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PHYTOCHEMICAL POTENCY OF CERTAIN MEDICINAL PLANTS AGANIST SOME PATHOGENIC MICROORGANISMS

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ABSTRACT

The herbs have potential clinical and therapeutic applications in the modern medical society productive for maintaining human health. The medicinal properties of the former have significant effects in biological activities ranging from infectious diseases to proliferating disorders. The present study was focused upon screening the phytochemical constituents and evaluating the antimicrobial effects of methanol leaf extracts against selected microorganisms at different concentrations respectively. The antimicrobial activity of the extracts against the microorganisms was determined by Kirby Bauer method. The results of the phytochemical analysis showed the presence of flavonoids, alkanoids, terpenoids, tannins, saponins and glycosides in the *Ocimum basilicum*, *Ocimum tenuiflorum*, *Cymbopogon citratus*, *Azadirachta indica* and *Psidium guajava* plant species. The antimicrobial results for different concentration, 100%, 75%, 50%, 25%, of the extracts against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Neisseria meningitidis*, *Bacillus subtilis* and *Corynebacterium diphtheria*, showed inhibition in some and none towards a few selected plant species. The positive result for antimicrobial activity of methanolic leaf extracts in the study encourages the traditional medicinal use comparative to synthetic multidrug therapy.

Keywords: Antimicrobial Activity, Medicinal Plants, Pathogenic Microorganisms, Phytochemical Components

INTRODUCTION

Plants have always been a source of medicinal purpose from ancient times. The healing property of herbs ranges from antimicrobial to anticancerous and other infectious diseases **[1]**. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs for medicinal purposes. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants **[2]**. The plants are investigated to better understand their properties, safety and efficiency. The leaves, seeds, flowers and roots are rich in volatile oils and other phytochemicals that act mainly on the digestive system, stimulating the appetite and relieving irritation. They also act as expectorant antimicrobial agents **[3]**.

Qualitative phytochemical investigation revealed that the extracts contained some phytoconstituents, saponins, tannins, alkaloids and flavonoids. These bioactive components including nitrate, chloride and sulphates beside other water soluble components which are naturally occurring in most plant materials, are known to be in nature thus conferring the anti-microbial property of plants **[4]**. Almost all of the study of antimicrobial properties has been done in laboratories under in vitro condition to observe the effects of both gram-positive and gram-negative microorganisms and other bacteria that cause a wide array of human and animal diseases.

A research on natural products, to increase the knowledge about the close relationship between the chemical structure of a certain compound and its biological properties, and to understand the animal and insect-plant interrelation with medicinal plants has been developed **[5]**. An important substances for the study of traditional use and verification of new pharmacological anti-infectious agents, have evaluated the in vitro antimicrobial activity of plant extracts against Gram-positive and Gram-negative bacterial strains isolated from human infections.

Plant based antimicrobial compounds have enormous therapeutical potential as they serve the purpose without any side effects that are often associated with synthetic antimicrobials. The methanol, ethanol, and chloroform form of plants extract such as *Azadirachta indica, Calotropis gigantean, Lawsonia inermis, Mimosa pudica, Ixora coccinea, Parthenium hysterophorus, Chromolaena odorata, Ocimum tenuiflorum, Cymbopogon* marginatus, *Psidium guajava etc* were subjected to a preliminary screening for antimicrobial activity against common human

pathogenic bacteria like *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Neisseria meningitidis, Bacillus subtilis, Corynebacterium diphtheria* and many others **[6]**. This study aimed at determining the phytochemical activity of the experimental plant extracts against various bacterial strains pathogenic to humans and animals.

MATERIALS AND METHODS

Collection of Plant Material

The experimental plants *Ocimum basilicum, Ocimum tenuiflorum, Cymbopogon citratus, Azadirachta indica, Psidium guajava,* were collected from various areas of Bhopal, Madhya Pradesh. The leaves of all the plants were air dried in shade for 10-15 days.

Preparation of Extracts

The dried materials were reduced into fine powder that was passed through 0.5mm pore size sieve. The dry powdered plant materials were soaked in organic solvent, methanol at room temperature for 48 hours, plant extracts was prepared. The extracts were filtered and concentrate was evaporated using a rotary evaporator with the water bath set at 40°C. All the extracts were concentrated and preserved in airtight bottle for further analysis of phytochemical compounds and anti microbial testing.

Test Microorganisms

Test microorganisms *Staphylococcus aureus, Staphylococcus epidermidis, Neisseria meningitidis, Bacillus subtilis,* and *Corynebacterium diphtheriae* were obtained from different sources and cultured, identified and preserved at Department of Microbiology, Barkatullah University, Bhopal on Brain Heart Infusion Medium (BHI).

Phytochemical Screening of Active Plant Extracts: phytochemical analysis of all the extracts were performed following the standard procedures Sthal (1969) **[7]** and Brindha (1981) **[8]**. **Terpenoids Determination (Salkowski Test) -** 0.5 gm of extract was treated with 2ml of chloroform and then with solution of concentrated sulphuric acid. Development of reddish brown color confirms the presence of terpenoids.

Alkaloids Determination - Wagner's reagent that is, 1.27gm of iodine and 2gm of potassium iodide dissolved in distilled water was added to the plant extract. Appearance of reddish brown color appeared, confirming the presence of alkaloids.

Flavonoids Determination - Two test were performed for the confirmation of flavonoids.

First- To each of the plant extract solution 5ml of dilute ammonia solution and 1ml concentrated sulphuric acid solution was

added. Solution turns yellow that disappear on standing indicates the presence of flavonoids.

Second - To each of the extract solution add 10ml of ethyl acetate and heat over the steam bath for 3 minutes, filter the mixture. 1ml dilute ammonium solution was added to 4ml of the filtrate. Appearance of yellow color indicates the presence of flavonoids.

Tannins Determination - 0.5gm of the extract was boiled in 10 ml of water in a test tube and then filtered. To the filtrated few drops of 0.1% of ferric chloride was added. A brownish green or blue black color develops confirming the presence of tannins.

Saponins Determination (Foaming Test) - The plant extract was shaken vigoursly with distilled water, persistent of foam confirms the presence of saponins.

Glucoside Determination - 0.5gm of extract was dissolved in 2ml of glacial acetic acid and 1 drop of 1% ferric chloride solution was added. The solution was further dissolved in concentrated sulphuric acid. A violet ring appear below the brown ring, while in the acetic acid layer a greenish ring is formed just above the brown ring and gradually spread throughout this layer.

Antibacterial Activity

The antibacterial assay was performed by well diffusion method originally described by Bauer *et al.,* (1966) **[9]**. Brain Heart Infusion Agar plates were prepared. The tested microorganisms were streaked on the plates. Wells was made of the same diameter (6mm), impregnated with the tested plant extracts. 100µl of the test plant extract was introduced. The plates were incubated at 37 °C for 24 hours. For each bacterial strain, negative controls were maintained where pure solvents were used instead of the extract. After the incubation period, the diameter of the inhibition zone around the plant extracts were measured and compared with the diameter of inhibition zone of the control plate **[10].**

RESULTS AND DISCUSSION

The phytochemical analysis reveled the presence of bioactive constituents, terpenoids, alkaloids, flavonoids, tannins, saponins and glucoside, in the methanolic extracts of the selected plants, *A. indica*, *O. basilicum*, *O. tenuiflorum, C. citratus, P. guajava. Terpenoids were present in all the selected plants. Flavinoids was present in all the selected plant extracts except P. guajava. Tannins and glycosides was present in all the plant extracts, they were absent in A. indica* and *P. guajava. Saponins was only present C. citratus, P. guajava,* whereas alkaloids was moderately present in all **(Table 1)**.

The results of antimicrobial activity of the experimental methanolic extracts have a wide

effect on the microorganisms. Varieties of herbal plants are in use as a substitute of synthetic medicines. The effect of extract on the selected plants varied against some common human pathogenic microorganisms, *S. aureus, S. epidermis, B. subtilis and C. diphtheria at different* concentrations as 100%, 75%, 50%, 25% and 0%. A constant amount of the extract was added to each well as 100µl. The diameter of the well measured was 6mm. The inhibition zone of each plant extract against each microorganism was measured. The maximum inhibition zone measured was 2.6mm against *S. aureus by O. basilicum and O. tenuiflorum* at 100% concentration of the extract. A minimum zone measured was 0.2mm against *S. epidermis and B. subtilis by O. tenuiflorum* at 50% and 25% and *N. meningitidis* by *C. citrates* at 50%. No inhibition zone appeared at certain concentration of the extracts and in a few at all the concentrations **(Table 2)**.

The use of plants to heal diseases, including infectious one, has been extensively applied by people. Data from the literature as well as results of this study revealed the great potential of plants for therapeutic treatment **[11]**. The methanolic extracts of the 5 selected plants were subjected to preliminary antimicrobial activity testing against 5 microorganisms. The study demonstrated that among all the plant extracts *A. indica* and *O. basilicum* had more pronounced antimicrobial activity against all pathogenic microorganisms. On treating all the microorganisms with the plant extracts, *S. aureus* and *C. diphtheria* offered inhibition zone at all the concentration of the tested extracts. Gram-positive bacteria, and particularly *Staphylococcus* species, are predominant among all the organisms that are responsible for infective complications following surgical vascular grafts or the implantation of prosthetic devices **[12]**. Treatment of postoperative infections is further complicated by the emergence of antibiotic-resistant pathogens, which has contributed significantly to the morbidity and mortality of hospitalized patients. Most *Staphylococcus* infections result in acute diseases. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms **[13].** There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the bioassay-guided fractionation to yield active principles. Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanism **[14]**. Plant based

antimicrobials often serve as therapeutical agents without any side effects and are associated with synthetic antimicrobials. The present study suggest that plant extracts which posses the phytochemical constituents also exhibit the antimicrobial activity and together they are responsible in inhibiting the microorganisms. The study can be beneficial in developing new herbal antibiotic drugs.

Table 1: Phytochemical Constituents of the Plant Extracts

Plant extracts	Terpenoids	Alkaloids	Flavonoids		Tannins	Saponins	Glycoside
			Test-1	Test-2			
Azadirachta indica	+	+	+	+	-	-	-
Ocimum basilicum	+	+ -	+	+	+	-	+
Ocimum tenuiflorum	+	+ -	+	+	+	-	+
Cymbopogen citrates	+	+ -	+	+	+	+	+
Psidium guajava	+	+	-	-	-	+	-

(+) Represents the presence of phytochemical, (-) Represents the absence of phytochemical

Table 2: Inhibition Zone Range Certain Microorganisms at Different Concentration of Plant Extracts

Microorganisms	Plant extract conc.	Inhibition zone of the selected plant extract (mm)				
		A	B	C	D	E
S. aureus	100%	2.0	2.6	2.6	1.5	1.1
	75%	1.5	1.5	1.3	1.1	1.0
	50%	1.3	1.5	1.3	1.0	0.8
	25%	0.5	0.8	1.0	1.0	0.8
	0%	-	-	-	-	-
S. epidermis	100%	1.7	2.5	1.8	0	0
	75%	1.5	1.3	0.5	0	0
	50%	0.6	1.4	0.2	0.4	0
	25%	0.6	0	0	0	0
	0%	-	-	-	-	-
B. subtilis	100%	1.5	1.3	0	1.1	0
	75%	1.3	0.5	0.5	0	0
	50%	0.4	0	0	0	0
	25%	0	0	0.2	0	0
	0%	-	-	-	-	-
N. meningitidis	100%	0	0.5	0	0	0
	75%	1.3	0.3	0	0	0

	50%	**0**	**0**	**0**	**0.2**	**0**
	25%	**0**	**0**	**0**	**0**	**0**
	0%	**-**	**-**	**-**	**-**	**-**
C. diphtheria	**100%**	**1.5**	**2.0**	**1.6**	**1.5**	**0.3**
	75%	**1.5**	**1.5**	**0.5**	**1.5**	**0**
	50%	**0.3**	**1.3**	**0.3**	**0.6**	**0.4**
	25%	**0**	**0.5**	**0.3**	**0.4**	**0.2**
	0%	**-**	**-**	**-**	**-**	**-**

A: ***Azadirachta indica,*** **B:** ***Ocimum basilicum,*** **C:** ***Ocimum tenuiflorum D: Cymbopogen citrates,*** **E:** ***Psidium guajava***

CONCLUSION

The traditional medicinal methods, especially the use of medicinal plants, play a vital role to cover the basic health needs in the developing countries, and moreover, the use of herbal remedies has risen in the developed countries.This work may provide essential information in the selection of plant extract for further isolation of constituents responsible for the activity against the studied species, thereby aiding to explore an antibacterial lead that is helpful in combating the diseases. These plant extracts can be used as raw materials for phototherapy, as therapeutical agents because of their antibacterial activities.

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