CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.6396021

Available online at: http://www.iajps.com *Research Article*

FORMULATION AND EVALUATION OF POLYHERBAL GEL CONTAINING ROSA ALBA, SANTALUM ALBUM LINN AND OCIMUM SANCTUM FOR TOPICAL FUNGAL INFECTION

[1]Antim Shobha Vora, [2]Dr. Sinil Shah, [3]Dr. C. K. Tyagi

[1]Sri Satya Sai University of Technology & Medical Sciences, Sehore (M.P.)

Article Received: January 2022 **Accepted:** February 2022 **Published:** March 2022

Abstract:

Plant based drugs provide outstanding contribution to modern therapeutics as a source of many valuable secondary metabolites which serves as plant defence mechanisms against predator such as microorganism, insects and herbivores which have been proved to be potentially active compounds. There is a tremendous increase in search of antimicrobial plant extracts due to the fact that the resistance offered against antibiotic by the microorganism, in short the effective life span of any antibiotic is limited. Candida albicans is common fungus responsible for the development of various forms fungal disease. In the present study anti-fungal gels were prepared using polymer carbopol 940 along with the hydroalcoholic extracts of flower of Rosa alba L, leaves of Santalum album Linn and Ocimum sanctum Linn and evaluated for their physicochemical properties, like pH, washability, extrudability, spreadability and viscosity. The formulations (PHG1-PHG6) were tested for the anti acne activity by well diffusion method against Candida albicans. Results showed that the gels were non-irritant, stable and posses anti-fungal activity. This study suggests that selected plants have potential against fungus and hence they can be used in topical anti-fungal preparations and may address the antibiotic resistance.

Keywords: *Rosa alba, Santalum album, Ocimum sanctum , Candida albicans, Carbopol, Physicochemical properties.*

Corresponding author:

Antim Shobha Vora

Sri Satya Sai University of Technology & Medical Sciences, Sehore (M.P.)

Antimvora30@gmail.com

QR code

Please cite this article in press Antim Shobha Vora et al, ***Formulation And Evaluation Of Polyherbal Gel Containing Rosa Alba, Santalum Album Linn And Ocimum Sanctum For Topical Fungal Infection.,*** *Indo Am. J. P. Sci, 2022; 09(3)*

INTRODUCTION:

Fungal infections are the leading cause of death in both advanced and developing countries. This is due to the use of immunosuppressive treatments, long term use of antibiotics, and longer survival of immunocompromised individuals [1]. There are numerous antifungal agents used clinically to treat fungal infections. Triazole antifungal agents like fluconazole and itraconazole came into play in the early 1990's, followed by amphotericin-B in the mid 1990's. These antifungal drugs can be broadly classified into five major classes, i.e. azoles, allylamines, echinocandins, griseofulvin, and flucytosine [2]. The emerging resistance of microbes to antifungal agents has serious implications in the management of infections. These antifungal compounds also act on targets found in mammalian cells which may result in toxicity or adverse drug interactions. Ketoconazole is one of the antifungal drugs used against both superficial and deep seated infections. However, its unpleasant side effects include nausea, abdominal pain, itching, toxicity, slow therapeutic response, and poor efficacy in immunocompromised patients [3]. Therefore, the discovery of novel antifungals is severely needed. Phytochemistry of various plant species has indicated that the phytochemicals could be a better source of medicine as compared to synthetically produced drugs. The use of plants as medicine goes back to early man. These traditional medicines based on medicinal plants have been used for centuries. Therefore one approach that has been used for discovery of antimicrobial agents is the evaluation of plant extracts [4].

Medicinal plants are of great importance to health of individuals and communities. This importance lies in their chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds include alkaloids, tannins, flavonoids, and phenolic compounds. The health effects of flavonoids include antioxidant, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic. Alkaloids show many useful effects like antihypertensive and anti-tumor. Alkaloid based drugs include caffeine, quinine, nicotine, artemisinin, cholchicine, and amblyopia. Tannins include corilagin and geraniin, which show anti-human immuno deficiency syndrome activity by inhibiting reverse transcriptase [5]. Herbal treatments applied topically have gained considerable attention due to their widespread use and ill-defined benefit/risk ratio. There are numerous medicinal plants which are widely used in the treatment of skin diseases and also known to possess antimicrobial activity.

The objective of this proposed study was to develop a polyherbal gel formulation containing flower of *Rosa alba* L, leaves of *Santalum album* Linn and *Ocimum sanctum* Linn to treat fungal infection as a safe, effective and an alternative therapy to the current conventional harmful antibiotics.

MATERIALS AND METHODS:

Material:

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade. The pathogenic microbes used in the current study are obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India.

Methods:

Extraction procedure:

Following procedure was adopted for the preparation of hydroalcoholic extract from the shade dried and powdered herbs [6-7].

Extraction by maceration process:

40.6 gm dried powdered of *Rosa alba* L, 53.7 gm dried powdered of *Santalum album* Linn and 58.9 gm dried powdered of *Ocimum sanctum* Linn has been extracted with hydroalcoholic solvent (ethanol: water; 80:20) using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 400C.

Determination of percentage yield:

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Screening

The chemical tests were performed for testing different chemical groups present in extract [8].

A. Alkaloids To the extract dilute hydrochloric acid was added. Then it was boiled and filtered.

i. ***Hager's test***

To 2-3 ml of filtrate, few drops of Hager's reagent were added. Formation of yellow precipitate indicated the presence of alkaloids.

B. Carbohydrates

i. ***Fehling's test*** (Reducing sugars): To 2 ml of extract, equal volume of mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes in boiling water bath. Formation of red or brick red coloured precipitate indicated the presence of reducing sugars.

C. Flavonoids

i. ***Lead acetate test***

Test solution with few drops of Lead acetate solution shows intense yellow precipitate colour.

ii. ***Alkaline reagent test:*** To 2 ml of test solution add 2 ml alkali, gives yellow color, which disappears on addition of dil. HCl it disappears, which indicates presence of flavonoids.

D. Proteins

i. ***Biuret's test*** (General test)

To 1 ml of test extract, 4% of sodium hydroxide solution and few drops of 1% copper sulphate solution were added. Formation of a violet red colour indicated the presence of proteins.

E. Saponins

i. ***Foam test***

The extract was shaken vigorously with water in a test tube. Formation of persistent foam indicated the presence of saponins.

F. Detection of proteins and aminoacids

i. ***Xanthoproteic Test***

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

G. Glycosides

i. ***Legals test***

To 2 ml of test solution, 1 ml of pyridine and 1 ml of sodium nitroprusside was added. Change in color to pink or red indicated presence of cardiac glycosides.

H. Phenol

i. ***Ferric chloride test***

Extract solutions were treated with 5% ferric chloride solution. Formation of blue colours indicated the presence of hydrolysable tannins and formation of green colour indicated the presence of condensed Phenol.

I. Diterpenes

i. ***Copper acetate Test***

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Quantitative studies of phytoconstituents:

Total flavonoids content estimation:

Determination of total flavonoids content was based on aluminium chloride method [9]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% AlCl3 solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Total phenolic content estimation:

The total phenolic content of extract was determined by the modified Folin-Ciocalteu method [10]. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25mg/ml was prepared in methanol. 1mg of dried extract was extracted with 10 ml methanol, filter, and make up the volume up to 10 ml. One ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min. for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Formulation development of polyherbal gel:

Method of preparation:

In a beaker, measured amounts of methyl paraben, glycerin, polyethylene glycol, hydroalcoholic extracts of *Rosa alba* L, *Santalum album* Linn and *Ocimum sanctum* Linn were dissolved in roughly 35 ml of water and swirled at high speed using a mechanical stirrer (or sonicator). Then, while stirring, Carbopol 940 was gently added to the beaker containing the aforementioned liquid. The solution was neutralized by progressively adding triethanolamine solution while stirring constantly until the gel was formed [11].

Carbopol 940 – Gelling Polymer

Triethanolamine- Gelling agent, pH Adjusting agent, Neutralizer

Methyl Paraben - Preservative

Distilled Water, Glycerin and Polyethylene Glycol-solvents

Table 1.1: Formulation of polyherbal gel

Ingredients (%)	PHG1	PHG2	PHG3	PHG4	PHG5	PHG6
Rosa alba L extract	1gm	1gm	1gm	1gm	1gm	1gm
Santalum album Linn extract	1gm	1gm	1gm	1gm	1gm	1gm
Ocimum sanctum Linn. extract	1gm	1gm	1gm	1gm	1gm	1gm
Carbopol 940	0.5mg	0.75mg	1.0 gm	1.25 gm	1.5 gm	2.0 gm
Polyethylene Glycol	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Methyl Paraben	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg
Triethanolamine	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Distilled Water (q.s)	100ml	100ml	100ml	100ml	100ml	100ml

(PHG= Polyherbal gel)

Evaluation of polyherbal gel:

Extrudability determination of formulations:

Polyherbal gel compositions were placed in collapsible metal tubes or collapsible aluminum tubes. The tubes were pushed to extrude the material, and the formulation's extrudability was tested by Benoy[12].

Determination of Spreadability:

Two standard-sized glass slides (6 ×2) one of the slides was covered with the polyherbal gel formulation that was to be tested for spreadability. The second slide was positioned above the first in such a manner that the formulation was sandwiched between them for a total distance of 6 cm down the slide. The polyherbal gel mixture between the two slides was traced uniformly to produce a thin layer by placing 100 grams of weight on the upper slide.

The excess of the polyherbal gel formulation clinging to the slides was scraped off and the weight was removed. The bottom slide was attached to the apparatus's board, and one end of the top slide was linked to a string to which a 20-gram force could be imparted using a simple pulley. The time it took for the upper slide to travel 6 cm and separate from the lower slide under the weight's direction was recorded. The experiment was performed six times, with the average of the results determined for each gel formulation.

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)
m = weight tied to the upper slide (20 grams)
l= length of glass slide (6cms).
t = time taken is seconds.

Determination of pH:

A digital pH meter was used to determine the pH of the polyherbal gels. One gram of gel was dissolved in 25 ml of distilled water, and the electrode was dipped in the gel mixture until a steady reading was obtained. It was also reported that she was always reading. Each formulation's pH readings were repeated two times [13].

Drug content:

1 gram of gel was placed in a 10 ml volumetric flask and diluted with methanol to assess the drug concentration. 1 ml of 2% AlCl3 solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm [14].

***In vitro* antifungal activity of polyherbal gel:**

To create diffused heavy lawn culture, a loop full of broth was collected and seeded onto sterile potato dextrose agar plates using a sterile cotton swab. Standard procedure [15] was followed to evaluate the antifungal activity of the polyherbal gel produced from hydroalcoholic extracts of *Rosa alba* L, *Santalum album* Linn and *Ocimum sanctum* Linn. utilizing the well diffusion technique. In antibiogram experiments, three concentrations of extracted phytochemicals were used: 25, 50, and 100 mg/ml. The placement of wells containing antibiotics on the surfaces of agar soon after inoculation with the organism examined is a key aspect. Inoculums made from undiluted overnight broth cultures should never

be utilized. After a 48-hour incubation period at 24-28°C, the plates were inspected for distinct zones of inhibition surrounding the wells impregnated with a specific drug concentration.

RESULTS AND DISCUSSION:

Herbal remedies from nature are well-researched and shown to be effective and safe natural treatments for a wide range of ailments. The goal of this study was to demonstrate preliminary chemical screening, gel formulation and antifungal activity of *Rosa alba* L, *Santalum album* Linn and *Ocimum sanctum* Linn. The dried extract was thoroughly cleaned under running tap water before being ground with an electric grinder. The powder was extracted using a hydroalcoholic extract solvent and the maceration technique. The phytochemical screening of *Rosa alba* L, *Santalum album* Linn and *Ocimum sanctum* Linn hydroalcoholic extract was one of the standardization criteria that were examined. The percentage yield of hydroalcoholic extract of *Rosa alba* L, *Santalum album* Linn and *Ocimum sanctum* Linn with 4.52, 5.63 and 10.41% w/w having yield respectively.

Table shows the findings of phytochemical screening of *Rosa alba* L, *Santalum album* Linn and *Ocimum sanctum* Linn hydroalcoholic extract. The phytochemical screening findings revealed that the phytochemical composition of hydroalcoholic solvents was generally comparable. However, physiological and biosynthetic processes occurring inside the plant are responsible for the lack of certain phytochemicals in one region of the plant sample and their presence in another.

The presence of phytochemicals (phenols and flavonoids) was investigated quantitatively. The total phenols and flavonoids concentration of *Rosa alba* L, *Santalum album* Linn and *Ocimum sanctum* Linn hydroalcoholic extract.

The prepared formulations were evaluated for clarity test and the study results show that, both the gels were clear and free of particles. The formulations exhibited a good homogeneity. The gel with carbopol 940 as gelling agent showed a good transparency. it was discovered that the freshly produced formulations were light green. Clogging was determined to be absent in all formulations, and all formulations PHG1, PHG2, PHG3, PHG4, PHG5, and PHG6 had acceptable homogeneity and texture. The ease and extent of washing with water were physically assessed after the formulations were applied to the skin. Because of their non-greasy characteristics, all of the formulations were easily washable and left no residues on the skin when washed with water. All of the gel formulations were determined to have average extrudability and washability.

Spreadability of the formulations PHG1, PHG2, PHG3, PHG4, PHG5, and PHG6 were studied and found to in the range of 24±5, 18±4, 20±9, 23±4, 13±2 and 25±5 respectively. The Formulation PHG5 showed the good Spreadability 6.93±8 among all formulation. The pH of the produced mixture was found to be comparable to the skin pH of 6.8 in the range of 7.1±0.5, 6.9±0.4, 7.2±0.3, 7.3±0.4, 6.8±0.2 and 7.5±0.7 respectively. Because the pH of all formulations was determined to be close to that of the skin, all formulations were classified as non-irritant.

The viscosity of different gel samples was measured in the aforesaid formulas, and it was discovered that the viscosity increased. The viscosity of the PHG5 formulation is excellent. The viscosity of formulations PHG1, PHG2, PHG3, PHG4, PHG5, and PHG6 were found to be 2214±2, 1954±7, 2141±5, 1825±6, 2324±8 and 2035±4 respectively. The phenolic content of prepared formulations was found to be 0.511±0.005, 0.760±0.003, 0.374±0.002, 0.562±0.005, 0.954±0.001 and 0.241±0.002 mg for formulations PHG1, PHG2, PHG3, PHG4, PHG5, and PHG6 respectively. The polyherbal gel produced from the plants was diluted to concentrations of 100, 50, and 25 mg per ml before being administered to the test organism through the well diffusion method. When compared to standard, the formulation PHG5 showed greater antifungal efficacy against *Candida albicans.*

Table 1.2: Physical characteristics of hydroalcoholic extract

Extracts	Consistency	Colour	Odour	% Yield (w/w)
Rosa alba L	Solid	Dark brown	Pungent	**4.52**
Santalum album L	Solid	Green	Pungent	**5.63**
Ocimum sanctum L	Solid	Green	Pungent	10.41

Table 1.3: Result of phytochemical screening of hydroalcoholic extract of *Rosa alba* L

S. No.	Constituents	Hydroalcoholic extract	Observation
1.	**Alkaloids** Hager's Test:	-ve	**Yellow coloured**
2.	**Glycosides** Legal's test	-ve	**Brown coloured**
3.	**Flavonoids** Lead acetate Alkaline Reagent Test:	+ve +ve	**Yellow coloured precipitate** **Light yellow coloured**
4.	**Phenolics** Ferric Chloride Test	+ve	**Black coloured**
5.	**Proteins** Xanthoproteic test	+ve	**Yellow coloured**
6.	**Carbohydrates** Fehling's Test:	+ve	**Red precipitate**
7.	**Saponins** Froth Test: Foam Test:	+ve +ve	**Layer of foam** **Foam**
8.	**Diterpins** Copper acetate test	+ve	**Emerald green coloured**
9.	**Tannins** Gelatin Test:	-ve	**White coloured**

(+ve= positive, -ve= negative)

Table 1.4: Result of phytochemical screening of hydroalcoholic extract of *Santalum album* Linn

S. No.	Constituents	Hydroalcoholic extract	Observation
1.	**Alkaloids** Hager's Test:	-ve	**Yellow coloured**
2.	**Glycosides** Legal's test	+ve	**Pink coloured**
3.	**Flavonoids** Lead acetate Alkaline Reagent Test:	+ve -ve	**Yellow coloured precipitate** **Green coloured**
4.	**Phenolics** Ferric Chloride Test	+ve	**Black coloured**
5.	**Proteins** Xanthoproteic test	+ve	**Yellow coloured**
6.	**Carbohydrates** Fehling's Test:	-ve	**Green coloured**
7.	**Saponins** Froth Test: Foam Test:	+ve -ve	**Layer of foam** **No Foam**
8.	**Diterpins** Copper acetate test	-ve	**Sky blue coloured**
9.	**Tannins** Gelatin Test:	-ve	**White coloured**

(+ve= positive, -ve= negative)

Table 1.5: Result of phytochemical screening of hydroalcoholic extract of *Ocimum sanctum* Linn

S. No.	Constituents	Hydroalcoholic extract	Observation
1.	**Alkaloids** Hager's Test:	+ve	**Yellow coloured ppt**
2.	**Glycosides** Legal's test	-ve	**Brown coloured**
3.	**Flavonoids** Lead acetate Alkaline Reagent Test:	-ve +ve	**White coloured** **Light yellow coloured**
4.	**Phenolics** Ferric Chloride Test	+ve	**Black coloured**
5.	**Proteins** Xanthoproteic test	+ve	**Yellow coloured**
6.	**Carbohydrates** Fehling's Test:	+ve	**Red precipitate**
7.	**Saponins** Froth Test: Foam Test:	-ve - ve	**No foam** **No Foam**
8.	**Diterpins** Copper acetate test	+ve	**Emerald green coloured**
9.	**Tannins** Gelatin Test:	+ve	**White coloured ppt**

(+ve= positive, -ve= negative)

Table 1.6: Estimation of total flavonoids and phenol content

	Rosa alba	
S. No.	**Total flavonoids content (mg/ 100 mg of dried extract)**	**Total phenol content (mg/ 100 mg of dried extract)**
1.	0.574	0.352
	Santalum album	
2.	0.741	0.514
	O. sanctum	
3.	0.684	0.435

Table 1.7: Results of evaluation of polyherbal gel

Formulation code	Extrudability	Spreadability (gcm/sec)	pH	Viscosity (cps)	Flavonoids content (mg/ 100 mg)
PHG1	Average	24±5	7.1±0.5	2214±2	0.511±0.005
PHG2	Average	18±4	6.9±0.4	1954±7	0.760±0.003
PHG3	Average	20±9	7.2±0.3	2141±5	0.374±0.002
PHG4	Average	23±4	7.3±0.4	1825±6	0.562±0.005
PHG5	Average	13±2	6.8±0.2	2324±8	0.954±0.001
PHG6	Average	25±5	7.5±0.7	2035±4	0.241±0.002

Table 1.8: Antifungal activity of polyherbal gel formulation (PHG5) against *Candida albicans*

S. No.	Standard /Formulation	Zone of inhibition (mm)		
		30mg/ml	**20 mg/ml**	**10mg/ml**
1.	Fluconazole	15±0.94	12±0.5	10±0.84
		100mg/ml	**50 mg/ml**	**25mg/ml**
2.	Polyherbal gel	14±0.47	10±0.74	9±0.57

CONCLUSION:

The present study was aimed to developed polyherbal gels for anti acne treatment using hydroalcoholic extracts of flower of *Rosa alba* L, leaves of *Santalum album* Linn and *Ocimum sanctum* Linn an aqueous based carbopol gel system and evaluated for their physicochemical properties, like pH, spreadability, viscosity and microbial assay. Our study reveals plant extracts to be good antifungals; their hydroalcoholic extracts formulated as hydrogels with satisfactory physicochemical parameters. Hence polyherbal formulation/preparations may be better than formulating a single herb.

REFERENCES:

1. Molero, G., Orejas, R.D., Garcia, F.N., Monteoliva, L., Pla, J., Gil, C., Parez, M.S., Nombela, C., 1998. Internation. Microbiol.1, 95-106.
2. Chen, S.C.A. and Sorrell, T.C., 2007. Antifungal agents. MJA.187, 404-409.
3. Lucca, A.J.D. and Walsh, T.J., 1999. Antifungal peptides: novel therapeutic compounds against emerging pathogens. Antimicrobial agents and Chemoth.43, 1-11.
4. Pyun, M.S. and Shin, S., 2005. Antifungal effects of the volatile oils from Allium plants against Trichophyton species and synergism of the oils with ketoconazole. Phytomed.13, 394-400.
5. Ozcelik, B., Aslan, M., Orhan, I. and Karaglu, T., 2005. Antibacterial, antifungal and antiviral activities of lipophylic extracts of *Pistacia vera*. Microbiol. Res.160, 159-164.
6. El-Ishaq A. Extraction of limonene from orange peel. Nutr Heavy Met 2015; 1–15.
7. Gotmare S. Orange Peel: A Potential Source of Phytochemical Compounds. Int J ChemTech Res 2018; 7(3): 231.
8. Arunkumar S, Muthuselvam M. Analysis of phytochemical constituents and antimicrobial activities of Aloe vera L. against clinical pathogens. World J Agric Sci 2009; 5(5):572–576.
9. Geeta Parkhe, Deepak Bharti. *In Vitro* Antioxidant Activity, Total Phenolic and Flavonoid Contents of Hydroalcoholic Extract of Leaves of Lagerstroemia Parviflora Roxb. Journal of Drug Delivery & Therapeutics. 2019; 9(4-A):708-711.
10. Tanaji Nandgude, Rahul Thube, Nitin jaiswal, Pradip deshmukh, Vivek chatap, Nitin hire. Formulation and evaluation of pH induced insitu nasal gel of salbutamol sulphate. Int J Pharma Sci & Nanotechnol 2008; 1 (2): 177-83.
11. Benoy Brata Bhowmik, Bhabani Shankar Nayak, Arkendu Chatterjee. Formulation development and characterization of metronidazole microencapsulated bioadhesive vaginal gel. Int J Pharma and Pharma Pract 2009; 1(1): 240.
12. Maria BR Queiroz, Natália B Marcelino, Marcos V Ribeiro, Laila S Espindola, Franciscor Cunha, Monica V da Silva. Development of gel with Matricaria recutita L. extract for topic application and evaluation of physical-chemical stability and toxicity. Lat Am J Pharma 2009; 28 (4): 574-9.
13. Panigrahi L, Ghosal SK, Pattnaik S, Maharana L, Barik BB. Effect of permeation enhancers on the release and permeation kinetics of lincomycin hydrochloride gel formulations through mouse skin. Indian J Pharm Sci (2006); 68: 205-11.
14. Srisombat Nawanopparatsakul, Jeeratikorn Euasathien, Chuwit Eamtawecharum, Porntip Benjasirimingokol, Sakdanai Soiputtan, Photchanart Toprasri, et al. Skin irritation test of curcuminoids facial mask containing chitosan as a binder. Silpakorn Uni versity J 2005; 5(1-2): 140-7.
15. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45(4):493-496.