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## Research Article

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# ESTIMATION OF ANTIMICROBIAL AND PHYTOCHEMICAL ACTIVITY OF CLITORIATERNATEA STEM AND LEAF EXTRACTS AGAINST SOME SELECTED BACTERIAL STRAIN

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### ABSTRACT

Focus on plant research has increased all over the world and there are clear evidences of medicinal plants being used in various traditional remedial systems. Antibacterial evaluation of methanolic, petroleum ether and chloroform extract of leaf and stem of Clitoriaternatea plant was carried out using the disk and well diffusion method. These were tested against two bacteria, Gram positive: *Bacillus subtilis*, *Staphylococcus aureus*; Gram negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*. These leaves and stem extracts exhibited anti-microbial activities with zones of inhibition ranging from 1.2 – 2mm in leaves and 0.5 – 1.5mm stem of methanol and petroleum ether in disk diffusion and in well diffusion bacteria. *Bacillus subtilis* showed the maximum zone of inhibition (2.5mm) in stems and leaves in methanol extract. Qualitative phytochemical analysis of this plant confirms the presence of various secondary metabolites tannins, carbohydrates, proteins, flavanoids, reducing sugars, glycosides and resins. Methanol was only present in all secondary metabolites. Hence *Clitoriaternatea* can be used to discover & develop natural products in the new pharmaceuticals and can be effectively used to cure numerous infectious diseases.

**Keywords:** Secondary metabolites, Flavonoids, Antibiotics, Synthetic drugs, Pathogens.

### INTRODUCTION

Medicinal plants are the alternative remedies with incredible healing properties and also as these are affordable to poor people. These herbal medicines can also be used to generate income, employment, foreign exchange for both developed and developing countries. Plants have been a good source of drugs for humanity from the most primitive time. There are about 800 plants that may possess medicinal properties according to ethnobotanical information. The development of the modern medicine or "Allopathy" remains rooted to the medicinal plants. 80% of the world population in developing countries primarily depended on herbal medicines. These medicinal plants are the richest bio-resource of folk medicine, traditional system of medicine, food supplements, nutraceuticals,

pharmaceuticals industries and chemical entities for synthetic drugs. Siddha, Ayurveda and Unani system of medicine had been developed in India, deals with the whole body, mind and spirit for the maintenance of health and disease prevention. The efficacy to prepare these traditional medicine depends on the use of proper plant parts and its biological potency depends upon the presence of required quantity and nature of secondary metabolites.

Secondary metabolites act as a defence mechanism against predation by many herbivores and insects. The study of secondary metabolites was started in the early 1800s by Fredrick Wilhelm Serturmer, when he isolated morphine from opium poppy. Plants secondary metabolites yields products that promote in the growth and development of plants but these are not required by the plants. Flavonoids are one

class of secondary metabolites that are also known as Vitamin P or as Citrin which we use as medicine, flavouring and recreational drug. The "flavonoid" research began in 1936 by the Hungarian scientist Albert Szent-Gyorgi, when he was uncovering a synergy between pure vitamin C and an unidentified co-factors from the peels of lemons called as "Citrin", and, later known as, "vitamin P". Flavonoids are low molecular weight bioactive polyphenols that plays a vital role in photosynthesising cells, are readily ingested by human beings and seems to display an important anti-inflammatory, anti-allergic and anti-cancer activity.

Antibiotics have been re-generated because the old ones are being resisted by the existing pathogens. Hence there is a need to develop new antibiotic which can be helpful for curing new emerging diseases. Plants are the natural and good resource of antibiotics with therapeutic potential. They have no side effects on the human body as compare to allopathic medicine's. Some chemical substances are present in the plants which are beneficial for the human body, commonly known as Phytochemicals. Phytochemicals are defined as the substances which are found in edible fruits and vegetables and had the potential for modulating human metabolism for the prevention of chronic diseases. Phenols, resins, flavonoids, carbohydrates, glycosides, tanins etc., are such phytochemicals that had been used as a major source in the Pharmaceuticals and Ayurvedic system.

*Clitoriaternatea* is distributed throughout tropical equatorial Asia and latter in South and Central America, East & West Indies, Bangladesh, China and India. It is also known as 'Butterfly pea' or 'Shankhpushpi' or 'Aprajita'. It is a perennial, twinning herb, stem terete, leaves imperinnate, deep-rooted, tall slender, climbing legume with five leaflets and a deep blue flower. *Clitoriaternatea* is the medicinal plant with all the secondary metabolites. It is well adapted to a variety of soil types (pH 5.5 – 8.9) and survive in both the extended rainfall regions and prolonged periods of drought. In Ayurveda of Indian medicine it is considered as a 'Medhya' drug which improve intelligence and enhance memory function. It is used for the treatment of jaundice, various skin diseases, diabetes, cancer, asthma, urinary infection, fever, migraine, constipation, indigestion, tumours, and central nervous system disorders. Many medicinal values are evaluated such as nootropic, anxiolytic, antidepressant,

anticonvulsant, sedative, antipyretic, anti-inflammatory and analgesic activities. The important use of *Clitoriaternatea* is in the treatment of cancer by chemotherapy. Because of its indigenous benefits it is being used in the pharmaceuticals for preparing medicines without any side effects as compare to allopathy or synthetic drugs.

#### **Materials and Method**

The leaf and the stem parts of *Clitoriaternatea* were collected from the College garden.

#### **Test microorganism and growth media**

The following Gram positive and Gram negative bacteria were used for anti-microbial activity studies: Gram positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*; Gram negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*. The bacterial strains were grown and maintained in Nutrient Broth in test tubes at 37°C for 24 h.

#### **Extract Preparation**

The various plant parts were prepared by drying in hot air oven at 150°C for 10h then the leaves and stems were grinded into fine powdered material. This material was then used for study.

#### **Preparation of Raw Material**

The dried form of powder of leaves and stems were weighed and then 100ml of methanol, petroleum and chloroform separately was added and evaporated in rotary vacuum evaporator. The dried powder was dissolved in petroleum ether, chloroform and methanol. Then the solvent was filtered by using Whatman filter paper no. 1 and it remained open for 24h till it was completely dried. Then 3ml of the solvent was added in the dried material and this was used for further study.

#### **Phytochemical Analysis**

The phytochemical analysis of leaf and stem extract of *Clitoriaternatea* were determined as follows:

##### **Tannins:**

0.5 ml of plant extracts and add 1ml of water and 1-2 drops of ferric chloride solution. Blue colour was observed for green black colour.

##### **Glycosides:**

To 2ml of plant extract, 1ml of glacial acetic acid and add few drops of ferric chloride and concentrated sulphuric acid was added. Reddish brown colour is formed.

**Flavonoids:**

2ml of plant extract, few drops of concentrated hydrochloric acid and magnesium ribbon was added. Pink tomato red colour indicates the presence of flavonoids.

**Reducing Sugars:**

0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

**Proteins:**

To 1ml of extract few drops of nitric acid were added by the sides of the test tube very gently. Yellow colour indicates the presence of protein.

**Carbohydrates:**

1ml each of Fehling's A and Fehling's B were added in diluted extract and heated for 30 min and observed for the formation of brick red colour.

**Resins:**

Distilled water was added to the extract and observed for turbidity which indicates the presence of resins.

**Antimicrobial Activity**

**By Disk Diffusion Method**

Antimicrobial activities of different plant parts extract were investigated by the disk diffusion method. The Muller Hinton Agar (MHA) plates, were pre -inoculated with the test organism and kept in incubator for 15 min. In this method paper disks, saturated with filter sterilized plant extract and also the tetracyclin antibiotic disk are placed onto the surface of a suitable solid agar medium. Then the plates were incubated for 24 h at 37°C. Zones of inhibition are then measured from the circumference of the disks to the circumference of the zone of inhibition. Diag. shown in Fig 1(1.2,1.3,1.4)

**By Well Diffusion Method**

Antimicrobial activities of different plant extract were investigated by well diffusion method. The Muller Hinton Agar(MHA) plates were pre-inoculated with the test organism and kept for 15 min .In this method well is punched with help of well puncher on the agar plate. A fixed volume of plant extract are then introduced into the wells. The plates are then incubated at 37°C for 24h. Then the plates were observed for the circumference of the zone of inhibition. Diag. fig.2 (2.1,2.2,2.3)

**Disk Diffusion Method** Fig 1(1.2,1.3,1.4)



Fig1.1

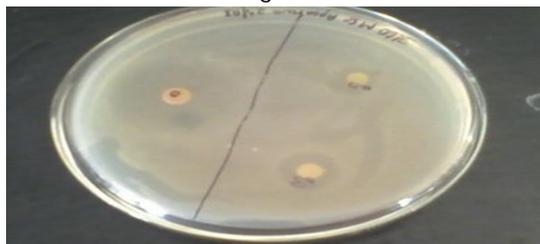


Fig 1.2



Fig1.3

**Well Diffusion Method** Fig.2 (2.1, 2.2, 2.3)



Fig 2.1



Fig 2.2



Fig 2.3



Fig 3.1 (proteins)



Fig3.2 (Carbohydrates)



Fig 3.3 (Resins)



Fig 3.4 (Tannins)



Fig 3.5 (Glycosides)



Fig 3.6 (Reducing sugars)

## RESULTS

The results of the phytochemical screening to test the presence of tannins, reducing sugars, glycosides, flavonoids, proteins, carbohydrates and resins in the plant extract of leaves and stem of *Clitoriaternatea* are shown in table 1.

**Table 1: Phytochemical constituents of Stem and Leaf extracts of *Clitoriaternatea***

SECONDARY METABOLITE	STEM			LEAF		
	P.E	M	C	P.E	M	C
Protein	-	+	+	+	+	+
Carbohydrate	-	+	+	-	+	+
Resins	-	+	-	-	+	-
Tannins	-	+	+	-	+	+
Glycosides	+	+	+	+	+	+
Reducing sugar	-	+	+	-	+	+
Flavonoids	-	+	+	-	+	+

+ Positive; - Negative; P.E petroleum ether; M Methanol; C chloroform

The results of the antimicrobial disk diffusion method of the extracts of leaves and stem of *Clitoriaternatea* are shown in Table 2.

The results of the antimicrobial well diffusion method of the extracts of leaves and stem of *Clitoriaternatea* are shown in Table 3.

**Table 2: Antimicrobial activity by disk diffusion method in *Clitoriaternatea***

Strains	Zone of growth inhibition					
	Stem (cm)			Leaf (cm)		
Gram positive bacteria	Ad	P	M	Ad	P	M
<i>Bacillus subtilis</i>	1.2	0.7	1.5	1	1.2	2
<i>Staphylococcus aureus</i>	1.8	-	1.5	2	-	1.5
Gram negative bacteria	Ad	P	M	Ad	P	M
<i>Escherichia coli</i>	2.3	-	0.7	2.5	0.5	1.5
<i>Pseudomonas aeruginosa</i>	1.8	-	-	2	1.5	0.6

Ad antibiotic disk; P petroleum ether; M methanol

**Table 3: Anti-microbial activity by agar well diffusion method in *Clitoriaternatea***

Strains	Zone of growth inhibition					
	Stem (cm)			Leaf (cm)		
Gram positive bacteria	P.E	M	C	P.E	M	C
<i>Bacillus subtilis</i>	0.5	2.5	0.4	1.2	1.5	1
<i>Staphylococcus aureus</i>	1.2	1.8	0.8	0.5	1.5	0.5
Gram negative bacteria	P.E	M	C	P.E	M	C
<i>Escherichia coli</i>	1.5	2.1	0.5	1.2	2.5	1.8
<i>Pseudomonas aeruginosa</i>	0.8	1.8	1.3	1.5	1.9	1.5

P.E petroleum ether; M Methanol; C chloroform

## DISCUSSION

There are various drugs for the treatment of new emerging diseases. Plants contain medicinal properties which had been used to treat human diseases and 50% of new chemical entities had been introduced. Recent technological advances and efforts should be made towards isolation and characterization of the active principles. Ayurvedic knowledge supported by modern science leads to find, characterize, and standardize the active compounds from herbal source. With the combination of modern and traditional system of medicine a better anti-depressant drugs can be produced with no side effects. Herbs are found all over the world with natural chemical compounds and can be used for future studies. The use of various parts of the medicinal plants helps to decrease the cost of medication and also be made locally available with lesser side effects as compared to synthetic drugs. Resins, proteins, carbohydrates, tannins, flavonoids, reducing sugars and glycosides are phytochemicals which is of great significance in therapeutic treatments. The qualitative phytochemical analysis of leaves and stem shows the complete presence of methanol in all the secondary metabolites. The leaves and stem has been evaluated for the medicinal values like

jaundice, migraine, sore throat, tumours, skin diseases, asthma, fever, urinary infections, constipation and indigestion and for central nervous system disorders. Herbal medicine had been used particular for the treatment of cancer and thus increases the survivability of a patients. The new emerging diseases are the major cause of mortality and morbidity all over the world. The number of multidrug resistant strains which reduced susceptibility to antibiotics are increasing had been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and epidermidis of human immunodeficiency virus (HIV) infections. These observation results in the new discovery of antibiotics from medicinal plants. In this present study, preliminary screening for antimicrobial activity showed, that methanol has showed more zone of inhibition than any other. Results obtained from this study indicates that the plant extracts showed the strongest antimicrobial activity than the commercially available antibiotics. Thus *Clitoriaternatea* plant can be used for various medicine preparation.

#### CONCLUSION

This study reveals that the extract obtained from the leaves and stems of *Clitoriaternatea* is important for preparation of the medicines with no side effects. The natural products act as a useful drugs in formulating a multi component herbal product and this would help in treatment of various neuro-degenerative disorders like anxiety and depression. The study of phytochemical help to identify the quality and purity of the drug. The study conducted with the *Clitoriaternatea* leaves and stem revealed different parameters that will be useful in scientific evaluation, identification and authentication of the drugs. The present study indicates that *Clitoriaternatea* of Fabaceae family have definitely some antimicrobial principles which are responsible for antibacterial activity. Thus, the activity of all these test extracts against bacterial pathogens, indicates that this plant are more resistant to bacterial attacks due to the presence of some biologically active substances, so these can be used in pharmaceutical and drug industries.

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