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# Physicochemical Phytochemical Studies and Acute Toxicity Studies of Extract of Roots of *Passiflora foetida* (Passiflorine)

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Article History:	ABSTRACT
Received on: 15 Jun 2022 Revised on: 02 Jul 2022 Accepted on: 03 Jul 2022 <i>Keywords:</i> Passiflora foetida, SGOT, ANOVA, Acute Toxicity	The Topic deal with biomedical, biological, medicinal, as well as economic fea- tures of the original substances as well as with their chemical components. The current study is one such effort to assess the physicochemical phyto- chemical studies and acute toxicity studies of extract of roots of <i>Passiflora</i> <i>foetida</i> (passiflorine). To study the antiarthritic activity of extract of roots <i>Passiflora foetida</i> . A present work demonstrated the estimation of serum SGOT (UV-Kinetic Method) and serum alkaline phosphate hydrolysis p-Nitro phenyl phosphate within presence of an oxidizing agent Mg <sup>+2.</sup> All these inter- action has been assessed just like directly absorbencies proportionate to an ALP action. The Physicochemical Standards, Preliminary Phytochemical Stud- ies and Haematological Parameter Express the values in mean $\pm$ SEM, (n=6), it when compared with the control, *P<0.05, **P<0.01, ***P<0.001 one way ANOVA accompanied through Dunnett's t-Test. However, we perform the Effect of ethanolic extract of whole plant of <i>Passiflora foetida</i> on mean changes in paw volume as well as Biochemical parameters evaluation of ethanolic extract of root of <i>Passiflora foetida</i> .

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#### INTRODUCTION

Pharmacognosy is bothered only with research like raw opioids like animal and vegetable beginnings. A phrase materia medica is being used to confer with

the whole materials in use with medicine such like organic chemical substances medicinal herbs, nutrient materials, but also biomedical arrangements such as vaccines but also sera. Pharmacognosy implicated the one comprehensive study like individual opioids as well as elaboration like basic principles. The term pharmacognosy was being utilized through c.a. sevdler through 1815 [greek; pharmakon=drug; gnosy=knowledge]. The topic deal with biomedical, biological, medicinal, as well as financial features of the original opioids but also their chemical components. At present pharmacognosy includes research like raw opioids but also with there organic derivative products. Thereby, digitalis but also its excluded glycosides, digoxin; datura as well as its excluded alkaloid atropine; opioid and also its cleansed compound morphine, are

all allowed to treat just like the topic like pharmacognosy [1].

In modern medicine vinca rosea is used which contains some 70 alkoloids the most important vinblastine and raubasine.

Medical plants as a whole appear to occupy a stable place in modern medicine; changes in respect of individual plants may be expected with the progress of scientific research. Industry is interested in synthesizing natural substances in order to combat supply shortages or reduce cost in comparison with the extraction of the active constituents(s) from natural raw materials.

Several of the crops used during herbal remedies contain active components whom impacts could be illustrated pharmacologically and also the activity of whole natural plant could generally be relevant with that of the excluded components. However, for some herbal remedies a scenario has been complicated even by prevalent use of such a variety of drugs together.

A herbal remedies were also inherent safety then a highly potent opioids, which frequently generate undesired side effects. There are extremely toxic plants in the plant kingdom which produce carcinogens, teratogens and other compounds which cause disease and sensitization. Thus, comfrey (Symphytum officinale), always considered a safe herb, has been found to contain small quantities of pyrrolizidine alkaloids which are known to be hepatotoxic and which, when administered to rats, cause liver cancer.

Similarly, reserpine, an alkaloid of rauwolfia serpentina, has been associated with breast cancer, but no such cases have been reported as a result of administration of the root extract. A number of cases of toxicity arising from the over-consumption of herbal remedies have been reported, and the principal danger appears to be that arising from the uncontrolled supply and administration of these products [2].

#### **Materials and Methods**

#### **Physicochemical Standards**

#### **Material Methods**

Such like total ash, acid insoluble ash, water soluble ash, resource extraction virtues have been ascertained individually such as air-dried powder form leaves of this plant according to the standard method [3].

#### **Determination of Total Ash**

About 2 to 3 grams (accurately weighed) of ground leaf extracts has been chosen to take inside a sil-

ica crucible earlier sparked as well as did weigh. This was vaporized through slowly increasing the warmth neither surpassing deep red steam (450 degrees centigrade) until unrestricted through the carbon, allowed to cool but also did weigh. The proportion like ash has been measured as regards to the air dried flour. A methodology has been reproduced 5 times to be get steady weight.

# **Determination of Water-Soluble Ash**

Here the ash content has been heated as for 25ml of liquid such as 5mins and it was filtrated through with an residue fewer filter paper (Whatmann No 41). This was accompanied through cleaning with heated water. A filtered paper has been combusted inside the silica crucible, allowed to cool and also the insoluble matter has been decided to weigh. A water-soluble ash has been measured through simply subtract insoluble matter as from total ash.

#### **Determination of Acid Insoluble Ash**

Here the total ash acquired has been heated such as 5minutes with 10% w/v dilute hydrochloric acid but also filtrated through with an ash fewer filter paper (Whatman No. 41). A filter paper has been combusted within silica crucible, allowed to cool acid non-soluble ash has been did weigh.

# **Determination Loss on Drying**

For a determination like deficit over dryers the subsequent procedure has been decided to follow. Approximately 1-2 gram of a powder form leaf has been weighed accurately inside a glass stoppered evaluating flask which would be initially solidified for 30 min inside the drier. Therefore the sample has been delicately rattled side wise even for distribution but also left to dry inside an oven about as 100 degrees centigrade of about 105 degrees centigrade through withdrawing a stopper. This was allowed to cool inside desiccators again and did weigh. A deficit over trying to dry has been measured with both the reference to the amount like air-dried flour.

# **Extractive Values**

# **Determination of Alcohol Soluble Extractive**

5 gm of flour has been macerated with 100 ml like alcohol of a defined strength inside a closed bottle such as 24 hrs, shaking oftenly throughout 6 hours as well as letting this to remain for 18 hours. This was filtrated quickly taking every precaution against lack of alcohol, as well as 25 ml like filtrate has been vaporized to dryness inside a tarnished flat-bottom shallow dish at 105 degrees centigrade but also did weigh. The proportion like alcohol solute extractive has been measured with reference to an air-dried powder [4].

### **Determination of Water-Soluble Extractive**

About 5 gms of a powder has been added to 50 ml like water about as  $80^{\circ}$  centigrade but also to this 2g like kieselguhr has been decided to add but also filtrated. 5ml of a supernatant has been transmitted to such a tarnished evaporating dish, an extract has been removed on such a steam bath, drying has been proceeded for 30 minutes, eventually this was left to dry in such a hot air oven for 2 hour shifts as well as did weigh, the proportion like water-soluble extractive has been measured just about air-dry opioid.

# **Soxhlet Method of Extraction**

A powder form opioid has been extracted as for solvent 70% ethanol utilizing Soxhlet equipment. Extraction was carried around until the extricate will become translucent.

A solute has been removed completely through the mare if before next separation was carried out. An extract was dried by rotary evaporator at  $40^{0}$  C.

Solvents have been far away from a extricate through evaporation below lower pressure.

A hardened extricate thereby acquired has been through desiccator until for much further research [Figure 1].

# Principle

Soxhlet extractor obtains components just using compressed water vapour came into contact only with specimen powder and also a soluble part in the powder gets mixed with the solvent [5].

#### Advantages

Simple and clear design production process consists as ease of visual maintains of its reuse after stripping and distillation.

#### **Phytochemical Screening**

The ethanol extract acquired through successive extraction method were confined to qualitative phytochemical analysis is to identify the character like components existing their Hibiscus

# **Ethanolic Root Extracts**

In the detailed analysis about just the phytochemical testing process even though follows:

Ethanolic, aqueous extract is subjected to numerous preliminary phytochemical analysis was conducted to test for such presence or absence of varied phytochemical constituents even by following techniques.

#### **Test for Alkaloids**

To extricate dilute hydrochloric acid will be decided to add as well as filtrated. A supernatant will be treated with different alkaloidal reactants.

# Mayer's Test

A supernatant will be allowed to treat as for Mayer's reagent appearance like cream coloring indicates presence like alkaloids.

# Dragendroff's Test

A supernatant will be allowed to treat as for Dragendroff's solvents looks like reddish-brown precipitate identifies the existence like alkaloids.

## Hager's Test

A supernatant will be allowed to treat with Hager's reagent appearance like yellow colour precipitate identifies the existence like alkaloids.

# Test for Carbohydrates and Reducing Sugar

A small amounts of a supernatant will be disintegrated through 4ml like deionized water but also filtrated. A supernatant would be made subject to [6].

# Molisch's Test

The tiny portion of a supernatant will be allowed to treat as for Molisch's reagent but also sulphuric acid. Forming of such a violet ring identifies the existence like carbohydrates.

# Fehling's Test

A extricate allowed to treat as for Fehling's reagent A and B. Demeanor like reddish orange coloring precipitate identifies the existence like reducing sugar.

#### **Benedict's Test**

A extricate allowed to treat as for benedict's reagent; Demeanor like reddish orange coloration precipitate identifies the existence like reducing sugar.

#### **Barfoed's Test**

A extricate allowed to treat as for barfoed's reagent and heated. Demeanor like reddish orange coloration precipitate identifies the existence like reducing sugars

#### **Test for Steroids**

# Libermann Burchard's Test

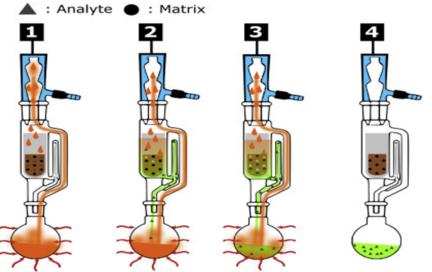
A extricate allowed to treat with 3ml of acetic anhydride. Few falls of glacial acetic acid accompanied by such a fall like conc sulphuric acid.

Demeanor like bluish green coloration identifies the existence like steroids [7].

## **Test for Proteins**

#### **Biuret Test**

The extricate treat with copper sulphate solution, accompanied besides addition like sodium hydroxide solution; Demeanor like violet coloration identifies the existence like proteins.



**Figure 1: Soxhlet Method of Extraction** 

# Millon's Test

The extricate allowed to treat as for millon's reagent; demeanor of pink colour identifies the existence of proteins.

#### **Test for Tannins**

The extricate allowed to treat as for 10% lead acetate solution; demeanor like white identifies the existence like tannins.

# **Test for Phenolic Compounds**

The extricate treated with neutral ferric chloride solution; Demeanor like violet coloration identifies the existence like phenolic compounds.

## **Test for Flavonoids**

5ml of extricate hydrolysed as for 10%sulphuric acid as well as allowed to cool. Then, it would be trying to extract with diethyl ether as well as split into three portions through different test tubes. Ml of diluted sodium carbonate. 1ml o 0.1N sodium hydroxide, as well as 1ml of powerful ammonia solution would be decided to add to first,  $2^{nd}$  and  $3^{rd}$ test tubes respectively. In every test tube. Advancement of yellow colour evidenced the existence of flavonoids.

# Shinoda's Test

A extricate disintegrated through alcohol, wih which few magnesium turnings might beaded accompanied through conc HCL drop wise but also heated, as well as demeanor like magenta coloration reveals existence of flavonoids.

#### **Test for Gums and Mucilage**

A extricate has been allowed to treat with 25ml of absolute alcohol, but also filtrated. A filterate might analyse for such swelling properties.

# Test for Glycosides

When a pinch the extricate has been allowed to treat as for glacial acetic acid and few drops like ferric chloride solution, followed by addition of conc. Sulphuric acid, formation of ring just at junction of the two fluids identifies the existence of glycosides.

#### **Test for Saponins**

Foam test: approximately 1 ml of a extricate has been diluted to 20 ml for deionized water as well as rattled well in such a test tube. A foaming froth within upper portion like test tube identifies the existence of saponins.

#### **Test for Triterpenoids**

A substance has been warmed as for tin but also thionyl chloride. Pink colour identifies the existence of triterpenoids.

# **Test for Calcium**

A few drops like dilute sodium hydroxide solution interact to shape a white precipitate with aluminium ions also with calcium ions. However, if excess sodium hydroxide solution seems to be decided to add: an aluminium hydroxide precipitate interacts to shape a colourless solution. The calcium hydroxide precipitate has been unaffected.

#### **Pharmacological Studies**

# Acute Toxicity Studies

# **Experimental Animals**

Inbreed Swiss albino rat (150-200 g) of male gender were acquired from animal house of SICRA labs Pvt Ltd Hyderabad. The rats have been retained in a well-ventilated room with 12:12 hour light/dark cycle through polypropylene cages. Standard pellet feed (Hindustan Lever Limited., Bengaluru) as well as drinkable water has been supplied ad libitum all through experimentation duration. Mice have been acclimatized to laboratory situations a week prior to the initiation like experimentations. Ethical review committee approval has been acquired through the AEC (Animal Ethics Committee) of CPC-SEA (REG.NO.769/2020/CPCSE (Committee for the purpose of control and supervision like experimentations through animals) [8].

# **Acute Oral Toxicity Study**

A protocol was conducted through utilising OECD 425 (Acute Toxic Class Method). An acute toxic class method is just a step wise methodology with four rats of such a single gender for each phase. Counting on the mortality but rather morbidity status of a rat and also the average 2 to 3 stages could be important to allow judgment upon that acute oral toxicity of a test compound. A methodology leads to usage variety of rats letting for allowable information based on scientific conclusion. The strategy used it to define dose levels [2000,1000,500,50,5mg/kg body weight] the outcomes enable a substance also to be ranked as well as categorized according to the globally harmonized process [GHS] for such categorization like chemicals where it lead to acute toxicity.

# **Experimental Procedure**

Male albino rats assessing 150-200gm has been used for research. A beginning dose level like ethanolic extricate like Passiflora foetida plant bodies are 100,600,1200,1800 and 2000mg/kg body weight P.O Dose volume has been administered of about overnight fasting conditions mice in were ad libitum. Nutrition has been withheld for another 3-4 hours after administering of the Passiflora foetida The as well as observed for signs for toxicity. body weights of the rats before and after administration were noted that changes in skin and fur, eyes, mucous membranes respiration, blood circulation, autonomic but also central nervous system but rather motor activity and behaviour pattern have been noticed but also sign of tremors, convulsions, salivation, lethargy, sleep as well as coma have been acknowledged. Onset of toxicity as well as signage of toxicity as well acknowledged [9].

#### Anti-Arthritic Activity

Evaluation of the anti-arthritic action through mice through complete Freund s Adjuvant procedure animals.

Inbreed healthy Wister albino male rats of weighing 150-200gm have been used in research. Each animal experimental has been clinically analyzed preoperatively about any diseases. The animals have been housed under analysis through research lab but also permitted to acclimatize for a week before experiment. Animals maintained through separate spacious clean cages under monitored room temperature  $[24+2^0c]$  diet and filter water. Well before experiment mice have been divided into five groups.

# Compound [Drugs to be Administered] Preparation

- 1. Extract; The extract was weighed according to rat body weight and suspended in 1%CMC solution.
- 2. Extract dose selection; Based on Acute toxicity studies, 250 and 500mg/kg B.W doses of EEPF were selected and administered against
- 3. Freund s adjuvant induced Arthritis. Starting point of study is animal selection and randomly dividing them into 4 groups [by considering animal body weights]
- 4. CFA; It contains 5mg mycobacterium tuberculosis [DIFCO] was suspended in heavy paraffin oil [MERCK] by thoroughly grinding with mortar and pestle to give a concentration of 5mg/ml.
- 5. Indomethacin; Indomethacin [10mg/kg B.W] was weighed according to rat body weight and dissolved in 1/CMC solution.

# Adjuvant Induced Arthritis

Wistar albino rats [150-200g] have been taken and classified into 5 groups, every group contains 6 animals. Animals have fasted overnight well before an experiment. On first day, they were infused through into sub plantar region of a left hind paw with 0.05ml like entire Freund s adjuvant. All this comprised of 5mg mycobacterium tuberculosis [DIFCO] beings suspended through bulky paraffin oil [MERCK] through extensively milling as for pestle and mortar to provide content of 5mg/ml dosage also with experiment compounds or even the standard was began the same day as well as proceeded for 12days. Paw volumes of the both sides as well as body weights have been documented on the day before infusion, whereby the paw volume was evaluated plethys mographically with equipment just like characterized within paw Edema tests. On 5th outing quantity of a infused paw has been evaluated again implying the first lesion and also impacting the chemotherapeutic drugs upon just process [12]. A consequences like severity of an influenced adjuvant illness has been accompanied through measuring system like a non-injected paw [peripheral nodules] with such a plethysmometer. From the day 13 to 21, a animals were not medicated with the

Table 1: Estimation of Serum Alkaline Phosphate				
Pipette	Sample			
Working reagent	1.0ml			
Sample	0.1ml			

# Table 1: Estimation of Serum Alkaline Phosphate

## Table 2: Estimation of AST and MDH

Pipette	Sample (µl)
Working reagent	50
Sample	10

# **Table 3: Physicochemical Standards**

S.no	Parameters	Values (%)	
1.	Total Ash (%)	16%	
2.	Water Soluble Ash (%)	2.60%	
3.	Acid Insoluble Ash	1.95%	
4.	Water Soluble Extract (%W/W)	17.25%	
5.	Alcohol Soluble Extract (%W/W)	4.58%	
6.	Loss on Drying	1%w/w	
7.	Moisture Content	4.99% w/w	

tested compounds or even the standard on  $21^{st}$  day, a body mass is decided again but consequences like peripheral nodules has been reviewed visibly as well as appraised according to the following sequence in Table 1.

# **Grouping of Animals**

Group1; Normal control [distilled water, P.O for 12 days]

Group2; positive control [Freund's adjuvant 0.05ml, sub-plantar region]

Group3; Freund's adjuvant-standard [Indomethacin 10mg/kg P.O for 12 days

Group4; Freund's adjuvant – low dose of EEPF [250mg/ kg] P.O for 12days

Group5; Freund's adjuvant- Hight dose of EEPF [500mg/kg] P.O for 12 days.

# **Evaluation of Parameters**

To Study the Anti Arthritic Activity of Ethanolic Extract of whole plant of *Passiflora foetida* [Passifloracea] by complete Freund's adjuvant method in rats though [13].

A) Body weights-by using weighing balance

B) Paw volume-by using plethysmograph

C) Haematological parameters-RBCcount, WBC count, HB% & ESR

D) Biochemical parameters-SGOT, SGPT ALP

# Freund's Adjuvant Method

Freund's adjuvant extract is a solution of antigen emulsified in mineral oil and used as an immunopotentiator.

# Percentage Inhibition

Average changes through infused paw Edema for initial paw volume, where measured through relevant days and % inhibition like paw Edema for un-treated groups has been measured by utilizing following formula.

I=  $[1-(\Delta V \text{ treated}/\Delta v \text{ untreated})]x100.$ 

I=% inhibition of paw Edema.

 $\Delta {\rm V}$  treated=mean change in paw volume of treated rat.

 $\Delta V$  untreated= mean change in paw volume of treated rat.

# Method for Collection of Blood Sample

Just on 21<sup>th</sup> day that the plasma (up to 5ml) obtained around cardiac puncture under the impact like ether anaesthetic. A few of the obtained plasma has been used to accomplish haematological study of about approximate RBC, WBC, HB% and ESR leftover plasma has been used to centrifuged at 3000rpm there as room temp to perform following biochemical studies.

# **Biochemical Parameters**

Biochemical parameters have been approximated

S. No	Physicochemical Tests	Absence/presence (-/+)
1.	Test for Carbohydrates	
A	Molisch's Test	+
3	Felling's Test	+
	Barford's Test	+
2.	Test for Glycosides	
A	Bontrager's Test	+
3	Modified Bontrager's Test	+
2	Legal's Test	+
)	Killer Kellan Test	+
3.	Test for Flavonoids	
A	Shinoda Test	+
3	Alkaline reagent test	+
1.	Test for Saponins	
A	Foam Test	+
5.	Test for Steroids	
A	Liberman – Buchard Test	-
3	Salkowski Test	-
2	Sulphur test	-
6.	Test for Alkaloids	
A	Hager's Test	-
3	Wager's Test	-
7.	Test for Fixed Oils and Fats	
4	Spot Test	-
3	Saponification Test	
3.	Test for Acidic Compounds	
A.	Extract +NaHCo3 Solution	_
1	Extract +Water, Warm, Litmus paper	
	Turns to Blue colour	
).	Test for Amino Acids	
A	Ninhydrin Test	-
3	Biuret Test	-
2	Xanthoproteic Test	-
LO.	Test for Tannins	
A	Extract + FeCl <sub>3</sub>	+
3	Lead Acetate Test	+
11.	Test for Phenols	
A	Extract + 4 drops of FeC <sub>3</sub>	+
12.	Test for Coumarins	
A	3ml of 10%NaOH was added to 2ml of Passiflora	+
	foetida root extract	
	Turns to yellow colour	

Sno	Treatment	RBC $(10^6$ cells	WBC	Hb (gm %)	ESR (MM/HR)
		/mm <sup>3</sup> )	(10 <sup>3</sup> cells/mm <sup>3</sup> )		
1.	-ve Control	$6.73 \pm 0.071$	$7.00{\pm}0.043$	$13.66 {\pm} 0.028$	2.76+0.069
2.	+ve Control	5.15±0.344**	9.25±0.043**	10.81±0.029**	6.52±0.150**
3.	Standard	6.17±0.022**	7.33±0.130**	13.71±0.052**	4.38±0.092**
4	EEPF-250mg/Kg	5.92±0.051**	8.25±0.117**	$11.25{\pm}0.056{**}$	5.81±0.131**
5	EEPF-500mg/Kg	6.03±0.077**	7.85±0.065**	12.00±0.175**	4.89±0.050**
	6, 6				

Table 5:	Ethanolic	Extract of	of Passiflora	Foetida
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Values are expressed in mean ±SEM, (n=6), when compared with control, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 one way ANOVA followed by Dunnett's t-Test [10].

#### Table 6: Paw Volume on Ethanolic Extract of Passiflora Foetida

S.no	Treatment and Dose	Paw Volume				
		0 week	$1^{st}$ week	$2^{nd}$ week	$3^{rd}$ week	
1.	-ve control	$0.216{\pm}0.1430$	$0.226{\pm}0.0105$	$0.201{\pm}0.0130$	$0.213{\pm}0.0095$	
2.	+ve control	$0.225{\pm}0.1708$	$0.825{\pm}0.0381^{**}$	$0.783 {\pm} 0.0247^{**}$	$0.706{\pm}0.0147^{**}$	_
3.	Indomethacin (10mg/kg)	0.283±0.0210	0.583±0.0401**	0.441±0.0351**	0.366±0.0307**	55.63
4.	EEPF- 250mg/kg	0.241±0.0300	0.691±0.0271**	0.575±0.0381**	0.483±0.0333**	41.45
5.	EEPF- 500mg/kg	0.233±0.0166	0.65±0.0150**	0.525±0.0335	0.446±0.0166	45.93

Values are expressed in mean  $\pm$  SEM, (n=6), when compared with control, \*P<0.05, \*\*P<0.01, \*P<0.001 one way ANOVA followed by Dunnnet's t-Test [11].

#### Table 7: Body Weight on Ethanolic Extract of Passiflora Foetida

S.no	Treatment and Dose	Body Weight (gms)			
		0 week	$1^{st}$ week	$2^{nd}$ week	$3^{rd}$ week
1.	+ve control	$189.17{\pm}8.002$	$192.50{\pm}7.610$	$194.17{\pm}6.379$	$198.33{\pm}5.110$
2	-ve control	$162.67{\pm}7.839$	$149.67{\pm}5.011$	$155.33{\pm}6.184$	$156.67{\pm}6.280$
3.	Indomethacin (10mg/kg)	187.50±6.677	180.83±7.236	186.33±5.136	195.17±4.214
4	EEPF-250mg/kg	$193.33{\pm}4.014$	$185.83{\pm}4.549$	$188.83{\pm}4.269$	$189.17 {\pm} 3.516$
5.	EEPF-500mg/kg	$161.67 \pm 3.870$	$155.83 {\pm} 3.005$	$158.00{\pm}2.436$	167.50±2.141

# Table 8: Biochemical Parameters on Ethanolic Extract of Passiflora foetida

S.no	Treatment	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)
1.	-Ve Control	$57.67 \pm 3.721$	$45.67{\pm}1.706$	$172.33{\pm}2.616$
2.	+Ve Control	101.67±1.282**	89.50±1.176**	196.50±2.540**
3.	Standard	72.50±1.176**	60.83±1.424**	$180.16 {\pm} 1.542 {*}$
4.	EEPF-25mg/kg	78.16±1.078**	69.67±2.261**	191.16±2.227**
5.	EEPF-500mg/kg	$84.50{\pm}1.384^{**}$	65.50±1.821**	$186.83{\pm}2.822$

Values are expressed in mean  $\pm$  SEM, (n=6), when compared with control, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 one way ANOVA followed by Dunnnet's t-Test

according to the standard protocol recommended even by manufacturer's instruction manual supplied with the biochemical kits. RobonikPvt. Ltd. India using semi autoanalyzer (ERBA Mannheim chem.-5plusv2, Germany) [14].

# Estimation of Serum SGPT (UV-Kinetic Method)

## Principle

SGPT polymerizes a transmit like amino group through the l-Alanine to2-Oxoglutarate with both the forming like pyruvate as well as l-Glutamate. A pyruvic acid as such constructed formed has been allowed to react as for NADH to provide l-lactate. The speed of such reaction has been monitored by such an indicator interaction paired with IDH inside the existence like NADH (Nicotinamide Adenine Dinucleotide). An oxidising like NADH inside this interaction seems to be monitored like a reduce within absorption like NADH at 340nm, which would be directly proportionate to SGPT activity [15].

 $\begin{array}{l} l-Alanine+2-oxoglutarate \xrightarrow{ALT} Pyruvate+\\ l-glutamate \end{array}$ 

 $\begin{array}{l} Pyruvate \ + \ NADH + H^+ \xrightarrow{LDH} L- \ Lactate \ + \\ NAD^+ \end{array}$ 

ALT: Alanine Amino Transferase

LDH: Lactate Dehydrogenase

#### Procedure

As discussed in above table blank, standard as well as sample has been prepared through going to consider 1.oml to work reagent as well as 0.1ml each one of distilled water, standard as well as specimen including both, eventually all the specimens have been incubated at  $37^{\circ}c$ , aspirated separately but also absorbance was taken at 340 nm [16].

#### Estimation of Serum SGOT (UV- Kinetic Method)

#### Principle

SGOT polymerizes a transmission like amino group through the l- Aspartate of about 2-oxo glutarate only with forming like oxaloacetate as well as lglutamate. The speed of this interaction has been surveilled by such an indicator interaction paired to malate dehydrogenase (MDH)through whereby oxaloacetate established has been decided to convert about malate ion inside the existence like NADH (Nicotinamide Adenine Dinucleotide).

An oxidizing like NADH inside this interaction has been evaluated as just a decrease inside the absorption like NADH at 340 nm, which really is directly proportionate to SGOT activity [17].

 $\begin{array}{c} Oxaloacetate + NADH + H^+ \xrightarrow{MDH} L-Malate + NAD^+ \end{array}$ 

AST: Aspartate Amino Transferase

MDH: Malate Dehydrogenase

## Procedure

As did mention inside the SGPT, the same methodology had already been decided to follow (Table 1).

# **Estimation of Serum Alkaline Phosphate (ALP)**

Principle Serum alkaline phosphate hydrolysis p-Nitro phenyl phosphate inside the presence of an oxidizing agent  $Mg^{+2}$ . Such an interaction has been assessed just like absorbencies directly proportionate to an alkaline phosphate action.

 $p - Nitrophenyl \ phosphate \ + \ H_2O \xrightarrow{ALP} P - Nitrophenol + Phosphate$ 

ALP: Alkaline phosphatase

# Procedure

As mentioned in the above table blank, standard as well as specimen has been able to prepare through considering 500  $\mu$ l like operating reagent as well as 10 (Table 2).

 $\mu$ l every one of deionized liquid, standard, specimen respectively, afterward the all specimens have been sub cultured at 37°C, aspirated separately as well as absorbance was taken at 405 nm [18].

#### **Statistical Analysis**

A statistical significance has been evaluated using one way analysis of variance (ANOVA) as well as tried to follow besides dunnet's comparison test using prism graph software. All data will be presented just like mean +SEM but also p< 0.05 has been considered as substantial (Table 3, Table 4 and Table 5).

#### RESULTS

#### **Physicochemical Standards**

#### **Body Weights**

Effect of ethanolic extract of whole plant of Passiflora foetida on mean changes in body weigh (Table 6).

#### **Paw Volume**

Effect of ethanolic extract of whole plant of passiflora foetida on mean changes in paw volume (Table 7) [19].

#### **Biochemical Parameters**

Biochemical parameters evaluation of ethanolic extract of root of Passiflora foetida (Table 8) [20].

# DISCUSSION

Rheumatoid arthritis is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attack synovial joints. The process produces an inflammatory response of the synovium (synovitis ) secondary to hyperplasia of synovial cell disease process often leads to the destruction of articular cartilage and ankylosis of the joints RA can also produce diffuse inflammation in the lungs pericardium in the lungs, pericardium, pleura, sclera and also nodular lesions, most common in subcutaneous tissue Although the cause of RA is unknown, autoimmunity plays a pivotal role in both its chronicity and progression, and RA is considered as systemic autoimmune disease FA increases then N-acetyltransferase (NAT) activity in blood and liver that not only shows the impact on the serum biochemical parameters like SGOT. SGPT and ALP but also on hematological parameter such as RBC, WBC and, Hb% and ESR.

Indomethacin is indeed a non-selective receptor like cyclooxygenase (COX) 1 and 2, enzyme certain take part in prostaglandin synthesizing through the arachidonic acid. Prostaglandins have been hormone-like molecule normally found inside the body in which they have a wide selection like effect, most of which led to suffering, viral infection, as well as inflammatory.

Chemical constituent present in MEPF extract indicates the presence are o flavonoids, coumarins, phenols, tannins, anthocyanidines.

# CONCLUSION

The physicochemical constituents like ash values, extractive values, loss on drying and the phytochemical studies such as preliminary phytochemical screening of Passiflora foetida will be helpful in pharmacopeial standards for the global acceptance. Root extract of Passiflora foetida exhibit an Anti-Arthritic effect due to the presence of phytochemicals, Flavonoids, Tannins, Coumarins Phenols. The above compounds consist of Hydroxyl groups which consists of anti-oxidants which is helpful for curing of arthritis which gives relief from joint pains, body pains. Preliminary phytochemical investigations on the EEPF were noted the presence of flavonoids, tannins, coumarins, phenols. No mortality or behavioural abnormality recorded in mice during experiment at the highest dose level of 5000mg/kg tested for LD<sub>50</sub> studies. Indomethacin exhibited a significant anti-arthritic activity. The high dose of EEPF exhibited a significant anti-arthritic activity by reducing serum biochemical parameters like ALP,

SGOT, SGPT levels and reduced the haematological parameters like ESR, and WBC, and increases the RBC and Hb levels in FA induced arthritis models in rats. These phytochemicals mostly possess antioxidant properties it helps in cellular damage and can also help to reduce inflammation which is cause of Rheumatoid Arthritis. The active constituent and isolation and further research on its activity can be useful for the prospective study, treatment of Rheumatoid Arthritis.

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#### **Conflict of Interest**

The authors attest that they have no conflict of interest in this study.

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