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Formulation of nano-encapsulated poly-herbal ointment for anti-inflammation

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ABSTRACT

Skin and soft tissue infections are one of the most common social problems in human community. Herbal medication is one of the best ways to treat such infections with least side effect. The main drawback of any topical ointment is poor penetration due to the barrier action of stratum corneum of the skin. To address such a problem we worked on formulation of nano-encapsulated antimicrobial ointment from herbal plants Plectranthus amboinicus and Hemigraphis colorata. These plants have antimicrobial, anti-inflammatory and anti-oxidant properties. Antimicrobial properties are for gram-positive, gram-negative bacteria and many fungal species. Anti-inflammatory properties are responsible for faster wound healing and anti-oxidant properties for scavenging free radicals from skin. It was found that SLN synthesized from 1:25 and 1:30 ratio were in optimal nano-range i.e. for 1: 30 - 77.63 nm and 1:25 - 48.51 nm. Combination of leaf extracts of both the plants were yielding a broad spectrum ointment and nano-encapsulation by formation of SLN will further enhance the penetration of drug into the system leading to efficient delivery with minimum side effects. Four different formulations were prepared and the consistency was found to be more in case of the formulation 4 with maximum preservative of 300 g for 2 ml of the plant extract. This work can serve as an effective new way for synthesis of ointments using Nanotechnology and combinations of compounds from herbal source in pharmaceutical industry and solve the problem associated with presently available ointments.

Keywords: Herbal ointment, *Plectranthus amboinicus, Hemigraphis colorata,* Antimicrobial activity, Anti-oxidant, Anti-inflammatory, SLN (Solid Lipid Nanoparticles), Formulation.

INTRODUCTION

The use of herbal medicines dates back to time immemorial. Ancient Egyptians, Indians, Chinese and other civilizations used local plants as medicines for treating diseases that affected their lives. The gradual learning process by trial and error over generations resulted in the evolution of a Medicare system which helped man from combat diseases with unique formulations based on plant parts. According to world health organization (W.H.O) 80% of the population of developing countries for their primary health care needs to depend on traditional medicines out of which mostly are plant drugs.

Skin infections are caused by the microorganisms which invade the skin tissues. Humans are natural hosts for many species of bacteria's which can colonize in the skin as normal flora. Dermatophytes and yeasts are harmless if present in small quantity but can cause fungal skin infections when they grow excessively. Fungal infections are mainly due to the organism *Candida albicans. Candida* is a type of opportunistic pathogen which is harmless to the body until the body's immune system is weakened.

Herbal plants were found to be a good source for a wide variety of compounds, such as phenolic compounds, terpenoids, and nitrogen containing compounds, vitamins, and secondary metabolites which possess antioxidant,

anti-microbial, anti-inflammatory, antitumor, anti-mutagenic, anti-carcinogenic and diuretic activities [1]. *Plectranthus amboinicus* is a member of family *Lamiaceae* has oregano-like flavor and odor and was known by different folk names such Ajwain patta (Hindi), Karpuravalli (Tamil) and Pani Koorka (Malayalam). It is a perennial plant which is highly aromatic and fleshy, extensively branched with leaves possessing a unique smell. The plant is well known for its antimicrobial and pharmacological activities. Antioxidant activity is reported to be mainly due to rosmarinic acid, chlorogenic acid and caffeic acid. Therefore this plant is useful in natural antibiotic formulation as an active ingredient. The plant *Hemigraphis colorata* commonly called as red ivy or purple waffle belongs to the family Acanthaceae, which is the native of tropical Malaysia. It is a prostrate growing plant with spreading, rooting stems. They are greyish green stained with red purple above and darker purple beneath. It is claimed in folk medicine that the plant has very good wound healing activity and therefore called *Murrikooti* (wound-healer) in Tamil language. The leaves are ground into a paste and applied on fresh cut wounds. However, very less pharmacological studies are carried out on this plant [2].

Inflammation is a part of the complex localized biological response of vascular tissues to harmful foreign stimuli, such as pathogens, damaged cells, or irritants. Free radicals only result in lipid peroxidation followed by membrane destruction and production of chemotactic factors and mediators. So, agents which can scavenge these ROS (Reactive oxygen species) can be used in the treatment of inflammatory disorders (3]. Drugs which are presently used for the management of pain and inflammatory conditions are narcotics (e.g. opioids), *non-narcotics* (e.g. salicylates) and *corticosteroids* (e.g. hydrocortisone). All of these drugs possess toxic and side effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars per molecule. On the contrary many medicines of plant origin had been used for a long time without any adverse effects [4].

Nano-encapsulation is a novel method of drug delivery, which means coating of the active ingredients within another material at Nano scale. Due to a number of advantages offered by SLN based drug delivery systems over conventional drug delivery systems they have recently turned up as a novel method for drug delivery. Main Advantages of SLN is that lipophilic molecule can be incorporated easily without any bio-toxicity problem of the carrier, efficient drug delivery is achieved, organic solvents can be avoided, drug is delivered to specific target site, stability of the drug is enhanced and scaling up is relatively easier than conventional drug delivery systems [5-6]. Topical drug application has been introduced to achieve several purposes on different levels (skin surface, epidermis, dermis and hypodermis). However, several problems have been reported with the conventional topical preparations e.g. absorption to the systemic circulation and low uptake due to the barrier function of the stratum corneum.

Herbal ointments which are made from plant sources have a number of advantages like easy availability, less side effects and effective cure. Most of the bacterial infections are deeply held; so selection of base during the preparation of ointment depends on the capacity of the drug to either penetrate or dissolve and release the drug effectively. There are different types of bases and each base has its own physical characteristics and therapeutic use which depends on the active components and hence are chosen accordingly.

MATERIALS AND METHODS

Source of Plant Materials: Leaves of herbs *Plectranthus amboinicus* and *Hemigraphis colorata* were collected from VIT Herbal Garden, Vellore, Tamil Nadu.

Extraction Methods: Plant extracts were obtained by a number of methods, we extracted through Sechelt extraction method with the help of (in the order) petroleum ether, benzene, chloroform, acetone, ethanol, methanol.

Decoction Preparation: 10g of fresh leaves were grinded and then boiled for 10 minutes in 100ml of distilled water. The obtained solution was filtered and then stored under refrigeration at 4°C [7].

Crude Extract Preparation: Fresh leaves were grinded and mixed in distilled water and centrifuged at 10000 rpm for 10 minutes. Supernatant was collected and stored at 4°C [8].

Solvent Extraction: Fresh leaves were collected and shade dried for 15-30. Dried leaves were grinded into fine powder. 5g of which was subjected to soxhlet apparatus using solvents (150ml) in increasing polarity as mentioned in Figure1 (Petroleum Ether, Benzene, Chloroform, Acetone and Water). After repeating several cycles the extract obtained was concentrated in Rotary Vacuum Evaporator. Percentage Yield was calculated and then extracts were stored in their respective solvents in vials at 4°C [4, 9].

Phytochemical Analysis:
Phytochemical Screening was done by the standard protocols described by Trease and Evans, [10] using different plant extracts of *Plectranthus amboinicus* and *Hemigraphis colorata* for tannins (responsible for anti-oxidant activity) and saponins, [9-11]. **Saponins**: About 0.2 g of the extracts was mixed well with 5 ml of distilled water and was heated until it boiled. Presence of saponins was indicated by Frothing (appearance of creamy miss of small bubbles). **Tannins:** Mixing of small quantity of extracts with water and heating on a water bath was carefully done. The obtained mixture was filtered and ferric chloride was added to the filtrate. The presence of tannins is indicated by a dark green solution.

DPPH Radical Scavenging Assay:
The primary reaction can be represented as:

$$Z^{\cdot}+AH = ZH+A^{\cdot}$$

Where,
$Z^{\cdot}$ is the DPPH Radical
AH is the donor molecule
ZH is the reduced form
A is free radical produced in this first step (Molyneux *et al*, 2004).

DPPH assay was carried out by taking the reaction mixture which contains 1ml of DPPH solution (200µM in ethanol) and different concentrations of the extract which were shaken and incubated for 20 minutes at room temperature. The resultant absorbance was recorded at 517nm. The percentage inhibition was calculated using the formula, [3, and 12].

In vitro Anti-inflammatory Activity: Membrane Stabilization Method
HRBC membrane stabilization method was used to study the anti-inflammatory activity in-vitro. Blood was collected from VIT University Health Center donated by healthy individual and was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). Blood samples were centrifuged at 3000 rpm and the packed cells were washed with isosaline (0.85%, pH 7.2) after which a suspension was made using isosaline (10% v/v). The assay mixture was prepared using 1ml of phosphate buffer (0.15M, pH 7.4), 2ml of hypo-saline (0.36%), and 0.5ml of HRBC suspension and 1ml of various concentrations of the extract. Diclofenac sodium was taken as a reference drug. For control, 2ml of distilled water was added instead of hyposaline. Then all the mixtures were incubated at 37°C for 30 min and centrifuged. The absorbance was read at 560 nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization was calculated using the formula described by [3, 13].

$$\% \text{ of Membrane Stabilization} = \frac{\text{Absorbance of Drug treated Sample}}{\text{Absorbance of Control}}$$

Synthesis of Solid Lipid Nanoparticles
Micro emulsion based SLN preparations:
For the preparation of Solid Lipid Nanoparticles (SLN), technique developed by Gasco and co-workers was followed which is based on the dilution of micro emulsions. SLN were made by stirring an optically transparent mixture at 65-70°C which is composed of a low melting fatty acid (stearic acid), an emulsifier (polysorbate 20, polysorbate 60, soy phosphatidylcholine, and sodium taurodeoxycholate), co-emulsifiers (butanol, sodium monooctylphosphate) and water. After stirring, the hot micro emulsion was dispersed in cold water (2-3°C) under stirring at around 400 rpm. Typical volume ratios of the hot micro emulsion to cold water are in the range of 1:25 to 1:50. Optimization was done by taking different ratios in this range. The dilution process is based on the composition of the micro emulsion needs to be optimized [14]. After that, the obtained micro emulsion was sonicated at a frequency of 22.5 KHz, intensity of 17 W/cm² & Pulse length of 10 seconds on and 10 seconds off for an interval of 10 minutes. Then sonicated solution is centrifuged at 13,000 rpm for 30 minutes to obtain the pellet containing the SLN. So, we took stearic acid, Tween 20 (Polysorbate 20), butanol and water in different ratios to find out the most optimum composition which leads to the synthesis of smallest and narrowly distributed particles.

Characterization of SLN prepared
AFM (Atomic Force Microscopy): A topological map was produced when probe tip with atomic scale sharpness rasteres across a sample and the map produced was based on the forces that play between the probe tip and the

surface of the sample. Ultrahigh resolution can be obtained using this approach, which has the ability to produce topological map of the sample according to their properties.

AFM Sample Preparation: 100µl of the pellet solution were dragged on clean glass slides and uniform smears were prepared from different batches of SLN synthesized. Then slide were kept in oven for overnight drying at 37°C.

Synthesis of Nano-encapsulated Particles: Loading of the most optimum set of extracts in the SLN can be done during synthesis by optimizing the amount to be added for various components. The methanolic plant extracts of both plants were added in a ratio of 1:1 before sonication step in the protocol of synthesis, [15].

In-Vitro Drug Release Studies: Dialysis Bag was soaked overnight in phosphate buffer Saline. The following day 5ml of Nano-encapsulated suspension was put into the bag and 0^{th} hour sample was collected. Samples were collected every hour until 8^{th} hour by replacing it with fresh PBS every time. Again samples were collected every 12^{th} hour until the 96^{th} hour, [16]. Release Time graph is plotted to find till what time the loaded particles were releasing the active ingredient.

Ointment Preparation:
Oily Base Preparation: Paraffin wax is melted on a hot plate and liquid paraffin is added to it followed by preservative benzoic acid. The mixture was transferred to pestle and mortar and Lipid Nanoshperes containing the plant extract was added and grinded at room temperature to obtain a fine paste like substance. The ratio of chemicals to be taken is optimized by hit and trial method. Flavoring agents were also added later, [17]. **Stability study of the ointment prepared:** The prepared ointment was taken in different containers and placed at different temperatures viz. room temperature, 4°C, 40°C for 1 week and physical parameters such as consistency, color, and odor were observed and results were recorded.

RESULTS AND DISCUSSION

Yield of different extracts: From our experimental results of extraction, it was found that maximum yield was obtained in the methanolic extract of *Plectranthus amboinicus* (14.4 %) followed by ethanol extract of *Plectranthus amboinicus* (13.2%) and least yield was obtained with chloroform extract of *Plectranthus amboinicus* (9.2%). While in case of *Hemigraphis colorata*, maximum yield was obtained in methanolic extract (19%) and lowest in acetone extract (14.2%). According to the previous researches, maximum yield obtained in *Plectranthus amboinicus* was 12%, [4] and in *Hemigraphis colorata* was 20%., in Table 1.

Table 1: % yield before storing for various samples:

Sample	Solvent	Amount of sample taken(g)	Amount of extract obtained(g)	%Yield (w/w)
Plectranthus amboinicus	Petroleum ether	5	0.57	11.4
	Chloroform	5	0.52	10.4
	Ethanol	5	0.66	13.2
	Water	5	0.46	9.2
	Methanol	5	0.72	14.4
Hemigraphis colorata	Methanol	5	0.95	19
	Petroleum ether	5	0.92	18.4
	Benzene	5	0.94	18.8
	Chloroform	5	0.83	16.6
	Acetone	5	0.71	14.2

***In-vitro* Anti-inflammatory Assay:** During inflammation, lysosomal enzymes are released which leads to variety of disorders. Acute or chronic inflammation is found to be related to the extracellular activity of these enzymes hence anti-inflammatory drugs (such as NSAIDs) act on these enzymes and stabilizes the lysosomal membrane [13]. The HRBC membrane is similar to the lysosomal membrane in its components and therefore percentage membrane stabilization of HRBC was taken as a measure of anti-inflammatory activity in-vitro. Maximum Percentage membrane stabilization was seen in ethanolic extract of *Plectranthus amboinicus* 62.61% (500 µl) followed by Methanolic Extract of *Plectranthus amboinicus* 54.05% (500 µl) and in *Hemigraphis colorata* methanolic extract 52.47% membrane stabilization was there in 500µl. The maximum percentage membrane stabilization obtained in the Drug Diclofenac was 66.09% (500 µl).
Anti-oxidant Assay (DPPH Assay): An increasing trend was observed with increasing concentration of all the extracts in percentage inhibition. But maximum percentage inhibition was obtained in combination of methanolic

extracts of *Hemigraphis* and *Plectranthus* in the ratio 1:1 which was 97.62% (500 µl) followed by Ethanolic, Methanolic extract of *Plectranthus* and Least was seen in Methanolic extract of *Hemigraphis*.

Phytochemical Analysis: Tannins are the compounds which are responsible for anti-oxidant activity. It was found that Tannins were present in ethanol, methanol & aqueous extracts of *Plectranthus amboinicus* and chloroform, acetone & methanolic extracts of *Hemigraphis colorata*.

SLN synthesis and Characterization: For Optimization of hot microemulsion. Water Ratio, SLN with different ratios (1:25, 1:30, 1:1, 1:2, and 1:20) was synthesized. Later, Characterization was done using AFM; it was found that SLN synthesized from 1:25 and 1:30 ratio were in optimal nano-range i.e. for 1: 30 - 77.63 nm and 1:25 - 48.51 nm (Figure 1.a, 1.b).

Figure 1.a. AFM result of SLN made from 1:30 Hot Micro- emulsion: Water Ratio

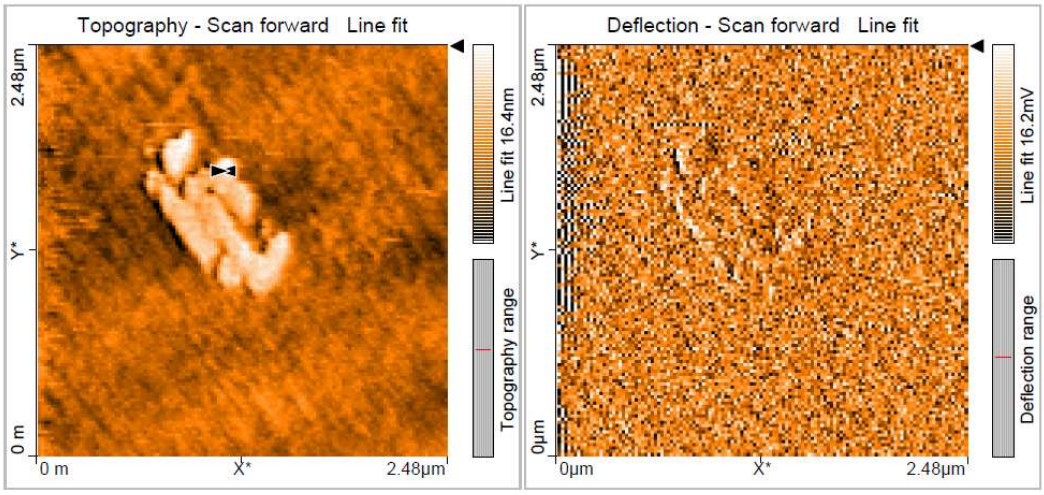


Figure 1.b. 3D Image obtained from AFM of SLN made from 1:30 Hot Micro-emulsion: Water Ratio.

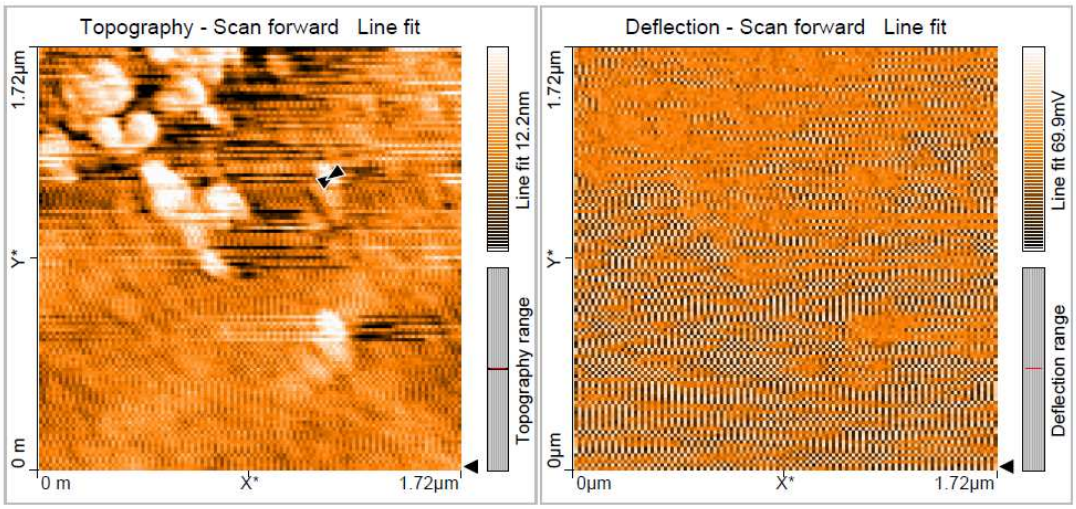
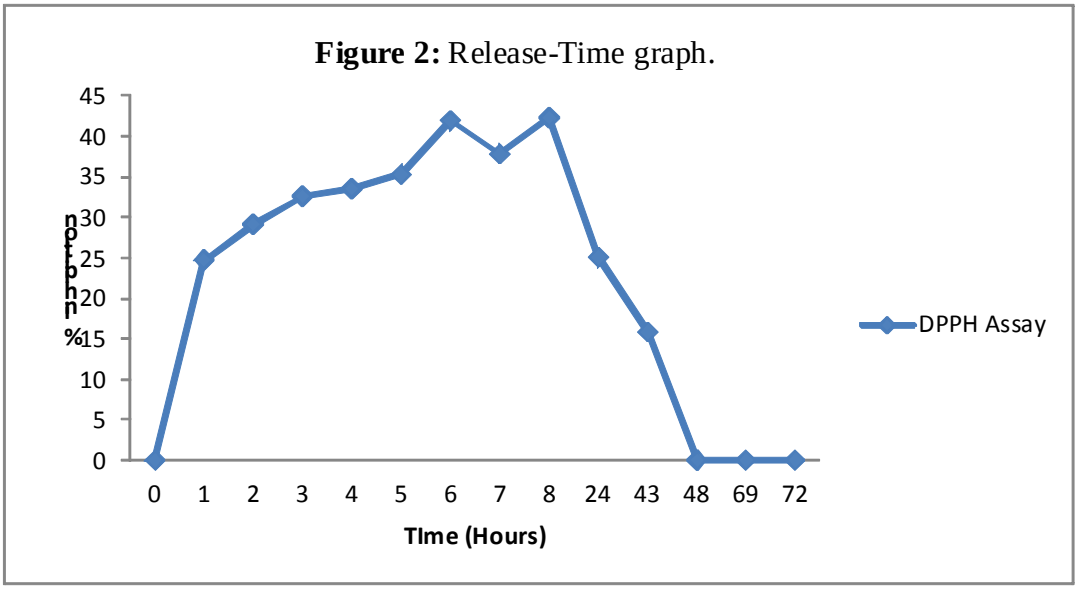


Figure 2: *In-vitro* drug release graph:



In-vitro Drug Release Studies: Release study of drug was done by calculating the anti-oxidant activity of the sample released over time (for 72 hours), which lasted for 48 hours. Maximum release was seen at 8th hour after which there was decline in the release of drug (Figure 2).

Ointment Preparation: Different formulations were made to optimize the composition of the ointment base and it was found that 1:3 ratio of paraffin wax: liquid paraffin showed the best consistency over the other ratios (1:1, 1:2, 2:1).

Table 2: Different formulations and their consistency.

Formulations	Hard Paraffin (g)	Liquid Paraffin (ml)	Preservative Benzoic acid (µl)	Plant Extract (ml)	Consistency
Formulation 1	10	5	100	1	--
Formulation 2	5	5	100	1	-
Formulation 3	5	10	200	1	+
Formulation 4	10	30	300	2	++

Stability Study of the prepared Ointment:
Stability study was done by measuring physical parameters (Color, Odor, Consistency, and Spreadability). In this study, the ointments were kept at different temperatures (4°C, room temperature, 40°C) for a week. After a week, desired physical parameters were observed in the ointments kept at room temperature, 40°C and 4°C except for the change in the consistency of the sample kept at 40°C.

CONCLUSION

The purpose of the study was to prepare a poly-herbal ointment using two different plants possessing remarkable pharmacological properties. It was also desired that the ointment should be free from the problems and deficiencies associated with conventionally used allopathic ointments. The major organisms responsible for skin infections are bacteria like *Staphylococcus aureus* and Fungus like *Candida spp*. The growth of these organisms was found to be inhibited by the plant extracts obtained from *Plectranthus amboinicus* and *Hemigraphis colorata*. Therefore, these plants were chosen for the preparation of ointment which can be used to treat dermatological infections. Results from the study indicate that combinations of plant extracts have enhanced antimicrobial, anti-oxidant and anti-inflammatory properties over the usage of single plant extracts of *Plectranthus* and *Hemigraphis.* Encapsulation of the plant extract with SLN was done as it can best address the problems of poor specificity and efficiency experienced in the case of general topical ointments.

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REFERENCES

[1]Arzu Birinci, P.K.Fatma, T. Arzu Ucar, *Asian Pacific Journal of Tropical medicine.*, **2013**, 6 (8), 616-624.
[2]J. Saravanan, W.R. Sheriff, N. Joshi. R. Varatharajan, V.G. Joshi, A.A. Karigar, *Research Journal of Pharmacognosy and Phytochemistry.*, **2010**, 2(1), 15-17.
[3]S. Lathaa, G.X. Fatima, S. Shanthi, S. Chamundeeswari, S. Seethalakshmi, Uma Maheswara Reddy, *Sri Ramachandra Journal of Medicine.*, **2011**, 4 (1), 23-25.
[4] A. Manjamalai, T Alexander, V.M. Berlin, *International Journal of Pharmacy and Pharmaceutical Sciences.*, **2012**, 4(3), 205-211.
[5] A. Dubey, P.Prabhu, J.V.Kamath. *International journal of pharmtech research coden.*, **2012**, 4(2), 705-714.
[6] S.R. Fathima, A. Vadivel, N. Samuthira, M. Sankaran, *Asian pacific journal of tropical medicine.*, **2012**, 1-10.
[7]D. Benito, B.N. Shringi, P. Dinesh Kumar, S.S.C. Nehru, J. Ashok Kumar, *Indo-Global Journal of Pharmaceutical Sciences.*, **2011**, 1(2), 186-193.
[8] Satish *et al*., *International Journal of Integrative Biology.*, **2010**, 9(1), 22-27.
[9] H. Nagalakshmi, S. Arijit Das, S. Bhattacharya, *International Journal of Green and Herbal Chemistry.*, **2012**, 1(2), 101-107.
[10] G.E. Trease, W.C. Evans. Pharmacognosy, Brailliar Tiridel and Macmillan Publishers, London.11th edition, **1989**.
[11] S. Arunkumar and E. Karthikeyan, *IJRAP*., **2011**, 2, 292-294.
[12] R. Sutharsingh, S. Kavimani, B. Jayakar, M. Uvarani, A. Thangathirupathi, *International Journal of Pharmaceutical Studies and Research.*, **2011**, 2(2), 52-56.
[13] V.R. Ravikumar, M. Dhanamani, T. Sudhamani, *Ancient Science of Life*, **2009**, 28(4), 7–9.
[14] S. Mukherjee, S. Ray, R.S. Thakur, *Indian J Pharm Sci.*, **2009**, 71 (4), 49-58.
[15] M.R. Gasco, *Pharm Tech Eur.*, **1997**, 9, 52-58.
[16] Wei Liu, Meiling Hu,Wenshuang Liu, Chengbin Xue, Huibi Xu, XiangLiang Yang, *International Journal of Pharmaceutics.*, **2008**, 364, 135–141.
[17]A. Chris, Alalor, I. Cecilia. Igwilo, P. Chukwuemeka, Azubuike, *Asian Journal of Biomedical and Pharmaceutical Sciences.***2012**, 2 (13), 15-19.