Research article **Available online www.ijrpsonline.com** **ISSN: 2249–3522**

International Journal of Research in Pharmacy and Science

Phytochemical screening and evaluation of anti-inflammatory activity of leaves extract of *Holoptelea integrifolia* roxb.

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ABSTRACT

The main objective of the present investigation is to phytochemical screening and Evaluation of Anti-inflammatory activity of Petroleum ether and Methanolic extract of Holoptelea Integrifolia Roxb. On Carrageenan induced paw oedema methods. Methanol and Petroleum ether extract (100 mg &200 mg/kg body weight) of leaves of *Holoptelea integrifolia* Roxb. was screened for anti-inflammatory activity by using carrageenan induced paw edema method. Reduced acute paw oedema volume induced by sub planter injection of carrageenan (0.1 ml of 1% of solution) in Wistar Albino rats using plethysmometer. Diclophenac sodium (10 mg/kg b.w, p.o) was as a standard drug (positive control). Petroleum ether and Methanolic extract of *Holoptelea Integrifolia* Roxb (100 &200 mg/kg bw) showed potent anti-inflammatory activity when compared to control and positive control drugs (diclophenac sodium) group. Values are expressed as mean ± S.D. statistical significances were determined using the student t-test. Values with $p < 0.01$ were considered significance. The present study indicates that oral administration of both extract of *Holoptelea Integrifolia* Roxb. Dose dependant improves the potent anti-inflammatory activity. The extract lowers the carrageenan induced rats paw oedema. Further pharmacological and biochemical investigation are essential to elucidate the mechanism of action.

KEY WORDS: Anti-inflammatory, Paracetamol, Holoptelea, Chromatography, Photochemistry

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INTRODUCTION

Natural product is a source for bioactive compounds and has potential for developing some novel therapeutic agent. Over the last decade there has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment[1]. *Holoptelea integrifolia* belongs to family Ulmaceae, commonly known as Indian Elm. The vernacular names of the plant *Holoptelea integrifolia* are in Hindi- chirmil, chilbil, Gujrati- charel, Marathi- Papara, Sanskrit- chirbilva, Tamil- ayi. Indian elm is a large deciduous tree, growing up to 18 m tall. It has with gray bark, covered with blister, peeling in corky scales on old trees[2,3]. Leaves of the Indian elm is used for treating oedema, diabetes, leprosy and other skin disease, intestinal disorders, piles and sprue. Decoction of bark is used for rheumatism. Oral application of the leaves is used for intestinal tumors. Dried bark is useful for pregnant ladies. Decoction of the leaves regulate the fat metabolism. Leaves are externally used to treat ringworm. Paste of the leaves applied to treat the inflammation. In Nepal, bark is externally used for swellings. Bark and leaf of *Holoptelea integrifolia* are applied externally on the white patches or leucoderma[4-6].

MATERIALS AND METHODS

CHEMICALS

All chemicals were of Highest available purity and were procured from E.merck, Mumbai, India, Himedia laboratories, Mumbai, India and SD fine chemicals, Mumbai, India.

PROCUREMENT OF PLANT

The leaves of *Holoptelea integrifolia* is Authentified by a qualified Taxonomist, Mr. Vinod Sharma Botanist, "Rajasthan University Botany Department" Jaipur (Authetification number: RUBL20661). A specimen was deposited in the institutional herbarium of Rajasthan university campus, India. The collected plant material was made thoroughly free from any foreign organic matter and a part of the material was dried under shade.

PREPARATION OF DRUG SOLUTIONS AND REAGENTS

Diclofenac Sodium was purchased from Central drug house, New Delhi. Normal saline solution (9% NaCl) and vehicle (1% Carboxymethylcellulose, CMC, 10 ml/kg) were prepared in laboratory and used.

ANIMALS

Wistar albino rats (150-180 g) of both sexes were selected for the anti-inflammatory study. The rats were given food and water ad libitum. All the animals were kept under laboratory conditions for an acclimatization period of 7 days before carrying out the experiments. All studies were carried out in groups of 6 rats each. Each rat was housed separately in a metabolic cage.

Pharmacological study was approved by Animal Ethical Committee of School of Pharmacy; Suresh Gyan Vihar University, with CPCSEA no 1234/a.08.

ANTI-INFLAMMATORY ACTIVITYS

CARRAGEENAN INDUCED PAW OEDEMA

Carrageenan induced paw oedema model was used to determine the anti-inflammatory activity of the extracts by the method of Winter et al. (1962). Paw oedema was induced injecting 0.1 ml of 1% carrageenan in physiological saline into the sub plantar tissues of the left hind paw of each rat[7]. 36 rats were allowed to fast for 18 h and divided into 6 groups of 6 animals each.

Group I served as Control received the vehicle (1% Carboxymethylcellulose, CMC, 10 ml/kg p.o.).

Group II served as Standard, received Diclofenac Sodium (10 mg/kg b.w).

Group III and IV served as test, received petroleum extract at doses of 100 and 200 mg/kg b.w. p.o. respectively Whereas group V and VI received methanolic extract (100 and 200 mg/kg b.w. p. o).

The animals pretreated with extract or Diclofenac sodium one hour before were injected with 0.1 ml of 1% Carrageenan (in 1% CMC) solution into the sub-plantar region of right hind paw. Paw volume was measured by dislocation of the water column in a Plethysmometer[8] immediately after Carrageenan application at 0, and 4 h after the stimulus. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response. The results were expressed as a percentage of inhibition of oedema.

$$\text{Percentage inhibition of oedema} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_c is the inflammatory increase in paw volume in control group of animals and V_t is the inflammatory increase in paw volume in drug-treated animals.

STATISTICAL ANALYSIS

All the values are expressed as mean ± standard deviation and analyzed for ANOVA and post hoc Dunnet's t-test. Differences between groups were considered significant at $P < 0.01$ levels. The statistical analysis was carried out using Graph pad Instat 3.0 software.

RESULTS AND DISCUSSION

Phytochemical screening of this plant of various extracts showed significant results (Table-1). pH of the extract is 1% and 10% solution is respectively 7.56 and 7.34. Reducing sugar present only in petroleum ether extract. Steroids and protein were resulted in all extracts except distilled water. Phenol present only in chloroform, methanol and distilled water extract. Alkaloid present only in methanol extract. Triterpenoid and anthroquinone present in methanol and distilled water extract. Flavones and amino acid present all extracts expect distilled water. Cathacin present in benzene and methanol extract. Tannin was resulted in petroleum ether, benzene, and distilled water extract.

FLUORESCENCE ANALYSIS

Fluorescence Analysis was done in Ultraviolet Fluorescence Analysis Cabinet. These systems offer the convenience of selecting UV wavelengths simply by pushing a button.These units combine separate built-in long wave and short wave UV light sources with specially designed specular aluminum reflectors to ensure maximum intensity and superior fluorescent contrast. In this process the herbal drugs was treat with different - different chemical reagent see the fluorescence of drugs under Ultraviolet light. And observe the fluorescence of drugs. When measured at the cabinet floor, the long wave UV source provides a typical peak intensity of 610 µW/cm2, while the short wave source has an intensity of 500 µW/cm2.

Table 1: Preliminary phytochemical analysis[10]

S.No.	Test		Powder drug	Pet ether	Methanol
1	Carbohydrates	Molish test Fehling test Benedicts test Iodine test	+ve +ve +ve +ve	-ve +ve -ve -ve	+ve +ve +ve +ve
2	Gum and Mucilage	Rhedanium red Swelling property	+ve +ve	-ve -ve	+ve -ve
3	Protein and Amino acid	Biuret test Ninhydrine test Heavy metal test Xanthoproteic test	+ve +ve +ve +ve	-ve -ve +ve -ve	+ve +ve +ve +ve
4	Fixed oils and Fats	Spot test Saponification test	+ve +ve	-ve -ve	-ve -ve
5	Alkaloids	Mayers test Dragendroff test Hager's test Wagnar test	-ve +ve +ve +ve	-ve -ve -ve +ve	-ve -ve -ve +ve
6	Glycosides	Legal test Bontrager test Killer killiani test Baljet test	+ve -ve +ve +ve	+ve -ve +ve -ve	+ve -ve -ve -ve
7	Phytosterol	Salkowaski test Libermanns buchard Libermann test	+ve -ve +ve	+ve -ve Slightly +ve	+ve -ve +ve
8	Flavonoids	Ferric chloride test Shinoda test Lead acetate Reaction with acid and base	+ve -ve +ve -ve	-ve -ve +ve -ve	-ve -ve +ve -ve
9	Tannin and Phenolic compound	Ferric chloride test Lead acetate test Pot. dichromate test Copper sulphate	+ve +ve +ve +ve	+ve -ve +ve +ve	+ve -ve -ve Slightly +ve
10	Saponins	Foam test	+ve	-ve	+ve
11	Volatile oils	Hydro distillation	-ve	-ve	-ve

Table 2: Fluorescence analysis

Chemical treatment	Fluorescence Observed	
	Under visible light	Under UV light
Powder as such	Green	Light green
Powder + 1N NaOH in methanol	Yellowish green	Pale green
Powder + 1N NaOH in water	Light red	Dark red
Powder + 50%HCL	Light yellow	Pale green
Powder + 50%HNO3	Light yellow	Pale green
Powder + 50%H2SO4	Dark green	Green
Powder + Petroleum ether	Dark green	Dark green
Powder + chloroform	Black	Dark green
Powder + picric acid	Green	Dark green
Powder + 5%Ferric chloride solution	Black	Light green
Powder + 5% Iodine solution	Brown	Light brown
Powder + Methanol	Dark brown	Black

P^H DETERMINATION

pH 1% solution: 1 gm of the accurately weighed drug is dissolved in water and filtered pH of filtrate is checked with a standardized glass electrode.

Table 3: P^H determination

	1 % solution	10 % solution
pH	7.56	7.34

pH 10% solution: 10 gm of the accurately weighed drug is dissolved in water and filtered. P^H of filtrate is checked with standardized glass electrode[9]. Results are shown in Table-3.

THIN LAYER CHROMATOGRAPHY

The technique of thin layer chromatography closely resemble those of column and paper chromatography. In thin layer chromatography, partition, however, occur on a Layer of finely divided adsorbent, which is supported on a glass plate.

This chromatography using thin layers of an adsorbant held on a glass plate or other supporting medium is known as thin layer chromatography. A glass plate is coated with a loose powder or with slurry of an adsorbant or anather finely ground materials. Slurries will adhere to the surface of the glass plate, after drying, as a thin layer. the unknown substance and reference materials are dissolved in

Table 4: TLC chromatography[11]

S.No.	Solvent system	For	Viazualisation	Rf value of pet ether extract	Rf value of Methanolic extract
1	Diethyl ether: Benzene (1:1)	Fatty acids	UV light at 60 F_{254}	0.4	0.25
2	Ethyl acetate : methanol (80:20)	Glycosides	UV light at 60 F_{254}	0.20	0.10
3	Ethyl acetate : formic acid : GAA : H_20 (100:11:11:2.7)	Flavonoids	UV light at 60 F_{254} and polyethylene glycol	0.10	0.5
4	Butanol : acetic acid : water (40:10:50)	Proteins and amino acid	UV light at 60 F_{254}	0.42	0.42
5	Chloroform : GGA : MeOH : H_20 (64:32:12:8)	Saponins	Anisaldehyde in sulphuric acid & UV light at 60 F_{254}	0.45	0.06
6	Dichloromethane: n-hexane : methanol	Steroids	UV light at 60 F_{254}	0.91	0.91
7	Toluene : Ethyl acetate: diethylamine (7:2:1)	Alkaloids	UV light at 60 F_{254} and	0.83	0.83
8	GGA : H_20 (2:98)	Tannins	Vanillin in HCl reagent	0.35	0.07

water or organic solvent and the solution is applied in a row of spot, 1-2 cm from the edge of the plate, with the help of a capillary micropipette or micro syringe. The chromatoplate is placed in a jar containing the solvent for development and the jar is lined inside with filter paper which acts as a wick and thus separates the atmosphere of the jar with solvent vapors. The jar is covered with an air tight lid. As the solvent ascend through the layer of capillary action, the sample is resolved in to friction. The plate is carefully withdrawn after the solvent front has migrated about 75% of the length of the plate. The plate is then dried and sprayed with a reagent for detection of components or more commonly exposed to iodine vapors. Solute position indicated by brown spots.

COLUMN CHROMATOGRAPHY

Solvents were choosen according to polarity from nonpolar to polar following solvent are Hexane, Petroleum ether, Ethyl acetate + Acetone, Acetone, Acetone + methanol, methanol + water and water. Sixty one friction were collected out of which first 4 friction from Hexane, 5 friction from pet ether 7 friction from ethyl acetate, 9 friction from ethyl acetate + acetone, 9 from acetone, 8 from acetone + methanol, 5 from methanol, 7 from methanol + water, 7 from water. Results are presented in Table-5.

Table 5: Chromatographic evaluation of plant extract

S.No.	Solvent system	Friction no.	R_F value	Remark
1	$CHCl_3$:GAA:MeOH:H_2O (64:32:12:8)	42	0.45	β-amyrin
2	Acetic acid: Acetonitrile (20:60)	51	0.91	Stigmasterol
3	Acetic acid: Acetonitrile (20:60)	27	0.83	β-sitosterol
4	Butenol: Acetic acid:H_2O (40:10:50)	35	0.2	Histamine
5	n-butenol: Acetic acid:H_2O	17	0.5	Histidine

ANTI INFLAMMATORY ACTIVITY

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation.

Table 6: **Effect of the petroleum ether and methanolic extract of *holoptelea integrifolia* leave against carrageenan induced paw oedema in rats**

Group	Paw edema volume (ml)					% Inhibition
	0h	1h	2h	3h	4h	
Control (0.1 ml of 1% carrageenan)	0.27± 0.03	0.43±0.04	0.58±0.02	0.72±0.03	0.87±0.01	-
Standard Diclofenac sodium (10 mg/kg)	0.23±0.02	0.34±0.03	0.28±0.01	0.25±0.01	0.27±0.02	93.33**
Petroleum Ether Extract (100mg/kg) + (0.1 ml of 1% carrageenan)	0.19±0.02	0.32±0.02	0.45±0.03	0.61±0.04	0.69±0.05	16.66^{ns}
Petroleum Ether Extract (200mg/kg) + (0.1 ml of 1% carrageenan)	0.23±0.02	0.31±0.03	0.42±0.03	0.56±0.02	0.64±0.04	31.66^{ns}
Methanolic extract (100mg/kg) + (0.1 ml of 1% carrageenan)	0.3±0.02	0.42±0.02	0.39±0.03	0.37±0.04	0.34±0.05	86.66**
Methanolic Extract (200mg/kg) + (0.1 ml of 1% carrageenan)	0.26±0.02	0.39±0.03	0.35±0.01	0.32±0.02	0.28±0.02	88.33**

**** Extremely significant (P<0.01), * Significant (p< 0.05), ns- Not significant (P> .05)**

All extracts exhibited anti-inflammatory activity when compared withcontrol group (T. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids which affect the system.

The most important mechanism of action of anti-inflammatory action of NSAIDs is considered to be inhibition of PG synthesis at the site of injury. The anti-inflammatory potency of different compound roughly corresponds with their potency to inhibit COX. However paracetamol is a potent anti-inflammatory and relatively strong COX inhibitor. PGs are only one of the mediator of inflammation; inhibition of COX dose not depress the production of other mediator like LTs ,PAF, cytokine etc.

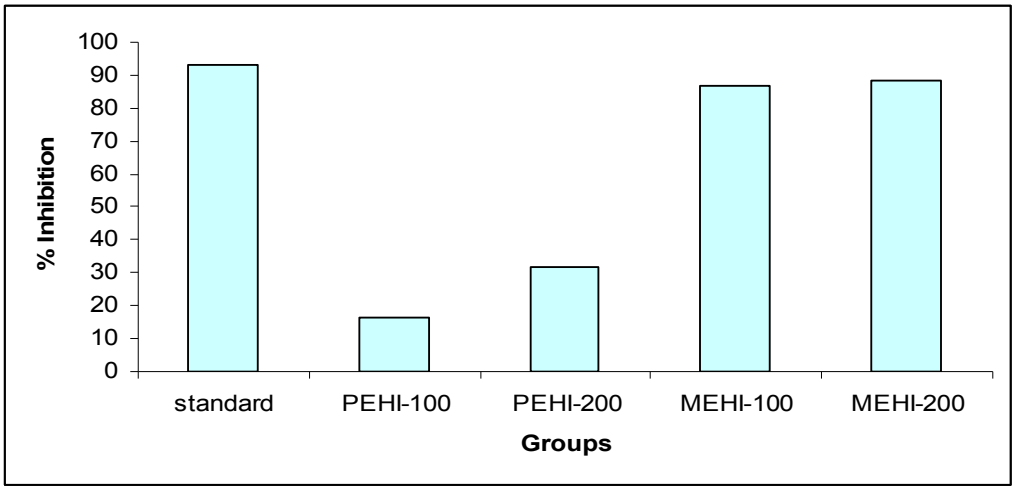

Figure 2: **effect of the petroleum ether and methanolic extract of *holoptelea integrifolia* leave against carrageenan induced paw oedema in rats PEHI- petroleum ether extract of *holoptelea integrifolia* and MEHI- methanolic extract of *holoptelea integrifolia***

CONCLUSION

Phytochemical studies were focused on preparation of petroleum ether and methanol extract of *Holoptelea integrifolia.* Both extracts and *Holoptelea integrifolia* leaf powder were studied to identify the presence of specific phytoconstituents in them. It was identified that *Holoptelea integrifolia* contains carbohydrates, flavanoids, glycocides, steroids, tannins, proteins and amino acid, alkaloids, saponins, etc. Whereas petroleum ether extract contains steroids, antraquinone glycocides, reducing sugar, flavanoids,amino acid and tannins . And methanol extract contains carbohydrates, proteins, amino acid, steroids, tannins, phenolic compound, flavonoids, saponins. Thin layer chromatographic investigations of both extracts reported presense of Alkaloids, glycocides, flavanoids, proteins and amino acids, saponins and steroids. Column chromatography investigation of plant extract reported the presence of β-amyrin, stigmasterol, β-sitosterol, histamine, histidine.

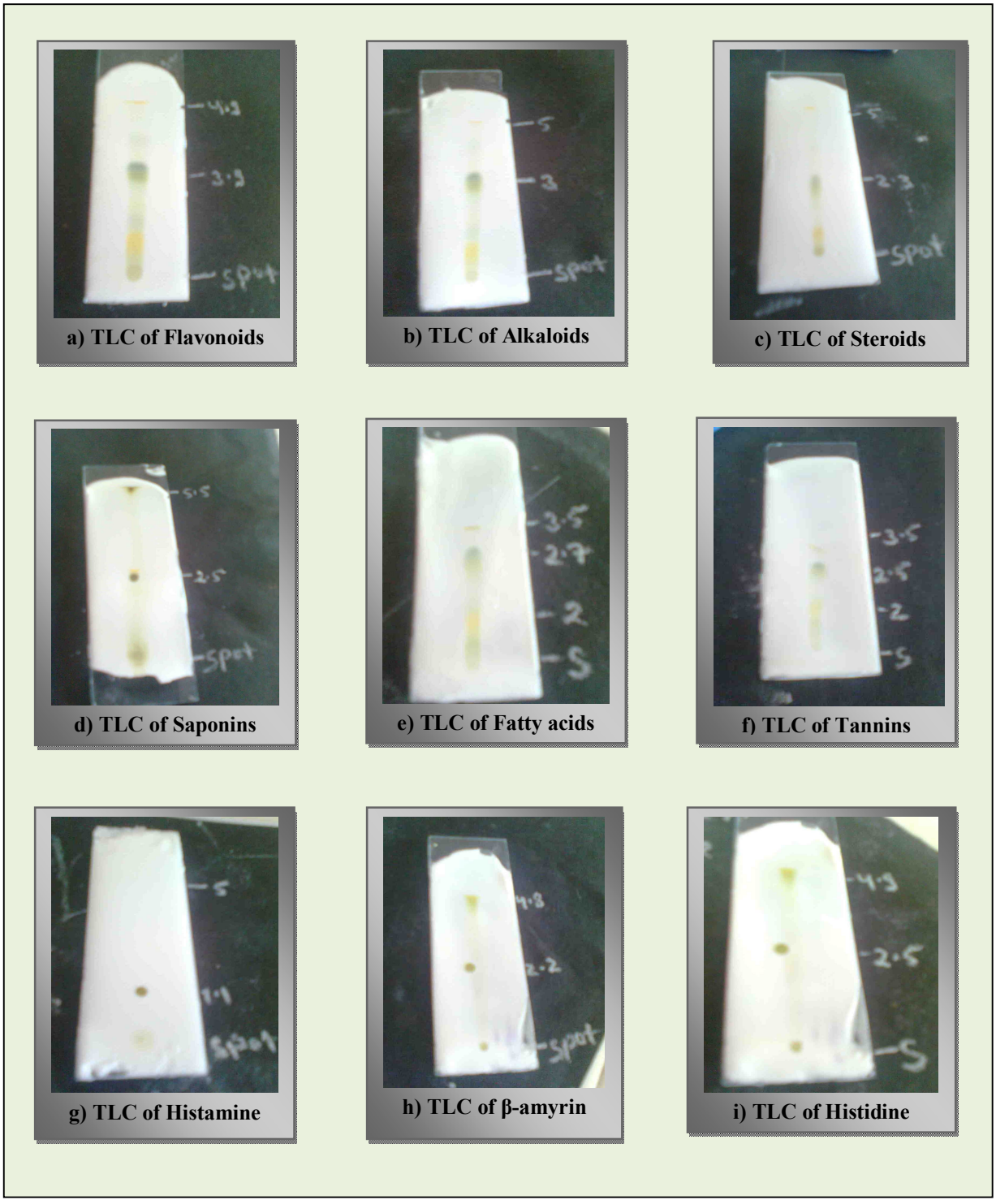

Figure 1: TLC of the plant extract of *holoptelea integrifolia*

Pharmacological study was focused on preparation of petroleum ether and methanol extract of *Holoptelea integrifolia.* It can be concluded from the present dissertation work that of Holoptelea *integrifolia* shows extremely significant positive Anti-inflammatory activity of both dose 100 mg/kg

and 200 mg/kg methanolic extract and non significant of both dose 100 and 200 mg/kg with petroleum ether extract when compared with Diclofenac sodium as standard anti-inflammatory drug. The activity may be due to presence of steroids and glycosides in methanolic extract and petroleum ether extract.

ACKNOWLEDGMENT

Authors are greatly thankful to Mr. Sunil Sharma , Chancellor and Dr. Sudhanshu Sharma, Chief Mentor Suresh Gyan Vihar University,Jaipur, for providing necessary facilities

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