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Phytochemical Analysis and Antimicrobial Activity of Various Indigenous Plant Species

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Abstract: ***Plants in the nature constitute various unidentified and excellent properties that can be used for various different purposes including creation of new drugs and therapies. The methanol extract of six medicinal plants i.e., Azadirachta indica (Neem) Calotropis procera (Aakh), Saraca asoca (Ashok), Ocimum tenuiflorum (Tulsi), Asparagus racemosus (Shatavari) and Withania somnifera (Ashwagandha) showed significant antibacterial activity against Escherichia coli, and antifungal activity against Aspergillus niger. Ocimum tenuiflorum(Tulsi) and Asparagus racemosus(Shatavari) leaf extract showed highest antimicrobial activity against E.coli and A.niger. The invitro antimicrobial activity was performed by agar disc diffusion method. The use of plant extracts with known antimicrobial properties, can be of great significance in therapeutic treatments. The study also prove that several plant extracts can be useful in preservation of food articles and constitution of several food preservatives. The presence of phytochemicals is medicinally important for formulation of many therapeutic drugs.***

Keywords: Antimicrobial Activity, Phytochemicals, Azadirachta indica, Calotropis procera, Saraca asoca, Ocimum tenuiflorum, Asparagus racemosus, Withania somnifera.

I. INTRODUCTION

India is an ancient land with vast biodiversity laid around. There are plants in the nature full of unexplored potential. There are many active compounds that are produced by the plants yet not much use by them until they are exposed to harsh condition differing their natural habitat. Phytochemicals being one of them is a secondary metabolite produced by plant species that is chemically active and play a vital role in development of various drugs in pharmaceutical industry. There are certain evidences of medicinal plant being a great source to obtain variety of drugs.

According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency [1].

In Indian system a large number of medicinal plants have been used for many centuries for treating various diseases. Medicinal plants have been as remedies for human diseases because of its chemical contents of therapeutic value. Most traditional medicines are developed from nature.

Thus, plants remain a major source of medicinal compounds. As of record around 20,000 plant species are in use for medicinal purposes across the globe and around 70 % of them are from Indian subcontinent [2]. Antibiotics or antimicrobial substances like saponins, glycosides, flavonoids and alkaloids etc are found to be distributed in plants, yet these compounds were not well established due to the lack of knowledge and techniques [3].

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds [4].

There are multiple proofs that several herbs that are found in nature can exhibit high antimicrobial activity. As per previous study done by Manish Dubey, Carum *carvi* (Common name: - Cumin) spice and herb extracts exhibited high antimicrobial activity against the bacteria, *E. coli* and the fungus, *Aspergillus niger*.[5]

Naturally occurring substances are of plants, animals and mineral origin. They are organic substances and could be obtained in both primary and secondary metabolic process; they also provide a source of medicine since the earliest time. The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facet of life have served a valuable starting material for drug development.[6]

The study here includes, some indigenous plant species found in Northern Indian Plains. We have focused to identify the Phytochemicals present in different species as well as analyse the antimicrobial activity of the Plant extracts (Methanol Extracts) on *E.Coli* and *A.Niger*. This work is done with some main objectives as below: -

1) To identify the Phytoconstituents of several Plant species including phytochemicals like terpenoids, flavonoids, Phlobatannins & Resins.
2) To identify the antimicrobial activity of all plant extracts taken, against two microbial strains namely *E.Coli* and *A.Niger*.
3) To compare the Antimicrobial activity of various extracts

II. MATERIALS AND METHODOLOGY

A. Place of Work

The experiments pertaining to the study were carried out in the Department of Biotechnology, R.B.S. Engineering Technical Campus, Agra, Uttar Pradesh, India.

B. Collection of Plants

For doing the same we collected the Plant materials from Agra region that are commonly available around. Plant Samples are collected in a Sunny day precisely between 10-11 A.M. The leaf of plant is taken with utmost care so that the active components does not get damaged or contaminated. The plant sample is collected and then washing is done gently under running water before letting it dry under shade.

Figure 1: - Some dried plant leaves dried and stored under shade.

C. Methanol Extract Preparation

The dried leaves are crushed into fine powder using the Grinding mixer so that we can obtain a fine powdered sample. The powder is then weighed using a weighing machine. Methanol is used as a solvent and ratio of 1:10 for sample and solvent is prepared. The sample is then transferred to a Conical flask and kept under shaking condition for 24 hours. After 24 hours the sample is collected and centrifuged for around 10 minutes on 10,000 rpm. The obtained Plant Extracts will be filtered up using a filter paper in an air tight container/Flask and kept preserved in Refrigerator for further use. We must keep in mind to bring back the temperature to normal before using the extracts for experimental purpose.

Figure 2: - Extracts of Plant leaves recovered.

D. Test for Phytochemical identification

We have tested upon the presence and absence of various phytochemicals with the help of previously stored leaf extracts. The absence and presence of phytochemicals like Flavonoid, Terpenoid, Resin & Phlobatannin are tested upon by the methods as below:-

1) *Test for Terpenoids:* An amount of 0.8 g of selected plant sample is taken in a test tube, then pour 10 ml of methanol in it, shaken well and filtered to take 5 ml extract of plant sample. Then 2 ml of chloroform is mixed in extract of selected plant sample and 3 ml of sulphuric acid is added in selected sample extract. Formation of reddish-brown color indicates the presence of terpenoids in the selected plants.
2) *Test for flavonoids:* For the confirmation of flavonoid in the selected plants, 0.5 g of each selected plant extract is added in a test tube and 10 ml of distill water, 5 ml of dilute ammonia solution is added to a portion of the aqueous filtrate of each plant extract followed by addition of 1 ml concentrated H2S04. Indication of yellow color indicated the presence of flavonoid in each extract.
3) *Test for Phlobatannins:* Plant powder sample is mixed with distill water in a test tube, then shaken well, and filtered to take plant extract. Then to each plant extract, 1% aqueous hydrochloric acid is added and each plant sample is then boiled with the help of Hot plate stirrer. Formation of red colored precipitate indicated the positive result.
4) *Test for Resins:* Few mg of extract is treated with caustic soda a red color is developed if resins are present.

E. Method for Antimicrobial activity test of various Plant Species:

The Antimicrobial activity is measured on a PDA- Potato Dextrose Agar media to analyze the growth of Fungi and Bacteria in the presence of Plant extracts. PDA media is prepared using standard laboratory protocol and then using the Agar disk diffusion method the MIC- Minimum inhibitory concentration of the plant extract is measured. The MIC plates are later incubated at 37oC in an incubator for 24-48 hours after proper disk placement and inoculation of strains. Later on, the Zone of inhibition is measured with the help of measuring scale preferably in millimeters and the result is noted down for the different extracts.

III. RESULTS AND DISCUSSION

A. Phytochemical Analysis

We have tested upon the presence and absence of various phytochemicals with the help of previously stored leaf extracts. The absence and presence of phytochemicals like Flavonoid, Terpenoid, Resin & Phlobatannin are tested and results are as below

S.No.	Sample	Flavonoid	Terpenoid	Resin	Phlobatannin
1.	Neem Leaf Extract	Present	Absent	Present	Present
2.	Aakh Leaf Extract	Present	Present	Absent	Present
3.	Ashok Leaf Extract	Present	Present	Absent	Present
4.	Tulsi Leaf Extract	Present	Absent	Absent	Present
5.	Shatavari Leaf Extract	Present	Present	Present	Present
6.	Ashwagandha Leaf Extract	Absent	Absent	Present	Present

Table 2- Qualitative Phytochemicals test results of various leaf extracts

B. Antimicrobial Activity

The antimicrobial activity was tested against two different strains namely *Escherichia coli* (Bacterium) & *Aspergillus niger* (Fungus) and showed very promising results. The MIC (Minimum inhibitory concentration) of various Leaf samples were measured in the Lab.

1) Aspergillus Niger

Samples	Concentration (mg/ml)			
	0.25	0.50	0.75	1
	Zone of Inhibition(mm)			
Neem Leaf Extract	0.2 mm	0.3 mm	0.5 mm	0.7 mm
Aakh Leaf Extract	0.00 mm	0.5 mm	0.9 mm	1.5 mm
Ashok Leaf Extract	0.1 mm	0.5 mm	0.7 mm	1.2 mm
Tulsi Leaf Extract	0.4 mm	1.0 mm	1.0 mm	1.3 mm
Shatawari Leaf Extract	0.2 mm	0.3 mm	0.5 mm	1.2 mm
Ashwagandha Leaf Extract	0.0 mm	0.0 mm	0.0 mm	0.2 mm

Table 3 – Zones of inhibition shown by different leaf extracts against different concentration under *A. Niger* Strains

2) Escherichia Coli

Samples	Concentration (mg/ml)			
	0.25	0.50	0.75	1
	Zone of Inhibition(mm)			
Neem Leaf Extract	0 mm	0 mm	0 mm	0.1mm
Aakh Leaf Extract	0.4 mm	0.8 mm	0.9 mm	1.2 mm
Ashok Leaf Extract	0.5 mm	0.6mm	0.8mm	1.2 mm
Tulsi Leaf Extract	0.1 mm	0.2 mm	1.2 mm	1.4 mm
Shatawari Leaf Extract	0.4 mm	0.5 mm	0.8 mm	1.2 mm
Ashwagandha Leaf Extract	0.1 mm	0.1 mm	0.3 mm	0.5 mm

Table 3 – Zones of inhibition shown by different leaf extracts against different concentration under *E. Coli* Strains

IV. CONCLUSION

From the above stdy we can conclude that the plants are a rich source of metabolites and rich extracts that are capable of fighting with various diseases that are present in the nature. The antimicrobial activity clearly indicates that the bacterial and fungal resistance of the plant samples are good enough and it can be helpful in the pharmaceutical formulation of drugs prior testing on trials effectively. The phytochemicals are of great importance to the Pharma establishment and if extracted can be commercially sold at a good rate and an effective pricing. The phytochemical screening for leaves, stem and roots show the presence of active component like saponins, tannins, flavonoids, terpenoids, glycosides and reducing sugars from aqueous and methanol extracts of utmost Pharmaceutical importance. It will provide experimental evidence that the extract preparation of leaves and stem of various indigenous species that are used as a traditional remedy or even not used traditionally, possesses antimicrobial property. It can be further used for extracting secondary metabolites that can serve as a therapeutic agent.

The above study can be extended by extracting the several phytochemicals that serve as active compounds possessing antimicrobial properties to formulate several drugs that might be effective, non- harmful and at the same time

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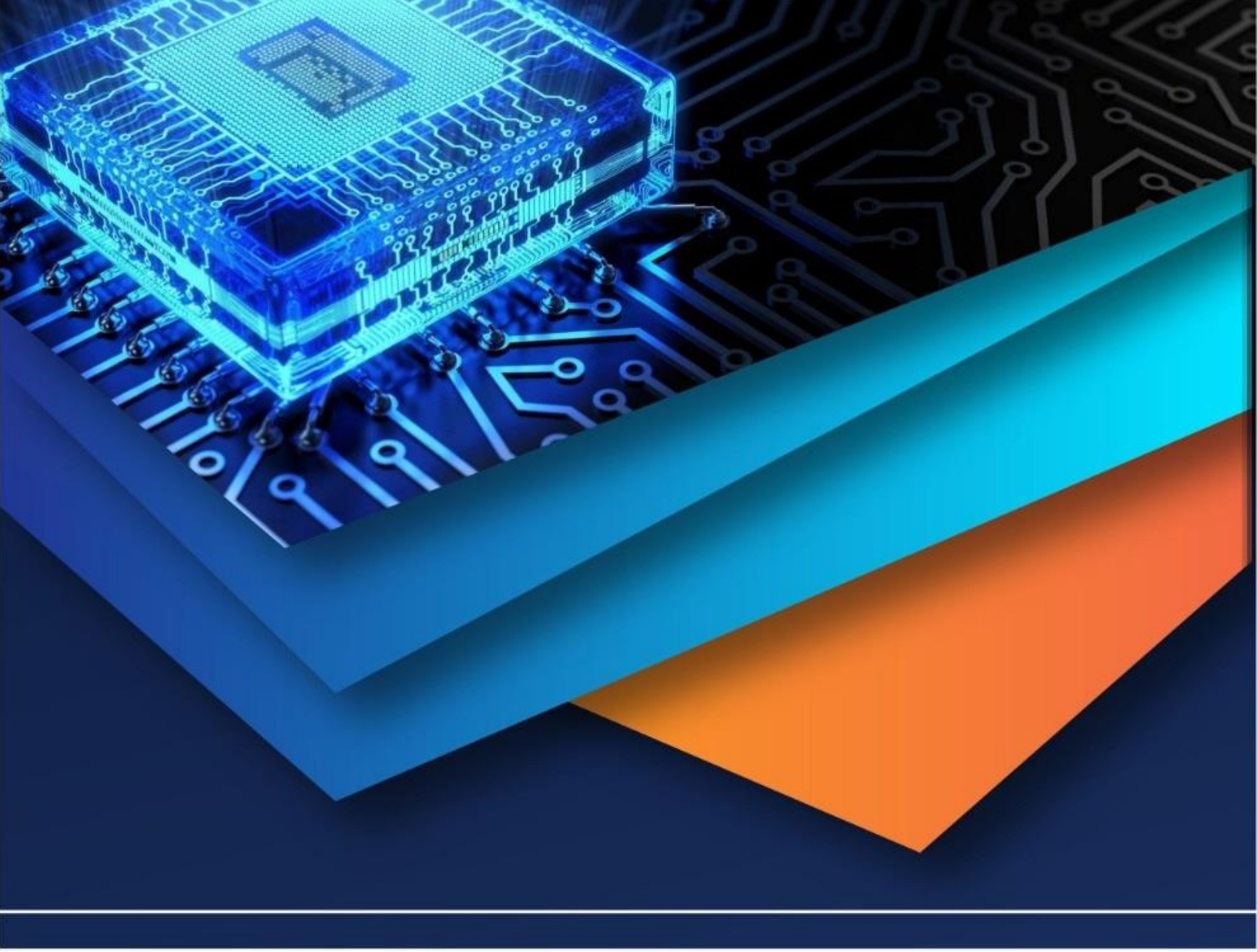

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