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ANTIBACTERIAL ACTIVITY OF HERBAL PLANTS AGAINST STAPHYLOCOCCUS AUREUS

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ABSTRACT

The in vitro antibacterial activity of various solvents and water extracts was assessed on *Staphylococcus aureus*. The zone of inhibition as determined by agar well diffusion method varied with the plant extract, the solvent used for extraction, and the organism tested. The results obtained in the agar diffusion plates were in fair correlation with that obtained in the minimum inhibitory concentration tests. The minimum inhibitory concentration of rose extracts was found in the range of 1.56-6.25 mg/ml for the multi-drug resistant Staphylococcus aureus isolates tested whereas higher values (6.25-25 mg/ml) were obtained against the multi-drug resistant isolates. Qualitative phytochemical analysis demonstrated the presence of tannins and saponins in all plants tested.

KEYWORDS: Agar well diffusion, S. aureus, Multi-drug resistant, Phytochemical analysis.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugsA wide range of medicinal plant parts is used for extract as raw drugs and they posses varied medicinal properties Medicinal plants are believed to be important source of new chemical substance s with potential therapeutic effects The secondary metabolites of plants were found to be source of various phytochemicals that could be directly used as intermediates for the production of new drugs. Traditional medicine should be able to play an even greater role in the modern primary healthcare system of the developing countries. The natural medicines are believed to be more acceptable to the human body, when compare to modern synthetic drugs. Thus the most important factor needed is to derive the maximum benefit from the traditional system of medicine for providing adequate healthcare service to rural people. Nature has long

been an important source of medicinal agents. An impressive number of modern drugs have been isolated or derived from natural source, based on their use in traditional medicine. The plants have been used traditionally for centuries and modern scientific studies have shown the existence of good correlation between the traditional or folkloric application of some of the plants further strengthens the search for pharmacological active components from plants leaves have been used as herbal medicine for their healing properties since ancient times. Some bioactive compounds within these plants are responsible for their medicinal value. The most prominent of these bioactive compounds are alkaloids, tannin, flavonoid and phenolic compounds. Their concentrations may vary in different plants which result in unique medicinal properties for a specific plant Leaves and bark of the guava plant are well recognized for the treatment of diarrhoea, gastrointestinal disorders, toothaches, colds, and swelling. Tea consumption (especially green tea) is considered to provide protection against lung, oesophagus, pancreas, liver, breast, colon, and skin cancers induced by chemical carcinogens. Neem leaves are capable of preventing hepatitis and controlling diabetes, and marigold leaf is known to be highly effective in healing of burns and bruises. During the last few decades, the global interest in the study of various medicinal plants has increased rapidly due to their antibacterial and antioxidant activities, low to costly synthetic drugs toxicity and the potential to be a cheaper alternative. The determination of antibacterial activities of different medicinal plants is of special interest these days due to the current global issue of increasing antibiotic resistance of microorganisms. It is assumed that the drug resistance in pathogenic microorganisms is developing due to indiscriminate use of commercial antimicrobial drugs. Antimicrobial resistance threatens the prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. Therefore, it is highly imperative to determine compounds which can be used to develop novel medicines with higher antimicrobial properties. The development of antimicrobial agents has been undeniably one of the greatest accomplishments of modern medicine. In recent years, multiple drug resistance in both human and plant pathogens has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The limited life span of antibiotics, has rendered a necessity to search for new antimicrobial substances from various sources such as medicinal plants. Plants used in traditional medicine are one of the most promising areas in the search for new biologically active compounds. Medicinal plants are well-known natural sources for the treatment of various diseases since antiquity. Furthermore, natural products, either pure compounds, or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the

unmatched availability of chemical diversity. Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential. Human infections particularly those involving microorganisms i.e. bacteria, fungi, viruses, they cause serious infections in tropical and subtropical countries of the world. In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. Over the past twenty years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Different extracts from traditional medicinal plants have been tested. Many reports have show the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks for modern medicine to attain new principles The increasing interest on traditional ethno medicine may lead to discovery of novel therapeutic agents. Medicinal plants are finding their way into pharmaceuticals, neutralceuticals.

METHODOLOGY

Collection of sample

S. no.	Name of plants	Scientific name
1	Neem	*Azadirachta indica* Family : Meliacea
2	Turmeric	*Curcuma longa* Family: Zingiberaceae
3	Rose	*Rose indic* Family : Rosacease
4	Bamboos	*Bambusoideae* Family : Grasses

Sample preparation

The samples were collected and then washed with double distilled water. Further, the samples were allowed for drying. The dried samples were grained and converted into powder**.**

Extraction of bioactive compounds

- The samples were dipped into polar and non-polar solvents in a 1:10 ratio, where 1 part belongs to the sample weight.
- The dipped samples were incubated for 48 hours in dark. Further, the samples were filtered and the solvents were evaporated at 50℃.
- Further, these samples were dissolved in DMSO

Antibacterial sensitivity test (AST)

- Prepare 45ml Nutrient agar media (NAM) and 3 petri plates.
- Autoclave (121℃ for 15min 15 psi) Pour media to plates
- Spread 20µl *Staphylococcus aureus* and prepare wail and load 45µl extract.
- Overnight in incubator and after that calculate Zone of inhibition.

Phyto-chemical test

T1 Alkaloid: Mayer's test

1ml of sample (acetone ext, methanol Ext, ethanol ext) +1 ml ammonia solution leave for 1-2min +3ml chloroform shake for 1-2min for proper mixing. Evaporate chloroform using a water bath. Add 1ml of Mayer's reagent appearing of a cream colour precipitate.

T2 Flavonoid: Alkaline reagent test

1ml of sample +1 ml NaOH become yellow colour. Few drops of dil HCL become colorless.

T3 phenol: Ferric chloride test

1ml of sample +1ml 10% ferric chloride appearance of a blue-green colour.

T4 Carbohydrate: Fehling reagent test

1ml FR 'A' +1 ml FR 'B' + 1ml sample place all the test tubes in a water bath for 5-10min creaming colour precipitate**.**

T5 Amino Acid: Ninhydrin test

1ml of sample +1ml 0.25%w/v Ninhydrin reagent. Place all test tubes in a water bath for 10min, blue/purple colours.

Ast of different concentration of plant extracts in combinations

- Prepare 60 ml Nutrient agar media (NAM)and 4 petri plates
- Autoclave (121℃ for 15min 15 psi)
- Pour media to plates

- Spread 20µl *Staphylococcus aureus* and prepare wail and after that load 45µl extract
- Overnight in incubator and after that calculate zone of inhibition of different plant extract.

RESULTS AND DISCUSSIONS

Collection of sample & sample preparation

The samples were collected from local area of Lucknow. Then washed thoroughly with distilled and allowed for sun dry. Further the samples were converted into powder by grinding in mixer.

Figure 1: Collection of sample & sample preparation.

Extraction of bioactive compounds

After this the samples were dipped into polar and non-polar solvents and incubated for 48 hours. When the solvents evaporate the remaining residue were collected into the micro-centrifuge tubes.

Filter leaf extract Collect filtrate, Dry leaf extract, Collect extract in eppendrof

Figure 2: Extraction of bioactive compounds.

Antibacterial sensitivity test of plant extract

After performing the antibacterial testing it was found that the best results were shown by each and every extracts while they are showing the clear zone (Zone of inhibition) as shown in below figure and table.

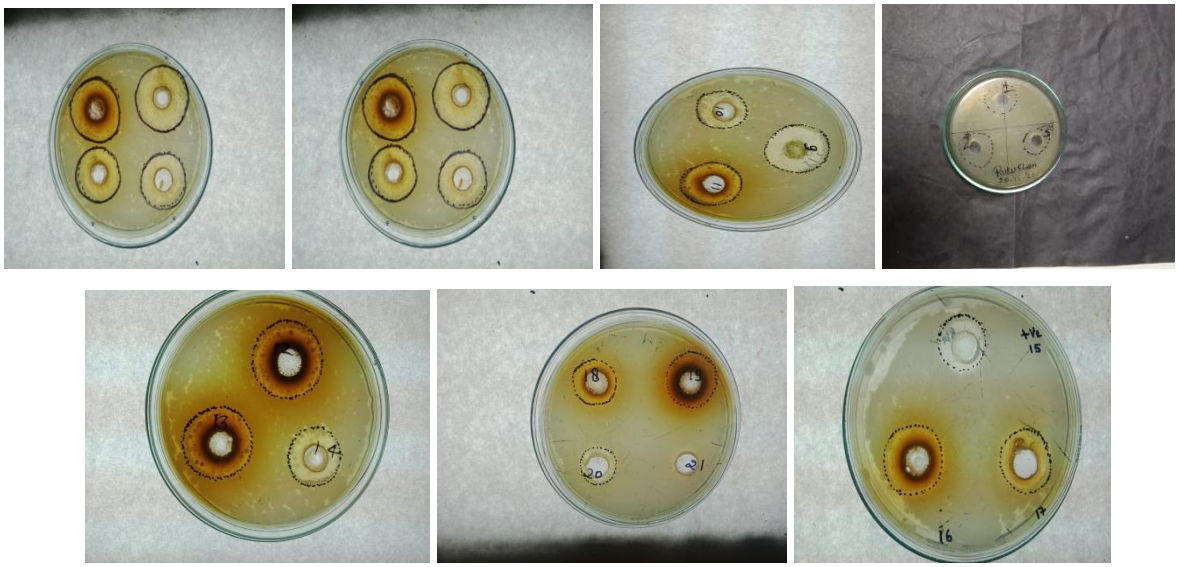


Figure 3: Antibacterial sensitivity test (AST) 11 +ve control & 12 –ve control.

Table 1: Antibacterial sensitivity test (AST).

S. no.	Sample no.	Name of Solvent and Plant extract
1	Sample no. 1	Rose + Acetone
2	Sample no. 2	Rose + Aq
3	Sample no. 3	Acetone + Neem
4	Sample no. 4	Neem + Aq.
5	Sample no. 5	Rose + Methanol
6	Sample no. 6	Turmeric +Aq.
7	Sample no. 7	Bamboos + Aq.
8	Sample no. 8	Acetone + Bamboos
9	Sample no. 9	Benzene + Turmeric
10	Sample no. 10	Methanol + Turmeric
11	Sample no. 11	Pt. ether + Turmeric
12	Sample no. 12	Acetone + Turmeric

Zone of inhibition of plant extract with bacteria

Table 2: Zone of inhibition of plant extract with bacteria.

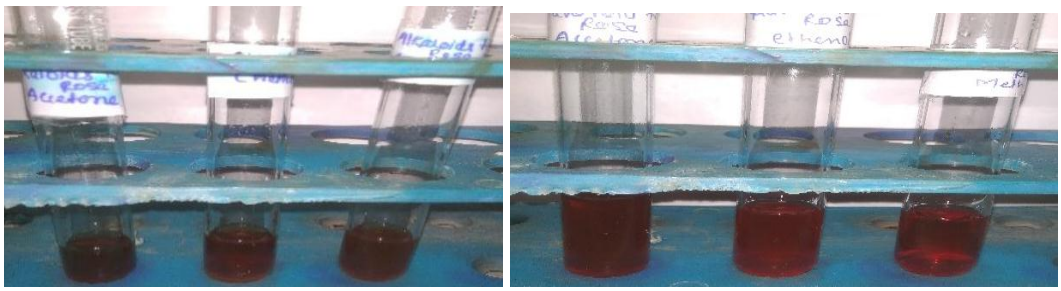
No. of sample	Zone of Inhibition	Name of Bacteria
Sample no. 1	67mm	*Staphylococcus aureus*
Sample no. 2	18mm	*Staphylococcus aureus*
Sample no. 3	5 mm	*Staphylococcus aureus*
Sample no. 4	13 mm	*Staphylococcus aureus*
Sample no. 5	24 mm	*Staphylococcus aureus*
Sample no. 6	50 mm	*Staphylococcus aureus*
Sample no. 7	21 mm	*Staphylococcus aureus*
Sample no. 8	26 mm	*Staphylococcus aureus*
Sample no. 9	24 mm	*Staphylococcus aureus*
Sample no. 10	11 mm	*Staphylococcus aureus*
Sample no. 11	6 mm	*Staphylococcus aureus*
Sample no. 12	5 mm	*Staphylococcus aureus*

Phytochemical test result

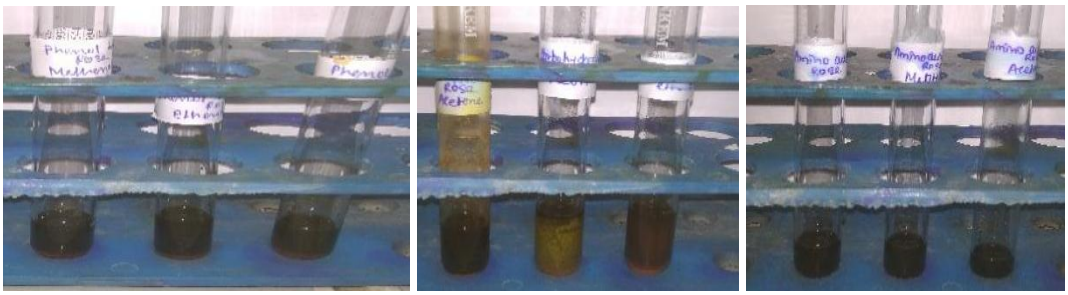
As the medicinal properties of the sample were shown the various phytocompounds, hence for the analysis of the phytochemicals the following tests were performed and the results were shown in below figures and tables.

Rose

T1 Alkaloid: Mayer's test T2 Flavonoid: Alkaline Reagent test



T3 phenol: Ferric Chloride test, T4 Carbohydrate: Fehling test, T5 Amino Acid: Ninhydrin

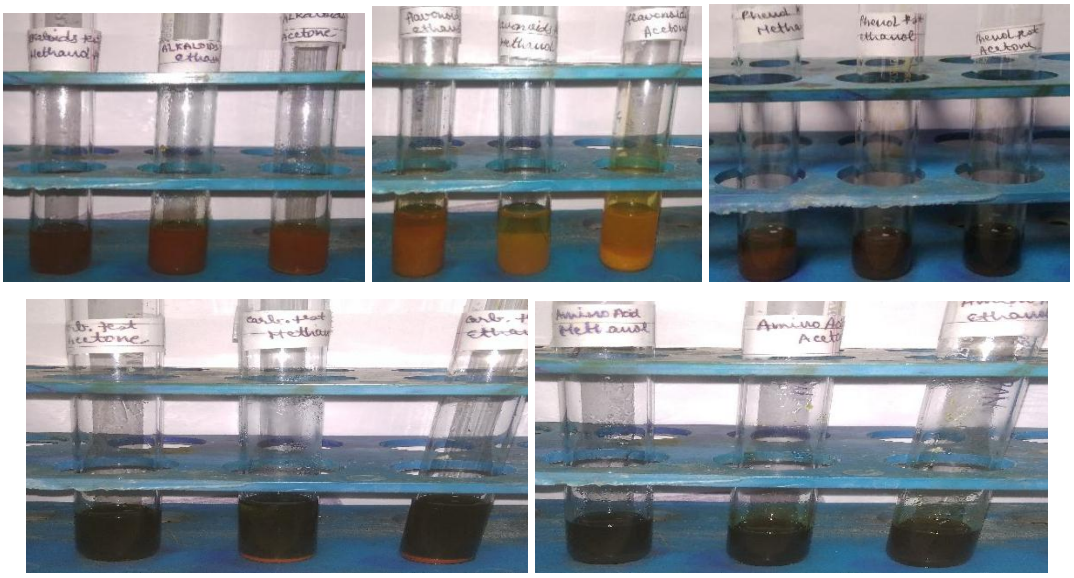


Plant name	Compounds	Methanol solvent	Ethanol solvent	Acetone solvents	Aquas
Rose extract	Alkaloid test	+ve	+ve	+ve	-ve
	Flavonoids test	+ve	+ve	+ve	-ve
	Phenol test	+ve	+ve	+ve	+ve
	Carbohydrate test	+ve	+ve	+ve	+ve
	Amino acid test	+ve	+ve	+ve	+ve

Figure 4: Phytochemical test result of rose +ve (Present test), -ve (Absent test).

Turmeric:

T1 Alkaloid: Mayer's test T2 Flavonoid: Alkaline Reagent test T3 phenol: F.A test

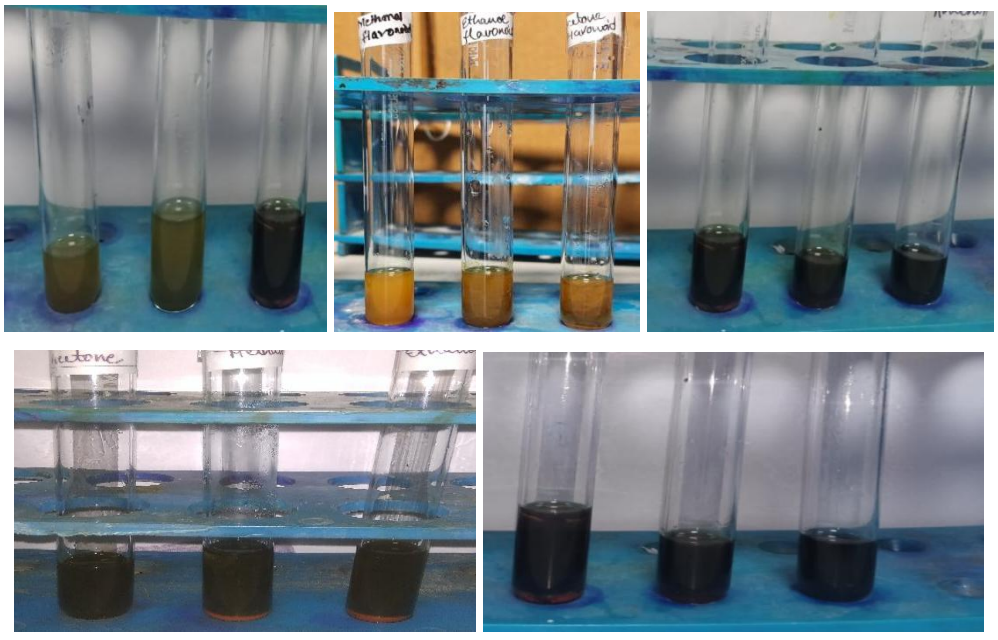


Plant name	Compounds	Methanol solvent	Ethanol solvent	Acetone solvents	Aquas
Turmeric extract	Alkaloid test	+ve	+ve	+ve	+ve
	Flavonoids test	+ve	+ve	+ve	-ve
	Phenol test	+ve	+ve	+ve	+ve
	Carbohydrate test	+ve	+ve	+ve	+ve
	Amino acid test	+ve	+ve	+ve	-ve

Figure 5: Phytochemical test result of turmeric +ve (Present test), -ve (Absent test).

Neem

T1 Alkaloid: Mayer's test T2 Flavonoid: Alkaline Reagent test T3 phenol test F.A test



T4 Carbohydrate: Fehling test T5 Amino Acid: Ninhydrin test

Plant name	Compounds	Methanol solvent	Ethanol solvent	Acetone solvents	Aquas
Neem extract	Alkaloid test	+ve	+ve	+ve	+ve
	Flavonoids test	+ve	+ve	+ve	+ve
	Phenol test	+ve	+ve	+ve	+ve
	Carbohydrate test	+ve	+ve	+ve	+ve
	Amino acid test	+ve	+ve	+ve	+ve

Figure 7: Phytochemical test result of neem +ve (Present test), -ve (Absent test).

AST of Different concentration of plant extracts in combination

S.no	Rose+ methanol	Neem +methanol	Turmeric+Aq.	Zone of inhibition
1	100µl	100 µl	100 µl	26mm
2	100µl	150 µl	50 µl	18 mm
3	150µl	50 µl	100 µl	26 mm
4	50 µl	100 µl	150 µl	20 mm
5	50 µl	50 µl	100 µl	19 mm
6	100 µl	50 µl	50 µl	17 mm
7	50 µl	100 µl	50 µl	27 mm
8	100 µl	0	100 µl	26 mm
9	+ve control	+ve control	+ve control	26 mm
10	0	100 µl	100 µl	96 mm
11	100 µl	100 µl	0	22 mm
12	150 µl	100 µl	0	32 mm
13	100 µl	0	150 µl	27 mm
14	0	150 µl	100 µl	13 mm
15	+ve control	+ve control	+ve control	12 mm
16	50 µl	50 µl	150 µl	18 mm
17	150 µl	50 µl	50 µl	12 mm
18	50 µl	150 µl	50 µl	13 mm
19	150 µl	0	0	18 mm
20	0	150 µl	0	3 mm
21	0	0	150 µl	1 mm

Figure 4: AST of Different concentration of plant extracts in combination.

CONCLUSION

The research work conclude that the herbal plants is responsible for various medicinal properties against the pathogenic micro-organisms. The herbal extracts extracted from the selected plants shows effective results against *S. aureus.* Further the phytochemical analysis was carried out and extract showed +ve for (alkaloid, flavonoid carbohydrate, phenol, amino acid) and -ve result waere shown by in the aquas solution.

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