

# Screening for Secondary Metabolites and Anxiolytic Effect of *Passiflora foetida*

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## Abstract

From the ancient time, plant medicine is considered an important part of healthcare system because of its relative accessibility, low cost, easy availability and acceptance by local communities. A variety of plants are employed in traditional medicine to treat nervous disorder; one among the genera is *Passiflora*. The objective of the present investigation was to screen the availability of secondary metabolites in methanolic extract of *Passiflora foetida* and to check its potential for the anti-anxiety activity using Elevated plus Maze (EPM) animal model. The crude extract showed the presence of Alkaloids, Coumarins, Flavonoids, Glycosides, Oils and fats, Phenols, Resin, Sterols, Steroids and Saponins, Tannins and Quinones which then subjected to anxiolytic activity at the doses of 200 and 400 mg/kg, per oral in mice and its efficacy was statistically compared with the standard anxiolytic drug, diazepam (3 mg/kg, Intraperitoneal). As a result, the extract increased the number of entries as well as the time spent in open arm as a dose dependent manner and proved to be statistically significant. These results provide support for the use of *Passiflora foetida* as traditional medicine for its Anxiolytic property.

**Keywords:** Anti-anxiety, Diazepam and Elevated Plus Maze

## 1. Introduction

The natural plant products have medicinal value and provides new source of drugs that can be used in traditional medicine. According to Eloff<sup>1</sup>, “The use of phytochemicals for pharmaceutical purpose and the derived bioactive compounds from medicinal plants were used effectively by 80% of individuals in developed countries”. Studies by Badessarini *et al.*<sup>2</sup> reported, “Anxiety as the major symptom of psychiatric disorders and is an unavoidable thing during medical and surgical conditions. Also the

symptoms of anxiety are commonly associated with depression”. Thakur and Rana<sup>3</sup> stated that, “Though there are lot of drugs available in chemotherapy, they have side effects. While natural medicine would be the best choice to avoid side effects with easy accessibility. There were a numerous medicinal plants reported to possess anxiolytic property”. Speroni and Minghetti<sup>4</sup> reported that, “*Passiflora carnata* is one among them, where the fresh or dried or whole plants of it was traditionally accepted for the treatment of nervous anxiety, which made a key interest to investigate the same effect in *Passiflora foetida*”.

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Several studies<sup>5-9</sup> have shown that, “Pharmacological studies of passion flower have antispasmodic, sedative, anxiolytic and hypotensive activity.

A research by Phengklai *et al.*,<sup>10</sup> suggested that, “*P. foetida* extracts possess antidepressant effects that could be used in the treatment of patients with depressive disorders”. Mohanasundari *et al.*,<sup>11</sup> studied that, “*Passiflora foetida* belongs to the family, Passifloraceae, is a fast growing and spreading vine [Mossukkattan or Poonaipudukku (in Tamil) and Stinking passion flower (in English)] found in riverbeds, dry forest floors, way side thickets, covered over the top of thorny shrubs and also grows near hamlets”. Patel *et al.*,<sup>12</sup> reported that, “The leaves are used for preparing tea to get relief from sleeping and nervous disorders” and Ingale and Hivrale<sup>13</sup> employed “leaves in baths for skin infections”. Expectorant for nervous conditions, spasms<sup>14</sup> and anti-inflammatory<sup>15</sup>. The research of Dassanayaki and Hicks<sup>16</sup> identified, “Major phytochemicals like alkaloids, phenols, glycosides, flavonoids and cyanogen compounds in *Passiflora foetida*” Further studies<sup>17-21</sup> showed, “The bioactive compounds such as C-glycosyl flavonoids apigenin, luteolin, chrysoeriol, kaempferol, isovitexin and vitexin”, Cyanohydrin glycoside tetraphyllin A, tetraphyllin B, tetraphyllin B sulphate, deidaclin, volkenin, fatty acids linoleic acid and lenolenic acid, alpha-pyrone named passifloricin”.

Even though a lot of reports are available on the phytochemical screening and pharmacological activities of *Passiflora foetida*, experimental report for the anxiolytic activity remained unexplored. Therefore, the present research was focused on the evaluation of anxiolytic activity of methanolic extract using animal models. The phytoconstituents may vary according to the location and season, so to confirm its presence and to know the phytoconstituents responsible for the anxiolytic activity, preliminary screening of secondary metabolites were carried out.

## 2. Materials and Methods

### 2.1 Collection of Plant Materials

*Passiflora foetida* (Figure 1) was collected and authenticated by the Botanical survey of India and the voucher specimen was registered with no: BSI/SRC/5/23/2014-15/Tech-1085.



**Figure 1.** Image of *Passiflora foetida*.

### 2.2 Preparation of Plant Extract

By the procedures of several studies<sup>22-25</sup>, “The leaves of *Passiflora foetida* were collected and washed with water. They were dried under shadow and powdered by pulveriser following extraction with methanol using maceration technique”. The extract was filtered and it was evaporated to dryness under reduced pressure using rotary evaporator, which was then used for phytochemical screening and anxiolytic activity.

### 2.3 Preliminary Screening of Secondary Metabolites

According to the standard procedures described by Sofowara, Trease and Harborne<sup>26-28</sup>, “the qualitative analysis of phytochemicals in the methanol extract of *Passiflora foetida* were screened.”

### 2.4 Experimental Animals

Adult male BALB/c mice weighing between 20–30g were housed as groups in polypropylene cages (11cm × 17cm × 28cm) under controlled conditions of light (12 h light–dark cycle) at temperature (25±2°C). They were fed with a standard pellet diet and water *ad libitum*. Prior to experimentation, the animals were acclimatized to

laboratory condition for one week and fasted overnight before the day of study. The experimental protocol was approved in accordance to the guidelines of IAEC (688/PO/Re/S/02/CPCSEA).

## 2.5 Experimental Design

According to the previous study<sup>29,30</sup>, “The animals were divided into four groups containing six in each group according to the study reported”. The dosage of test extract was selected in accordance with the acute toxicity studies<sup>31</sup> reported in mice which revealed that, “there was no alteration in the general behaviour of mice with no mortality even after 3 days at a highest dose of 2000 mg/kg”. The grouping was done as follows,

Group I :Control (Normal saline 10 ml/kg, *Per oral*)

Group II :Standard (Diazepam 3 mg/kg, *intraperitoneal*)

Group III :Test I (Methanolic extract of *Passiflora foetida* 200mg/kg, *Per oral*)

Group IV :Test II (Methanolic extract of *Passiflora foetida* 400mg/kg, *Per oral*)

## 2.6 Elevated Plus Maze Model of Anxiety

### 2.6.1 Elevated Plus Maze

As per the procedure<sup>32</sup>, “The elevated plus-maze comprised of two open (16cm × 5cm) and two enclosed (16 cm × 5cm × 12cm) arms that radiated from a central platform (5cm × 5cm) to form a plus sign. The maze was constructed using black painted wood. Slight raised edge on the open arms (0.25 cm) was provided with additional grip for the animals and the plus - maze was elevated to a height of 25 cm above floor level by a single central support”. “Four 25 W red fluorescent lights arranged as a cross at 50 cm above the maze were used as the source of illumination. The experiment was conducted during the dark phase of the light cycle (9:00–14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. The number of entries after 30 minutes of drug administration into each of the two types of arm was counted during a 10 minutes test period. The percentage of open arm entries was used as indices of anxiety. An arm entry was defined when all four paws of the mice were in the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels. All behavioral recordings were carried out with the neutral

blind observer unaware of the treatment that the mice had received”<sup>33</sup>.

## 2.7 Statistical Analysis

“The results have been expressed as mean ± standard deviation (S.D.). The test doses were compared among themselves and also with diazepam and control by one-way analysis of variance (ANOVA) followed by Dunnett’s “t” test”<sup>34</sup>.

## 3. Results and Discussion

### 3.1 Qualitative Screening of Secondary Metabolites in METHANOL Extract of *Passiflora foetida*

The qualitative screening of secondary metabolites is presented in (Table 1).

The qualitative screening of secondary metabolites had shown the presence of various phytochemicals such as Alkaloids, Coumarins, Flavonoids, Glycosides, Oils and fats, Phenols, Resin, Sterols, Steroids, Saponins, Tannins, and Quinones whereas Phlobatannins and Terpenoids found to be absent (Table 1). It was found that the flavonoids were abundant in the screening which added strong support to the report of Patil *et al.*, 2013<sup>35</sup>. Next to the flavonoids, glycosides and saponins were the major phytoconstituents present in the methanolic extract of *Passiflora foetida*. In the current study, when the animals were placed on the Elevated Plus Maze, the fear due to height induced anxiety which decreased the motor activity and made preference to remain at safer places.

### 3.2 Anxiolytic Effect using EPM for Methanol Extract of *Passiflora foetida*

The Anxiolytic effect using EPM for Methanol extract of *Passiflora foetida* is given in (Table 2).

According to Kumar and Sharma<sup>36</sup>, “Antianxiety agents were expected to increase the motor activity which could be measured in terms of the time spent by the animal in the open arms”. In such a way, methanol extract of *Passiflora foetida* with concentration (200mg/kg and 400mg/kg) had shown a dose dependent increase in number of entries as well as the time spent in open

**Table 1.** Screening of secondary metabolites in methanol extract of *Passiflora foetida*

S.No.	Secondary metabolite	Result
1	Alkaloids	Present
2	Coumarins	Present
3	Flavonoids	Present
4	Glycosides	Present
5	Oils and fats	Present
6	Phenols	Present
7	Phlobatannin	Absent
8	Resin	Present
9	Sterols	Present
10	Steroids	Present
11	Saponins	Present
12	Tannins	Present
13	Terpenoids	Absent
14	Quinones	Present

**Table 2.** Anxiolytic effect using EPM for Methanol extract of *Passiflora foetida*

S.No.	Experimental group	Dose (mg/kg)	Number of Entries Mean±S.D		Time spent (in Seconds) Mean±S.D	
			Open arm	Closed arm	Open arm	Closed arm
1	Control -Normal saline	10ml	8.5 ±0.32	7.62±0.14	155.63± 1.62	145.13±5.55
2	Standard drug (Diazepam)	3	7.32±0.58	5.66 ± 0.26*	256.58±9.60**	12.62±0.95***
3	Test group I (Methanol extract)	200	7.61±0.62	6.72 ± 0.34	177.32 ±7.33	98.64 ± 6.3*
4	Test group II (Methanol extract)	400	9.50±0.61	7.85 ±0.42	195.66 ±5.56*	54.33±0.13**

n=6; The data is expressed as Mean ± S.D.;\*P< 0.05, \*\*P < 0.01 and \*\*\*P < 0.001: Statistically significant when compared to standard.

arm compared to control group without causing any neuromuscular side effects (Table 2). The current research had given a strong experimental evidence for the

anxiolytic property of *Passiflora foetida* and it is believed that as per the studies<sup>37-41</sup>, “the phytoconstituents present in the methanolic extract had revealed such an activity

especially due to the presence of flavonoid, apigenin, kaempferol, Isovitexin and Saponin, which were reported earlier in other plants to support the current study". Although a variety of plants were reported to possess anxiolytic action<sup>42,43</sup>, the present study had paved the way to include *Passiflora foetida* in the list of anxiolytic plants.

## 4. Conclusion

The study revealed an experimental proof on anxiolytic property of *Passiflora foetida* with the constituents responsible for it. Further researches are essential to isolate, identify, quantify, characterize and elucidate the structure of the particular bioactive compounds responsible for the action. Also the compound responsible can be subjected to know the target of action and by performing docking studies, the interaction between the drug and the target could be studied for the better understanding.

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