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EXTRACTION, SCREENING, FORMULATION AND EVALUATION TEST FOR GEL PREPARATION OF ETHANOLIC LEAF EXTRACT OF ACALYFA INDIA LINN FOR ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

Acalypha indica is the fourth largest genus of the euphorbiaceae family. Most of the Acalypha species are used as medicinal plants. It has various traditional uses such as cure for stomach ache, dyspepsia, venom antidote, rheumatism and dermatitis. Present study involves ethanolic extract of the leaves of Acalypha indica. it was subjected to the phytochemical screening. The plant contains carbohydrates, steroids, glycosides, flavanoides, tannins and alkaloids. Plant extract was subjected to the anti-inflammatory activity by carrageenan induced rat hind paw method. The response is as potent as standard drug indomethacine and predenisolone. The alcoholic extract was formulated into gel and carried various evaluation tests such as PH test (6.7), spreadability 7.5cm, viscosity, In vivo diffusion test and the results were upto IP Standard. The GEL was subjected to the skin sensitivity test. The test is passed and there is no skin irritation was observed. The gel formulated can be used for analgesic and anti-inflammatory purpose for treat pains and strains.

INTRODUCTION

Acalypha is the fourth largest genus of the Euphorbiaceae family. It consists of 450 species and it is a green shrub , trees and annuals, mainly grown in the tropical regions of Africa, America and Asia. Most of the *Acalypha* species are used as medicinal plants. It has various traditional uses such as cure for stomachache, dyspepsia, venom antidote, rheumatism and dermatitis. Additionally, the leaf infusion is used for the treatment of stomach problems and swellings of the body, while the leaf maceration is applied in eye infections and its decoction is consumed in Tanzania to treat epilepsy. The plant has several biological values concerning to its antioxidant, anti-inflammatory, wound healing and cytotoxic properties. Indian Copperleaf is a small erect herb, growing up to 60 cm or more. The ascending branches are angled and velvet-hairy. Leaves are broadly ovate, nearly triangular rather coarsely toothed. Leaf stalks are as long as or longer than the 3-5 cm long blades. The plants belongs to kingdom plantae, subkingdom tracheobiota, superdivision spermatophyte, division magnaliophyta , dicotyledons, order euphorbials, family euphorbiaceae. Ethanolic extract was prepared and subjected to the anti-inflammatory activity, the obtained extract was prepared in the form gel and evaluation test are performed. The

leaves are Alternate, ovate, rhomboid-ovate, sometimes obovate, subacute or obtuse at apex, cuneate or tapering at base, serrate only in the upper part along the margins, dark-green above, pale-green below, glabrous or thinly hairy, petioles 3-8 cm long, hairy, stipules small. The leaf extract was used for the treatment of scabies and other skin infections. It is also useful in bronchitis, pneumonia. Gels are the semi solid preparations which are intended for the external purpose. The sustained acalyphamide gel, which contains the anti-infalmmation, anti-diabetic, anti analgesic and cytotoxic activity. To enhance the antiinflammation activity of acalyphamide in acalypha indica by using extraction process using different solvents(ether, chloroform water, hydro- ethanolic) . the main aim to ensure the inflammation activity by different solvents . The clinical trails are performed on the rats approved by the CPCSEA board. The ethanolic extract of acalyphamide shows the more potent nature than indomethacin , prednisolone which are taken as standards.

Materials and methods

Dried leaves and seedlings of Acalypha Indica (Local area of Manglagiri) authenticated by Acharya Nagarjuna University. Animal ethical committee of Nirmala college of pharmacy was approved the work. Healthy adult Swiss albino rats (150-200g, male) were used for the study. The animals were acclimatized to standard laboratory conditions (temperature 25±5°C0, humidity (55±5%) and maintained on 12-h light : 12-h dark cycle. They provided with regular rat chow and drinking water at libitum. Solvent ethanol, normal saline solution, carrageenan, Tween 80, sodium lauryl sulphate, carbapol(950) grade all the chemicals are from Sigma Aldrich. .The equipments used are plethysograph, frans diffusion cell, membrane preparations.

Preparation of Plant material:

Acalypha indica L. Was collected at flowering stage from Guntur district, Andhra Pradesh identified at the following collection, the whole plant was shade dried for 7 days and made into a coarse powder by grinding.

Preparation of ethanol extract:

900 ml of ethanol was taken and plant product is placed in soxhalet apparatus, then soxhalation is carried for 4 days at temperature below 40°C. The ethanol extract is concentrated by means of simple distillation.

Preparation of test material: The extracts (ethanol) were suspended in saline solution containing 1% Tween 80 such that each ml of suspension contained 125mg and 250mg of the extract.

PHYTOCHEMICAL SCREENING:-

TEST FOR CARBOHYDRATES:

(a) Molishs Test: To 2-3 ml. Aqueous extract, add few drops of alpha-naphthol solution in alcohol, shake and conc. H2SO4 from sides of the test tube. Violet ring is formed at the junction of two liquids.

TEST FOR REDUCING SUGARS:

Fehling"S Test: Mix 1ml. Fehling"s A and add1ml. Fehling"s B solutions, boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 mins. First a yellow, then brick red ppt is observed.

TEST FOR NON-REDUCING POLY SACCHARIDES:

(a) Iodine Test: Mix 3 ml. Test solution and few drops of dilute iodine solution. Blue colour appears, it disappears on boiling and reappears on cooling

(b) Tannic Acid Test For Starch: With 20% tannic acid, test solution give ppt.

TEST FOR PROTIENS:

(a) Biuret Test: To 3 ml. T.S. add 4% NaOH and few drops of 1%$CuSo_4$ solution . violet or pink colour appears.

TEST FOR AMINO ACIDS:

(a) Ninhydrin Test: Heat 3 ml. T.S. add 3 drops 5% ninhydrin solution in boiling water bath 10 mins. Purple or bluish colour appears.

TEST FOR STEROIDS:

(a) Salkowski Reaction:

To 2ml Of extract, add 2ml chloroform and 2 ml Conc H2SO4. Shaken well chloroform layer appears red and acid layer shows greenish yellow fluorescence.

TEST FOR CARDIAC GLYCOSIDES:

(a) Baljet'S Test:

A thick section shows yellow to orange colour with sodium picrate.

TEST FOR ANTHRAQUINONE GLYCOSIDES:

(a) Borntragers Test For Anthraqunione Glycosides:

To 3 ml. Extract, add dil.h2so4. boil and filter. To cold filterate. Add equal volume benzene or chloroform. Shaken well. Separate the organic solvent. Add ammonia. Ammonical layer turns pinkor red.

TEST FOR SAPONIN GLYCOSIDES:

(a) Foam Test:

Shaken the drug extract or dry powder vigoursly with water. Perisistent foam observed.

(b) Haemolytic Test:

Add drug extract or dry powder to one drop of blood placed on glass slide. Haemolytic zone appears.

TEST FOR FLAVONOIDS:

(a) Shinoda Test:

To dry powder or extract add 5ml. 95% ethanol, few drops conc. HCl and 0.5 g magnesium turnings. Pink colour observed. To small quantity of residue, add lead acetate solution yellow coloured precipitate is formed. Addition of increasing amount of sodium hydroxide to the residue shows yellow colouration which decolourises after addition of acid.

EXPERIMENTAL PROCEDURE:

Animal activity.

For plant extract: Animals were divided into five groups, each containing 2 animals. Group I served as normal control. Group II was injected with carragenan + standard drug Indomethacin (10mg/kg body weight) Group III was injected with carragenan + standard drug Prednisolone (10mg/kg body weight) Group IV was injected with carragenan + plant extract (125mg/kg body weight) Group V was injected with carragenan + plant extract (250mg/kg body weight)**.** The results are showed in the table no 2, 3 and 4. Fig 1, 2, 3. The pharmacokinetics was studied by using the results of pharmacological data**.**

EVALUVATION TEST FOR THE GEL: PHYSICO-CHEMICAL TESTS:

pH Test: The pH of gel which is initially prepared by the mechanical process. The ph was gel was checked on the preparation was noted as 6.07. After 15 days the Ph was noted as 7.2. This test is majorly to detect the stability of P^H of the product.

IN – VITRO **DIFFUSION STUDIES:** Diffusion studies can be done by frans diffusion cell.

PREPARATION OF SEMI PERMEABLE MEMBRANE: take the egg by making the hole in the egg remove all residues of egg and wash with distilled water twice. place the egg hard shell in 50 ml of conc.HCl to remove the calcareous part of shell. after melting of shell the membrane was obtained used in frans diffusion shell.

PROCEDURE: place the 10 ml of phosphate buffer in the canal and the gel was applied on the membrane and placed on magnetic stirrer. Collect the samples of 1 hour at 1,2,3,4,5,6,12 hours respectively. Measure the absorbance at 232 nms in uv specctrometry.results were represented in table no 5, fig no 4. The apparatus consists of two slides in which one slide is firmly fixed in a wooden frame while the other slide can easily slide over the surface of the fixed one. Anexcess of gel was placed between the two slides of the apparatus. A weight of 1Kg was allowed to rest on theslide

for 5 minutes so that a uniform film of gel wasformed and the air between the slides was expelled. The excess gel was removed carefully from the edges of the slides. The bottom slide was properly anchored and the top slide was subjected to a pull of 80 gms weight.

Table no 1: Phytochemical constituent of *Acalypha*

S.No	Type of constituent	Ethanolic extract
1	Carbohydrates	+
2	Steroids	+
3	Proteins	-
4	Amino acids	-
5	Glycosides	+
6	Flavanoids	+
7	Tannins	+
8	Alkaloid	+

Treatment	Dose (mg/kg body weight)	Increase in paw volume			
		1 h	2 h	3 h	4 h
Normal	---	0.4±0	0.4±0	0.4±0	0.4±0
Control(saline)	---	1.15 ±0.29	1.52±0.29	1.71±0.29	1.8±0.29
Indomethacin	10	0.45±0.048	0.5±0.048	0.55±0.048	0.45±0.048
Prednisolone	10	0.6±0.05	0.6±0.05	0.6±0.05	0.5±0.05
Chloroform extract	125	0.55±0.065	0.65±0.065	0.6±0.065	0.5±0.065
Chloroform extract	250	0.55±0.065	0.65±0.065	0.6±0.065	0.5±0.065

Table 2: Antiinflammatory effect of chloroform extract of Acalyphaindic L In carrageenan-induced rat paw inflammation

Treatment	Dose (mg/kg body weight)	Increase in paw volume			
		1 h	2 h	3 h	4 h
Normal	---	0.4±0	0.4±0	0.4±0	0.4±0
Control(saline)	---	1.15 ±0.29	1.52±0.29	1.71±0.29	1.8±0.29
Indomethacin	10	0.45±0.048	0.5±0.048	0.55±0.048	0.45±0.048
Prednisolone	10	0.65±0.06	0.6±0.06	0.6±0.06	0.5±0.06
Ethanolic extract	125	0.60±0.57	0.60±0.57	0.52±0.57	0.55±0.57
Ethanolic extract	250	0.6±0.06	0.6±0.06	0.5±0.06	0.5±0.06

Table 3: Antiinflammatory effect of ethanolic extract of Acalyphaindica L In carrageenan- induced rat paw inflammation

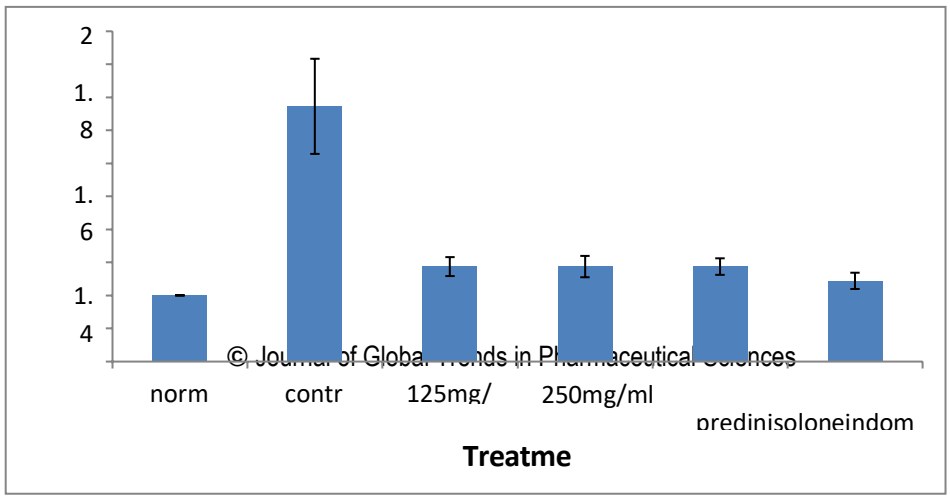
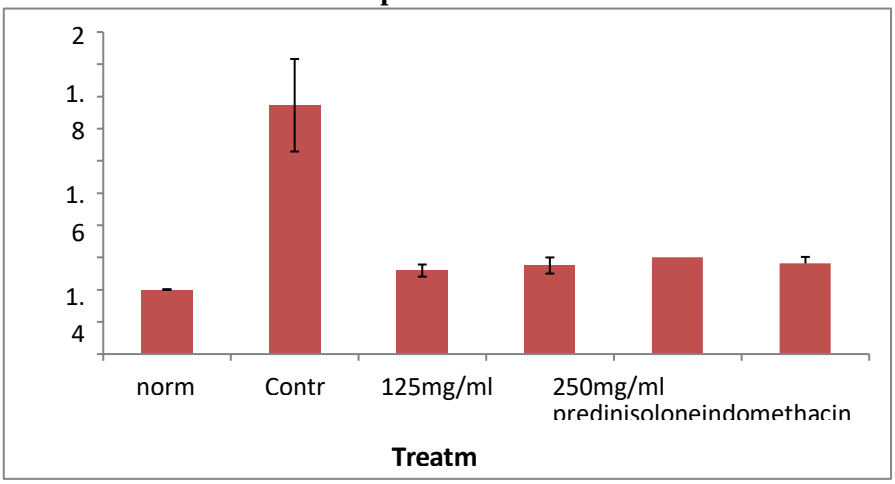


Table 4: Antiinflammatory effect of hydroalcoholic extract of Acalyphaindica L In carrageenan-induced rat paw inflammation

Treatment	Dose (mg/kg body weight)	Increase in paw volume			
		1 h	2 h	3 h	4 h
Normal	---	0.4±0	0.4±0	0.4±0	0.4±0
Control(saline)	---	1.15 ±0.29	1.52±0.29	1.71±0.29	1.8±0.29
Indomethacin	10	0.55±0.05	0.6±0.05	0.6±0.05	0.5±0.05
Prednisolone	10	0.65±0.04	0.6±0.04	0.6±0.04	0.55±0.04
Hydroalcoholic extract	125	0.5±0.03	0.6±0.03	0.5±0.03	0.5±0.03
Hydroalcoholic extract	250	0.6±0.05	0.6±0.05	0.6±0.05	0.5±0.05

Fig 2: Antiinflammatory effect of Hydroalcoholic extract of Acalyphaindica L In carrageenan-induced rat paw inflammation



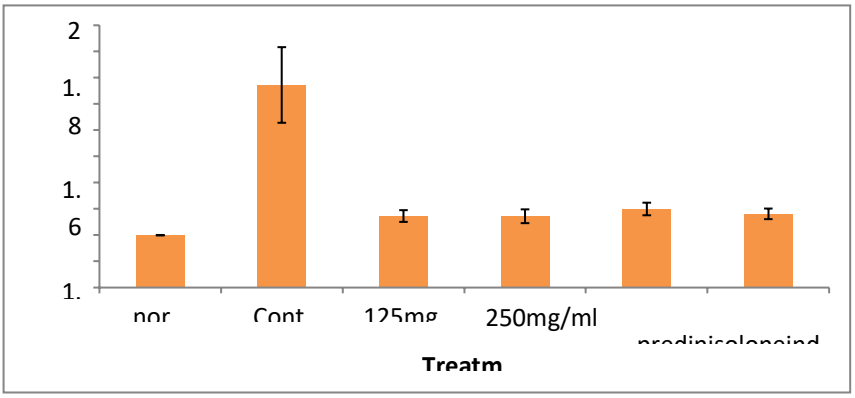


Fig 3: Antiinflammatory effect of Ethanolic extract of Acalyphaindica L In carrageenan- induced rat paw inflammation

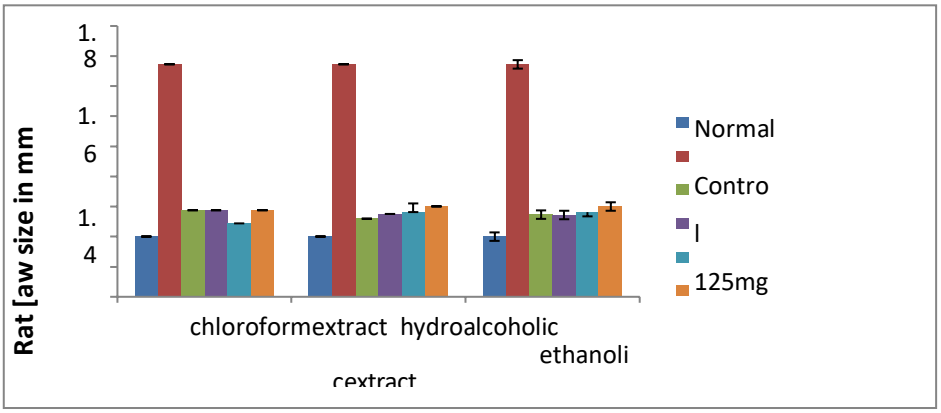
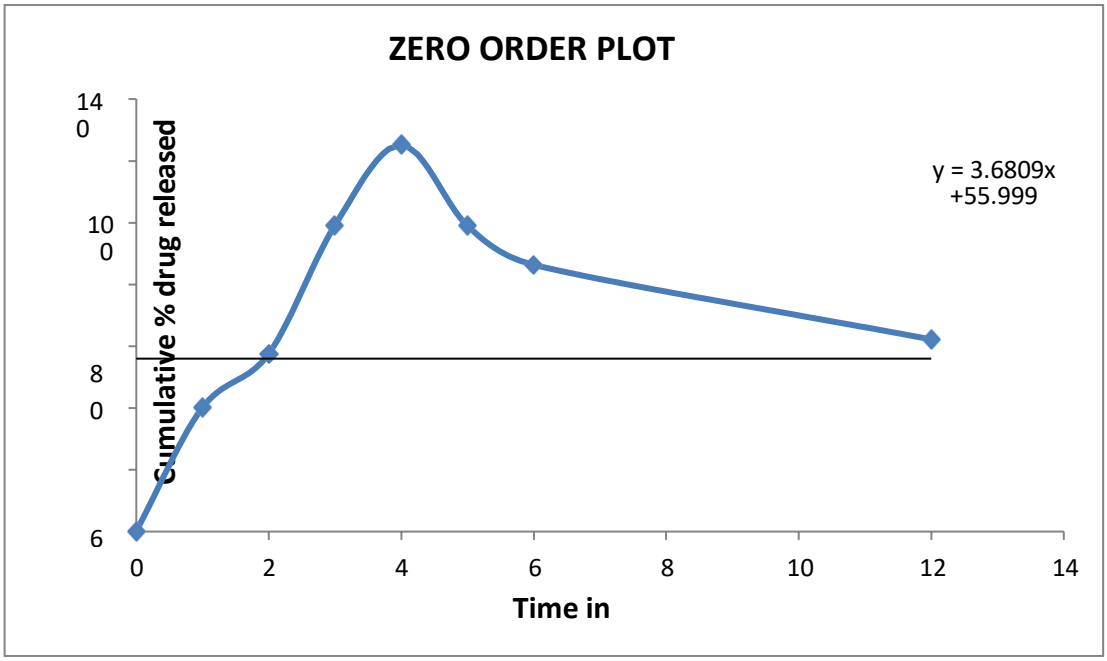


Fig 4: Comparitive study of Antiinflammatory effect of extracts of Acalyphaindica L In carrageenan-induced rat paw inflammation



Graph 1: Zeo and First order plot of Acalypha indica linn gel formulation

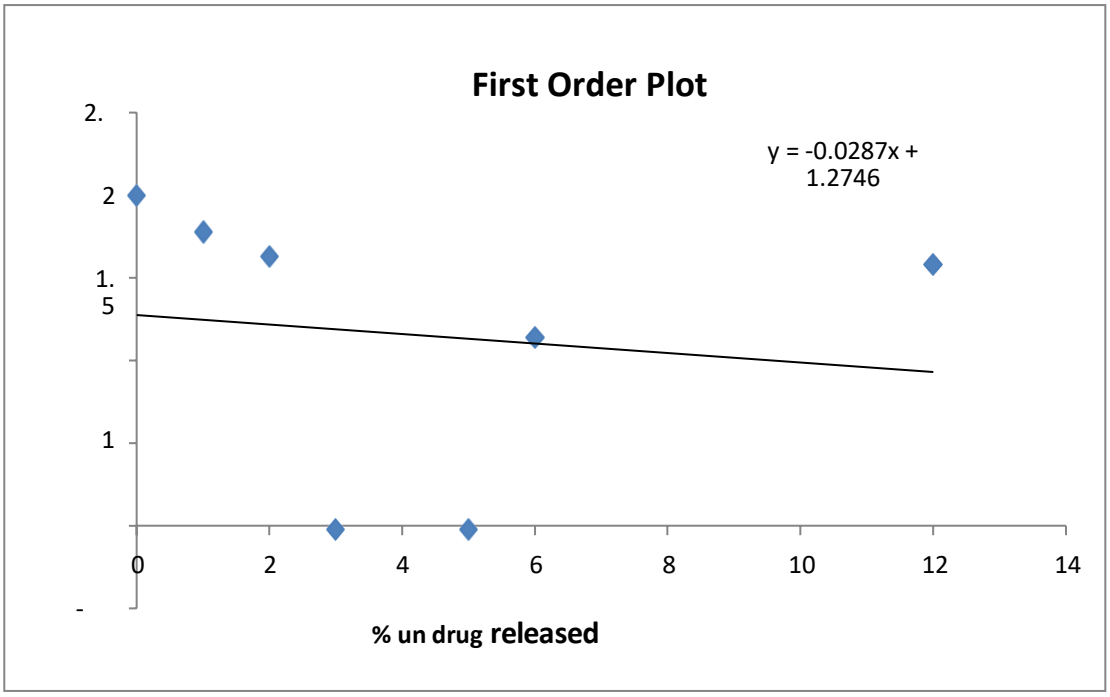
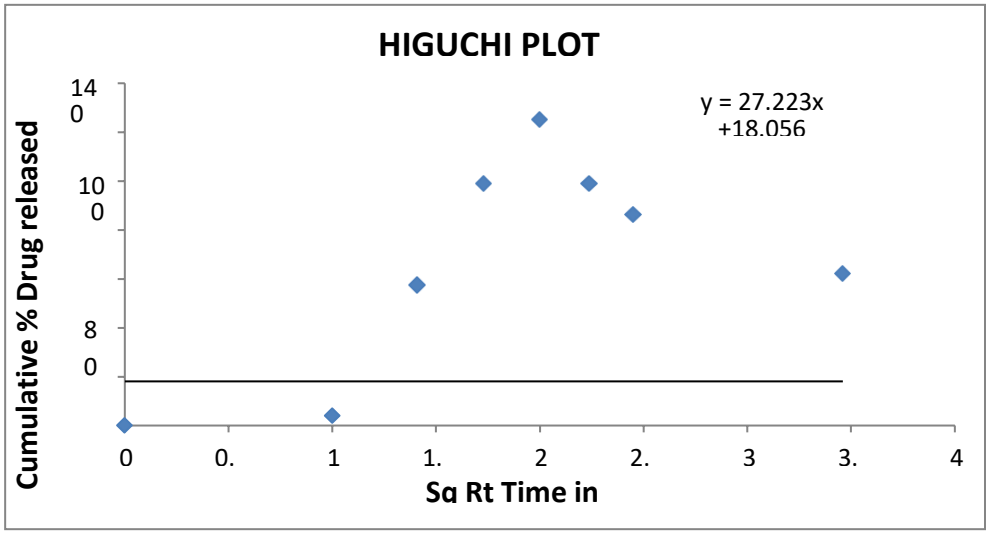
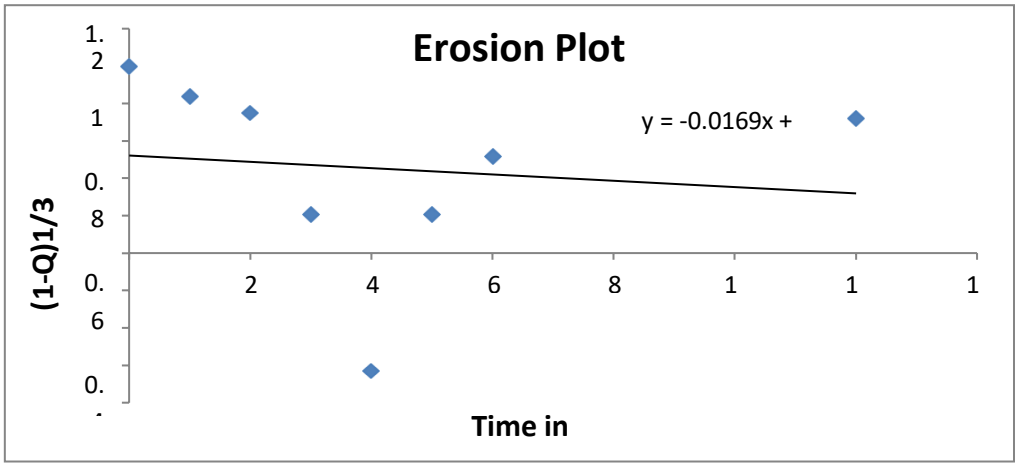
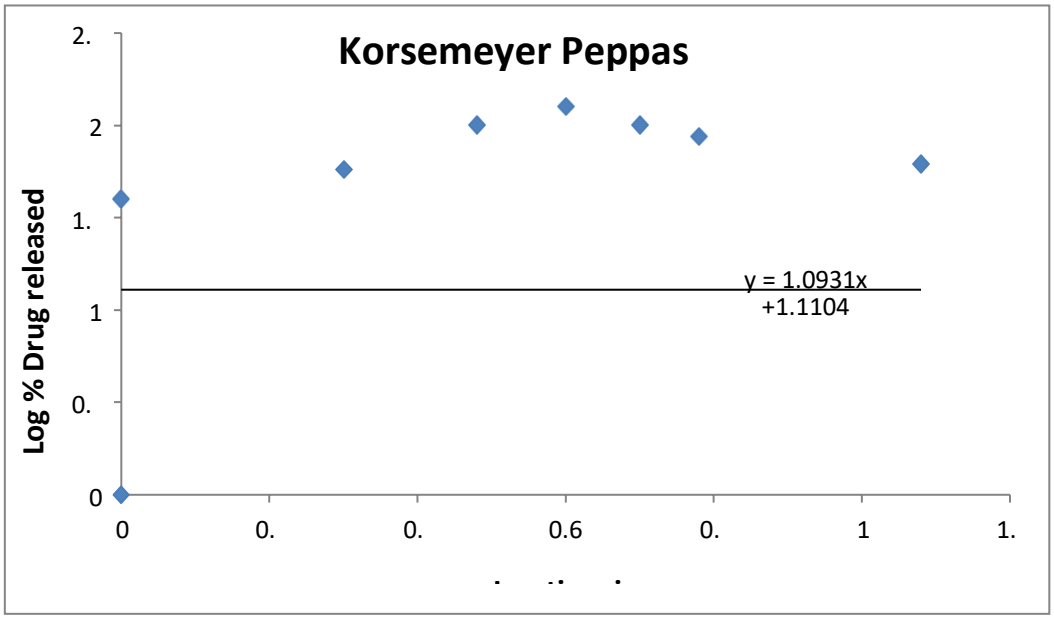


Fig no 6 Graph 3: Higuchi and erosion and peppas plot of Acalypha indica linn gel formulation







EVALUVATION TEST FOR THE GEL:

Table 5 - Absorbance vs time

S.NO	TIME (HOURS)	ABSORBANCE
1	1	0.1517
2	2	0.2175
3	3	0.3748
4	4	0.4739
5	5	0.3748
6	6	0.3267
7	12	0.2354

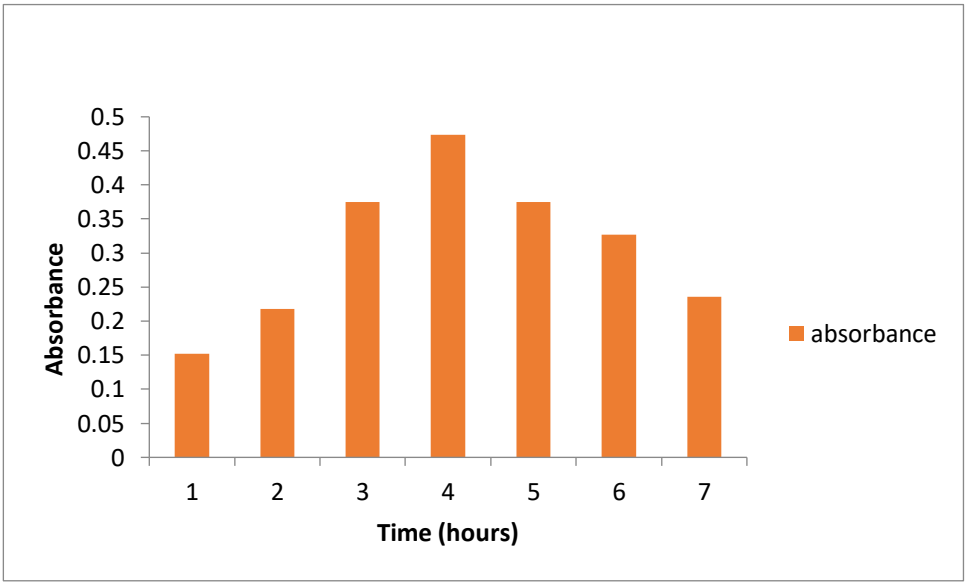


Fig no 4 - Bar graph on absorbance vs time

Table no 6: Viscosity vs rpm

RPM	CENTIPOISE	TORQUE
0	0	24.4
0.3	1.22e	60.9
0.6	680e	68
1.5	0000	0000
0.5	614e	51.2
1	446e	74.6
2	0000	00000

Fig:9 Bar graph on viscosity of gel

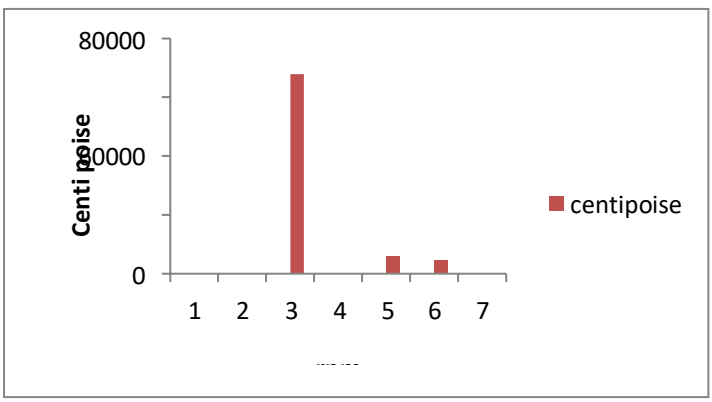
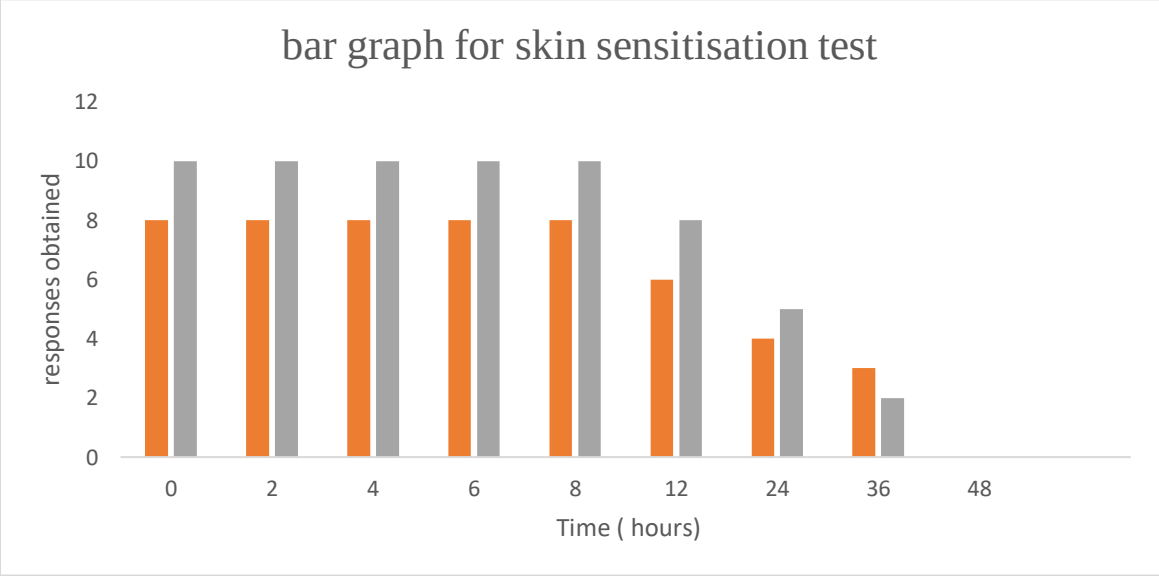


Fig 10 - Response graph

Tab no 7 values representing responses at a particular period of time

TIME(hours)	TEST RESPONSE (mm)	STANDARD RESPONSE (mm)	CONTROL RESPONSE (mm)
0	8	10	10
2	8	8	10
4	8	8	10
6	8	8	10
8	8	7	8
12	6	8	6
24	4	5	5
36	3	2	3
48	0	0	0



The time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability. Spreadability was then calculated using the following formula: S = M × L/ T. Where, S = is the spread ability, M = is the weight in the pan (tied to the upper slide), L = is the length moved by the glass slide, T = represents the time taken to separate the slide completely.

3. Viscosity: viscosity is determined by Brookfield viscometer.

4. Skin Irritation Test:

Procedure:- 1% solution of carrageenan was prepared. The test solution was given in the proportion of 50:50 in a complex mixture of (0.5 ml of carrageenan + 0.5 ml of saline solution. The solution was injected to the sub marginal vein of rats to induce the inflammation. The inflammation was indicated by the redness. The rats were devided into three groups each contain 3 animals. The carrageenan-saline solution was injected to the intra ocular muscle of rat to induce inflammation on the test and standard rats, after obtaining the redness, the standard gel (diclofenac gel) and test gel (acalyphamide gel) was applied on the injured rats, for the successive time intervals such as 0,2,4,6,8,10,12,24,36,48 hours respectively . The response shown by the test and standard gel was recorded by measuring the distance covered by gel which mimics the therapeutic activity of test formulation. By comparing the responses the msc can be evaluated with help of scale. Results were represented in table no 7, fig no 10.

RESULT AND DISCUSSION

The ethanolic extract was subjected to the phytochemical screening it contains alkaloids, carbohydrates, steroids, glycosides, flavanoides, tannins, alkaloids. Ethanolic extract was subjected to the Anti inflammatory activity was performed by carrageenan induced rat hind paw method the percent inhibition for first hour was 0.45-0.5mm, second hour 0.5-0.6, third hour 0.45-0.5mm, fourth hour 0.45-0.5mm. The extract was formulated into gel and evaluation test was performed. P^H tested it was 6.07, spreadability was 7.5cm, viscosity was in between 1.22-680e. Invitro diffusion cell the results are satisfactory. The gel was subjected to skin sensitivity test, there was no occurance of skin irritation.

CONCLUSION:

Acalypha indica is a wild plant. It is having many medicinal uses. Ethanolic extract was collected and was subjected to the phytochemical screening it contains steroids, carbohydrates, flavanoides, glycosides, tannins and alkaloids. Anti inflammatory activity was performed by carrageenan induced rat hind paw method. The percent inhibition was inbetween 0.4-0.6mm which is equipotent when compared to the standard. The ethanolic extract was formulated into gel and subjected to the evaluation test. The results of PH test , spreadability, viscosity, invitro diffusion studies were according to the IP limit. The gel was subjected to the skin sensitivity test, there was no irritation produced during test period. It was concluded that the formulated gel can be used for treatment of inflammatory conditions.

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