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ANTI - MICROBIAL ACTIVITY OF LEAF EXTRACT OF *PASSIFLORA INCARNATA* LINN WITH QUALITATIVE PHYTOCHEMICAL SCREENING

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ABSTRACT

The antimicrobial activity of *Staphylococcus aureus* was tested in an ethanolic extract of the leaves of *Passiflora incarnate*, a species of the Passifloraceae family. When compared to the aqueous extract, the ethanolic and aqueous leaf extracts of P. incarnate exhibited the highest degree of antibacterial activity. The presence of phenolic compounds in the plant material which gives it its antibacterial activity, according to a phytochemical examination of both extracts.

Keywords: Antimicrobial activity; Passiflora incarnate; Passifloraceae; Phytochemicals

INTRODUCTION

In many countries' traditional medical systems, *Passiflora incarnata* Linn. (family Passifloraceae), commonly referred to as maracuja, passion flower, or maypops, was used primarily as a sedative, anxiolytic, anti-convulsant, asthma, bronchitis, analgesic, antispasmodic, and other respiratory disorders **[1, 2]**.

According to reports, the plant contains carbo line alkaloids of harmala group **[3]**, flavonoids **[4]**, Coumarin glycosides **[5]** as well as benzopyrene derivative maltol **[6]**. To confirm the activity and determine the characteristics related to it, plants that may have antimicrobial action should be evaluated against a suitable microbal model. Effects of plant extract on bacteria have been the subject of numerous studies carried out globally **[7]**. In India, considerable work has been done on ethnomedicinal plant **[8]**. There is now more interest in several vintage natural products. Aqueous and ethanolic extracts from plants used in allopathic medicine were proposed as possible sources of antimicrobial, antiviral, and antitumoral compounds **[9]**. During the initial phases of screening programs, the utilization of crude plant extracts may demonstrate greater efficacy than the assessment of pure chemicals derived from natural sources.

Passiflora incarnata, a member of the Passifloraceae family, can reach lengths of up to 10 meters. The plant has numerous documented medicinal applications, including its sedative properties for alleviating nervous anxiety and hysteria. The desiccated herb is commonly utilized as a therapeutic infusion. A sedative-infused chewing gum has been developed. It is utilized as a local application for ulcers, sores, anxiety, shingles, menstrual disorders, asthma, mood disorders, epilepsy, cardiac issues, hypertension, insomnia, hysteria, neuralgia, nicotine dependence, pain, sexual dysfunction, and spasms **[10-12]**. Infectious diseases remain the principal cause of elevated mortality rates in developing countries. The alarming global prevalence of antibiotic resistance in contemporary medical practice necessitates the development of novel compounds. Investigating Passiflora incarnate L.'s prospective phytochemical components and antibacterial action is the goal of the current study.

MATERIALS AND METHODS

2.1 Collection, identification, and authentication of Plant material: After being gathered from a cultivated source, the leaves of *Passiflora incarnata* L. (Passifloraceae) were mechanically ground into a coarse powder and allowed to dry in the shade. Dr. Alok Srivastav, Associate Professor in the Department of Plant Science at M.J.P. Rohilkhand University in Bareilly, Uttar Pradesh, India, where voucher specimen no. RU/PS/2023/01 has been deposited, verified the identity of the plant material.

2.2 Preparation of leaves powder: *Passiflora incarnata* leaves were gathered and allowed to dry in the shade. After being dried for 24 hours at 35°C in an oven, the materials

were mechanically ground into a powder. These powdered materials have been utilized for additional research **[13]**.

2.3 Preparation of Extract: Using a Soxhlet extractor, 25 g of dried Passiflora incarnata powder was placed in a thimble and extracted gradually with ethanol over 18 hours **[14]**. Using a rotary flash evaporator, all of the extracts were concentrated and stored in an airtight bottle at 5°C until they were needed. Each extract underwent antimicrobial activity testing and phytochemical analysis.

2.4 Phytochemical analysis

A small amount of dry extract has been used for phytochemical screening test **[13, 15]**, which tested for glycosides using Baljet's and Legal's test and alkaloids using Dragendroff's and Wagner's reagent.

2.5 Test organism:

The organism was employed for this study as test organism:

2.5.1 Bacteria:

Staphylococcus aureus

2.6 Antimicrobial activity:

Antimicrobial activity assay was conducted of ethanolic extract of *Passiflora incarnata* Staphylococcus aureus. These microbial cultures have been collected from BIU College of Pharmacy's Microbial Laboratory at Bareilly International University in Bareilly, Uttar Pradesh, India.

2.6.1 Requirements:

- **Chemicals:** NaCl (0.5 percent), Mueller-Hinton agar (5 percent), meat extract (0.5 percent), distilled water, tap water.
- **Glassware:** Beaker, glass rod, measuring cylinder, petri dishes, conical flask, cotton plug, aluminium foil,
- **Apparatus:** Hot air oven, B.O.D. incubator, Autoclave

2.6.2. Procedure:

- Sterile all glassware by acetone/ethanol and keep them in a hot air oven to dry (heat sterilization)
- Measure 5% Mueller-hinton agar powder, 0.5% NaCl, 0.5% meat extract, 5-10 ml tap water and dissolved it in 100 ml distilled water with continuously stirring with glass rod.
- A pale yellow color texture appears and some light yellow precipitates were formed as visible in above picture.
- Conical flask filled with agar media were closed with a cotton plug and wrapped above by aluminium foil.
- Conical flask then kept in the autoclave at 121°C for 30 minutes
- After autoclave moist heating, pour agar media from conical flask to different petri dishes carefully.

- A pale yellow, slightly viscous liquid then formed as depicted in picture below of petri dish.
- Put all petri dishes in B.O.D incubator rapped with aluminium foil with little air space at 37.C for 24 hours.
- After 24-48 hours, bacterial colonies were visible on the above layer of agar culture **(Figure 1a,b)**.
    - For antimicrobial sensitivity, well diffusion method was used for identification of zone of inhibition of microbial growth by test substance **(Figure 2a, b)**.
- Test substance was applied in three different concentrations which are 50 mg, 100mg and 200 mg.
- After 24 hours of applying test substance via well diffusion method, its appears in **(Figure 3 a,b,c)**.
- Measurement of the diameter of zone of inhinition/ dark appearance around the well had given the following data: **(Table 2)**

1(a) **1(b)**

Figure 1 (a, b): Bacterial colonies visibility in agar culture

2(a) **2(b)**

Figure 2 (a,b): Sensitivity to antimicrobial activity by diffusion method

Determination of minimum inhibitory concentration (MIC): Antibacterial inhibition at a specific time point can be used to gauge the strength of antibacterial activity. MIC, the lowest concentration in nutritional media at which no growth occurs, can be used to calculate it. The MIC was determined using a micro broth dilution. A 96-well microtitre plate was filled with 100µL of each of the test substance's different concentrations (30, 50, 80, 100, and 120 mcg/ml) that have been prepared by two-fold serial dilution by utilizing distilled water. Each well, containing varying extract concentrations, was administered an equal volume (100 µL) of overnight-cultivated bacterial cultures standardized to a 0.5 McFarland turbidity, resulting in a total volume of 200 µL per well. Bacterial culture without sample and BHI broth with extract were added to separate wells as a control. For twenty-four hours, plates have been incubated at 37°C. The plates were examined for turbidity, indicating bacterial growth, in the wells. MIC value was defined as the lowest concentration of the test substance that prevented the bacteria from growing visibly (without turbidity). Every experiment was conducted in triplicate. MIC of ethanolic extract is 50mcg/ml.

3. RESULT AND DISCUSSION:

3.1. Phytochemical screening of extract of *P. incaranata* leaves:

All of the extracts' phytochemical analyses showed that the ethanolic extracts of Passiflora incarnate contained alkaloids, glycosides, saponins, phytosterols, flavonoids, phenolic compounds, and tannins **(Table 1)**. Phenolic compounds are accountable for the antimicrobial activity, as indicated by further phytochemical analysis of ethanolic extract. Phytochemical analysis of Passiflora incarnata leaf extract indicates the presence of phenolic compounds, alkaloids, flavonoids, as well as tannins. This activity was demonstrated against test organisms using plant extracts.

3.2. Antimicrobial assay

Ethanolic extracts were tested against bacteria. The leaf of Passiflora incarnata was effective against bacteria **(Table 2) (Figure 1)**.

Table 1: Qualitative Phytochemical screening of aqueous and ethanolic extract of *P. incaranata* leaves (successive extracts)

Sr. No.	Phytoconstituents	Tests	Observe successive extraction	
			Aqueous	Ethanol
1.	Alkaloids	Meyer's reagent	-	++
		Dragendroff's reagent	+	+
		Wagner's reagent	+	+++
		Hager's reagent	-	+
		Muroxide test	-	+
2.	Glycosides	Baljet' s Test	-	+
		Legal's Test	-	+

		Borntrager's Test	+	+
3.	Saponins	Foam Test	-	+++
		Haemolysis Test	+	++
4.	Phytosterols	Liebermann-burchard test	+	+
		Salkowski reactions	-	-
		Liebermann-burchard test	+	+
5.	Flavonoids	Shinoda test	+	+++
		II	+	++
6.	Phenolic Compounds and Tannins	5%$FeCl_3$ solution	-	+
		10% Lead acetate solution	+	++
		Gelatin solution	-	++
		Dil.HNO_3 solution	+	+

Where, +++ High, ++ Moderate, + Slight, - Negative

Table 2: *In-vitro* antimicrobial activity in ethanolic extract of *P. incarnata* leaves after 24 hrs

Sr. No.	Name of organism	Zone of inhibition (mm) at 50 mg (Figure:-3a)	Zone of inhibition (mm) at 100 mg (Figure:-3a)	Zone of inhibition (mm) at 200 mg (Figure:-3a)
1.	*Staphylococcus aureus*	8	12	18

3 (a) 50 mg 3 (b) 100mg 3 (c) 200 mg.

Figure 3 (a, b, c): Sensitivity to antimicrobial activity by diffusion method in test substance after 24 hrs

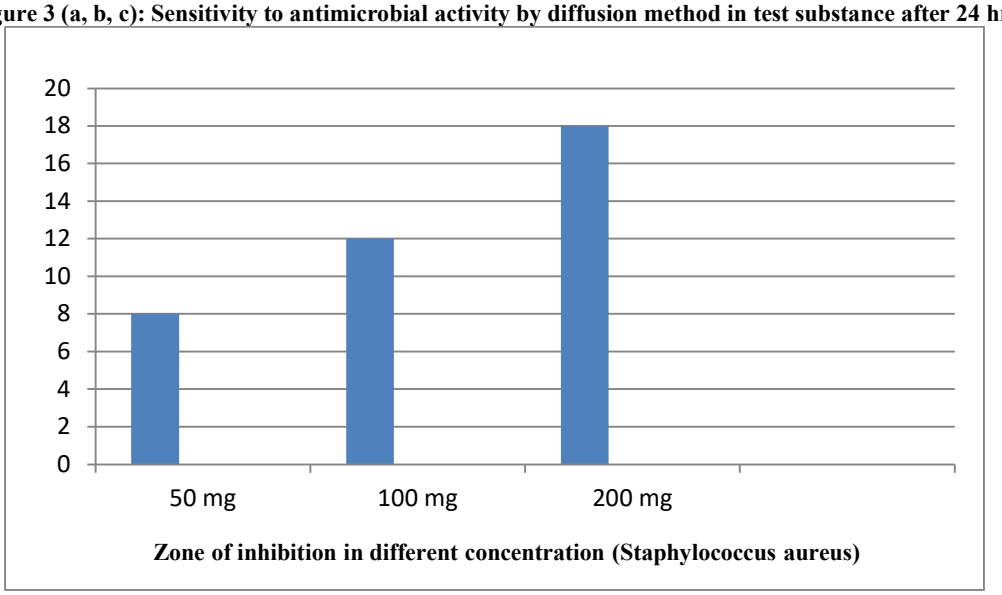

Figure 4: In-*vitro* antimicrobial activity in ethanolic extract of *P. incarnata* leaves after 24 hrs

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