

## Phytochemical analysis & in-vitro cytogenetics assay of chromosomal aberration on peripheral human blood lymphocytes by the leaf crude extract- *Passiflora foetida* L

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### ABSTRACT

The present investigation is to evaluate the analysis preliminary photochemical screening and *In-vitro* cytogenetic assay measuring chromosomal aberration frequencies induced by the leaf crude extract *Passiflora foetida* L. as an active ingredient in peripheral human blood lymphocyte. Whole blood lymphocytes were cultured *in-vitro* using a standard protocol and the treatment with the leaf crude extract. The plant extract and fractions revealed preliminary phytochemical compounds with no toxicity. The present investigation revealed that no chromosomal aberration was found in the test sample. And also this study supports, the traditional medicines (herbal extracts) to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases. The toxicological data are used to evaluate the safety and efficacy of new chemical entities (NCE). One of the most important aspects of safety pharmacology is the evaluation of genotoxicity potential of the NCE. Genotoxicity studies are conducted both at non-clinical and clinical levels.

**Keywords:** *Passiflora foetida* L., Methanol extracts, Genotoxicity, RPMI-60, Mytomycine C, Chromosomal Aberration.

### INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. This traditional knowledge forms the codified systems of medicine and exists in the forms of Ayurveda, Unani, Siddha and Swariga (Tibetan) systems of medicine. The flora and fauna are used for medicinal purposes and they have important cultural roles and as well as vital roles in forest ecology, such as pollination, seed predation and dispersal, seed germination, herbivory and predation on potential pest species (Perumalsamy, *et al.*, 2007). Ethno medicinal study deals with the study of traditional medicines. Since ancient times mankind has been using herbal plants, organic materials as

well as materials from the sea, rivers etc. for its betterment. These substances have been used as food, medicine etc. Amongst them, the substances having medicinal value have been extensively used for treating various disease conditions. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Various parts of the plants like roots, leaves, bark, exudates etc. are used as per medicinal properties (Mehta kavit, *et al.*, 2013). Botanical name: *Passiflora foetida* L., Tamil name: Punaipazham, Common name: Love-in-a-mist, Stinking passionflower. The genus *Passiflora* belongs to Passifloraceae family includes the passion fruit, is the largest and the most widespread genus of tropical flora. In India it is used as an herbal medicine and called 'Punaipazham'.

**Figure-1. *Passiflora foetida* L.**

Leaves of the plant utilized as folk medicine for treatment of anti-anxiety, stress and insomnia. Additional, they are also useful for the treatment of hysteria, skin inflammation, cough and fever. Chemical constituents in *P. foetida* L. include hydrocyanic acid, groups of flavonoids and Harman alkaloids. In the Asia Continent, the leaves decoction of this plant is used in India as emmenagogue and to treat asthma, biliousness, hysteria whereas in America, Brazilians use the herbs in the form of lotions or poultices for erysipelas and skin diseases with inflammation (Dhawan *et al.*, 2004). Some pharmacological properties of *P. foetida* have been studied. It is found to have anti-parasite, anti-bacterial, anti-fungal and antioxidant activities (Rasool *et al.*, 2011).

Furthermore, this plant exhibited hepatoprotective, anti-depressant, anti-carcinogenic, analgesic and anti-inflammatory properties (Balasubramaniam *et al.*, 2010). The use of *P. foetida* L. in the treatment of women infertility suggests that this plant could have some estrogenic and/or anti-estrogenic properties. Since synthetic estrogens are known to cause endometrial or breast cancer and other adverse effects (Kellen *et al.*, 1996), the use of plants as new natural sources of estrogens is investigated and encouraged.

## METHODOLOGY

### Plant Collection

Leaves of the plant *Passiflora foetida* L. were identified and collected in the winter season at Loyola college campus. Fresh healthy leaves were separated from stems, thoroughly washed

2-3 times with water and dried in shade at room temperature. The dried plants were milled to a fine powder with the help of a blender and stored at room temperature in closed containers in the dark until used.

**Figure-2. Leaf crude powder**

### Preliminary Phytochemical Analysis

Phytochemical tests were carried out to detect the presence of particular compounds using standard procedure.

#### Detection of Tannins:

##### *FeCl<sub>3</sub> test:*

2ml filtrate was added to 2ml FeCl<sub>3</sub>; blue- black precipitate indicates the presence of tannins.

#### Detection of Alkaloids:

##### *Dragondroff's test:*

200mg leaf material was taken in 10ml methanol and filtered. 2ml filtrate was added to 1% HCl, steam for 10 minutes. To this add 6 drops of Dragondroff's reagent; Reddish brown precipitate indicates the presence of alkaloids.

#### Detection of Saponins:

##### *Frothing test:*

1ml of methanol extract was diluted with 20ml distilled water and shaken well for 15 minutes and observed for formation of froth in the upper layer. The presence of froth indicates the presence of saponins.

#### Detection of Cardiac Glycosides:

##### *Keller-kiliani test:*

2ml of methanol extract was added to 1ml glacial acetic acid, to this mixture few drops of FeCl<sub>3</sub> and one drop of conc. H<sub>2</sub>SO<sub>4</sub> was added. Green blue colour indicated the presence of Cardiac glycosides.

**Detection of Steroids:*****Liebermann-Burchard reaction:***

2ml filtrate was taken, to this 2ml acetic anhydride and few drops of conc. H<sub>2</sub>SO<sub>4</sub> were added. Blue-green ring indicates the presence of steroids.

**Detection of Flavanoids:*****NaOH solution test:***

2ml of methanol extract was added to 2ml of 10% NaOH solution. Yellow to orange colour indicates the presence of flavanoids.

**Detection of Proteins:*****Xanthoproteic test:***

1ml of extract was added to 1ml of HNO<sub>3</sub>, boil in a water bath. Orange colour indicates the presence of proteins.

**Detection of Triterpenes:*****Salkowski test:***

2ml of filtrate was mixed with a few drops of conc. H<sub>2</sub>SO<sub>4</sub>. The solution slowly turns red, indicates the presence of triterpenes.

**Detection of Carbohydrates:*****Molisch's Test:***

Extract was dissolved individually in 5 ml distilled water and filtered. The filtrate was treated with 2 drops of alcoholic  $\alpha$ -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

***In-vitro* Cytogenetic Assay:**

Take 6 sterile 25 cm<sup>2</sup> capacity disposable T flask labeled test with concentration of 5mg, 2.5mg, 1.25mg, positive control, negative control, and normal control. 9 ml of the growth medium was taken in each of the labeled sterile T flask, 0.8 ml of heparinized whole blood collected freshly from healthy adult male/female donor was added. To this, 0.1 ml of PHA solution, 0.1ml of FBS was added. The flasks were then transferred to CO<sub>2</sub> incubator. The culture was incubated at 37  $\pm$  1 °C for 48 h. After 48h incubation, add 0.1 ml of leaf extract for subject, 5 mg, 2.5 mg and 1.25 gm concentration of leaf extract in three flasks, positive mutagen for positive control,

distilled water for normal control, and DMSO for negative control. These cultures were incubated at 37 °C for 3-6 hours.

After 3 hrs, these cultures were transferred to centrifuge tubes, centrifuged at 1600rpm at 10-15min. Then the supernatant was removed gently. The pellet of the centrifuge was taken and a freshly made working growth medium containing all ingredients was added and mixed gently. No PHA was added. The total volume of the cultures was made up to 10 ml using the culture medium. These cultures were incubated at 37  $\pm$  1 °C and for 18-21 h. 1 h prior to the completion of incubation, colchicine (0.4  $\mu$ g/ml) was added to stop culture growth. Culture was harvested.

**Harvesting of Cells:**

The tubes with the collected aspirates and PBS were added drop by drop, then the tubes were spun at 1600 rpm for 5-10 min. in a centrifuge at room temperature. After centrifugation, the supernatant will be carefully aspirated out using a Pasteur pipette. A freshly prepared hypotonic solution (pre incubated at 37 $\pm$ 1°C) was added drop by drop to the cell pellet to make it to a volume of 5-10ml with same hypotonic solution (KCl) and tubes with cells in hypotonic solution were incubated for 10-15 minutes at 37 $\pm$ 1 °C. At the end of incubation time few drops (1-2 ml) of freshly prepared fixative (3:1 methanol and glacial acetic acid) were added.

Following the hypotonic treatment, the tubes were again centrifuged at 1600 rpm for 5-10 min. at room temperature. The supernatant was aspirated out carefully using a Pasteur pipette without disturbing the cell button at the bottom. The above processes of washing the cells were repeated using fixative until the cells button becomes white in color.

**Slide Preparation & Staining:**

Immediately, slides will be air dried or placed on slide warming table maintained between 40-50°C. After drying, the slides were labeled as tests concentration 5mg, 2.5mg, 1.25mg, positive control, negative control, and normal control.

All the slides were stained with freshly prepared Giemsa working solution for 5-10 minutes at room temperature. The prepared slides were observed under compound microscope at 100 x magnification.

## RESULT

### Preliminary phytochemical analyze:

The present studies revealed (Table-1) which explained the phytochemical tests were carried out to test the presence of preliminary phytochemical compounds. Tests revealed the presence of alkaloids, flavonoids, Cardiac glycosides, proteins, tannins, carbohydrates, and steroids in *Passiflora foetida L.* tested extract. In addition to this, the presence of leaf extract of alkaloids, flavonoids, proteins, carbohydrates, and steroids compounds were present in high quantity. The tannins and glycosides compounds were present in low quantity and the saponins and triterpenes were absent.

The present study of preliminary phytochemical analysis showed the presence of alkaloids, flavonoids, Cardiac glycosides proteins, tannins, carbohydrates, and steroids. It has been reported that alkaloids and flavonoids were responsible for the anti-inflammatory and antinociceptive activity of prostaglandin synthetase inhibition. Therefore anti-inflammatory and antinociceptive activity of the plant extract may be attributable to the existence of alkaloids and flavonoids either in single form or in combination. Furthermore, presence of alkaloids, flavonoids, and sterols in medicinal plants has been associated with antidiarrhoeal action.

**Table-1 Phytochemical tests revealed the presence of following compounds tested in the *Passiflora foetida L.***

Compounds	Tests	ME
Alkaloids	Dragondroff's test	++
Carbohydrates	Molisch's test	++
Glycosides	Keller-kiliani test	+
Flavanoids	NaOH solution test	++
Proteins	Xanthoproteic test	++
Saponins	Frothing test	-
Steroids	Liebermann-Burchardreaction	++
Tannins	FeCl <sub>3</sub> test	+
Triterpenes	Salkowski test	-

- = Absence compound

+ = Low presence compound

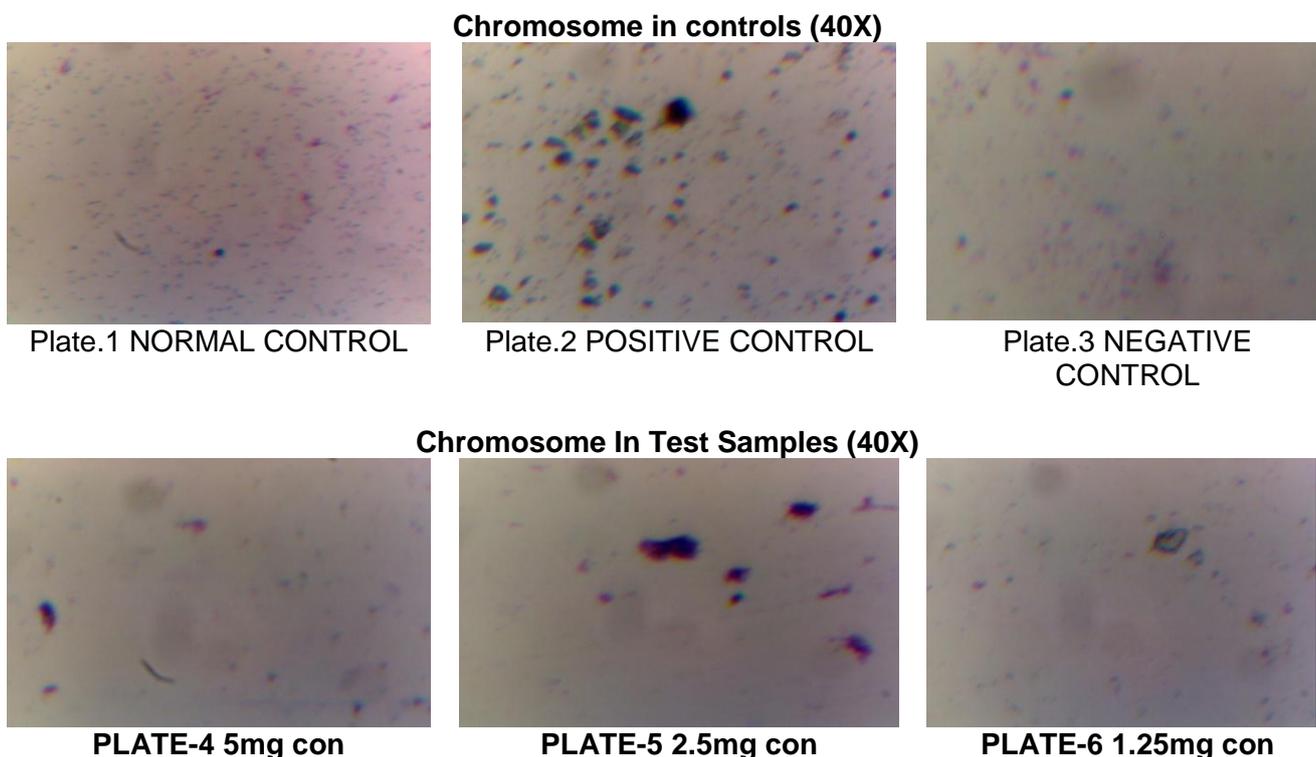
++ = High presence compound

Therefore the presence of individual or combinations of flavonoids and alkaloids may give the plant extract its possible antinociceptive and anti-inflammatory effects. Alkaloids, as well as flavonoids may also be accountable for the anti-diarrhoeal, anti-arrhythmic, anti-cholinergic, Stimulant, Adenosine receptor antagonist, cough, analgesic, remedy for gout, antiprotozoal agent, sympathomimetic, vasodilator, antihypertensive, analgesic, Stimulant, Nicotinic acetylcholine receptor agonist, inhibitor of acetylcholinesterase, anti-arrhythmic, antipyretics, antimalarial, antihypertensive, Muscle relaxant, antitumor, vasodilating, antihypertensive, Stimulant, Aphrodisiac activities of *Passiflora foetida L.*

**Table-2. *In-vitro* cytogenetic assay of chromosomal aberration-*Passiflora foetida L.* leaf crude extract.**

STEPS	Conc. 0.5 mg	Conc. 2.5 mg	Conc. 1.25 mg	normal	-ve control	+ve control
BLOOD ml	0.8	0.8	0.8	0.8	0.8	0.8
Conc.0.5 ml	0.1	-	-	-	-	-
Conc.2.5 ml	-	0.1	-	-	-	-
Conc.1.25ml	-	-	0.1	-	-	-
Dis.H <sub>2</sub> O ml	-	-	-	0.1	-	-
DMSO ml	-	-	-	-	0.1	-
Mitomycin C.	-	-	-	-	-	0.1
Observation	Non-mutagen	Non-mutagen	Non-mutagen	No chromosomal aberration	No chromosomal aberration	chromosomal aberration present

**Figure-3. Giemsa-stained sections of chromosomal aberration effect of *Passiflora foetida L* leaf crude extract on peripheral human blood lymphocytes.**



### Chromosomal Aberration:

#### *Negative control:*

The analyzed data (Table-2, Plate-3, and Plate-7) confirmed that the cells and chromosomes appeared normal and there was no effect due to DMSO, which is a solvent/vehicle used to dissolve the plant extract. This indicates that there will no carcinogenic affect due to DMSO which will in turn not affect the integrity of the study.

#### *Positive control:*

The present study data (Table-2, Plate-2, and Plate-8) depicts that the cells and chromosomes appeared abnormal in condition there was formation of structural aberration leading to breaking of chromatids and centromeres which resulted due to addition of carcinogen mitomycin C.

#### *Normal control:*

Interpretation of data (Table-2, Plate-1, and Plate-9) confirmed that availability chromosomes of chromatids and centromeres appeared in normal condition. There were no

changes found in normal control of peripheral human blood lymphocyte along with centromeres. There was no aberration found which confirmed that the blood was withdrawn from healthy individual.

#### *Test samples:*

The examined data (Table-2, Plate-4, Plate-5, Plate-6 Plate-10 Plate-11 and Plate-12) showed the No chromosomal aberration was found in the test sample (*Passiflora foetida L.*) with a dose ranging from 5mg, 2.5mg, and 1.25mg. (5mg-high dose, 2.5mg – mid dose, 1.25mg – low dose). This indicates that the plant extract was safe and will not cause any adverse effects in human lymphocytes leading to cancer.

Chromosome abnormalities are considered to be one of the most important cytogenetic parameters for the manifestation of genotoxicity. Recently, it was reported that persons with high frequency of CA develop cancer twice as frequent as others. During the present investigation plant extract from *Passiflora foetida L.*, a non-mutagenic even at the dose levels ranging from 5 to 1.25mg/culture was

.....Figure-3. Giemsa-stained sections of chromosomal aberration effect of *Passiflora foetida* L leaf crude extract on peripheral human blood lymphocytes.



PLATE.7 CHROMOSOME IN NEGATIVE CONTROL (100X)

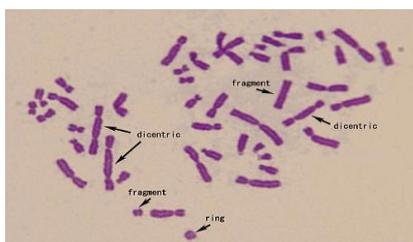


PLATE.8 CHROMOSOME IN POSITIVE CONTROL (100X)



PLATE.9 CHROMOSOME IN NORMAL CONTROL (100X)



PLATE.10 CHROMOSOME IN 5mg CONCENTRATION (100X)



PLATE.11 CHROMOSOME IN 2.5mg CONCENTRATION (100X)



PLATE.12 CHROMOSOME IN 1.25mg CONCENTRATION (100X)

measured by the *in-vitro* chromosomal aberration assay using human blood lymphocyte. Plants have been used throughout history as remedies in most cultures and were the basis for many pharmaceuticals currently which are in use. It was estimated that 80% of the tropical and subtropical populations of the world depends on herbal remedies to treat diseases and sources of new, safer, and effective compounds with medicinal properties were investigated.

## DISCUSSION

Therapeutic uses of cardiac glycosides primarily involve the treatment of cardiac failure. Their utility results from an increased cardiac output by increasing the force of contraction. By increasing intracellular calcium as described below, cardiac glycosides increase calcium-induced calcium release and thus contraction. Protein deficiency and malnutrition can lead to variety of ailments including mental retardation and kwashiorkor.

The previous study Flavonoids was showed to have a wide range of biological and pharmacological activities in *in-vitro* studies. Examples include anti-allergic (Yamamoto, *et*

*al.*, 2001), anti-inflammatory (Yamamoto, *et al.*, 2001; Cazarolli, *et al.*, 2008) , antioxidant (Cazarolli, *et al.*, 2008), anti-microbial, antibacterial (Coutinho, , *et al.*, 2013; Manner, *et al.*, 2013), antifungal and antiviral (Friedman, *et al.*, 2007), anti-cancer and anti-diarrheal activities (Cazarolli, *et al.*, 2008; Sousa, *et al.*, 2008). It reduce cardiovascular disorders (Manach *et al.*, 2008), diabetes mellitus (Babu, *et al.*, 2013), and celiac disease (Ferretti, *et al.*, 2012). Flavonoids have also been shown to inhibit topoisomerase enzymes (Esselen, *et al.*, 2009; Bandelete, *et al.*, 2008) and to induce DNA mutations in the mixed-lineage leukemia (*MLL*) gene in *in-vitro* studies (Barjesteh, *et al.*, 2008). However, in most of the above cases no follow up *in vivo* or clinical research has been performed, leaving it impossible to say if these activities have any beneficial or detrimental effect on human health. But in present studies that the plant extract *Passiflora foetida* L. is non-mutagenic at the dose levels ranging from 5 to 1.25mg/culture as measured by the *in-vitro* chromosomal aberration assay using human lymphocyte.

The previous study, when incubated with red grape juice and red wines with a high content of

condensed tannins, the poliovirus, herpes simplex virus, and various enteric viruses were inactivated (Bajaj, 1988). In tissue-cultured cell assays tannins have shown antiviral (Lu, *et al.*, 2004), antibacterial (Akiyama, *et al.*, 2004), and antiparasitic effects (Kolodziej, *et al.*, 2004). Tannins isolated from the stem bark of *Myracrodruon urundeuva* may offer protection against 6-hydroxydopamine-induced toxicity (Nobre, *et al.*, 2007) Souza *et al.* discovered that the tannins isolated from the stem bark also have anti-inflammatory and antiulcer activity in rodents, showing a strong antioxidant property with possible therapeutic applications (Souza, *et al.*, 2006). Therefore the present studied *Passiflora foetida L.* also anti-inflammatory, antioxidant, and antiulcer antiviral, antibacterial, and antiparasitic effects activity possible therapeutic applications.

The present study of preliminary phytochemical analysis showed the presence of alkaloids, flavonoids, Cardiacglycosides, proteins, tannins, carbohydrates, and steroids. It has been reported that alkaloids and flavonoids were responsible for the anti-inflammatory and antinociceptive activity of prostaglandin synthetase inhibition. Therefore anti-inflammatory and antinociceptive activity of the plant extract may be attributable to the existence of alkaloids and flavonoids either in single form or in combination. Furthermore, presence of alkaloids, flavonoids, and sterols in medicinal plants has been associated with antidiarrhoeal action. Therefore the presence of individual or combinations of flavonoids and alkaloids may give the plant extract its possible antinociceptive and anti-inflammatory effects. Alkaloids, as well as flavonoids may also be accountable for the antidiarrhoeal action of *Passiflora foetida L.* (Asadujjaman, *et al.*, 2014).

Plants have been used throughout history as remedies in most cultures and were the basis for many pharmaceuticals currently in use. MEPP contains alkaloids, flavonoids, tannins, steroids, and glycosides and the extract possesses analgesic, antidiarrhoeal and cytotoxic activities. Results of the experiment tend to suggest that the plant could be a good source of alternative

medicine for rheumatism, inflammation, abdominal pain and diarrhoea. Further research is essential to identify active principle(s) and explore the mechanisms involved for their bio-activities. Overall, the findings of this research support some of the traditional uses of *P. foetida* in different ailments. This work indicates a need to pursue the isolation of bioactive compounds from MEPP, as well as investigate the pharmacological utility of these isolates (Asadujjaman, *et al.*, 2014).

Chromosome abnormalities are considered to be one of the most important cytogenetic parameters for the manifestation of genotoxicity. Brogger, *et al.*, (1990) have reported that persons with high frequency of CA develop cancer twice as often as others. During the present investigation plant extract *Passiflora foetida L.* is non-mutagenic at the dose levels ranging from 5 to 1.25mg/culture as measured by the in-vitro chromosomal aberration assay using human blood lymphocyte. The background frequency of CA matched very well with those reported for control in various investigated by (Yadav and Thakur 2000). The present study of chromosomal abnormalities did not detected levels ranging from 5 to 1.25mg/culture among the *Passiflora foetida L.* compared to control.

The in-vitro chromosomal aberration assay using human blood lymphocyte test was a cost-effective and accurate procedure, which can be easily carried out for genotoxicity-based studies. CA test was better indicator for genotoxicity damage SCE than MN (Ramakrishnan, *et al.*, 2011). The present study clearly indicates that the plant extract *Passiflora foetida L.* is non-mutagenic.

Based on the above results, it was discussed that the plant extract *Passiflora foetida L.* was non-mutagenic at the dose levels ranging from 5 to 1.25mg/culture as measured by the in-vitro chromosomal aberration assay using human lymphocyte. The previous study of oral acute toxicity study of *P. foetida L.* leaves indicated that this plant did not induced any mortality or change of behavior in female adult Wistar albino rats for the doses less than 5000 mg/kg. The

median lethal dose (LD50) of the *Passiflora foetida* L. extracts must be above 5.000 mg/kg. According to (Schorderet, 1992), substances with LD50 values greater than 5000 mg/kg are classified as substances with low toxicity. Thus, the aqueous extract, hexane extract and methanol extract of *P. foetida* L. leaves can be considered as substances with low toxicity (Bleu, *et al.*, 2012).

Previous investigation before any chemical compound can be approved as a pharmaceutical drug or any food can be labeled with a health claim, it must undergo extensive *in vitro*, *in vivo*, and clinical testing to confirm both safety and efficacy. National and international regulatory authorities like the US Food and European Food Safety Authority (EFSA) are responsible for assessing this evidence and granting such approval. At the current time, neither the FDA nor the EFSA has approved any health claim for phytochemical compounds of flavonoids, or approved any phytochemical compounds of flavonoids other as pharmaceutical drugs (FDA, 2013; Health, 2013; EFSA, 2010). Moreover, several companies have been cautioned by the FDA over misleading health claims (Inspections, 2013; Inspections, 2013; Lipton, 2013; Fruits, 2013).

Therefore present investigation (Plate-6 and Plate-7) that the plant extracts *Passiflora foetida* L. was non-mutagenic at the dose levels ranging from 5 to 1.25mg/culture as measured by the in-vitro chromosomal aberration assay using human lymphocyte. The previous study of oral acute toxicity study of *P. foetida* L. leaves indicated that this plant did not induced any mortality or change of behavior in female adult Wistar albino rats (Venkatasubbaiah *et al.*, 2013; Bleu, *et al.*, 2012; and Ashok J Patil, 2014). So the future that plant *Passiflora foetida* L. was used to detect new drugs medicinal plants of *Passiflora foetida* L. were also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases.

## CONCLUSION

The present study showed that phytochemical analysis of alkaloids, flavonoids, cardiac glycosides, proteins, tannins, carbohydrates, and steroids. Therefore, the present studies on *Passiflora foetida* L. suggest that the plant could be a good source of alternative medicine for rheumatism, inflammation, abdominal pain, diarrhea, oxidant etc. It is used as a therapeutic agent for antiviral, antibacterial, antimalarial, antiparasitic, agent, antiarrhythmic, antitumor, antiulcer, antihypertensive, anticholinergic, stimulant, adenosine receptor antagonist, cough, analgesic, remedy for gout, sympathomimetic, vasodilator, muscle relaxant, aphrodisiac activity. Based on the above results, it is concluded that the plant extract (*Passiflora foetida* L.) is non-mutagenic at the dose levels ranging from 5 to 1.25mg/culture as measured by the in-vitro chromosomal aberration assay using human lymphocyte.

Therefore, genetic composition of CA, SCE and MN must be studied to determine if they contain specific genes associated with carcinogenesis as suggested by concept of field carcinogenesis. The results of such studies could have a significant impact on the future use to detect new drugs medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases.

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