INTERNATIONAL JOURNAL
OF PHARMA AND BIO SCIENCES

Internationally Indexed Journal

Indexed in Chemical Abstract Services(USA),Index Coppernicus ,Ulrichs Directory of Periodicals,Google scholar ,Cabi ,DOAJ ,PSOAR, EBSCO ,SCOPUS, EMBASE etc.

Rapid Publishing

The International Journal of Pharma and Bio Sciences (IJPBS) is an international journal published quarterly. The Aim of IJPBS is to publish peer reviewed research and review articles in less time without much delay in the developing field of Pharmaceutical and Biological sciences. One week from the date of manuscript submission author gets the decision of acceptance and if accepted the manuscript will be processed within 3 weeks (approx.) for publication.

Pharmaceutical Sciences

Branches

- Pharmaceutics
- Novel drug delivery sys
- Nanotechnology
- Pharmacology
- Pharmacognosy
- Analytical chemistry
- Pharmacy practice
- Pharmacogenomics
- Polymer sciences
- Biomaterial sciences
- Medicinal chemistry
- Natural chemistry
- Biotechnology
- Pharmacoinformatics
- Biopharmaceutics

Biological Sciences

Branches

- Biochemistry
- Biotechnology
- Bioinformatics
- Cell biology
- Microbiology
- Allied Sciences
- Molecular biology
- Neurobiology
- Cytology
- Pathology
- Immunobiology

ELSEVIER

Impact Factor 0.47*

**Refer Instruction to authors available at www.ijpbs.net*

Indexed In Elsevier Bibliographic Database (SCOPUS, EMBASE and Sciverse)

Journal Home page
www.ijpbs.net

FOR INSTRUCTION TO AUTHORS VISIT
www.ijpbs.net
FOR ANY QUERIES EMAIL TO

- editorofijpbs@yahoo.com
- editorijpbs@rediffmail.com
- prasmol@rediffmail.com

Int J Pharm Bio Sci 2012 Oct; 3(4): (P) 161 - 170

Research Article **Novel drug delivery system**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

DESIGN OF AKKALKARA (*SPILANTHES ACMELLA*) FORMULATIONS FOR ANTI-MICROBIAL AND TOPICAL ANTI-INFLAMMATORY ACTIVITIES

NITIN GUPTA, ANKIT R. PATEL AND RAVINDRA R. P*.

NMIMS-SPTM, Mumbai- Agra Highway, Babulde, Shirpur (Dhule), Maharashtra, India- 425405

ABSTRACT

For centuries, *Spilanthes acmella* (L.) Murr. (Fam. Compositae) has been recommended in traditional medicine for treatment of toothache, rheumatism and fever. Of the reported phytoconstituents of the plant, Spilanthol is known to possess antibacterial and anti inflammatory activity. The aim of the current investigation is to evaluate the potential of novel vesicular carrier, ethosomes, containing the methanolic extract of *Spilanthes acmella*, for anti inflammatory action via transdermal route and to test the potential of the herbal extract for antimicrobial property when formulated as an oral mucoadhesive gel. Drug loaded ethosomes prepared using phospholipid and ethanol, were optimized and characterized for entrapment efficiency, vesicular size, shape, *in vitro* skin permeation, and stability. The ethosomal vesicles were incorporated in carbopol gel base and its anti-inflammatory efficiency was compared with the marketed diclofenac gel. Mucoadhesive oral gels containing extract were formulated using different concentrations of polymers and extracts and tested for parameters like viscosity and mucoadhesive behavior. The optimized formulation was evaluated for antimicrobial activity against different microorganisms responsible for causing tooth decay using well diffusion method. The results fully validate the claims of the traditional medicine about the use of Akkalkara for local anti-inflammatory and anti-microbial properties and confirm the suitability of ethosomes for transdermal delivery of active constituents of this herbal formulation. Both formulations show results comparable to that of standard allopathic formulations.

Keywords: ***Spilanthes acmella,*** **Transdermal, Ethosomes, Mucoadhesive Gel, Anti-microbial**

RAVINDRA R. P
NMIMS-SPTM, Mumbai- Agra Highway, Babulde, Shirpur (Dhule), Maharashtra, India-

Corresponding author

INTRODUCTION

The World Health Organisation (WHO) has estimated that 80% of the population of developing countries relies on plant-based traditional medicines to maintain their primary health care needs. High treatment cost and side effects along with drug resistance are major problems associated with synthetic drugs. Since traditional medicine is not only easily accessible but also affordable, there is an increased emphasis on the use of plants to treat human disease. Therefore, the global markets are turning to plants as a potential and realistic source of ingredients for healthcare products. [1, 3]

Spilanthes acmella [SPA] is an indigenous herb belonging to the family Compositae. It is an important medicinal plant commonly known as Akkalkara plant with rich source of therapeutic constituents. It is called the toothache plant because by chewing the leaves or flowers, it produces a numbing effect on the tongue and gums. The flower heads of *S. acmella* can be used to relieve toothache and also as anti inflammatory and analgesic. [2]

In the past few decades, considerable attention has been focused on the development of novel drug delivery system (NDDS) for herbal drugs. The novel carrier should ideally fulfill two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body, over the period of treatment. Secondly, it should channelize the active entity of herbal drug to the site of action. Conventional dosage forms including prolonged-release dosage forms are unable to meet these criteria.

Ethosomes have been shown to exhibit high encapsulation efficiency for a wide range of molecules including lipophilic drugs. This could be explained by multilamellarity of ethosomal vesicles as well as by the presence of ethanol in ethosomes which allows for better solubility of many drugs. Ethosomes were reported to improve *in vivo* and *in vitro* skin delivery of various drugs. Contrary to deformable liposomes, ethosomes are able to improve skin delivery of drugs both under occlusive and non-occlusive conditions. [4]

Bioadhesive dosage forms have been used to target local disorders at the mucosal surface to reduce the overall dosage required and to minimize the side effects that may be caused by the systemic administration of the drugs. Bioadhesive formulations use polymers as the adhesive component. These polymers are often water soluble and when used in a dry form, they attract water from the mucosal surface and this water transfer leads to a strong interaction. These polymers also form viscous layers when hydrated with water, which increases the retention time over the mucosal surfaces and leads to adhesive interactions. The objective of the present study is to design appropriate dosage forms containing the extract of *Spilanthes acmella* for topical and oral use and finally to evaluate them for enhanced anti-inflammatory and antimicrobial activity resp. [5, 6]

MATERIALS AND METHODS

Materials: Soya Lecithin (Merck Ltd.), Sodium CMC (Merck Ltd.), Carbopol 940 & 934 (Merck Ltd.), HEC (Merck Ltd), Methyl & Propyl Paraben (Suprim Chemicals), Sodium Metabisulphite(Suprim Chemicals), Glycerin (Rankem Chemicals Ltd.), Triethanolamine (Rankem Chemicals Ltd.), Carrageenan (Analytical Grade), Ethanol (Merck Ltd.). All the materials used in this study were of analytical and pharmaceutical grade.

Methods

Preparation of the extract: Fresh aerial parts of the SPA were collected from the herbal garden of the SPTM NMIMS Shirpur identified and authenticated. The plants were cleaned, dried under shade. 1 kg. of powdered flower heads was extracted with petroleum ether (b/p 60-80^0 C) for 12 hrs. After vacuum evaporation of petroleum ether the residue (16gm.) was

treated with methanol. So obtained Spilanthol was purified with successive washings with acetone. The yield of purified Spilanthol crystal was 407 mg/kg 90% dry powder. [7]

Phytochemical Studies: Freshly prepared SPA extract was subjected to phytochemical screening tests for the detection of various constituents including Spilanthol.

Preparation of Ethosomes Containing Extract: Ethosomes were prepared by solvent dispersion method. Soya Lecithin (up to 2-3%) ,2.5 gm of extract were taken and dissolved in (30-40%) of 90% ethanol by use of magnetic stirrer (Remi Motors Mumbai), to this solution fine stream of distilled water (100%) was added by use of syringe very slowly, then whole system was stirred for 30 minutes at 700 rpm. Ethanol and Soya Lecithin concentration was changed; for example initially 2 gm of Soya Lecithin was taken and treated separately with 30 and 40% ethanol, the same was repeated by taking 3gm of lipid. [8, 9]

Characterization of Ethosomes

Image analysis of ethosomes by motic microscope: Visualization done by Compound Digital Biological Microscope (DM -111/ Motic microscope, Shanghai, China). The optical microscope is attached with the software Digipro V 4.0, through which image analysis was done, photographs were captured.

Measurement of Ethosomal size distribution

Drug Content: The drug content is calculated by the formula

$$\frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100$$

Entrapment Efficiency: Entrapment Efficiency was determined by centrifugation method. The vesicles were separated in a high speed cooling centrifuge at 15,000 rpm for 30 minutes in the temperature maintained at 4oC. The sediment and supernatant liquids were separated; amount of drug in the sediment was determined by lysing the vesicles using methanol. From this, the entrapment efficiency was determined by the following equation. Entrapment efficiency is calculated by the formula. [8]

$$\frac{DE \times 100}{DT}$$

DE — Amount of drug in the ethosomal sediment
DT— Theoretical amount of drug used to prepare the formulation

Preparation of Topical gel containing ethosomes: The ethosomal formulation having high entrapment efficiency and smaller vesicular size was centrifuged in the temperature 4^{o}C at 15,000 rpm for 90 minutes to separate the ethosomal vesicles. The Ethosomal sediment which contains only the entrapped drug was collected and dispersed in the carbopol 940 (1%) gel base with gentle stirring. [9]

Preparation of Mucoadhesive Gel:

Carbopol 934 and Sodium CMC were used as a gelling agent. Carbopol 934 was dispersed in preserved water (Sodium metabisulphate 0.05 %) and glycerin overnight. The extract was dissolved in above solution and stirred for 10 min, and neutralized by triethanolamine to pH 6.4 and then mixed at 300 rpm for 10 min. [5]

Evaluation of Gel:

Spreadibility: The spread ability of the gel formulation was determined, by measuring diameter of 1 gm gel between horizontal plates (20×20 cm2) after 1 minute. The standardized weight tied on the upper plate was 60 gm. It is calculated by the formula

$$S = \frac{M \times L}{T}$$

Where, S = Spreadibility, M = Weight in the pan (tied to the upper slide),
L = Length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other

pH: *1* gm of gel was taken and it was diluted up to 10 ml and triplicate reading was noted using pH meter (Pro Lab - 1000 - Bench Top Ph Meter) and the average of three readings was taken.

Homogeneity: All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Viscosity: The viscosity of gel was determined by using a Brookfield viscometer DVII model with a spindle in combination with a helipath stand having the spindle S 64.

In vitro diffusion study: It is calculated with the help of Franz diffusion gel.

Mucoadhesion Measurement

An agar plate which contained agar at 1.5% w/v was prepared by weighing 1.5 g of agar powder, then adding pH 6.8 phosphate buffers into the agar, and finally gently heating a mixture with a water-bath. The hot solution was poured into the plate with diameter of 9 cm. [6]

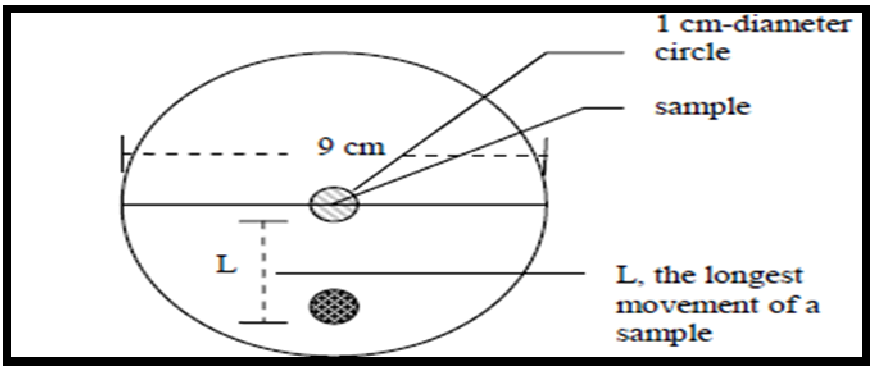

Figure: 1
In vitro Mucoadhesive Measurement of gels by agar method

The agar was then allowed to cool to a setting point. An equal amount of each sample was placed onto the center of the agar plate and a circle with a diameter of 1 cm was made as shown in Fig. 1. The longest movement distance of the sample (L) at room temperature was determined by slanting the plate at 30˚. At 1, 2, 3 and 4 h, the movement distance of the sample was measured and recorded.

Stability Studies: The Stability Study was performed as per ICH guidelines. The formulated gel were kept at different temperatures and RH, viz. 25ºC ± 2ºC / 60% ± 5% RH, 40ºC ± 2ºC / 75% ± 5% RH and 4ºC

for a period of one month and will be studied for appearance, pH, viscosity and spreadability

Skin irritation study: This study was carried out on healthy albino rats of wistar strain. The animals were divided into two groups i.e. control and test. The back skin of area 5 cm^2 was shaved before one day of starting the study. The study was carried out for 4 days. At the end of study, the animals were observed for any skin irritation like erythema or rashes and score were given as per the irritation. [8]

Anti-inflammatory Activity: Topical gel containing *Spilanthes acmella* was evaluated for anti-inflammatory activity by carrageenan-induced rat paw edema method. Albino rats of wistar strain (150-200 g) were randomly distributed into three groups of six animals each. The first group served as a control, second group served as the standard (Diclofenac sodium topically), while the third group received topical gel (1%) formulation. After 1 h, 0.1 ml of 1% w/v suspension of carrageenan was injected into the sub-plantar region of the right hind paw to all the three groups. The paw volumes were measured using plethysmometer (UGO Basile, 7140 Italy) every hour till 3 h after carrageenan injection, and mean increase in paw volumes were noted. Thus edema volumes in control (Vc) and in groups treated with test compounds (Vt) were calculated. The percentage inhibition was calculated by using the formula. [10]

Determination of antibacterial activity

Bacterial culture preparation: The bacterial cultures used in the study were *Escherichia coli, Streptococcus mutants, Bacillus cereus and Lactobacillus aureus.* These bacterial cultures were maintained on nutrient agar slants at first being incubated at 37ºC for about 18-24 hours and then stored at 4ºC as stock cultures for further antibacterial activity.

Well Diffusion Method: After the plates solidified the freshly prepared microbial broth culture suspension (about 0.1 ml) was spread over the agar media using L shaped sterilized glass spreader separately under aseptic condition using laminar air flow. Then wells were made in each plate with the help of borer of 8 mm diameter. In these well, about 0.1 ml of each flowers extracts individually was loaded. Petri plates were incubated for 24 hrs at 37ºC in the incubator. After incubation, the diameter of clear zone of inhibition produced around the well or holes were measured in mm and compared with standard drug. [11]

RESULTS AND DISCUSSION

Visualization of vesicles, size and entrapment efficiency: Ethosomal formulations were prepared by classic solvent dispersion method were translucent and had uniform dispersion of ethosomal vesicles. The ethosomal vesicles of selected formulations were evaluated for vesicular size and the results listed in table 1. The vesicular size of the ethosomes decreased with increase in ethanol concentration and increased with increase in phospholipid concentration. At the same time, the content of ethanol and phospholipid had significantly positive effect on the entrapment efficiency of the ethosomal carriers. Increase in the contents of ethanol and phospholipid caused increase in entrapment efficiency. [8]

Table: 1
Composition, entrapment efficiency and average particles size of ethosomes

Ethosomes	Extract	Average object area	Entrapment Efficiency
EA1.	2.5%	0.2Sqμ	79 %
EA2.	5.0%	0.2Sqμ	85 %
EA3.	10.0%	0.4Sqμ	77 %

Evaluation parameter of Topical Gel: The developed herbal gel was yellowish in color, translucent in appearance and showed good homogeneity with absence of lumps. The formulated F3 preparation was much clear and transparent as compared to F4 and F5 formulation.

Table: 2
Evaluation parameters of topical gel

Batch	Color	Appearance	Spreadibility (g.cm/sec)	Viscosity (dynes/cm^2)	pH
F3	Yellowish	Homogenous	29.72	47448	6.8
F4	Yellowish	Homogenous	32.28	49456	6.5
F5	Yellowish	Homogenous	31.63	52546	6.6

In vitro skin permeation studies: The In vitro drug release at the end of 3rd hour was found to be (84.24±0.69).

Skin Irritation studies: Since there was no irritation, erythema or rashes found at the end of 4th day, the formulation was found to be quite safe.

Anti inflammatory activity

Table: 3
Comparison of % Inhibition of paw volume by carrageenan paw edema

Sr. No.	Treatment	0 hr		1 hr		2 hr		3 hr	
		EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)
1	Control	1.79± 0.21	-	1.83± 0.33	-	1.94±0.24	-	1.82±0.11	-
2	Diclofenac Sodium Gel	1.11± 0.14	37.98	0.92± 0.70	49.72	0.84± 0.21	56.7	0.79± 0.42	56.59
3	Formulation F3	1.13± 0.21	36.87	0.95± 0.11	48.09	0.92± 0.47	52.5	0.86± 0.15	52.75

Values are mean ±SEM, (n=6), P<0.01; EV=Edema Volume, EI=Edema Inhibition

By Carrageenan paw edema method there was significant reduction in paw volume was observed when compared to the standard diclofenac gel. The % Inhibition in paw volume was determined and it was compared with the standard formulation i.e. Diclofenac Sodium gel. Hence the formulation is quite effective for treating the inflammation. [9]

II) Design of oral mucoadhesive gel

In all, six gel formulations were prepared by dissolving the extract of *Spilanthes acmella* in different polymers and their combinations. A comparison their composition, pH, viscosity and spreadability is given in Table 4 below.

Evaluation parameter of Oral Gel

Table: 4
Spreadability, pH and viscosity of oral gel formulations

Formulation code	Polymer type	Spreadability (gm*cm/sec) ± SD (n= 3)	pH	Viscosity (cP)
F1	CARBOPOL (3%) + SCMC (1%)	34.09 ± 0.985	6.6	31712
F2	SCMC (2%)	33.60 ± 2.140	6.8	31526
F3	CARBOPOL (2%) + SCMC (1%)	33.00 ± 0.771	6.5	31733
F4	CARBOPOL (3%) + SCMC (1%)	34.09 ± 0.985	6.6	31712
F5	SCMC (2%)	33.60 ± 2.140	6.8	31526
F6	CARBOPOL (2%) + SCMC (1%)	33.00 ± 0.771	6.5	31733

The values of surface pH were within the range of neutral or slightly acidic pH, this indicates formulations can be used without any irritation in the oral cavity. Spreadability of gel formulations (F1-F3) were measured, which were found to be in the acceptance range of 32- 39 gm*cm/sec. In the all formulation (F1-F6), when increased the speed of spindle in rpm, the torque of all formulation is increased with decreasing the viscosity of all formulations, which indicates good viscosity of all formulation.

The mucoadhesive property of the formulations was determined by the movement distance on the afar plate the results of which are demonstrated in Table 5 below.

Table: 5
Mucoadhesive measurement

Formulation		Movement Distance (cm)			
		1 hr	2 hr	3 hr	4 hr
F1	CARBOPOL (3%) + SCMC (1%)	0.6	0.8	1.1	1.2
F2	SCMC (2%)	0.9	1.3	1.4	1.4
F3	CARBOPOL (2%) + SCMC (1%)	0.7	0.9	1.1	1.1
F4	CARBOPOL (3%) + SCMC (1%)	0.6	0.8	1.1	1.2
F5	SCMC (2%)	0.9	1.3	1.4	1.4
F6	CARBOPOL (2%) + SCMC (1%)	0.7	0.9	1.1	1.1

The shorter the movement distance, the better adhesion between the polymer and the agar plate was obtained. The movement distance of the formulation appeared to be inversely related to the polymer concentration. The results suggest that the increase in polymer concentration will provide better adhesion and hence longer residence time. Among the all formulation, formulation F6 shows best results at 1 hr and after 4 hrs and hence was selected for further studies as an optimized formulation. The composition of the optimized formulation is given in Table 6 below.

Table: 6
Optimized Formula

Ingredients	% weight
Carbopol 934	2%
SCMC	1%
Triethanolamine	0.2%
Glycerol	0.1%
Sodium metabisulphite	0.05%
Distilled water	100%

In vitro* drug release*: The diffusion study of the optimized formulation (n =3) revealed that release rate of the drug was controlled for a period of time with 77.64 ± 0.35 % released around 2hrs. See Fig. 2.

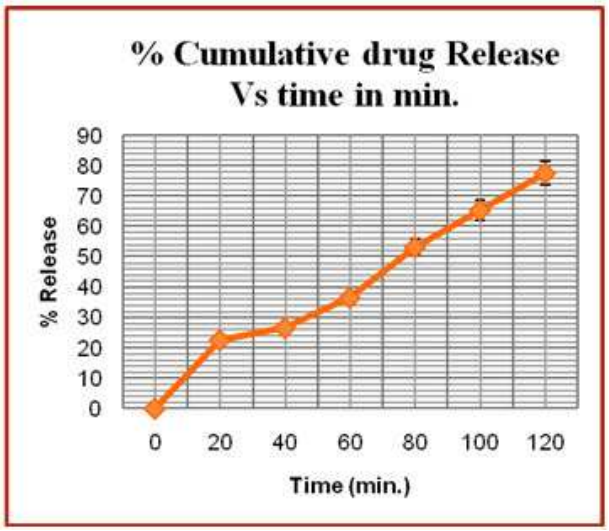

Figure. 2
% Cumulative release vs. time (n= 3)

Antimicrobial Susceptibility test

Table: 7
Zone of inhibition of herbal extract of Spilanthes acmella

Test Organism	Zone of Inhibition (mm)								
	2.5 %			5.0 %			10.0 %		
	M	E	S	M	E	S	M	E	S
E.coli	07	16	20	08	17	21	09	17	25
Streptococcus	08	18	22	07	20	23	07	19	22
Bacillus cereus	08	20	28	05	23	27	09	25	28
Lactobacilli	06	25	31	09	26	28	10	26	30

M = Methanol, E = Extract of Herb, S = Moxifloxacin

Different concentrations of methanolic extract (2.5, 5.0 and 100 %) were screened for antibacterial activity against *E.coli*, *streptococcus*, *Bacillus cereus, Lactobacilli.* In the table above, it is shown that after 5% concentration of herbal extract, the size of zone of inhibition remains constant. There is no significance difference in zone of inhibition after increasing the Spilanthol concentration beyond 5%. Hence 5% is optimum concentration of extract of *Spilanthes acmella* for anti-microbial action against all four types of microorganisms implicated in oral infections. The extract at this concentration shows pronounced anti-microbial action, comparable to the standard modern drug (moxifloxacin).

CONCLUSION

Spilanthes acmella is rich in various phytoconstituents. Spilanthol is responsible for the anti inflammatory and antibacterial activity. Ethosomes have been considered as a possible vesicular carrier for transdermal delivery of *Spilanthes acmella*. The enhanced accumulation of *Spilanthes acmella* via ethosomal carrier within the skin might help to

optimize targeting of this drug to the epidermal and dermal sites Mucoadhesive Oral Gel was successfully prepared using natural herbal extract which can be targeted in oral cavity disease like toothache, tooth decay and mouth ulcer and increase bioavailability of herbal extract that will result in better patient compliance with minimum side effects.

Thus *Spilanthes acmella* has a wide application, in which Spilanthol can be used as a biomarker and can act as a lead compound creating new opportunities in modern pharmaceuticals for the treatment of anti inflammatory and anti microbial conditions.

REFERENCES

1. Chopra, R.N., Nayara, S.L., Chopra, I.C. (1956). *Glossary of Indian medicinal plants*; Council of Scientific & Industrial Research: New Delhi; 168-169.
2. Chakraborty, A., *et al.* (2004). Preliminary studies on anti-inflammatory and analgesic activities of Spilanthes acmella in experimental animal models. *Indian. J. Pharmacology*., 36 (3): 148 – 150.
3. Gokhle, V. G., *et al.* (1945). Chemical investigation of *Spilanthes acmella* (Murr.). *J. Chemical Society*. 1, 250-252.
4. Dave Vivek et al., 2010, Ethosome for Enhanced Transdermal Drug Delivery of Aceclofenac. *Intnl. J. Drug Del*. 2. 81-92Saraf, D.K., Dixit, V.K., (2002). *Spilanthes acmella* Murr, study on its extract Spilanthol as larvicidal compound, *Asian J Exp Sci.* 16(1-2): 9-19.
5. Gandhi R. B., *et al.* (1994). "Oral cavity as a site for bioadhesive drug delivery," *Adv. Drug Deliv. Rev.,* vol. 13, 43-74.
6. Tangri, P., *et al.* (2011). Oral mucoadhesive drug delivery systems: a review. *Int. J. Biopharm.,* 2, 36-46.
7. Saraf, D.K., Dixit, V.K., (2002). *Spilanthes acmella* Murr, study on its extract Spilanthol as larvicidal compound, *Asian J Exp Sci.* 16(1-2): 9-19.
8. Donatella P. et al., 2005, Ethosomes for skin delivery of ammonium glycyrrhizinate: in vitro percutaneous permeation through human skin and in vivo anti-inflammatory activity on human volunteer. *J. Control. Release*. 106:99–110.
9. Krijavainen M. et al., 1996; Interaction of liposomes with human skin in vitro- the influence of lipid composition and structure. 1304: 179-189.
10. Jain A. K. et al., 2010, Evaluation of Antinociceptive and Anti-inflammatory Activity of Leaves of Cassia grandis Linn. *Intnl J Pharm and Clinical Res*.2 (3). 106-108.
11. Pandey, H.K., *et al.* (2007). A herbal formulation for toothache and related disorders and a process for preparation thereof. IN Patent 2004DE00260; [Chem. Abstr., 147, 350526].
12. Manish K.Chourasia et al., 2011, Nanosized ethosomes bearing ketoprofen for improved transdermal delivery. Results inPharmaSciences.1, 60–67.
13. Basha B. et al., 2011, Formulation and evaluation of Gel containing Fluconazole-Antifungal Agent. *Int. J. Drug Dev. & Res.,* 3 (4): 109-128.
14. Foldvary M. et al., 1993, Topical liposomal local anesthetics: design, optimization and evaluation of formulations. *Drug Dev. Ind. Pharm.;* 19: 2499–2517.
15. Barry, A.L., (1976). The Antimicrobial Susceptibility Tests: Principles and Practices, Lea and Febiger, Philadelphia; 92-104.
16. 16. Heng, P.W.S., *et al.* (2003). Buccal Delivery Systems. *Drug Dev. Ind. Pharm*., 29, 821-832.