

**Research Article**

**Antimicrobial activity of traditionally used herbs against human pathogens**

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**Abstract**

Antimicrobial agents are effective in curing diseases because of their selective toxicity against pathogenic microbes without causing any harm to the cells of the host. Antimicrobial activity of three herbs *Amaranthus spinosus*, *Solanum nigrum* and *Orchis muscula* were tested using methanol, hexane, chloroform and acetone extracts against six human pathogenic microbes. Agar well diffusion method was used for preliminary screening of extracts against microbes. The micro-dilution method was used for the determination of the minimal inhibition concentration (MIC). Phytochemical screening of plant extracts was done for the presence of various secondary metabolites. The results were analysed by using zone of inhibition and it was observed that methanol extracts of *Orchis muscula* and *Amaranthus spinosus* were effective against all the human pathogens tested.

**Keywords:** *Staphylococcus mutans*, antimicrobial activity, Phytochemical, *Orchismuscula*, Zone of inhibition

**1. Introduction**

Antibiotic drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This has forced scientists to search for new antimicrobial medicines from medicinal plants. Bacterial infections, especially those produced by *Staphylococcus aureus* are very difficult to manage<sup>1</sup>. Medicinal plants possess potent medicinal value that is due to the presence of a variety of phytochemical constituents in the plant tissues which cast a definite physiological action on the human body<sup>2</sup>. Antimicrobial properties of certain Indian medicinal plants have been reported on the basis of folk information<sup>3,4,5</sup>. Furthermore, it is expected that the wide use and extension in the utilization of such local agricultural products would increase and stabilize the income of farmers in the rural areas<sup>6</sup>.

*Amaranthus spinosus* (*Amaranthaceae*) grows annually as an erect, monoecious herb up to 100–130 cm tall. Plant grows abundantly from July to October in moist places. It is an annual weed that is widely distributed in the humid zone of the tropical countries. The leaves and roots are applied to relief bruises, abscesses, burns, wound, inflammation, menorrhagia, gonorrhoea, eczema and inflammatory swelling<sup>7</sup>. It is known to play regulatory role on different hormones and is used for treatment of cancer, hepatotoxicity and protection of cardiovascular system<sup>8,9</sup>.

The plant *Solanum nigrum* belongs to Family *Solanaceae*. *Solanum nigrum* is known to have anticancer,

antioxidant, neuroprotective, antimicrobial, and antipyretic properties<sup>10,11</sup>. *S. nigrum* has been used as the important ingredient for herbal formulations in India.<sup>12</sup>

*Orchis muscula* is an herbaceous plant with stems up to 50–60 cm long, green at the base and purple on the apex. The use of *Orchismuscula* has been very widely used as an alternate to arrowroot and Coffee. This orchis is really a blessing for the geriatric patients and for many disorders of gastro-intestinal tracts and is a potent aphrodisiac and tonic. *Orchid muscula* has a broad spectrum of medicinal properties. Although this plant is used by traditional healers, but to our knowledge there is no report regarding scientific proof of antimicrobial activity of *O. muscula*.

The present study was undertaken to evaluate antimicrobial activity of different traditionally used plants using different solvents and to determine their phytochemical constituents.

## 2. Materials and Methods

### 2.1 Procuring of bacterial strain

Pure cultures of test organisms were procured from IMTECH, Chandigarh, India. The bacterial strains used in the study were *Staphylococcus aureus* (ATCC96), *Streptococcus mutans* (ATCC890), *Bacillus subtilis* (ATCC6633), *Bacillus amylolique faciens* (ATCC23350), *Aspergillus fumigates* (ATCC1022) and *E. coli*(ATCC483).

### 2.2 Preparation of plant extracts

The leaves of plants *Amaranthusspinosus*, *Orchismuscula* and *Solanumnigrum* were washed thoroughly 2- 3 times with running tap water and then with sterile water. The leaves of the plants were air dried and powdered and macerated with organic solvents for 3-7 days at room temperature. For extraction, 50g of powdered plant material was dissolved in water and in organic solvent viz. acetone, hexane, methanol and chloroform (Sigma Aldrich ltd.) to make 10-18 ml of each extract. The solution was kept undisturbed for 3-7 days to avoid external contact and then subjected to filtration through sterilized Whatman's filter paper. After filtration, the acetone, methanol, hexane and chloroform extracts thus obtained were immediately evaluated for their antibacterial and antifungal activity using modified agar well diffusion method.

### 2.3 Antimicrobial assay

#### 2.3.1 Agar well diffusion assay:

The antimicrobial activity of 4 crude extracts(hexane, acetone, chloroform and methanol) of the plant parts against all bacterial and fungal strain were evaluated by using well diffusion method. For bacteria nutrient agar (Hi-media, Mumbai) growth medium and for fungal activity potato dextrose media and czapek media (Hi-media, Mumbai) plates were prepared. The plates were poured with 100µl of standardized inoculums (1.5x10<sup>8</sup> CFU/ml) of each microorganism and spread with sterile swabs. Wells were made into plates containing the microbial inoculums. 100µl volume of the plant extract was poured into the well of inoculated plates. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the medium. Then the plates were kept for incubation for 24 hrs at 37°C.

After the incubation, the plates were observed for zone of inhibition surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimetres.

#### 2.3.2 Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was calculated according to the standard broth micro dilution method<sup>13</sup>. The fractions and compound were dissolved in water and 2% dimethyl sulfoxide (DMSO). The initial concentrations of fractions were 1 mg/ml and for compound it was 0.5 mg/mL. The initial test concentration was serially diluted two-fold (96-well plate). Each well was inoculated with 5 mL of suspension containing 10<sup>8</sup> CFU/mL of bacteria. Antibacterial agent Streptomycin and antifungal agent nystatin were included in the assays as positive control. The plates were incubated 24hr. at 37°C.

### 2.4 Phytochemical screening

The freshly collected extract fractions of plants *Amaranthus spinosus*, *Solanum nigrum*, *Orchis muscula* were tested for the presence of phytochemical constituents. These were identified by characteristic colour changes using standard procedures<sup>14</sup>.

### 2.4.1 Test for tannins

To 2 ml of each extract, a few drops of 10% lead acetate were added. The appearances of white precipitate indicate the presence of tannins.

### 2.4.2 Test for saponins (Frothing Test)

To 1 ml of each extract taken in a measuring jar, 9 ml of distilled water was added and shaken vigorously for 15 s and extracts were allowed to stand for 10 min. Formation of stable foam (1 cm) indicates the presence of saponins.

### 2.4.3 Test for steroids

10 ml chloroform was added to 2 ml of all the extracts. To these extracts, 1 ml of acetic anhydride was added, followed by 2 ml of concentrated sulphuric acid along the sides of the test tube. The appearance of blue-green colour at the junction indicates the presence of steroids.

### 2.4.4 Test for flavanoids

5 ml of dilute ammonia was added to a portion of an aqueous filtrate of the extract. 1ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added. Yellow colourations that disappear on standing indicate the presence of flavanoids.

### 2.4.5 Test for terpenoids:

The test for terpenoids was same as that for steroids. The appearance of red, pink or violet color at the junction is an indication for the presence of terpenoids.

## 3. Results

The antimicrobial activity of *A. spinosus*, *Solanum nigrum* and *Orchis muscula* leaves extracts against pathogenic microorganisms is depicted in Table I. The extracts showed considerable antimicrobial activity against all the strains tested. The results from MIC indicated that *E. coli* was most sensitive microbe tested; showing the largest inhibition zones (28 mm) for leaves extracts. The leaves extracts were comparable with the standard drugs, streptomycin and nystatin. Out of the three leaf extracts, methanol extract of *Orchis muscula* was found to be more effective on all the microbes tested with zone of inhibition ranging from 11.5 to 17.4 cm. followed by methanol leaves extract of *Amaranthus spinosus* with zone of inhibition ranging from 8.6 to 15.3. The chloroform extracts of all three leaf extracts failed to give any zone of inhibition.

**Table I. Antimicrobial activity of extracts of medicinal plants against bacterial strains.**

Plants	Solvent	<i>B. Amyloliqefaciens</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. mutans</i>	<i>S. aureus</i>	<i>A. Fumigates</i>
		Diameter of zone of inhibition in millimetres					
<i>A. spinosus</i>	Acetone	7.4 ±0.05	9.1±0.02	12.5±0.13	13.5±0.03	12.5±0.02	10.4±0.1
	Hexane	9.4±0.1	12±0.09	–	–	–	7.2±0.11
	Chloroform	–	–	–	–	–	–
	Methanol	8.6±0.08	15.3±0.1	11±0.11	14.1±0.12	13.1±0.05	12.4±0.07
<i>S. nigrum</i>	Acetone	7.9±0.04	–	14.1±0.07	10.5±0.12	14±0.12	11.1±0.07
	Hexane	10.2±0.02	13.2±0.09	–	–	10±0.11	10.3±0.02
	Chloroform	–	–	–	–	–	–
	Methanol	11±0.02	–	12.1±0.05	14.9±0.08	11.5±0.02	13.1±0.02
<i>O. muscular</i>	Acetone	10.5±0.05	10.1±0.11	13.3±0.2	14.5±0.11	14.2±0.05	11.4±0.2
	Hexane	7.9±0.05	12.5±0.11	10.1±0.19	11.1±0.2	9.6±0.05	10.3±0.19
	Chloroform	–	–	–	–	–	–
	Methanol	11.5±0.02	13.2±0.1	12.5±0.11	17.4±0.02	14.5±0.03	12.2±0.11

Values are mean ± SD of three samples analyzed individually in triplicate. Diameter of inhibition zone (mm) including disc diameter of 6 mm and MIC in µg/ml. Controls used are streptomycin and nystatin for bacterial and fungal strains, respectively.

All the plant extracts were evaluated for the presence of phytochemicals and the results are tabulated in Table II. The methanol extracts of all plant extracts showed the presence of higher tannins and flavonoid concentration. Only small concentration of terpenoids were present in chloroform extract of *Orchis muscula* while chloroform extract of *Amaranthus spinosus* and *Solanum nigrum* failed to show presence of any phytochemical. The phytochemical content in methanol, hexane and acetone extracts of *Orchismuscula* were found to be highest.

**Table II Phytochemical screening test results of *A. spinosus*, *S.nigrum*, *O. muscula***

Phytochemical constituents	Acetone			Hexane			Methanol			Chloroform		
	<i>A. spinosus</i>	<i>S. nigrum</i>	<i>O. muscula</i>	<i>A. spinosus</i>	<i>S. nigrum</i>	<i>O. muscula</i>	<i>A. spinosus</i>	<i>S. nigrum</i>	<i>O. muscula</i>	<i>A. spinosus</i>	<i>S. nigrum</i>	<i>O. muscula</i>
Tannins	+	-	+	-	-	+	+	+	+	-	-	-
Saponins	-	-	+	+	-	+	-	-	-	-	-	-
Steroids	+	-	-	+	-	+	-	+	-	-	-	-
Terpenoids	+	-	+	-	-	+	+	-	+	-	-	+
Flavanioids	+	-	-	-	-	-	+	+	+	-	-	-

+ Represents presence of the phytoconstituent; - represents absence of the phytoconstituent.

#### 4. Conclusion and Discussion

Antibiotics resistant is increasing day by day among micro organisms due to the spread of resistant genes via plasmid throughout other species, eventually limiting the efficacy of various drugs. The priority for the next generation or decades must be focused in the development of alternative drugs and the recovery of molecules that would allow the consistent and proper control of micro organisms which cause diseases. Although function of many phytochemicals classified as secondary metabolites is unknown, but certain phytochemicals have structural, functional and general defence against plant pathogens. The results of our investigation confirmed the rationale for the medicinal use of the studied plants. This study demonstrated that methanol, acetone and hexane extracts of *A. spinosus*, *S. nigrum* and *O. muscula* plants have different degree of antimicrobial activity. The remarkable aspect of the results was that all the extracts inhibited the growth of both gram positive and gram negative bacteria as well as fungus at their concentrations tested. Among all extracts, the methanol extract of *Orchis muscula* showed highest activity against gram positive *S. aureus* and gram negative *E. coli*. Therefore we would like to state that constituents of extract of *A. spinosus*, *S. Nigrum* and *O. muscula* may serve as a potential source of industrial drugs useful in chemotherapy of some bacterial and fungal infections.

From our investigation, the results obtained confirm the therapeutic potency of *Amaranthus spinosus*, *Solanum nigrum* and *Orchis muscula* used in traditional medicine. In addition, these results form a good basis for selection of the plant for further phytochemical and pharmacological investigation. The results of the present study supports the folkloric usage of the studied plant and suggests that the plant extract possess certain constituents with antibacterial and antifungal properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

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