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Phytochemical extraction and antimicrobial activity of some medicinal plants on different microbial strains

Rekha Jadon [1*], Shruti Dixit [2]

1. Ethnopharmacology Student, FET, RBS College, Agra, Uttar Pradesh, India.
   [Email: rekhajadon328@gmail.com]
2. Student, FET, RBS College, Agra, Uttar Pradesh, India.
   [Email: shrutidixit003@gmail.com]

The following study was conducted to investigate the Phytochemical extraction and antimicrobial activity of some medicinal plants on different microbial strains. Juicy extracts of different medicinal plants including, *Zingiber officinale* (Ginger), *Allium sativum* (Garlic), *Allium cepa* (Onion), *Emblica officinalis*(Amla) and *Benincasa hispida* (Petha) were tested using the agar-well diffusion method for their antimicrobial activity against the common bacterial pathogens *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* and fungal pathogens *Aspergillus niger* and *Penicillium chrysogenum.* The susceptibility of the microorganisms to the extracts of these plants was compared with each other and with selected antibiotics by measuring the diameter of the inhibition zones. The antimicrobial activities of these plants were discussed according to their phytochemical components. Among five plants tested *Allium sativum* (Garlic) was found to be the most effective against all pathogens at 100 ul/well. *Emblica officinalis* (Amla) extract exhibited activity against *E. coli*, *B. subtilis* and *S. aureus* while the remaining medicinal plant extracts showed less or no activity. A qualitative phytochemical analysis was performed for the detection of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars. The present study will be successful in identifying a candidate plant with different antimicrobial activity which could be further exploited for isolation and characterization of the novel phytochemicals in the treatment of infectious disease, especially in light of the emergence of drug-resistant microorganisms and the need to produce more effective antimicrobial agents.

Keyword: Antimicrobial activity, zone of inhibition, phytochemicals, susceptibility, drug resistance.

1. Introduction

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is found in “Rigveda”, which is said to have been written between 4500 -1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medical science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of the science of life and the art of healing [2]. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country.

Medicinal plants have played important role in the traditional and orthodox system of medicine in the curing of different types of diseases. Analysis of different species of medicinal plants for biological active components known to have pharmacological properties have been conducted and most of the studied plants have shown antimicrobial property [3, 4].

As a result of resistance development by pathogenic organisms against antibiotics, side effects and consumer demand, scientific interest are directed to extraction, characterization of the potentially active ingredients and subsequent development of drugs, herbal products as supplement [5], topical products and varieties of surfactants for internal use [6, 7]. The medicinal properties of the plants could be credited to the presence of one or more of the active constituents of the plant. It has been reported that the antimicrobial activities of medicinal plants can be due to the presence of phytochemicals such as alkaloids, flavonoids and terpenoids. In recent years in order to discover novel antimicrobial drugs, screening of plants has been accelerated. Therefore the preliminary screening of medicinal plants for antimicrobial activity and for the presence of phytochemicals can establish a flat form for further development on the research of this area.

This study was focused on the analysis of phytochemical compounds and the screening of their biological activity using Ginger *(Zingiber officinale)*, Garlic *(Allium sativum)*, Onion *(Allium cepa)*, Amla *(Emblica officinalis)* and Petha *(Benincasa hispida)*.

2. Materials and methods

2.1 Medicinal plants

Ginger (*Zingiber officinale*), is an important commercial crop belonging to the family Zingiberaceae grown for its aromatic rhizomes which are used both as a spice and medicine. Ginger has been found effective in multiple studies for tearing nausea caused by sea sickness, morning sickness and chemotherapy, though ginger was found superior over a place for post-operative nausea.

Garlic (*Allium sativum*) is a perennial, erect, bulbous plant and belongs to the family Liliaceae. The juice of bulb is used to treat skin diseases, ear ache and cough, treatment of fever, bronchitis, cough, arthritis, digestive complaints, and also this plant have anticancer, antidiabetic and antimicrobial properties.

Onion *(Allium cepa* Linn.*)* is a member of the Liliaceae, which consists of over 250 genera and 3700. Onion is used to decrease cancer tumor initiation, promote healing of stomach ulcers, reduce the cholesterol, blood pressure and symptoms associated with diabetes mellitus, inhibit platelet aggregation (involved in thrombosis) and prevent inflammatory processes associated with asthma.

Amla *(Emblica officinalis)* belonging to the family Euphorbiaceae possesses anti-viral, antibacterial, anti-cancer, anti-allergy and anti-mutagenic properties. Commonly known as amla, it is highly valued in traditional Indian medicine.

Petha *(Benincasa hispida)* belongs to the family of Cucurbitaceae also called as the winter melon or white melon. The juice can also be an effective mouthwash, and gargle, helping to soothe mouth ulcers, gingivitis (bleeding gums) and will protect the teeth and gums from bacteria.

2.2 Stock Culture Preparations and Condition

The stock culture of each bacterial and fungal organism was prepared by taking nutrient agar slants and potato dextrose agar slants. Bacterial strains were incubated for 18-24 hrs and fungal strains were incubated for 48-72 hrs at 37 ºC. All cultures were maintained 4 ºC and transferred every 15 days to maintain viability.

2.3. Sample collection and preparation of Medicinal Plants

All the plants were purchased from the local market of Agra. They were properly cleaned with sterile distilled water and two extracts were prepared. Aqueous extract of respective plant parts was prepared fresh. Washed plant parts were macerated in a sterile, ceramic mortar. The homogenate was then filtered off with a sterile, muslin cloth and used directly for the sensitivity test. The extracts were stored in air tight vials until used for the assay.

Aqueous decoction extract was prepared by boiling 20 g dry powder of plant parts in 100 ml sterile distilled water for 15 minutes. The flasks allowed to cool and then filtered. The filtrate was used as the aqueous decoction. This extract was considered as the 100% concentration of the extract. The concentrations at 1:10 were made by diluting the concentrated extract with appropriate volumes of sterile distilled water.

2.4 Determination of the Antimicrobial Activity

2.4.1. Preparation of the Inocolumn

For the preparation of the inocula, test bacteria and fungi were taken from the stock culture and were grown separately in nutrient broth and potato dextrose broth and incubated at 37 ºC for 24 hours on an orbital shaker at 200 rpm respectively. This culture was used for the antibacterial assays.

2.4.2. Antimicrobial activity testing using Agar well diffusion assay

The selected bacterial cultures (*E. coli, B. subtilis and S. aureus)* and fungal cultures (*A. niger*, *P. chrysogenum)* were swabbed on the surface of sterile nutrient agar and potato dextrose agar plates using a sterile cotton swab. Agar wells were prepared with the help of sterilized cork borer with 10 mm diameter. Using a micropipette, 100 ul of different extracts were added to the wells in the plate and antibiotic was used as a control. The plates were incubated in an upright position at 37 ºC for 24 hours. The diameter of inhibition zones was measured in mm and the results were recorded.

2.5. Identification tests for active compounds

The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids, flavonoids, reducing sugar, saponins and tannins by the following procedure:

2.5.1. Alkaloid

Alkaloid solution produces brown colour precipitate when a few drops of Wagner reagent (dissolve 2 gm of Iodine and 6 gm of Potassium Iodide in 100 ml water) are added.

were then plugged and removed from heat and

2.5.2. Glycoside

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

2.5.3. Terpenoid

About 0.5 gm of each extract in 2 ml of chloroform. Conc. H_2SO_4 carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive result for the presence of terpenoid.

2.5.4. Flavonoid

About 0.2 gm of each plant extract was dissolved in dil. NaOH and HCl was added. A yellow solution that turns colourless indicates the presence flavonoids.

2.5.5. Tannins

To 0.5 ml of extract solution 1 ml of water and 1 - 2 drops of ferric chloride solution were added. The Blue color was observed for gallic tannins and green black for catecholic tannins [8].

2.5.6. Reducing sugar

To 0.5 ml of extract solution, 1 ml of water and 5 - 8 drops of Fehling's solution were added at hot and observed for brick red precipitate.

2.5.7. Saponins

About 0.2 gm extract was shaken with 5 ml of water, then heated to boil. Frothing (appearance of a creamy mass of some bubbles) shows the presence of saponins.

3. Results

In the present study, we identified the antimicrobial activity of five plants such as Ginger (*Zingiber officinale)*, Garlic *(Allium sativum)*, Onion *(Allium cepa)*, Amla *(Emblica officinalis)*, and Petha *(Benincasa hispida)*. The gram positive bacteria chosen for the study was *S.*

aureus and *B. subtilis* and gram negative bacteria were *E. coli*, and the fungal strains *A. niger* and *P. chrysogenum*. Pure juice extracts, decoction extracts and their dilution of 1:10 of these plants were taken and the ZOI values were determined (Table 1). The juicy extract of *Allium sativum* was most effective against all the target pathogens *S. aureus*, *E. coli*, *B. subtilis*, *A. niger*, *P. chrysogenum* having ZOI 13 mm, 14 mm,12 mm, 17 mm, 16 mm respectively. *Emblica officinalis* was found to be most effective against *S. aureus* having ZOI 12 mm. Whereas, *Zingiber officinale* was found to be effective against *S. aureus* and *B. subtilis* with ZOI 11mm and 12 mm respectively. Among all these plants tested, *Allium cepa* and *Benincasa hispida* was ineffective against all the pathogens. The decoction extracts was not more effective against all pathogens.

In terms of sensitivity against standard antibiotic produced higher inhibitory effect on *S. aureus* and followed by *B. subtilis* and *P. chrysogenum*. *A. niger* and *E. coli* was found to be least sensitive against Ampicillin. A qualitative phytochemical analysis was performed for the detection of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars.

Table 1: Zone of inhibition showed by plant extracts and sensitivity assay-

Micro-organisms	**ZOI (100 µl/well) in mm**										
	Zingiber officinale		***Allium sativum***		***Allium cepa***		***Emblica officinalis***		***Benincasa hispida***		***Ampicillin* (30 µl/ml)**
	Pure juice	**Dil. (1:10)**	**Pure juice**	**Dil. (1:10)**	**Pure juice**	**Dil. (1:10)**	**Pure juice**	**Dil. (1:10)**	**Pure juice**	**Dil. (1:10)**	
E. coli	--	--	14 mm	11 mm	--	--	9 mm	8 mm	--	--	Insensitive
B. subtilis	--	12 mm	12 mm	10 mm	9 mm	--	9 mm	12 mm	--	--	sensitive
S. aureus	9 mm	11 mm	13 mm	9 mm	--	--	11 mm	8 mm	--	--	sensitive
A. niger	--	--	17 mm	12 mm	6 mm	--	9 mm	--	--	--	Least sensitive
P. chrysogenum	--	--	16 mm	15 mm	5mm	--	8 mm	6 mm	--	--	Sensitive

Table 2: Phytochemical constituents of the plants extract

Plant juices	**Alkaloid**	**Glycosides**	**Terpenoid**	**Saponins**	**Flavonoid**	**Tannins**	**Reducing sugars**
Zingiber officinale	-	-	+	+	+	-	-
Allium sativum	+	-	+	+	+	-	+
Allium cepa	+	-	+	+	-	-	+
Emblica officinalis	+	+	+	-	-	+	+
Benincasa hispida	-	+	+	-	-	-	-

4. Discussion

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant, although; it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct [9]. Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds, especially against bacterial pathogens. *In vitro* studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness varied.

The antimicrobial activity of many plant extracts has been previously reviewed and classified as strong, medium or weak [10]. The medicinal plants like Ginger (*Zingiber officinale),* Garlic *(Allium sativum),* Onion *(Allium cepa),* Amla *(Emblica officinalis),* Petha *(Benincasa hispida)* are being used traditionally for the treatment of inflammation, carminative, cough, toothache, antiseptic expectorant, stomatitis and some fungal infection like candidiasis. The antibacterial activity has been attributed to the presence of some active constituents in the juices.

Phytochemical screening of the *Allium sativum, Emblica officinalis* and *Allium cepa* in the present study revealed the presence of terpenes and glycosides. However, a glycoside appeared to be the major bioactive component that offers antisecretory and antiulcer effects [11]. Plant glycosides, which are not normally toxic when ingested orally, are known to inhibit chloride transport in the stomach [12].

Garlic *(Allium sativum)* is believed to have medicinal properties, such as antibacterial antifungal and anti-diabetic. The inhibition produced by the plant extracts against a particular organism depends upon various extrinsic and intrinsic parameters. Due to variable diffusibility in agar medium, the antibacterial property may not be demonstrated as ZOI commensurate to its efficacy.

Intensive use of antibiotics often resulted in the development of resistant strains [13], thus create a problem in treatment of infectious diseases, furthermore, antibiotics sometimes associated with side effects [14] whereas there are some advantages of using antimicrobial compounds of medicinal plants such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to a long history of use and being renewable in nature [15]. Because of this, the search for new antibiotics continues unabated. These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources. Awareness of local communities should be enhanced incorporating the traditional knowledge with scientific findings.

5. Conclusions

The present study has provided some comparative biochemical information on the proximate, mineral element and phytochemistry of *Zingiber officinale, Allium sativum, Allium cepa, Emblica officinalis,* and *Benincasa hispida* support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity. This antimicrobial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. There are indications that all medicinal plants are good sources of nutrients, mineral elements and phytochemicals. Therefore, their use as nutritional supplements is highly promising.

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