JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF *PASSIFLORA INCARNATA* LINN.

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Abstracts

Plants have been one of the important sources of medicine since the beginning of human civilization. Always microbes threatening the entire world especially in and around of human beings, so we are spending lot of time to identify the microbes and control microorganism of disease for human. During the thousands of years of human existence many natural material were identified for combating human ailments either by instinct or intuition or trial and error. More than 90% of drugs used in traditional systems of medicine in a country like India come from plants. In this study, we selected medicinal climber *Passiflora incarnate* fruits used to cure historically in treating anxiety, insomnia, seizures and hysteria, which leaves containing phytochemical compounds and its antimicrobial activity were screened against selected pathogens.

Introduction

The microbes are exceptionally diverse and are indispensable components of our ecosystem. Few of the microorganism that abound in nature are disease producing or pathogenic. They affect the human society in countless ways[1]. Deadly microbial diseases undoubtedly played a major role in historical events[1]. A firm basis for the casual nature of infectious disease was established only in the latter half of the nineteenth century. Fungi, being larger than bacteria, were the first agents to be recognized Agostino Bassi (1773–1856) demonstrated in 1835 that a silkworm disease called muscardine was due to a fungal infection. Berkeley (1803 – 1889) proved that the great potato blight of Ireland was caused by a fungus. Following his success with the study of fermentation, Pasteur was asked by French government to investigate the pebrine disease of silkworm that was disrupting the silk industry. He showed that the disease was due to a protozoan parasite after several years of work [2].

Many new biological pathogen have been recognized in the past 25 years which are responsible for causing numerous deadly microbial diseases[3]. The control of microorganism is critical for the prevention and treatment of disease. The subject of 'drug' is as old as disease. The world's oldest known pharmacological and therapeutic writing came from India and China. Plants have been one of the important sources of medicine since the beginning of human civilization. During the thousands of years of human

existence many natural material were identified for combating human ailments either by instinct or intuition or trial and error. More than 90% of drugs used in traditional systems of medicine in a country like India come from plants[4].

During world war II, the demand for chemotherapeutic agents to treat wound infections led to the development of a production process and the beginning of an era of antibiotic research. The discovery of antibiotics has been one of the landmarks in the history of medicine[1].

Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more plants[5,6].

Only a small proportion of the world flora has been examined for pharmacologically active components. Over 75 different chemical compounds of known structure derived from higher plants are represented in medicinal prescriptions[7, 8].

In India the use of different parts of several medicinal plants to cure specific ailments has been only in vague from ancient time. Spreading and preserving the knowledge on medicinal plants and their uses has become important for human existence[9]. Undoubtedly the plant kingdom still holds many species of plants containing substances of medicinal values which has yet to be discovered, hence the study and preservation of plants and herbs is becoming more and more essential[10].

Passiflora incarnata L. the plant used for the present study was selected on the basis of their medicinal uses in traditional medicine. It has varied therapeutic action such as sedative, hypnotic, antispasmodic and anodyne. Having all the above said views in mind, the present work was designed with the qualitative analysis of phytochemicals and antimicrobial activity.

The plant is indigenous to an area from the southeast U.S. to Argentina and Brazil. It is cultivated in Europe. A garden plant in common Indian gardens in south India and North America [11]. The axillary pedicle grows up to 8cm and bears 1 flower. The flowers are androgynous and rayed with a diameter of 5 to 9cm and have an involucre. The 5 sepals are green on the out side, white on the inside and tough. The 5 petals are white to pale red. There is a secondary corolla inside the petals, white to pale red[12]. The Passion Flower is a perennial vine on a strong, woody stem reaching up to about 10m in length. The vine is initially angular, later gray and rounded with longitudinally striated bark. The leaves are alternate, petiolate, serrate and very finely pubescent. The under surface is hairier than the upper surface. There are bumpy extra-floral nectaries on the leaf blades. Stipules and tendrils grow from the leaf axils [13].

Passion Flower herb consists of the fresh or dried aerial parts of *Passiflora inicarnata*. The flowering shoots are cut 10 to 15cm above the ground, usually after the formation of the first apple-sized fruit. The harvest is dried in a hay drier or in the air. For a maximum flavonoid content in the flowering shoot, twice yearly harvest is recommended, opinions are not, however, unanimous [14]. Passion fruit is propagated either by seed or by semi-hard wood cuttings, 3-4 m, in length. Rooted cuttings are said to come to bearing earlier than seedlings [4]. The vines may he manure once a year with well-rotted compost or cattle manure and a little of chemical fertilizers [15]. Pruning is not generally practiced in South India,

but as the fruits are borne on new wood, pruning of diseased and overcrowded vines, up to the main branches, during February-March is recommended, to secure a good crop [16].

Sedative, hypnotic, anti-spasmodic and anodyne [11]. Neuralgia, seizures, hysteria, nervous tachycardia and spasmodic asthma [11]. *Passiflora incarnata* leaves and root is considered as an alternative to castor oil in scrofulous and venereal diseases. A bath with the plant extract is used in mania, while the root boiled in oil is used for rheumatism. The plant has great reputation as a febrifuge [17].

It has been widely shown that many plant derived compounds present significant anti-inflammatory effects. For this reason, they represent potential molecules for the development of new drug, especially designed for the treatment and/or control of chronic inflammatory states such as rheumatism, asthma, inflammatory bowel disease and atherosclerosis. This review focuses on the naturally occurring compounds with antimicrobial and pharmacological properties.

MATERIALS AND METHODS

Collection of Plants

The plant *Passiflora incarnata* L. was collected from Jamunamarthur, Jawadhu Hills, Tiruvannamalai district, Tamil Nadu, India. Collected plant was carefully examined and identified with the help of regional flora [18,19]. Specimen was further confirmed with reference to Herbarium sheets available in the Department Herbarium, Government Arts college, Tiruvannamalai, Tamil nadu, India.

Extraction of plant material

Various extracts of the study plant was prepared according to the methodology of Indian Pharmacopoeia[20]. The leaves were dried in shade and the dried leaves were subjected to pulverization to get coarse powder. The coarse powder material was subjected to Soxhlet extraction separately and successively with ethanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C). Both the extracts were stored in a refrigerator in air tight containers. Both the extracts were analysed for phytochemical screening of compounds, antimicrobiolal and pharmacological activity.

3.3 Qualitative phytochemical studies

Qualitative phytochemical analyses was done by using the procedures of Kokate *et al.* (1995)[21]. Alkaloids, carbohydrates, tannins, phenols, flavonoids, gums and mucilages, phytosterol, proteins and amino acids, fixed oils, fats, volatile oil and saponins were qualitatively analysed.

3.3.1 Carbohydrates

300mg of aqueous and alcoholic extracts were dissolved separately in distilled water and filtered. The filterate was boiled with Fehling's and Benedict's solution. Formation of brick red precipitate in Fehling's and Benedict's solution showed the presence of reducing sugars and non reducing sugars, respectively.

3.3.2 Alkaloids

The extracts were dissolved separately in dilute hydrochloric acid and filtered. The filterate was tested carefully with various alkaloidal reagents such as Mayer's, Dragendorff's, Hager's and Wagner's

reagent separatively. Appearance of cream, orange, brown, yellow and reddish brown precipitates in response to the above reagents respectively, indicate the presence of alkaloids.

3.3.3 Steroids

The test solution was treated with minimum amount of chloroform to which three drops of acetic anhydride and two drops of concentrated sulphuric acid were added. Appearance of purple colour then changed to blue or green denotes the presence of steroids.

3.3.4 Tannins and Phenols

Small quantity of alcohol, chloroform and aqueous extracts were dissolved in water separately and to that ferric chloride solution (5%) or gelatin solution (1%) (or) lead acetate solution (10%) was added. Appearance of blue colour with ferric chloride (or) precipitation with other reagent indicates the presence of tannins and phenols.

3.3.5 Saponins

1ml of each extract were dissolved separately in 20ml of water and shaked in graduated cylinder for 15 minutes. Formation of one cm layer of foam indicate the presence of saponins.

3.3.6 Fixed oils and fats

A drop of concentrated extract was pressed in between two filter papers and kept undisturbed, oils stains on the paper indicate the presence of oils and fats.

3.3.7 Gums and Mucilages

About 10ml of extract was slowly added to 25ml of absolute alcohol under constant stirring. Precipitation indicates the presence of gums and mucilages.

3.3.8 Proteins and Free aminoacids

A small quantity of each extracts were dissolved separately in a few ml of water and subject the solution to Millon's or Biuret tests, red or pink-purple colour indicates the presence of proteins.

3.3.9 Flavonoids

The extract was mixed with few ml of alcohol. It was heated with magnesium and then conc. HCl was added under cooling. Appearance of pink colour indicates the presence of flavonoids.

The extract with few ml of aqueous NaOH, form yellow colour and changes to colourless with HCl, indicate the presence of flavonoids.

3.3.10 Volatile oils

For the detection of volatile oil, 50g of powdered plant material was taken in a volatile oil estimation apparatus and subjected to hydrodistillation. The distillate was collected in the graduated tube of the assembly in which the aqueous portion is automatically separated from the volatile oil, if it is present in the drug and returned back to the distillation flask.

3.4. Antimicrobial studies

3.4.1 Media preparation

3.4.1.1 Bacterial media (Muller Hindon Media)

36g of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plates were pored with 5mm dia cork porer. The plates with wells were used for the antibacterial studies.

3.4.1.2 Fungal Media (PDA)

200gm of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were pored with 6mm dia cork porer.

3.4.2 Bacterial strains

The bacterial and fungal pathogenic strains were obtained from the Microbial Type Culture Collection (**MTCC**), The Institute of microbial technology. Sector 39-4, Chandigarh, India.

Bacterial strains were *Klebsiella pneumoniae* (MTCC-2653), *Staphylococcus aureus* (MTCC-737), *Staphylococcus epidermis* (MTCC-435), *Streptococcus pyogenes* (MTCC-1923) and *Serratia marcescens* (MTCC-2645).

3.4.3 Fungal strains

Fungal strains were *Aspergillus niger* (MTCC-1344), *Aspergillus flavus* (MTCC-1973), *Aspergillus fumigatus*, *Ganoderma lucida* (MTCC-1039) and *Mucor indicus* (MTCC-918).

3.4.4 Antibacterial activity of the plant extract

The aqueous and ethanolic extract of dried leaves of *Passiflora incarnata* L. was used throughout the study. The ethanolic and aqueous extract of 100,200 &500mg were tested against different bacterial pathogens such as *Klebsiella pneumoniae*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococcus pyogenes* for their antimicrobial activity. It was demonstrated by well diffusion assay.

3.4.5 Antifungal activity of the plant extract

The ethanolic and aqueous extract of 100,200 & 500mg were tested against different fungal pathogens such as *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Ganoderma lucida* and *Mucor indicus* for their antifungal activity. It was demonstrated by well diffusion assay.

3.4.6 Antimicrobial activity of the standard drugs

The test was carried out by using commercially available antibiotic disks viz., Sparfloxacin (5µg), Vanomycin (30µg), Amikacin (10µg), Amoxycillin (10µg), Ofloxacin (2µg) as standard drugs for all the bacterial pathogenic strains. Fluconazole (10µg), Nystain (10µg), Clotriomazole (10µg) and Amphotericin (10µg) were tested against fungal pathogenic strains. The sensitivity patterns were recorded and the reading were interpreted according to the critical diameter given by National Committee for Clinical Laboratory Standards [22].

3.4.7 Well diffusion method

Antibacterial and antifungal activity of the plant extract was tested using well diffusion method [23]. The prepared culture plates were inoculated with different selected strains of bacteria and fungi using streak plate method. Wells were made on the agar surface with 5mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37±2°C for 48 hours for fungal activity and for 24 hours for bacterial activity. The plates were observed for the zone formation around the wells.

The aqueous and ethanolic extract of the dried leaves of *Passiflora incarnata L.* w*as* used throughout the study. The ethanolic extract was dissolved in sterile distilled water to form dilution such as 100, 200 and 500mg. Each concentrations of the drug were tested against different bacterial pathogens. It was demonstrated by well diffusion assay [23].

The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

Results and Discussion

For all human ailments herbal medicines are available in our environment itself. The present investigation had been undertaken to find out the quality of phytochemicals, antimicrobial and pharmacological effectiveness of the aqueous and ethanolic extract of *Passiflora incarnata* L.

Phytochemical Studies:

Carbohydrates, alkaloids, steroids, tannin, phenol, saponins, fixed oils and fats, gums and mucilage, protein, flavonoids and volatile oils were qualitatively evaluated in the alcoholic extracts of *Passiflora incarnata* L. Steroids was absent among the tested phytocompounds. The results of the study was tabulated and presented in table 1.

The plant used for this study was not chosen at random, but on the basis of their medicinal uses in traditional medicines. It is used to treat various diseases [24,25]. The medicinal plants have curative properties due to the presence of various complex chemical substances of different compositions, which are found as secondary plant metabolites [6].

Table- 1
Qualitative phytochemical screening of ethanolic extract of *Passiflora incarnata* L

++++ - Rich amount
+++ - Moderate amount

S.No	Name of the compounds	Name of the test	Status of the substance
1.	Carbohydrates	a. Fehling's b. Benedicts	++++ ++++
2.	Alkaloids	a. Mayer's b. Hager's c. Wagner's d. Dragen Dorfff's	++ - - ++
3.	Steroids	Chloroform + acetic acid + . H_2SO_4	-
4.	Tannin &Phenols	a. 10% Lead acetate b. 5% Ferric Chloride c. 1% gelatin d. 10% Sodium chloride	+ - + -
5.	Saponins	Foam test	+++
6.	Fixed oils & Fats	Spot test	++++
7.	Gums & Mucillage	Alcoholic precipitation	++
8.	Proteins	Biuret test	++
9.	Flavonoids	Na OH / HCL	++++
10.	Volatile oils	Hydro distillation method	+++

++ - Minimum amount
\+ - Present
\- - Absent

Biochemically active substances such as phenols and tannins were present in the extracts. Many disinfectants used in the health care centers such as lysol, cresol and dettol contain phenol as their active ingredient [26]. Several recent papers reported that the presence of antibacterial activity are due to flavonoids [27,28]. Alkaloids are important components of the plant, which act against pathogenic organisms [29]. Many alkaloids have been identified to impair the release of acetocoids in inflammation [30].

Essential oil from different plants species are known to exhibit various kinds of biological activity including antifungal, antimicrobial, cytostatic, insecticidal, allelopathi and antioxidant. It also acts as bio-

regulator [31,32]. Essential oils and their components have been claimed to possess antibacterial [33,34]., antifungal, antiseptic, antihelminthic [35] and germicidal [36] properties.

The extract of *Passiflora incarnata* showed the presence of volatile oil also [37]. Flavonoids had been reported to exhibit multiple biological effects. The presence of flavonoids may be responsible for anti-inflammatory and antioxidant activity [38].

Antimicrobial Study:

The work on screening of antimicrobial potential of *Passiflora incarnata* is very limited. Both the aqueous and ethanolic extract of *Passiflora incarnata* L. showed different sensitivity responses in different pathogenic organism. The ethanolic extract exhibited higher response than that of aqueous extract.

Antibacterial Activity

The aqueous and ethanolic extract showed moderate activity against all tested bacterial pathogens. The different concentration of both aqueous and ethanolic extract were inhibited the bacterial pathogens in different manner. Higher concentration of the extracts show high potentiality of lysing capacity in all the five selected bacterial pathogens except *Staphylococcus epidermis* and *Serratia marcescens* in which it show slightly lesser activity. The result of the present antibacterial study was shown in (Table- 2).

Antifungal Activity

Antifungal activity of aqueous extract of *Passiflora incarnata* showed higher amount of inhibition on the *Mucor indicus* whereas lesser activity was observed against *Aspergillus niger*. The ethanolic extract of plant showed enormous antifungal activity against on all the selected fungal pathogens except *Aspergillus niger*.

Table-2

***In vitro* antibacterial activity of standard antibiotic and extracts of *Passiflora incarnata* L.**

S.No	Drug and its concentration	Zone of inhibition (mm)				
		SM	SA	KP	SE	SP
1	Sarfloxacin(10µg)	18	-	18	15	15
2	Vanomycin(30µg)	-	-	-	-	-
3	Amikacin (5µg)	19	-	20	-	18
4	Amoxycillin (10µg)	-	-	-	-	-
5	Ofloxacin(5µg)	25	19	21	10	22
6	Aqueous (100mg)	22	21	23	10	18
7	Aqueous (200mg)	23	25	28	15	28
8	Aqueous (500mg)	29	35	38	22	35
9	Ethanol (100mg)	15	20	25	16	25
10	Ethanol (200mg)	20	25	30	24	30
11	Ethanol (500mg)	29	30	35	27	35

SM- ***Serratia marcescens***
SA- ***Staphylococcus aureus***
KP-*Klebsiella pneumonia*
SE-*Staphylococcus epidermis*
SP-*Streptococcus pyogenes*

Table-3

***In vitro* antifungal activity of standard antibiotic and extracts of *Passiflora incarnata* L.**

S.No	Drug and its concentration	Afl	Afu	Ani	Glu	Min
1	Fluconazole(10µg)	-	-	-	-	-
2	Nystain(100µg)	09	**07**	**11**	**12**	-
3	Clotrimazole**(10µg)**	11	**07**	-	**12**	-
4	Amphotericin(100µg)	10	**08**	**12**	**19**	**17**
5	Aqueous (100mg)	14	**11**	**06**	**15**	**19**
6	Aqueous (200mg)	21	**15**	**10**	**19**	**31**
7	Aqueous (500mg)	22	**18**	**05**	**25**	**42**
8	Ethanol (100mg)	15	**12**	**09**	**11**	**14**
9	Ethanol (200mg)	20	**20**	**11**	**17**	**21**
10	Ethanol (500mg)	30	**22**	**12**	**22**	**25**

Afl- ***Aspergillus flavus***
Afu- ***Aspergillus fumigatus***
Ani- ***Aspergillus niger***
Glu- ***Ganoderma lucida***
Min- ***Mucor indicus***

The result of the antifungal activity was shown in (Table – 3). Generally both the aqueous and ethanolic extract of *Passiflora incarnata*, exhibited higher antibacterial activity than antifungal activity. Antimicrobial activity of higher plants was well documented [39,40,41]. These different extracts exhibited antimicrobial activity against different organism in different manner.

In this study, the selected microbes were human pathogenic organism. In the same way most of the researchers choose the human pathogens for this type of studies. Different aspects of antimicrobial activity of some medicinal plant extract against some pathogenic microbes were studied[42] .

Antimicrobial and antifungal activities of 85% ethanol and water extracts of *Alismae rhizome* were tested against *Bacillus subtilis, Staphylococcus aureus* and *Aspergillus niger* showed activity on the

concentration dependent manner. Antimicrobial activity of essential oil of *Toddalia asiatica* leaves were active against *E.coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus* and *Aspergillus niger* [42].

The antibacterial activity of *Andrographis paniculata* was well documented. In addition, research suggests that andrograghollids have a direct antibacterial effect and a direct antiparasitic effect[43]. The geographical distribution of this plant has led to its traditional use in Ayurveda (Indian), Thai and Chinese medicine. According to these traditions, *Andrographis paniculata* dispels heat (i.e., is antipyretic) and removes toxin, which makes it, a good treating agent for infections and fever causing diseases. It has been used in bacterial dysentery, arresting diarrhea and in upper respiratory infections tonsillitis, pharyngitis, laryngitis, pneumonia, tuberculosis, pyelonephritis [44].

Herbs are staging a come back and a herbal renaissance is blooming across the world. They have been prized for their medicinal, flavouring and aromatic qualities for centuries and yet for a while they were over shadowed by the synthetic products of the modern civilization. But once having realized their serious side effects, people are going back to nature with hopes of safety and security. Today, herbs are finding diverse uses in the society from medicine to manure, insecticides, pesticides and many articles of daily uses.

Summary

In India medicinal plants are unaccountable national treasure. Recently, the lot of side effects of long term use of synthetic drugs created an awareness among the people to switch over from synthetic medicine to naturally based traditional medicine.

A laborious research should have been carried out to evaluate the potentialities of herbal based medicine. It has been estimated that higher plants produce more than 100,000 secondary metabolites of which only 15-20% have been chemically characterized. As a preliminary step, in the present study the medicinal plant *Passiflora incarnata* was selected and the major phytochemicals present in this plant was analysed using standard qualitative tests. The antimicrobial activity of the aqueous and ethanolic extract of the plant was studied against five selected bacterial and five selected fungal pathogens.

Eventhough aqueous and ethanolic extract of the leaves were more powerful in to control microorganisms, the exact mechanism of action of the extract to produce the observed effect is needed to be elucidated in detail in future.

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