

*Full Length Research Paper*

# Exploring *Passiflora incarnata* (L.): A medicinal plants secondary metabolites as antibacterial agent

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Accepted 1 July, 2010

Different solvent extract of *Passiflora incarnata* (L.), known for antibacterial activity, were investigated for antibacterial efficacy against *Staphylococcus aureus* (oxacillin resistance strain) and *Escherichia coli* by disc diffusion method. The secondary metabolites extracted in methanolic extract were found to be more potent as compared to ethyl acetate, as it shows minimal inhibitory concentration (MIC) at extreme lower dilutions. Thin layer chromatography (TLC)-bioautography indicated passicol as a major active compound in ethyl acetate and flavonoid in methanol extract.

**Key words:** *Passiflora incarnata* (L.), passicol, secondary metabolites, antibacterial activity.

## INTRODUCTION

Plant compounds are of interest as a source of safer or more effective substitutes than synthetically produced antimicrobial agents. One such plant is *Passiflora incarnata* (L.), which is a perennial creeping vine, used both as an edible and for medicinal purposes. Passion flower is widely employed by herbalist and natural health practitioners around the world today. Phytochemical investigation of *P. incarnata* and *Passiflora edulis* and occasional analysis of other species revealed that the members of this genus contain alkaloids, phenols, cyanogenic compounds and glycosyl flavonoids. The majority of the active components in this plant are C-glycosyl flavones based on apigenin and luteolin, while harman alkaloids are found in trace amounts, sugar (sucrose) and trace of volatile oil (Bradley, 1992; Leung and Froster, 1996; Newall et al., 1996).

Birner and Nicolls (1973) showed that the antimicrobial active principle, now named Passicol, in *Passiflora mollissima*, *Passiflora caerulea* and *P. edulis* fruit rinds, had ultraviolet absorption characteristic of the polyacetylenic group of compounds. While various pharmacological studies have conformed sedative, antispasmodic and anxiolytic activity of several chemical fractions definitive attribution to an active component has not been achieved.

Natural products play an important role in drug development programme of pharmaceutical industry (Baker et al., 1995). Thus our study was aimed to explore active

principle of various solvent extracts from *P. incarnata* (L.) leaves, which are claimed to be responsible for antimicrobial properties.

## MATERIALS AND METHODS

### Collection of plant material

The plant material that is leaves of *P. incarnata* (L.) were locally collected from Tapovan Area, Amravati (M.S) India. The plants were authenticated by using standard flora and are cross checked with herbarium records at the institute as *P. incarnata* (L.).

### Test microorganisms

Culture of bacteria viz. oxacillin resistance strain *Staphylococcus aureus* (ORSA) and *Escherichia coli* were obtained from Department of Biotechnology, SGB, Amravati University, Amravati and are maintained on nutrient agar for further use.

### Preparation of plant extract

The plant extract were prepared using ethyl acetate, methanol and aqueous phase. The leaves were cut into circular shape (about 1 cm) with sterile blade and washed with distill water and then by 70% alcohol. The extract were prepared from 100 gm cut leaves in 100 ml sterile distill water in 250 ml conical flask. The system was kept at 37°C and at 80 rpm. Harvesting was conducted by removing diffusate and replacing with an equal volume of sterile distilled

**Table 1.** *In vitro* antibacterial activity of secondary metabolites (Passicol) isolated from of *P. incarnata* (L.) in ethyl acetate.

Dilution of ethyl acetate extract	Conc. ( $\mu\text{g/ml}$ )	Test microorganism (Zone of inhibition in mm)	
		<i>S. aureus</i>	<i>E. coli</i>
00			
1:1	250	9	9
1:2	125	7	6
1:4	62.5	6	<6
1:8	31.25	5	4
1:16	15.62	> 4	3
1:32	7.81	> 4	3
1:64	3.90	> 4	3
1:128	1.95	4	3
1:256	0.97	> 3	>2
1:512	0.48	3	>2
1:1024	0.24	3	<2
1:2048	0.12	3	No zone
Control	Ethyl acetate	No zone	No zone

water. For preparing the ethyl acetate extract, acidify the diffusate with 12N  $\text{H}_2\text{SO}_4$  to pH of 1 to 1.5 and again extract with 0.35 volume of ethyl acetate using separating funnel for 10 min. It was further centrifuged and the pooled organic phase was evaporated to concentrate the extract. The methanolic extract were prepared from 2 gm fresh leaves which was grinded in pestle mortar, extracted in 5 ml methanol on sonicator (Biologics Model 150 V/T) for 20 min. The extra solvent was again evaporated to concentrate the extract.

The aqueous leaves extract were prepared by crushing 1 gm fresh leaves in blender for 5 min, together with the 1 ml of distilled water. After filtering through membrane filter (0.2  $\mu\text{m}$ , Nalgene<sup>R</sup>) the raw extract were obtained. To predict the activity of dried leaves, they were dried in shade for 15 days and reduced to coarse powder and then extracted with distilled water. Each solvent extract prepared above was tested further for its antibacterial activity against *S. aureus* (ORSA) and *E. coli*.

#### Determination of minimal inhibitory concentration

The antibacterial potency of extract was determined in terms of minimum inhibitory concentration (MIC), by using the filter paper disc diffusion method (Pelczar et al., 1998). The lawn of test bacterium that is *S. aureus* (ORSA) and *E. coli* were prepared using glass spreader. The filter paper disc (3 mm) which were presoaked in respective extract and their dilutions for 20 min (excess solvent air dried) were kept onto the bacterial lawn. The plates were left on bench for one hr before incubation at 37°C for 24 h to allow prediffusion of extract (Esimone et al., 1998). The zone of inhibition (in mm) was measured using zone measuring scale and compared with control set (disc with solvent). The MIC value was taken as the lowest concentration of the extract showing complete lack of growth after the incubation period (EUCAST, 2000a).

#### Thin layer chromatography fingerprinting

The presence of active metabolites in *P. incarnata* (L.) extracts was done on Thin Layer Chromatography (TLC) plates. The 10  $\mu\text{l}$  of

each test sample were spotted onto the silica plates. The plates were developed in saturated chromatographic chamber, saturated by 100 ml solvent system (ethyl acetate: chloroform-2:1) for ethyl acetate extract, (chloroform : methanol: water- 8:2:0.3) for methanolic extract and (chloroform : methanol: 10%NH<sub>3</sub> -80:40:1.5) for aqueous phase and dried leaves extract. The development of plate required about 45 - 50 mins which then visualized under a ultraviolet (UV) transilluminator (Photodyne). Different plant components show different colored spots, whose retardation factors (Rf) were measured against solvent front and were compared with the standard retardation factor values given in literature as Passicol standard is yet not available.

## RESULTS AND DISCUSSION

The antibacterial activities of secondary metabolites of *P. incarnata* (L.) extracted in ethyl acetate and methanol was summarized in Tables 1 and 2. The result indicates that, the MIC of ethyl acetate extract was 0.12  $\mu\text{g/ml}$  for *S. aureus* (ORSA) and 0.24  $\mu\text{g/ml}$  for *E. coli*. while MIC for methanol extract was found out at 0.0037  $\mu\text{g/ml}$  for *S. aureus* (ORSA) and 0.0009  $\mu\text{g/ml}$  for *E. coli*, while aqueous extract and dried leaves extract do not possess any antibacterial activity. As the secondary metabolites shows highest antibacterial activity in methanolic extract as compare to ethyl acetate against *S. aureus* (ORSA) and *E. coli* (shown in Figures 1 and 2), to substantiate the claim, for the presence of active metabolites, TLC was performed.

The TLC of ethyl acetate extract on silica plate separates the sample into fourteen colored bands (spots) having different Rf shown in Table 3 and Figure 3a. The green spot having Rf 0.79 to 0.81 (strongly fluorescent) was scraped off, eluted in water shows bromine water

**Table 2.** *In vitro* antibacterial activity of secondary metabolites (Flavonoid) isolated from of *P. incarnata* (L.) in methanol.

Dilution of ethyl acetate extract	Conc. (µg/ml)	Test microorganism (Zone of inhibition in mm)	
		<i>S. aureus</i>	<i>E. coli</i>
Pure	1000	No zone	No zone
1:1	500	No zone	>10
1:2	250	No zone	10
1:4	125	No zone	10
1:8	62.5	No zone	10
1:16	31.25	No zone	< 10
1:32	15.62	No zone	<10
1:64	7.81	No zone	10
1:128	3.90	10	>10
1:256	1.95	12	>10
1:512	0.97	13	>10
1:1024	0.48	14	11
1:2048	0.24	>10	11
1:4096	0.12	18	11
1:8192	0.06	23	11
1:16384	0.03	13	11
1:32768	0.0	13	12
1:65536	0.0075	11	13
1:131072	0.0037	>10	15
1:262144	0.0018	No zone	14
1:524288	0.0009	No zone	11
1:1048576	0.00045	No zone	No zone
Control	Methanol	No zone	No zone

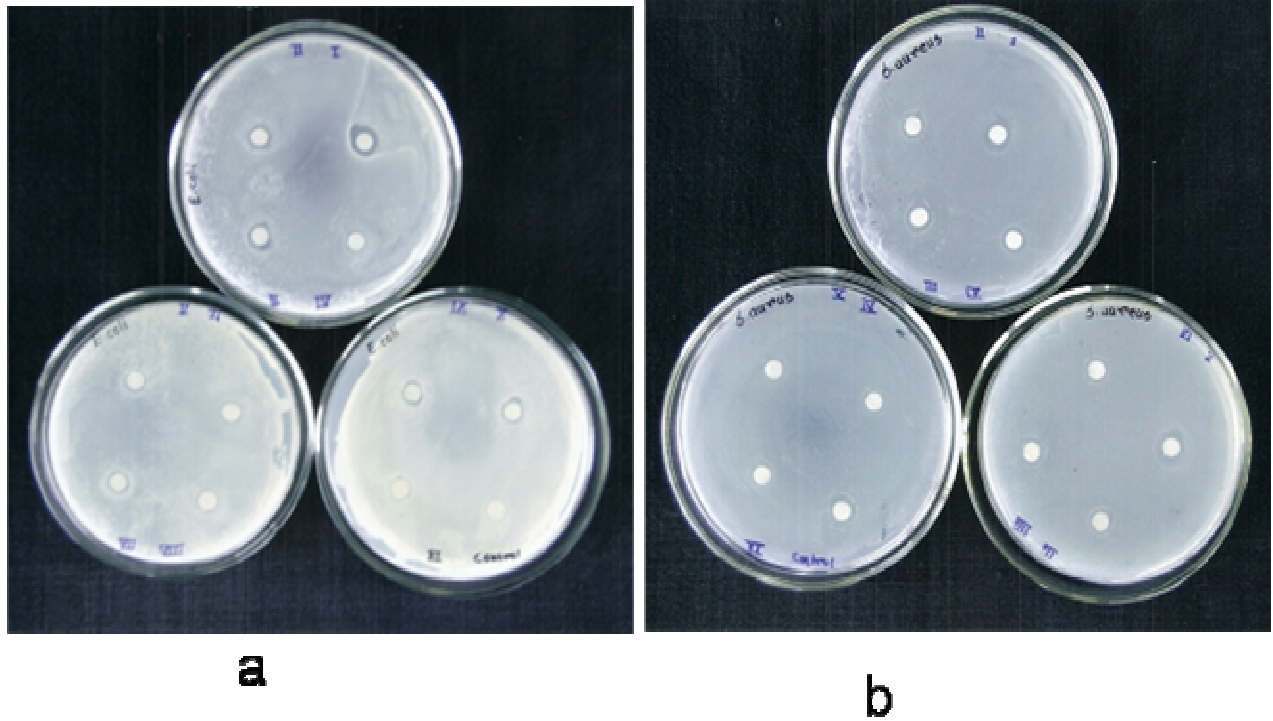
test positive. When small quantity of compound are spread on surface of silica plate and exposed to air shows instability. This indicates the presence of polyacetylene group of compounds in ethyl acetate extract. The presence of such compound as antimicrobial agent was reported by Nicolls et al. (1973) with *r<sub>f</sub>* 0.79 and 0.81 and unstable acetylene group named as "Passicol". Birner and Nicolls. (1973); Bendini et al. (2005) and Yuldasheva et al. (2005) support these facts in other species also.

The TLC of methanolic extract has five different colors spots having different *R<sub>f</sub>* shown in Table 3 and Figure 3b. According to Wagner et al. (1996) Passifloraceae is characterized by six to eight yellow-green zones of flavone-c-glycosides between the start and *R<sub>f</sub>*-0.65: isorientin as major zone (*R<sub>f</sub>*-0.45), the green zone of isovitexin and vitexin. Isovitexin-2"-0-glucoside (*R<sub>f</sub>*-0.2) and additional zones above and below. Li et al. (1991) identified schaftoside, isoschaftoside, Isovitexin-2"-0-glucopyranoside and iso orientin-2"-0-glucopyranoside as four major c-glycosidic flavonoid from *P. incarnata* (L.) on the basis of mass spectral and <sup>13</sup>C NMR data. So the review confirms the antimicrobial activity of methanol extract may be attributed due to the presence of

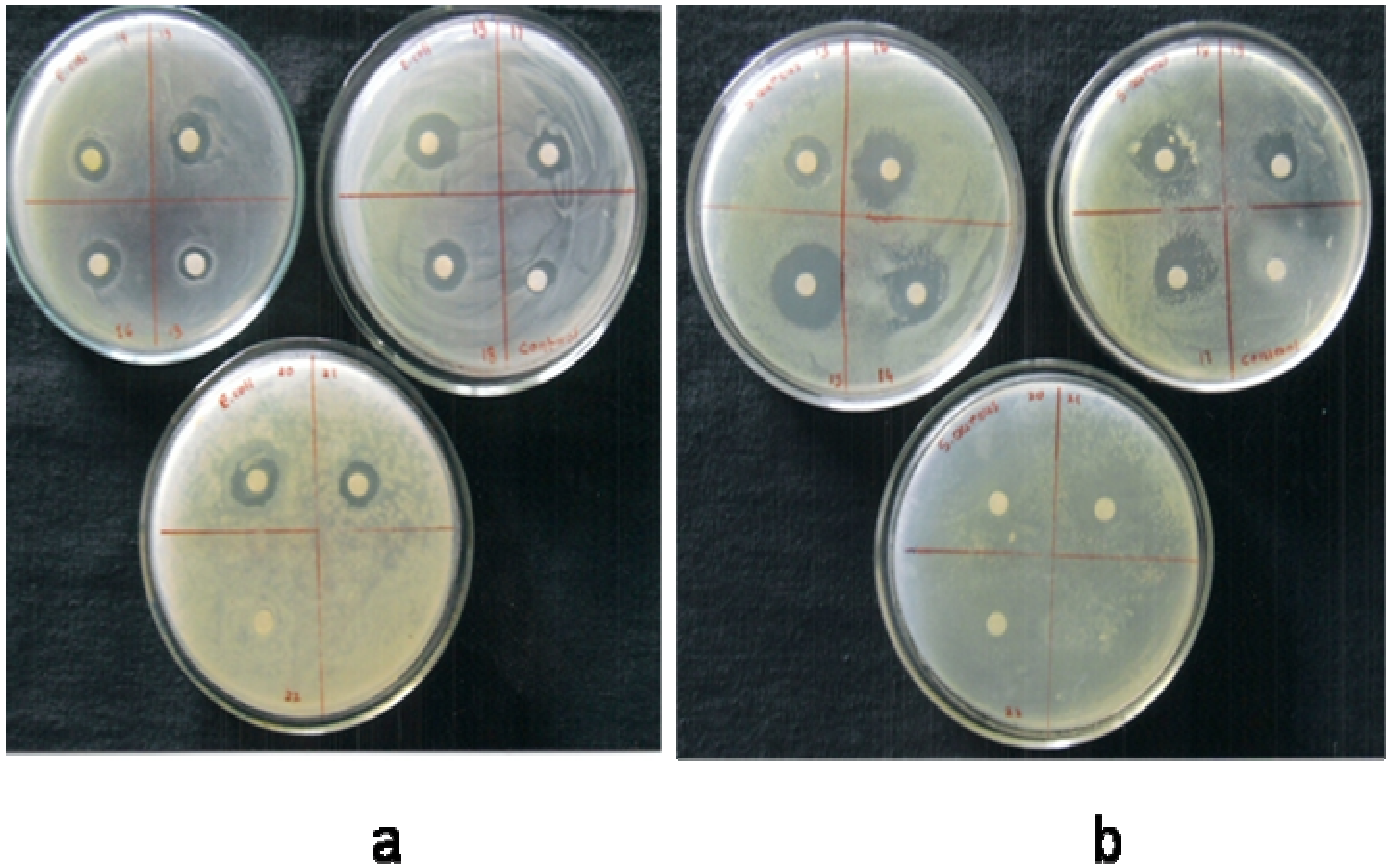
"Flavonoid".

It was also confirmed that the activity of Passicol remained high indefinitely in ethyl acetate extract, it is gradually lost in aqueous solution and quickly lost (usually within 1 or 2 days) in the dried residues (Nicolls et al., 1973). Acetylenic and polyacetylenic compound are present in some families of higher plant and higher fungi and some of them exhibit antibiotic activity (Sorensen, 1961; Thomas and Allen; 1970; Lechner et al., 1970). The presence of acetylenic group was conformed by bromine water test (Jamode et al., 1998), which was found to be positive for Passicol (ethyl acetate extract) and negative for Flavonoid (methanol extract). Wagner et al. (1996) showed the plain water infusion is effective in removing harmine and less of other plant components. Thus in present study water phase extraction does not show any separation of active principle in aqueous and dried leaves extract and also had negative antimicrobial activity.

On the basis of literature, it confirms that presence of Passicol in ethyl acetate extract and Flavonoid in methanolic extract, which contributes to antimicrobial activity. It has been proposed by some investigators (Birner and Nicolls, 1973) that Passicol present in ethyl



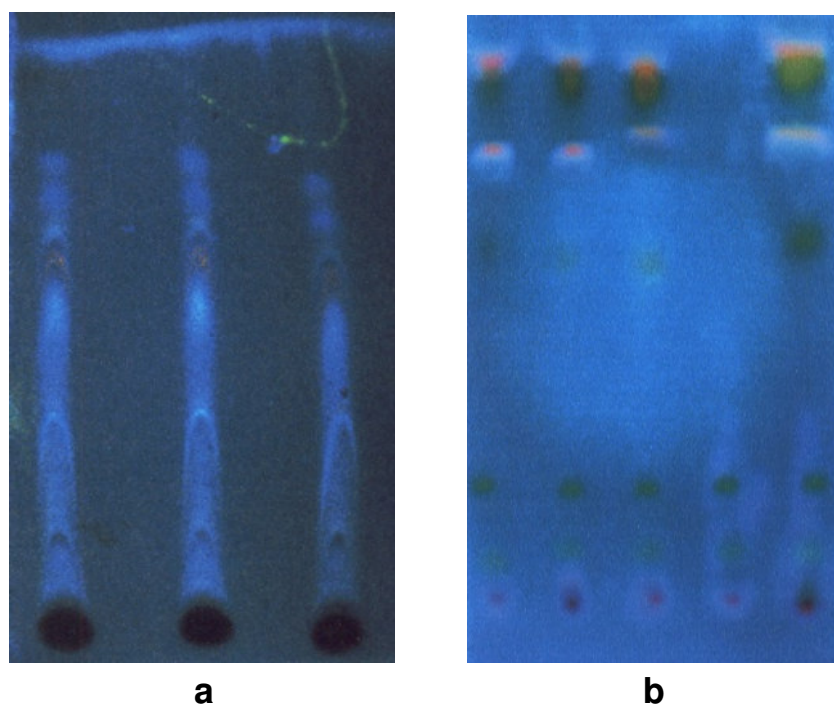
**Figure 1.** Inhibitory activity of ethyl acetate extract of *P. incarnata* (L.) against (a). *S. aureus* and (b). *E. coli*.



**Figure 2.** Inhibitory activity of methanolic extract of *P. incarnata* (L.) against (a). *S. aureus* and (b). *E. coli*.

**Table 3.** Comparative Rf values and analysis of *P. incarnata* (L.) extracts.

No. of band	Ethyl acetate extract	Methanolic extract	Aqueous extract	Standard Rf value	Possible component/color of spot
Solvent front (cm)	16.9	15	15.2	Ethyl acetate extract Rf = 0.81 - 0.83 (Nicoll et al., 1973)	Passicol is found to present in ethyl acetate
1	0.81	0.6	-	Methanol extract	
2	0.79	0.5	-	Iso-orientin 0.45;	Iso-orientin and isovitexin 2''-0-glucoside, was found to present in methanol extract
3	0.66	0.4	-	Isovitexin 2''-0-	
4	0.65	0.2	-	Glucoside=0.2	
5	0.53	0.06	-	Wagner et al., 1996)	
6	0.52	-	-		
7	0.51	-	-		
8	0.44	-	-		
9	0.42	-	-	Aqueous extract	
10	0.29	-	-	Harmine-0.1	
11	0.23	-	-	Harmala-0.75	Harmala alkaloid was not found
12	0.14	-	-	(Aoyagi et al., 1974;	
13	0.12	-	-	Soulimani, et al., 1997)	
14	0.11	-	-	-	

**Figure 3.** Separation of active metabolites from *P. incarnata* (L.) a). ethyl acetate extract and b). methanolic extract by TLC.

acetate extract was found to be antimicrobial but we found that methanol extract has the antimicrobial com-

ponent, which was more potent than ethyl acetate extract.

## ACKNOWLEDGEMENT

The research project was supported by the grants from Department of Science and Technology (DST), New Delhi under FAST TRACK no. SR/FT/LS-127/2008.

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