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ANTIMICROBIAL ACTIVITY OF LEAF EXTRACT OF PASSIFLORA INCARNATA L.

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ABSTRACT : Ethanolic extract of leaves of leaves of Passiflora incarnate included in Passifloraceae family were screened for phytochemical constituents and antimicrobial activities towards nine bacteria eight fungi. Among all extracts, ethanolic leaf extract of P. incarnate showed the highest antimicrobial activity when compared with other extracts. Phytochemical analysis of all the extracts revealed that the antimicrobial activity of the plant material is due to the presence of phenolic compounds.

Key words: Antimicrobial activity ; Passiflora incarnate ; Passifloraceae ;Phytochemicals.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Over 50 % of all modern clinical drugs are of natural product origin (Stuffness .M et al., 1982) and natural products play an important role in drug development programs in the pharmaceutical industry (Baker JT et al.,1995).Various medicinal plants have been used for years daily life to treat disease all over the world. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines .Higher plants, as sources of medicinal compounds , have continued to play a dominant role in the maintenance of human health since ancient times (Forombi Eo 2003). Plants with possible antimicrobial activity should be tested against an appropriate microbal model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extract on bacteria have been studied by a very large number of researches in different parts of the world (Reddy PS et al.,2001).Much work has been done on ethanomedical plants in India(Erdogrul OT et al., 2002). Interest in a large number of traditional natural products has been increased. It has been suggested that aqueous and ethanolic extracts from plants used allopathic medicine are potential sources of antiviral, antitumoral, and antimicrobial agents (Ates DA et al., 2003). The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps then the screening of pure compounds isolated from natural products (Maheshwari JK et al.,1986). Passiflora incarnata belonging to the family passifloraceae grows up to 10m in length .It is a perennial vine on a strong, woody, stem .The leaves are alternate, petiolate, serrate and very finely pubescent .The under surface is hairier than the upper surface. There are bumpy extra-floral nectarines on the leaf blades. Stipules and tendrils grow from the leaf axiles(Krishan Marg et al.,2003). White or lavender coloured flowers with nectar glands, versatile, sweet smelling yellow fruits. Many medicinal uses have been recorded for the plant, it is reported to possess sedative to treat nervous anxiety and hysteria . The dried herb is frequently used as medicinal tea. A sedative chewing gum has been produced .It is used as local application for ulcer , sores, anxity, asthma, epilepsy, heart problems, hyper tension, hysteria, insomnia, menstrual disorders, mood disorders, neuralgia, nicotine addiction, pain, sexual dysfunction, shingles, spasms,(Herbclip 1996, Daniel B et al.,1993)In developing countries, infectious diseases remain the main cause of the high mortality rates recorded. In modern medical practice, the alarming world wide incidence of antibiotic resistant cause an increasing need for new compounds. Therefore the aim of present work is to investigate the potential phytochemical constituents and antimicrobial activity of Passiflora incarnate L.

MATERIAL AND METHODS

Collection and identification of plant material

The plant Pqassiflora incarnate L was collected from Ooty hills during different period. The collected plant was carefully examined and identified with the help of regional flora (Gamble,J.S.,1967), (Mathew,K.M.,1983). Specimen was further confirmed with reference to Herbarium sheets available in the Rapinat Herbarium , St. Joseph college, Thiruchirapalli.

Preparation of leaf powder

Leaves of Passiflora incarnata were collected and dried under shade. The dried materials were mechanically powdered after keeping them in an oven at 35°C for 24 hours . These powder materials were used for further investigation(Harborne J.B .,1973).

Preparation of extract

25g of powder was filled in the thimble and extracted successively with ethanol using a soxhlet extractor for 18hrs(Prakash A et al .,1975).All the extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight bottle until further use. All the extracts were subjected to phytochemical analysis and antimicrobial activity assay.

Phytochemical analysis

A small portion of the dry extract was used for phytochemical screening test (Trease G E et al.,1983), (Harborne J B.,1973).Dragendorffs reawere used to test for alkaloids, ferric chloride for tannins , while Bebedict´s solution was used to test for saponins.

Test organisms

The following organisms were employed for this study as test organisms:

Bacteia

Klebsiella pneumoniae , Staphylococcus aureus , Staphylococcus epidermis, Streptococcus pyogenes , Serratia marcens , Citrobactor divercence , Proteus vulgaris , E. coli , Aeromonas hydrophylla .

Fungi

Saccaromysin servicia , Aspergillus fumigatus , Aspergillus flavus , Rhizopus, Fusarium, Aspergillus niger , Aspergillus candicus , Candida albicans.

The bacteral and fungal pathogenic strains were obtained from the Microbial Type Culture Collection (MTCC), The Institute of Microbial Technology, sector39-4, Chandigarh , India(Bauer , A.W et al.,1996).

Antimicrobial activity(Collins ,C.H et al.,1970)

Antimicrobial activity assay was conducted against Klebsiella pneumonia,Staphylococcus aureus, Staphylococcus epidermis Streptococcus pyogenes, Serratia marcescens , Citrobactor divercence, Proteus vulgaris , E.coli, Aeromonas hydrophylla and fungus like Saccaromysin servicia, Aspergillus fumigatus, Aspergillus flavus , rhizopus, Fusarium, Aspergillus niger, Aspergillus candicus, candida albicans.These microbial cultures were obtained from culture collection from the Institute of Microbial Technology , Chandigarh, India.

The sterile Nutrient Agar(NA) medium(Peptone 5g;Beef extract 3g;NaCl 2g and Agar 15g/litre;Ph 7) and Potato Dextrose Agar (PDA)medium (200g Potato extract , Dextrose 20g/Agar 16g/litre ;pH6) were used as basel media for growing these pathogenic bactria and fungus respectively.Inoculums of the pathogen for the assay were prepared in liquid media of the respective compective composition . One ml of the broth inoculums was mixed with medium poured into the Petri plates and allowed for solidification. After solidification 6mm diameter duplicate well was made with the help of a sterile cork borer in the medium. In each well 100ml of the filtrate was poured. All the plates were incubated at room temperature and the zone of inhibition was recorded. For bacteria , the plates were incubated for 24 hours and fungi 48 hours. Solvents used for extraction served as control.

RESULT AND DISCUSSION

Phytochemical analysis of all the extracts revealed that the presence of alkaloids, carbohydrates, phytosterols , fixed oils and fats , phenolic compounds,tannins, flavonoids, proteins, aminoacid, saponins, gums, mucilage and volatile oils in etanolic extracts of Passiflora incarnate(Table-1).Further phytochemical analysis of ethanolic extract revealed that the antimicrobial activity is due to the presence of phenolic compounds.

The phytochemical analysis of the Passiflora incarnate leaf extract shows the presence of tannins, alkaloids, flavonoids and phenolic compounds .Tannins have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of the cell protein synthesis (Hagerman AE et al., 1981).This activity was exhibited against test organisms with the plant extracts.

Table-1 Qualitative phytochemical screening of leaf (successive extracts)

S.No	Tested for	Reagents used	Pet ether	Benzene	Chloroform	Alcohol	Water
1.	Carbohydrate	Felling's	+	+	+	+	+
2.		Molisch's	+	+	+	+	-
3.		Benedict	+	+	+	+	-
4.	Alkaloids	Dragendraff's	+	+	+	+	+
5.		Wagner's	+	-	+	+	-
6.		Hager's	+	+	+	+	+
7.		Mayer's	+	-	+	+	-
8.	Tannins & Phenol	Lead acetate	-	-	-	+	-
9.		$FeCl_3$	-	-	-	+	-
10.	Phytosterol	LB test	+	+	+	+	-
11.	Saponins	Foam test	+	+	+	+	+
12.	Gums & Mucilage	Alcohol ppt.	+	+	+	+	+
13.	Flavanoids	Zn + Con. HCl	+	+	+	+	+
14.		Shinoda test	+	+	+	+	+
15.	Fixed oils & fats	Spot test	+	+	+	+	-

(+) Presence (-) Absence

Antimicrobial assay

Ethonolic extracts were tested against bacteria and fungi. The leaf of Passiflora incarnata were effective against bacteria and fungi(Table-2,3)(Figer-1-2).Apart from antimicrobial activity exhibited by tannins, they also react with proteins to provide the typical tanning effect.

Medicinally ,this is important for the treatment of inflamed or ulcerated tissues(Mota MLR et al.,1985).Tannins have important roles such as stable and potent antioxidants(Trease GE et al .,1983) Herbs that have tannins as their main component are astringent in nature and used for treating intestinal disorders such as diarrhoea and dysentery(Raffauf RF et al., 1996) , thus exhibiting antimicrobial activity. One of the largest group of chemical produced by plant are alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications.

Passiflora incarnata is used for the treatment of inflammation , wound healing, antitumor and antinelgesic , hence different formulation could be prepared for clinic trials. It is hoped that this sudy would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

Table-2 Invitro antibacterial activity in ethanolic extract of Passiflora incarnata leaf.

S.No	Name of the organisms	Zone of inhibition (mm)		
		50mg	100mg	200mg
1	Citrobacter divercence	1.68	2.00	2.25
2	Serratia marcercens	1.57	2.81	3.37
3	Staphylococcus aureus	1.68	1.08	2.00
4	Staphylococcus pyogenes	-	-	-
5	Staphylococcus epidermis	-	-	-
6	Proteus vulgarin	-	-	-
7	Escherichia coli	-	-	-
8	Klebsiella pnemoniae	-	-	-
9	Aeromonas	-	-	-

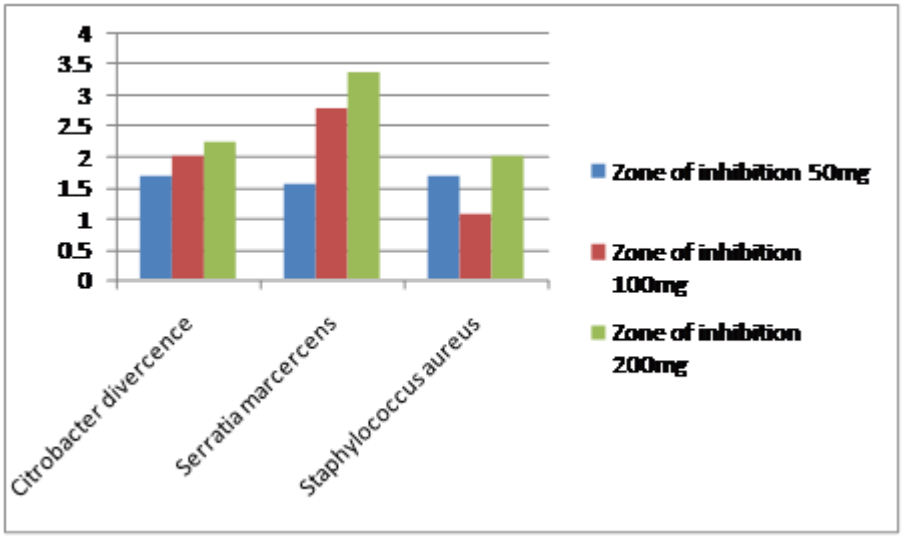

Fig-1 Invitro antibacterial activity in ethanolic extract of Passiflora incarnata leaf.

Table-3 Invitro antifungal activity in ethanolic extract in Passiflora incarnate leaf.

S.No	Name of the organisms	Zone of inhibition (mm)		
		50mg	100mg	200mg
1	Saccaromysin servicia	1.68	1.07	2.81
2	Aspergillus fumigatus	1.57	2.00	2.25
3	Aspergillus flavus	0.78	1.68	2.25
4	Rhizopus	-	-	-
5	Fusarium	-	-	-
6	Aspergillus niger	-	-	-
7	Aspergillus Candicus	-	-	-
8	Candida albucans	-	-	-

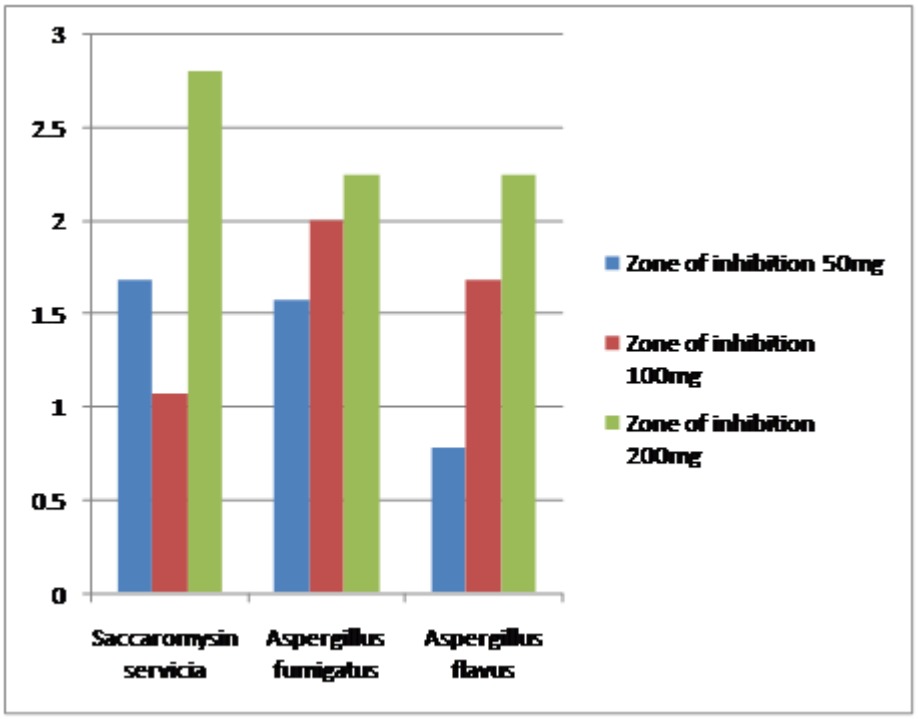

Fig-2 : Invitro antifungal activity in ethanolic extract in Passiflora incarnate leaf

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