inocula were prepared and store at 4 ^{0}C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of inoculums.

15. Determination of MBC: The MBC was determined by serial sub-cultivation of 2 ul into micro liter plates containing 100ul of broth per well and further incubation 72 hours at 28 ^{0}C. The lowest concentration with no visible growth was defined as MBC, indicating 99.5% killing of the original inoculum. The reading was taken and compared with the standard streptomycin for bacteria as the positive controls. All the experiments were performed in duplicate and repeated three times.

16. Determination of MFC: The MFC was determined by serial sub-cultivation of 2 ul into micro liter plates containing 100ul of broth per well and further incubation 72 hours at 28 ^{0}C. The lowest concentration with no visible growth was defined as MFC, indicating 99.5% killing of the original inoculums. The standard Greisofluvin was used as the positive controls (1-3000ug/ml). All the experiments were performed in duplicate and repeated three times.

2.1 Observation

In the present investigation, the inhibitory effect of different extracts (methanol, ethanol, petroleum ether and water) of plant *Scilla indica* Baker evaluated against both fungicidal and bacterial strains. The antimicrobial activity was determined using agar well diffusion method and micro dilution method summarized in the table. The activity was quantitatively assessed on the basis of inhibition zone and their index is also calculated along with minimum inhibitory concentration (MIC).

2.2 Measurement of antimicrobial activity

The antimicrobial potential of the experimental compound of the plant was evaluated according to their zone of inhibition against various pathogens and the results were compared with the activity of the standard streptomycin (1mg/disc) and griseofluvin (1mg/disc). The

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result reveals that all the extracts are potent antimicrobial agent against the microorganisms studied among the different solvent extract studied.

Table 1. Antimicrobial activity (zone of inhibition, mm) of various extracts of *S. indica* bulb against pathogen (Bacteria and Fungi).

Microorganism bacteria	**Ethanol**	**Methanol**	**Petroleum ether**	**Aqueous (water)**	**Standard (streptomycin)**
B. cereus	13.4	18.9	10.1	14.5	23..5mm
S. aureus	12.6	16.5	11.4	13.6	18.3mm.
E.c oli	18.9	14.6	11.7	ND	21.4mm
P. aeruginosa	13.2	14.3	12.4	ND	20.5mm
fungi	**Ethanol**	**Methanol**	**Petroleum ether**	**Aqueous (water)**	**Standard (griesoflvin)**
A. niger	16.4	13.1	12.4	ND	17.53mm
A. flavus	13.7	15.5	14.7	11.3	15.33mm
R. stolonifer	12.9	13.3	11.4	12.3	13.76mm

ND: not detected the growth of microorganism

Table 2. Minimum inhibitory concentration MIC (µg/ml) and MBC of the different various extracts of *S. indica* bulb.

Microorganism bacteria	**Ethanol**		**Methanol**		**Petroleum Ether**		**Aqueous (water)**		**Standard**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
B. cereus	26.4	43.8	22.4	45.7	32.4	55.4	19.8	54.6	39.5	58.9
S. aureus	18.8	51.3	34.5	65.8	21.6	45.3	14.4	53.7	25.6	62.3
E. coli	19.8	38.7	13.7	42.1	17.3	33.7	22.10	47.6	31.8	65.6
	11.2	53.6	17.4	48.3	19.4	55.8	20.2	58.3	27.3	72.1

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P.aeruginosa										

Table 3. Minimum inhibitory concentration MIC (µg/ml) and MFC of the different extracts of *S. indica* bulb.

Micro organism Fungi	**Ethanol**		**Methanol**		**Petroleum Ether**		**Aqueous (water)**		**Standard**	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A. niger	19.2	39.5	21.6	42.7	18.7	45.3	16.5	32.4	22.7	47.8
A. flavus	22.3	31.3	16.4	53.4	25.3	58.9	22.1	43.6	26.5	59.6
R. stolonifer	14.2	32.5	11.2	21.7	16.3	28.0	17.3	25.1	19.9	34.4

III. RESULTS AND DISCUSSION

Traditional medicinal plants have indefinite therapeutic value worldwide. The World Health Organization estimated that up to 80% of the world population still realize on traditional remedies (Arun Kumar and Muthu Selvan, 2009) with more than 35 thousand plants are being use for medication purposes in various human culture (Koshi et al., 2009). The emergence of antibiotic resistant microbial strains and increasing failure of the modern chemotherapeutics made the search for microbiologically active medicinal plant necessity (Parekh and Chandra, 2007). The emergence of drug resistance pathogen is making the treatment of infectious diseases more difficult among the common drug resistant (MDR-T.V), methicillin-resistant Stephylococcus auries (MRSA) and Vancomycin resistant interoeocci in 2013 alone, there were about 5 lacks new cases of MDR TV has been identified in hundred countries (WHO report 2015).

Traditional medicinal plant has long history of application as alternative medicine. In current studies the unsaponified compound of *Scilla indica* displayed promising antibacterial and antifungal activities against medically important microbial strains viz. *B. cereus, S. aureus, E. coli, P. aeruginosa* (bacteria), *A. niger, A. flavus, R. stolonifer* (fungi).

It was observed that all ethanol, methanol, petroleum ether, and aqueous extracts of *Scilla indica* (bulb) Baker were active against the bacterial and fungal strain. Susceptibility of extract was tested by serial dilution method (MIC) and agar well diffusion method was determined.

The alcoholic extracts viz. ethanol and methanol extract of *Scilla indica* showed the significant antimicrobial activity against the different strains. These extracts showed maximum activity against *E. coli* and *B. cereus*.

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The study also revealed that petroleum ether extract show moderated activity against different bacterial and fungal strain and aqueous extracts showed the minimum antimicrobial activity.

The observations of the MIC study have been tabulated in table 1-3 and it was found to be varying in different extracts. In the present study the bacterial and fungal strain which showed the maximum value of MIC and MBC/MFC for the different extracts showed the less antimicrobial activity and the different bacterial and fungal strain which shows the minimum value of MIC and MBC/MFC show the maximum antimicrobial activity. If the organism which showed maximum value has the less potential of growth in the antimicrobial agent and which showed the low MIC and MBC/MFC value for the other bacteria is an indication of the efficacy of the extract. The analysis suggests that the extract were bacteriostatic at lower concentration but bactericidal at higher concentration.

Similarly it was reported earlier that plants viz., *Cuminum cyminum, Jasminium officinale, Thymus capitatus, Viscum album, Tanecetum sorbfolium, Pimpinella anisum, Galega officinalis, Liquidamber orintalis, Thus coriaria, Alnus quutinosa, Pinmenta officinalis, Camelia sinensis, Artemisia indica, Medicago falcate, Tecoma stans,, Heliotropium bacciferum, Cassia alata, Nigella sativa, Momordica charantia, Lantana camara, Accacia nilotica and Justicia zelanica etc.* showed high antibacterial and antifungal activities against different strains of bacteria and fungi tested (Dabur et al., 2007, Jagessar et al., 2008, Omar, 2010, Javid et al., 2015, Ahmad et al., 2015, Batool and Azish, 2013, Bacha, 2016).

IV. CONCLUSION

The conclusion, of the present study showed the potential antimicrobial component that may be used for the therapy against various bacterial and fungal diseases. The study indicates that can be studied for the further assay to evaluate effectiveness as antimicrobial agent. Further, the potential of this plant must be further explore, in order to develop an alternative therapy for the treatment of infections cause by different bacterial and fungal organisms. By developing new and more effective antibacterial and antifungal herbal drugs.

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