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Formulation and Evaluation of Herbal Gel Containing Centipeda Minima Leaves Extract and Study of its Anti-Microbial and Anti-Inflammatory Activities

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ABSTRACT:

The leaves of Centipeda minima were selected for the proposed study. The leaves were shade dried, extracted by decoction method and the prepared herbal extract powder was evaluated for phytochemical screening tests such as, physical evaluation, test for alkaloids, test for proteins, test for glycosides, test for carbohydrates and sugars, test for tannins and phenolic compounds, test for flavonoids, test for steroids and test for fixed oils and fats. The Centipeda minima extract was subjected to preformulation studies such as drug -

excipient interaction study by FT-IR and identification of chemical compounds by GC-MS analysis.The present study was undertaken to formulate centipeda minima herbal gel using three different gelling agents such as Carbopol 934, HPMC K 100 M and Xanthan gum. A total of six formulations were prepared. The formulated gel were evaluated for physical appearance, pH, spreadability, estimation of drug content, *in-vitro* diffusion study, stability study anti-microbial and anti-inflammatory activity. Formulation F6 containing centipeda minima along with xanthan gum as gelling agent showed better spreadability(29.65%), maximum drug content (73.82%), faster drug release (78.4%), promising anti-microbial and anti-inflammatory activity. Based on the study of pH, spreadability, drug content, stability, *in-vitro* drug release, anti-inflammatory and anti-microbial activity, formulation F6 containing centipeda minima and xanthan gum emerged as the best formulation for topical application.

Key words: Centipeda minima, Anti-microbial, Anti-inflammatory, Carbopol 934, Xanthan gum, HPMC K 100 M.

INTRODUCTION:

Natural products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs have been developed from them. The first written records on the medicinal uses of plants appeared in about 2600 BC from the Sumerians and Acadians[1]. According to World Health Organization(WHO), 80 % of the people living in rural areas depend on medicinal herbs as primary healthcare system[2]. An herbal medicine is defined as a plant-derived product used for medicinal and health purposes.

The leaves of Centipeda minima plant was selected for the study. It is a prostrate or ascending slender, leafy herb, somewhat wooly or nearly smooth, with numerous branches spreading from the root and 8-20cm long. The leaves are oblong-obovate to oblanceolate, are 1cm long or less. It has anti-inflammatory, anti-allergic, sunscreen and cell renewal activity. It is used in various skin disorders including relief of itching, dry skin, psoriasis and also used in whooping cough, nasal allergy, malaria and asthma[3].

A gel is a semisolid system of at least two constituents, consisting of a condensed mass enclosing and interpenetrated by a liquid. A gel consists of a natural or synthetic polymer forming a three dimensional matrix throughout a dispersion medium or hydrophilic liquid. After application, the liquid evaporates leaving the drug entrapped in a thin film of the gel – forming matrix physically covering the skin. Different classes of polymeric materials have been used in the formulation of gels to achieve rate controlled drug delivery[4]. The mechanism of drug release depends upon the physicochemical properties of the drug and polymer.

The objective of the work is to extract centipeda minima leaves by decoction method, to identify their chemical compounds, to formulate centipeda minima herbal gel by using different gelling agents such as carbopol 934 (synthetic polymer), xanthan gum (natural polymer) and HPMC K100 M (semi-synthetic polymer) and to evaluate their physico-chemical parameters, anti-inflammatory and anti-microbial activity.

MATERIALS AND METHODS:

Materials:

The mature fresh leaves of centipeda minima were collected from Namakkal, Tamilnadu and authenticated by Dr. N. Senthil kumar, Head and Associate professor of Botany, Ayya Nadar Janaki Ammal College, Sivakasi. Xanthan gum was procured from Madhu hydrocolloids Pvt. Ltd., Gujarat, India. Carbopol 934 was procured from Maruti chemicals, Ahmedabad, India. HPMC K 100 M was procured from Sun bulkactive Pvt. Ltd., Mumbai. Triethanolamine was procured from Ultimate chem India Pvt. Ltd., Maharashtra. Propyl paraben was procured from Unicorn Petroleum Industries Pvt. Ltd., Mumbai. Propylene glycol was procured from Meru chem Pvt. Ltd., Mumbai, menthol oil from Everest Flavours Pvt. Ltd., Mumbai, methyl paraben from Akil healthcare Pvt. Ltd., Gujarat and Purified water was procured from Andavar plus drinking water, Chennai.All other chemicals and reagents used were of analytical grade.

METHODS:

Extraction of Centipeda minima Leaves by Decoction Method:

Decoction is a method of extraction by boiling herbal or plant material to dissolve the chemicals of the material, which may include stems, roots, bark and rhizomes. This procedure is suitable for extracting water-soluble, heat stable constituents. This process is typically used in the preparation of Ayurvedic extracts called “quath” or “kwath”. In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further[5].

PREFORMULATION STUDY:

Phytochemical Screening Study of Centipeda minima Extract:

The leaves of centipeda minima were shade dried, extracted by decoction method and reduced to coarse powder and stored in air tight container for further use. The organoleptic properties like color, odour and taste of the crude drug were evaluated[6].

The physico-chemical characters of centipeda minima leaves extract such as ash value, acid insoluble ash, water soluble ash and loss on drying were determined.

Phytochemical screening study of centipeda minima such as test for alkaloids, proteins, glycosides, carbohydrates and sugars, tannins and phenolic compounds, flavonoids, steroids and test for fixed oils and fats were determined[7].

The chemical composition of the extract obtained from Centipeda minima was analysed by Gas Chromatography-Mass Spectroscopy.

FT-IR studies of the pure Centipeda minima, gelling agent and combination of crude drug and gelling agent containing highest proportion were carried out to found any interaction between drug and excipients used in the formulation. FT-IR study was performed using IR spectroscopy (SHIMADZU)[8].

FORMULATION OF CENTIPEDA MINIMA HERBAL GEL:

Preparation of Centipeda minima Herbal Gel using Carbopol 934:

Accurately weighed carbopol 934 was taken in a beaker and dispersed in 10 ml of distilled water. Keep the beaker aside to swell the carbopol for half an hour and then stirred using mechanical stirrer at 1200 rpm for 30 min. Take propylene glycol in a separate beaker and add weighed quantity of propyl paraben and methyl paraben to it and stirred properly. Add the dispersed carbopol solution dropwise into 0.2 gm / 0.3 gm of centipeda minima extract and stirred well. To this add propylene glycol mixture with constant stirring. Finally the volume made upto 20ml by adding remaining purified water and triethanolamine was added drop wise to the formulations for adjustment of required skin pH and to obtain the gel at required consistency[9].

The same above procedure was followed for the preparation of centipeda minima herbal gel prepared using HPMC K 100 M and xanthan gum as gelling agents. The composition of centipeda minima herbal gel formulations were presented in Table-1.

Table 1: Composition of Centipeda minima Herbal Gel Formulations

S.No	INGREDIENTS	F1	F2	F3	F4	F5	F6
1	Centipeda minima extract(g)	0.2	0.3	0.2	0.3	0.2	0.3
2	Carbopol 934(%)	2	2	-	-	-	-
3	HPMC K 100 M(%)	-	-	2	2	-	-
4	Xanthan gum(%)	-	-	-	-	2	2
5	Methyl paraben(g)	0.3	0.3	0.3	0.3	0.3	0.3
6	Propyl paraben(g)	0.15	0.15	0.15	0.15	0.15	0.15
6	Propylene glycol(ml)	3	3	3	3	3	3
7	Menthol oil(drops)	2	2	2	2	2	2
8	Triethanolamine (ml)	q.s	q.s	q.s	q.s	q.s	q.s
9	Purified water	Upto 20ml	Upto 20ml	Upto 20ml	Upto 20ml	Upto 20ml	Upto 20ml

EVALUATION OF CENTIPEDA MINIMA HERBAL GEL FORMULATIONS:

Physical Appearance:

The prepared gel formulations containing centipeda minima are inspected visually for their color, homogeneity, consistency and phase separation.

Measurement of pH:

The pH of developed gel formulations was determined using digital pH meter. 1gm of gel was dissolved in 100 ml distilled water and kept aside for two hours. The measurement of pH of each formulation was done in triplicate and average values are calculated.

Spreadability:

The spreadability test was determined for all centipeda minima herbal gel formulations.Two sets of glass slides of standard dimensions were taken for the study. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides. 100g weight of gel was placed on the upper slides so that the gel was between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only upper slides to slip off freely by the force of weight tied on it. A 20 g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated for three times and the mean time was taken for calculation.

Spreadability was calculated using the, following formula:

$$S = M \times L / T$$

Where,

S = Spread ability,

M = Weight tied to upper slide,

L = Length of the glass slide,

T = Time (in sec).

Estimation of Drug content:

100mg of centipeda minima gel was dissolved in sufficient quantity of pH 6.8 phosphate buffer to get the clear solution and the volume was made up to 100ml with pH 6.8 phosphate buffer. 1ml of the solution was diluted to 10ml with pH 6.8 phosphate buffer and the absorbance of the resultant solution was measured at 365 nm using UV spectrophotometer. The amount of centipeda minima was determined from the standard calibration curve and the percentage drug content was determined[10].

***In Vitro* Diffusion Study:**

Modified apparatus with cellophane membrane was used to study the *in vitro* release of gel formulations. The cellophane membrane, previously soaked in pH 6.8 phosphate buffer was tied to one end of an open end glass cylinder. The dissolution medium used was phosphate buffer (pH 6.8). Centipeda minima gel containing

100mg of drug was placed into this assembly. The cylinder was connected to a stand and suspended in dissolution medium. The dissolution medium was kept at 37±0.5°C temperature and stirred at 100 rpm using magnetic bead. 5 ml aliquots were withdrawn at every 5, 10, 15, 20, 30 and 45 minutes. 5ml dissolution medium was added to maintain equal volume of receptor medium. The sample was diluted and measured using UV spectrophotometer at 365 nm[11].

Stability Study:

The formulated gel were filled in the collapsible tubes and stored at 25°C ± 2°C/ 60% ± 5% RH for a period of three months and studied for appearance, pH and spreadability for initial period and after every 45 days[12].

Anti-microbial Study:

The antimicrobial activity of the centipeda minima gel was evaluated by agar well diffusion method. Bacteria were grown in Muller Hinton broth. After inoculation, plates were dried for 15 minute and the wells were punched using sterile corn borers. Once wells were formed, they were filled with 100 µL gel and blank water. Commercially available diclofenac was used as a positive control in this study. Plates were incubated for 24 h at 37 °C to allow gel to diffuse through the agar media to form zones of inhibition. The diameters of the zone of inhibition for different leaf extracts against different bacteria were measured in millimeter for further analysis. An agar well (6 mm) showing no zone of inhibition was considered as no antimicrobial activity. All experiments were done in triplicate and the average values were used for drawing bar diagrams[13].

Anti-inflammatory Study:

Animals:

Albino Wistar rats either sex, weighing 150–200 g were used. They were housed in standard environmental conditions and fed with standard rodent diet with water ad libitum. All animal procedures were followed. Five groups (group1, group2, group3, Test and Standard) were used. Six animals in each group were used for the experiment.

The experiments were designed and conducted in accordance with ethical norms approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPSCEA) and Institutional Animal Ethical Committee (SBCP/2020-21/CPCSEA/IAEC/I/(1)/F16/158 dt.14/12/2020).

Carrageenan-Induced Rat Paw Edema:

Animals were fasted for 24 hrs before the experiment with free access to water. Approximately 50 µl of a 1% suspension of carrageenan in saline was prepared 1 h before each experiment and was injected into the plantar side of right hind paw of rat. 0.2 g of herbal gel containing 1% Centipeda minima extract was applied to the plantar surface of the hind paw by gently rubbing 50 times with the index finger. Rats of the control groups receive the plain gel base and 0.2 g of Diclofenac sodium gel containg 1% Diclofenac sodium was used as a standard. Drugs or placebo were applied 1 h before the carrageenan injection. Paw volume was measured immediately after carrageenan injection and at 1, 2, 3 and 4 hrs intervals after the administration of the noxious

agent by using a plethysmometer[14]. Anti-inflammatory activity was measured as the percentage reduction in edema level when drug was present, relative to control.

The % inhibition of edema induced by carrageenan was calculated for each group using the following equation:

% inhibition in edema thickness = {1- (Tt/Tc)} x 100

Where:

Tt = Mean increase in thickness of carrageenan paw edema of treated groups,

Tc = Mean increase in thickness of carrageenan paw edema of control groups.

RESULTS AND DISCUSSION:

Organoleptic Properties:

The organoleptic properties like color, odour and taste of the crude drug were evaluated. The color of centipeda minima was found to be dark dreen powder. A slightly aromatic odour was observed in the study and the taste was found to be slightly pungent and bitter.

Ash Value, Acid Insoluble Ash, Water Soluble Ash and Loss on Drying Values of Centipeda minima Extract:

Physico-chemical values are helpful to determine the quality as well as purity of crude drug, espeecially when the drug is present in powdered form. The ash value, acid insoluble ash, water soluble ash & loss on drying values of centipeda minima leaf extract was found to be 5.28%, 1.2%, 3.3% and 11.8% respectively.

Phytochemical Screening Study of Centipeda minima:

The results of phytochemical screening study of centipeda minima leaves extract demonstrated the presence of glycosides, carbohydrates, flavonoids, tannins and polyphenols in aqueous extracts.

GC-MS Analysis:

The chemical composition of the extract obtained from Centipeda minima was analysed by Gas Chromatography-Mass Spectroscopy. Seven compounds were identified in centipeda minima by GC-MS analysis and their spectra and spectral data are presented in Figure - 1 to 4.

Centipeda minima plant contains several chemical compounds. In GC-MS study, seven chemical compounds were separated and identified as octadec-9-enoic acid, hexadecenoic acid, cis-vaccenic acid, benzene, 2- tert - butyldimethyls, 5- methyl - 2 - phenylindolizine, 2, 4, 6 - cycloheptatrien -1- one and octodecanoic acid.

FT-IR Spectral Study:

FT-IR spectral studies indicated that the drug is compatible with all the excipients. The FT-IR spectrum of physical mixture showed all the characteristic peaks of centipeda minima thus conforming that no interaction of drug occurred with the components of the formulation.

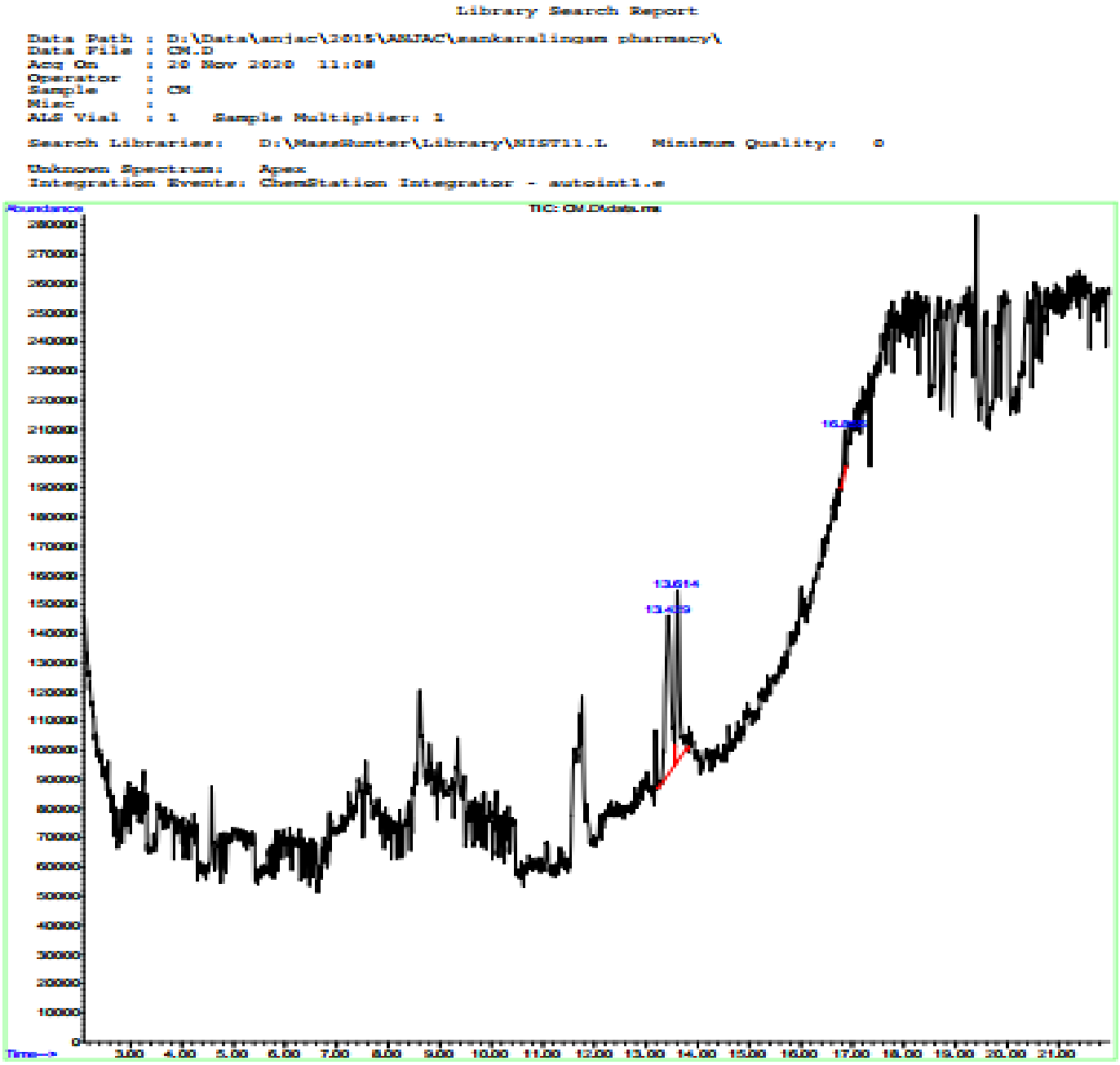
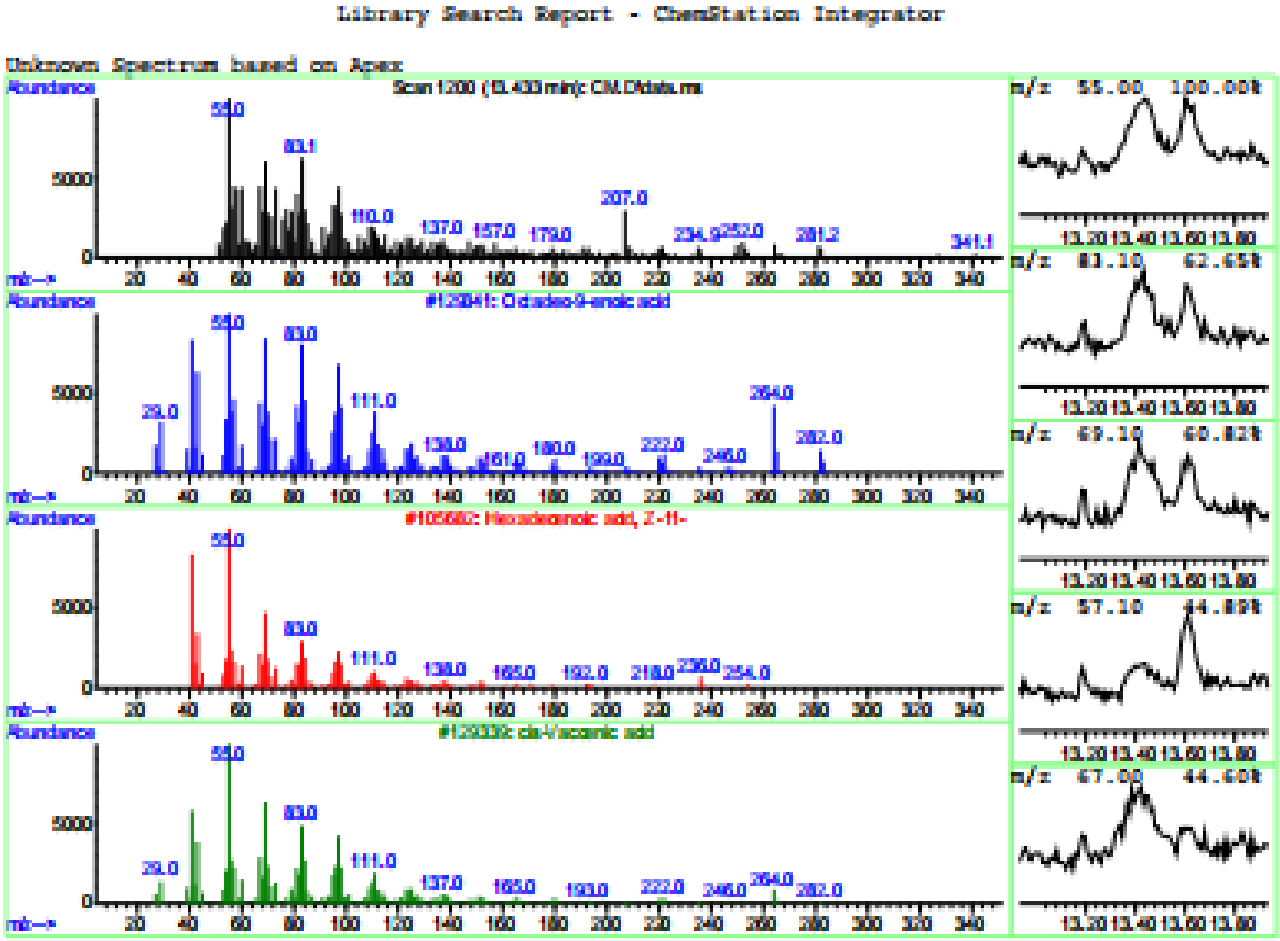


Figure 1 : Gas chromatography mass spectroscopy spectra of centipeda minima



Data File: D:\Data\anjac\2015\ANJAC\sankaralingam pharmacy\CM.D
Sample : CM

Peak Number: 1 at 13.433 min Area: 4114612 Area % 56.44

The 3 best hits from each library.	Ref\#	CAS\#	Qual
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1 Octadec-9-enoic acid	129341	1000190-11-7	91
2 Hexadecenoic acid, Z-11-	105682	002416-20-8	70
3 cis-Vaccenic acid	129339	000506-17-2	55

Figure 2 : GC-MS spectra of octadec-9-enoic acid, hexadecenoic acid and cis-vaccenic acid

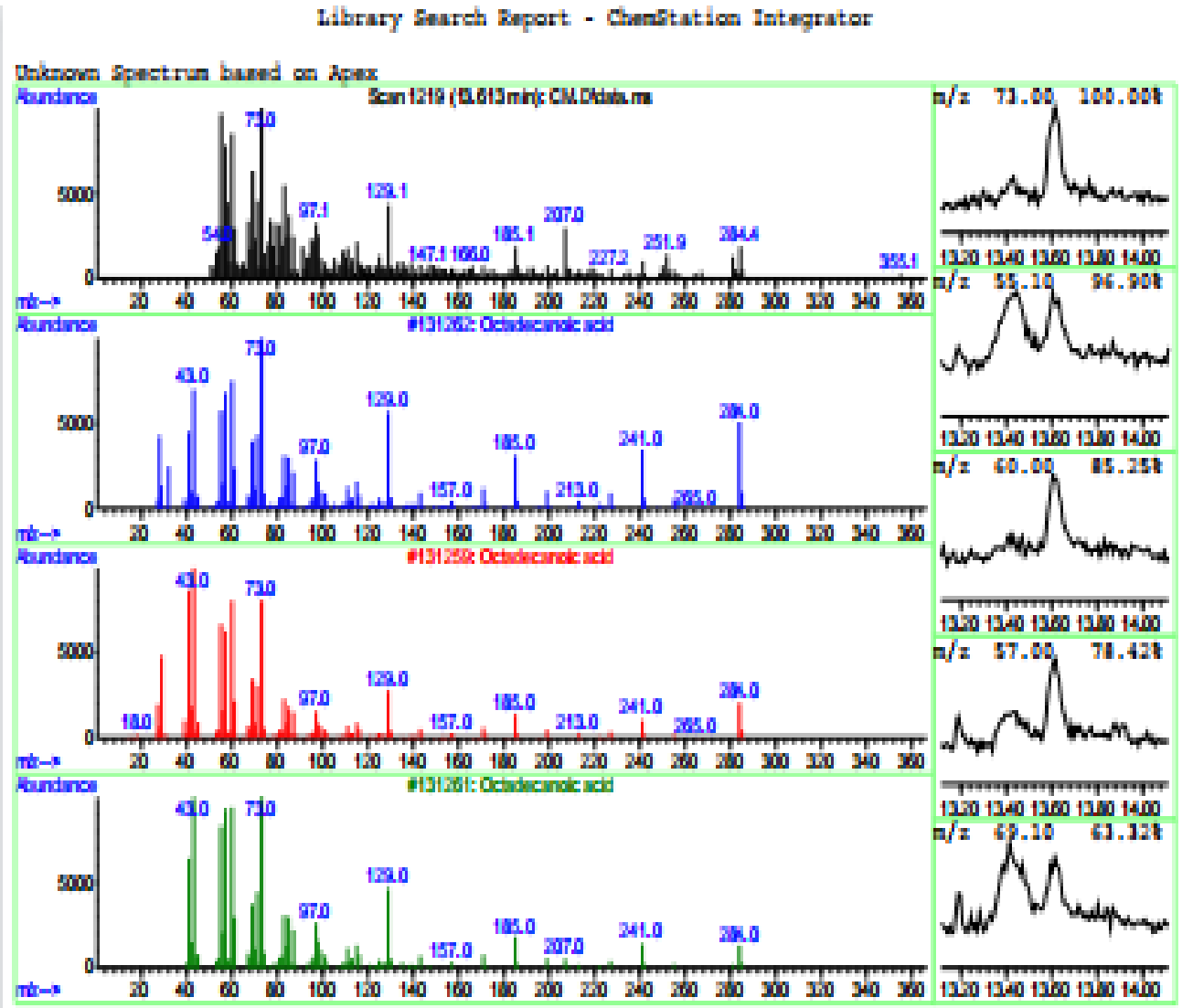
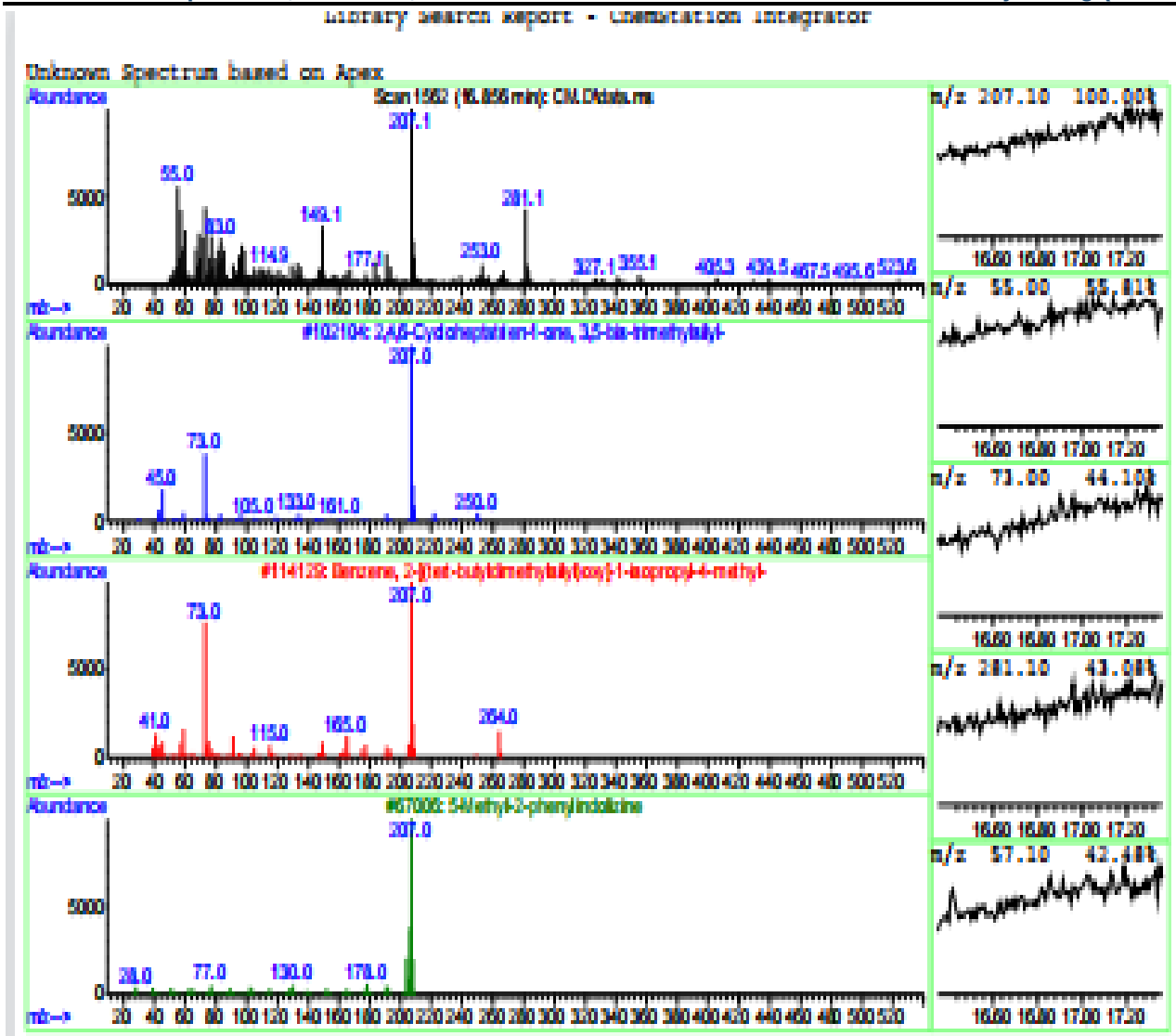


Figure 3 : GC-MS spectra of octadecanoic acid



Data File: D:\Data\anjac\2015\ANJAC\manikaralingam pharmacy\CN.D
Sample : CN

Peak Number: 1 at 16.856 min Area: 177904 Area % 5.18

The 3 best hits from each library.	Ref\#	CAS\#	Qual
D:\MassHunter\Library\NIST11.L			
1 2,4,6-Cycloheptatrien-1-one, 3,5...	102104	1000161-21-8	42
2 Benzene, 2-[(tert-butyldimethyls...	114129	330455-64-6	35
3 5-Methyl-2-phenylindolizine	67006	036944-99-7	27

Figure 4 : GC-MS spectra of 2, 4, 6, cycloheptatrien -1- one, benzene, 2- tert - butyldimethyls and 5- methyl - 2 - phenylindolizine

Physico-Chemical Evaluation of Centipeda Minima Herbal Gel Formulations

The physico-chemical evaluation parameters of centipeda minima herbal gel was presented in Table - 2.

Table 2: Physico-Chemical Evaluation Parameters of Centipeda minima Herbal Gel Formulations

Formulation code	pH*	Spreadability* (gm.cm/sec)	Drug Content* (%)
F1	6.8	27.65	67.98
F2	6.7	27.72	69.13
F3	7.0	15.23	64.51
F4	6.8	15.67	67.63
F5	6.8	28.78	71.13
F6	7.0	29.65	73.82

*** Values mentioned are the average of three determinations**

The pH values of centipeda minima herbal gel formulations are found in the range of 6.7 to 7.0. The pH values of all formulations were found in the close range of neutral pH (7.4) and hence suitable for topical formulations. Formulation F2 showed minimum pH value (6.7) and formulation F3 and F6 containing HPMC K 100 M and Xanthan gum as gelling agent, exhibited high pH value (7.0).

Spreadability values of centipeda minima herbal gel formulations was found in the range of 15.23 to 29.65 gm cm / sec. Formulation F5 and F6 containing xanthan gum as gelling agent exhibited high spreadability (28.78 and 29.65 gm cm / sec).

The content of centipeda minima estimated by UV spectrophotometric method in all formulations were found in the range of 64.51 % to 73.82 %. Formulation (F6) prepared using xanthan gum showed maximum drug content (73.82%) than other formulations.

***In Vitro* Diffusion Study:**

The results of *in vitro* drug release study of formulation F1 to F6 are shown in Table - 3 and percentage drug release Vs. time profiles were represented graphically in figure - 5.

Table 3 : *In Vitro* Drug Release Studies of Centipeda minima Herbal Gel Formulations

S.No	Time in minutes	Percentage drug release* (%)					
		Formulation code					
		F1	F2	F3	F4	F5	F6
1	5	20.78	25.32	27.21	24.1	25.12	25.38
2	10	35.28	34.21	40.6	35.13	40.5	42.13
3	15	48.6	50.16	56.3	44.10	52.12	55.12
4	20	59.2	62.3	66.12	56.27	60.3	68.10
5	25	65.1	70.2	74.3	77.32	75.49	78.4

*** Values mentioned are the average of three determinations**

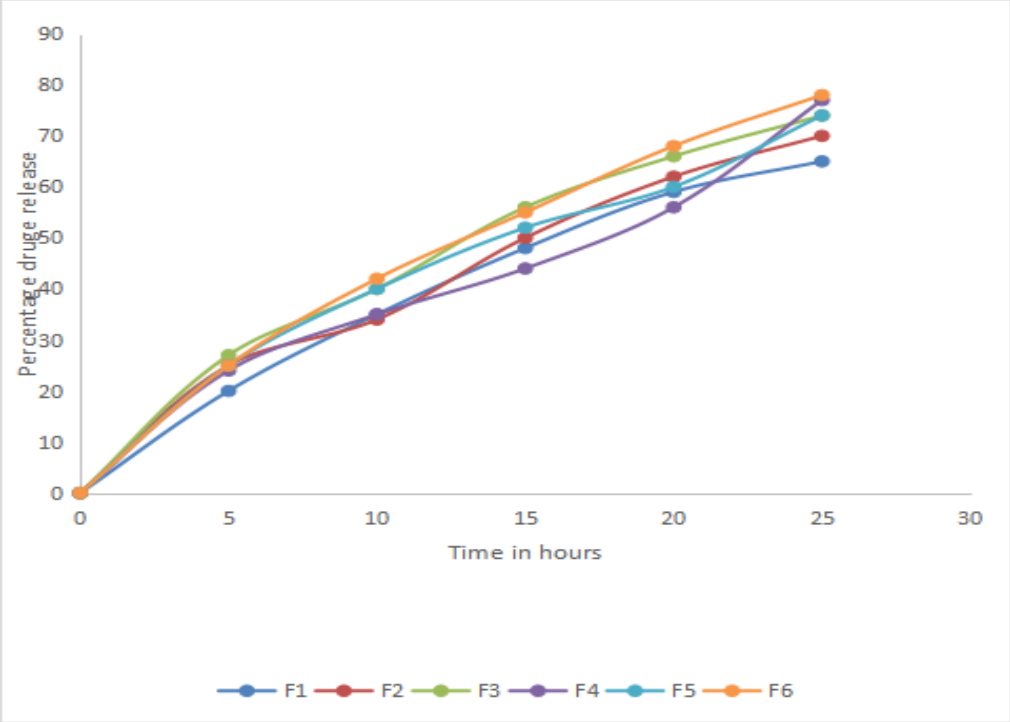


Figure 5 : I*n vitro* drug release profiles of centipeda minima herbal gel formulations

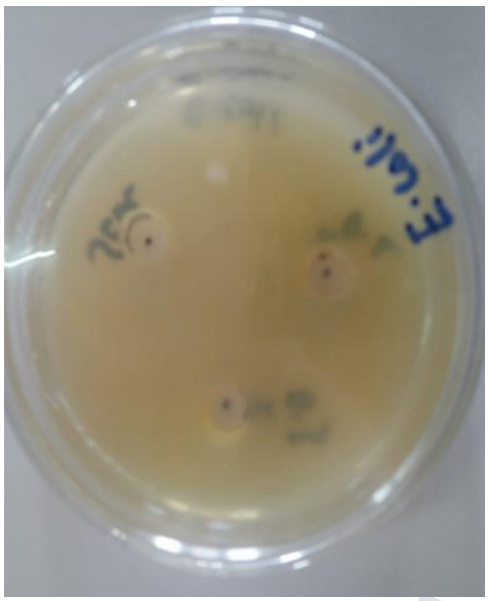
Formulations F1 and F2 prepared using carbopol 934 as gelling agent exhibited slow drug release (65.1 % and 70.2 %) at the end of 25 minutes. Formulations F5 and F6 prepared using xanthan gum as gelling agent exhibited faster drug release (75.49 % and 78.4 %) at the end of 25 minutes.

Stability Studies:

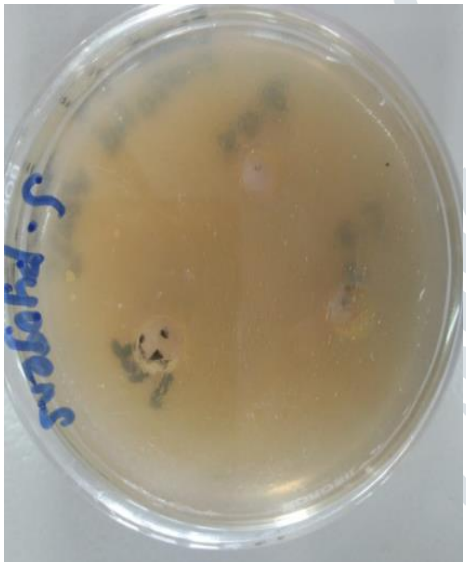
Stability studies revealed that there was no significant changes found in physical appearance, pH, spreadability values of centipeda minima herbal gel formulations during the period of three months after stored at 25ºC ± 2ºC/ 60% ± 5% RH. The study revealed that centipeda minima herbal gel formulations was stable at 25ºC ± 2ºC/ 60% ± 5% RH even after stored for three months.

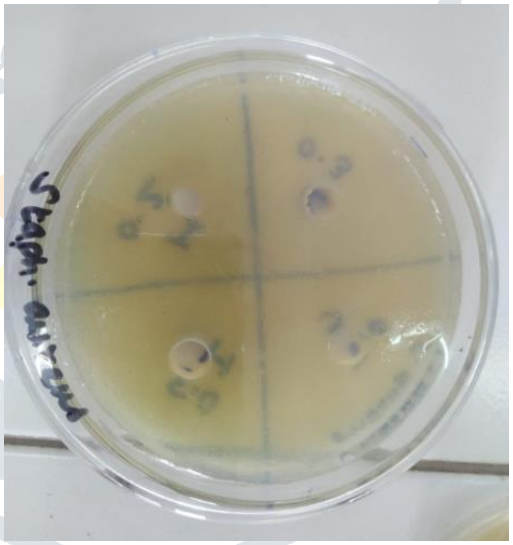
Anti-microbial Studies:

Gram positive bacteria, *Streptococcus pyogenes* and *Staphylococcus aureus* and gram negative bacteria *E.coli* and *Proteus vulgaris* were used for the study (Agar well diffusion method)[17]. The zone of inhibition exhibited by centipeda minima herbal gel formulations were shown in figure 6 and 7.



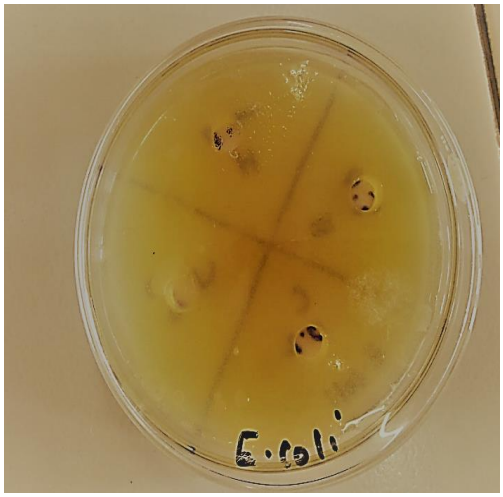
E.coli **Proteus vulgaris**



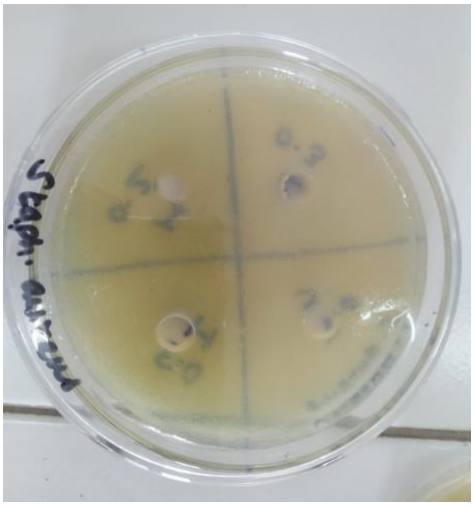


Streptococcus pyogenes **Staphylococcus aureus**

Figure 6: Zone of inhibition exhibited by centipeda minima herbal gel formulations (F1, F3 and F5)



E.coli



Staphylococcus aureus

Streptococcus pyogenes

Proteus vulgaris

Figure 7 : Zone of inhibition exhibited by centipeda minima herbal gel formulations (F2, F4 and F6)

In this study two gram positive and two gram negative microorganism were used in agar well diffusion method. Formulations (F1, F3, F5) containing 0.2 gm centipeda minima showed no zone of inhibition (or) minimum zone of inhibition against gram positive and gram negative microorganism. Formulation (F2, F4, F6) containing 0.3 gm of centipeda minima showed maximum zone of inhibition against all microorganism. Hence formulations (F2, F4, F6) Containing 0.3 gm of centipeda minima were selected for Anti-inflammatory study based on their Anti-microbial activity. Formulation F5 and F6 containing xanthan gum as gelling agent exhibited maximum anti-microbial activity than other formulations.

Anti-inflammatory Studies:

The present study indicates the efficacy of centipeda minima herbal gel as an efficient therapeutic agent in acute anti-inflammatory conditions.The percentage inhibition of edema exhibited by centipeda minima herbal gel formulations are shown in figure -8.

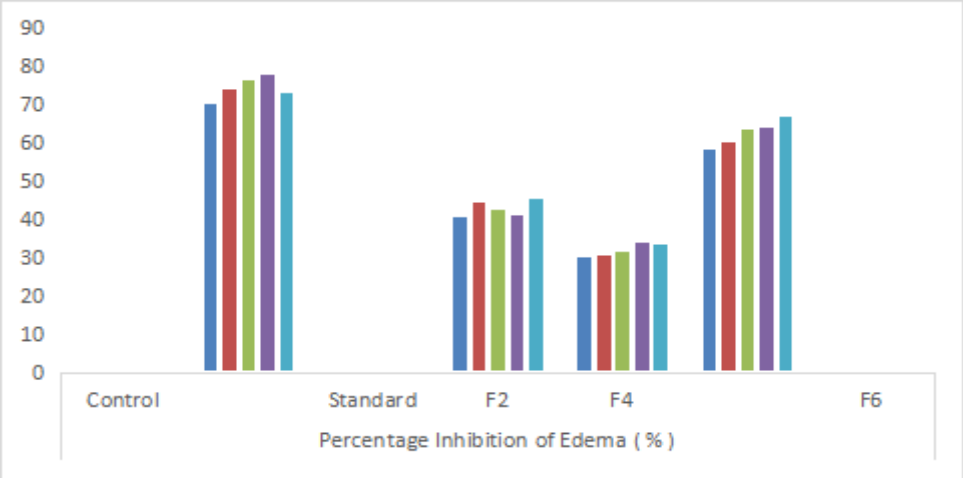


Figure 8 : Percentage inhibition of carrageenan induced paw edema by diclofenac sodium and centipeda minima herbal gel formulations(F2, F4 and F6)

The percentage inhibition of edema of marketed sample (standard), was found to be 73.21% at 4 hours, formulation F2,F4 & F6 was found to be 45.36 %, 33.44 % and 66.72 % respectively at the end of 4 hours. Standard exhibited maximum percentage inhibition of edema (73.21%) compared with formulations F2, F4 & F6. Formulation F6 containing centipeda minima along with xanthan gum as gelling agent exhibited maximum percentage inhibition of edema (66.72%) at the end of 4 hours compared with other formulations (F2 and F4) and showed better anti-inflammatory activity.

CONCLUSION:

Formulation and evaluation of herbal gel containing centipeda minima leaves extract and study of its Anti-inflammatory and Anti-microbial activities are successfully carried out. Phytochemical screening studies of crude drug, identification of chemical compounds by GC-MS, drug-excipient interaction study by FT-IR, formulation of centipeda minima herbal gel and various evaluation parameters were performed. The results revealed that the centipeda minima herbal gel formulations are good in appearance, easily spreadable, showed better release profile and stable even after stored at 25^{o}C ± 2^{o}C/ 60% ± 5% RH for 3 months.

Among all gel formulations, formulation F6 containing centipeda minima along with xanthan gum as gelling agent showed better spreadability, maximum drug content (73.82%), faster drug release (78.4%), promising anti-microbial action against gram positive and gram negative micro organism and exhibited significant anti-inflammatory activity.

The study concludes that the developed formulation F6 consisting 0.3 gm of centipeda minima with xanthan gum was found to be promising herbal gel for the treatment of local inflammation.

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