WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 11, 1018-1030. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF HERBAL LIPOSOMAL GEL

***[1]P. V. Thushara, [2]V. M. Ananya, [3]C. Suma and [4]C. K. Amritha**

[1]Assistant Professor Department of Pharmaceutics Malik Deenar College of Pharmacy, Kasaragod.

[2]Assistant Professor Department of Pharmaceutical Analysis.

[3]Assistant Professor Department of Pharmaceutical chemistry.

[4]Assistant Professor Department of Pharmacology.

Article Received on
29 July 2020,

Revised on 19 August 2020,
Accepted on 09 Sept. 2020,

DOI: 10.20959/wjpr202011-18710

***Corresponding Author**
P. V. Thushara
Assistant Professor
Department of
Pharmaceutics Malik
Deenar College of
Pharmacy, Kasaragod.

ABSTRACT

Plants are nature's cures and have been utilized by individuals on earth for food and drug. Herbal medicine is also called botanical medicine or phytomedicine, refers to using a plant's seeds, fruit, flowers, bark, leaves, root, for medicinal purpose. Herbal medicine are very important in the developing world since they are safe, inexpensive, effective with minimal side effect. The combination of herbal drug with novel drug delivery system (NDDS) enhances the therapeutic activity and reduces the toxicity.Several common plants are used for many of the acute and chronic diseases widely. In our study, we are formulating a gel by incorporating novel drug carrier such as liposome containing herbal extract. Liposome can be used to for localized drug action since their small size and which have high penetrability through the epithelium .The liposome are more stable in gel formulation which will improve will the contact time of the drug.

KEYWORDS: Herbal medicine, Liposome, Gels.

INTRODUCTION

Liposomes are small spherical shaped vesicles that can be made from cholesterol and natural nontoxic phospholipids**.** Because of their hydrophobic and hydrophilic character (besides liposomes are promising frameworks for medication conveyance. Liposome properties contrast extensively with lipid piece, surface charge, measure, and the technique for preparation Besides, the component of bilayer segments decides the flexibility an rigidity.

Formulating a gel by incorporating novel drug carrier such as liposome containing herbal extract. Liposome can be used to for localized drug action since their small size and which have high penetrability through the epithelium The liposome are more stable in gel formulation which will improve will the contact time of the drug.

MATERIALS AND METHODS

Table 1:

Sl. No.	Materials/solvents	Manufactures/Suppliers
1	Ethanol	Changshu Hongsheng Fine Chemical Co. Ltd
2.	Fresh plant material	Prasad pharmacy, Trikaripur
3	Chloroform	Medlise Chemicals ,Kannur
4	Cholesterol	Medlise Chemicals ,Kannur
5	Soyalecithin	Medlise Chemicals ,Kannur
6	Sodium hydroxide	Medlise Chemicals ,Kannur
7	Carbapol-934	Burgoyne Burbidges &Co. Mumbai,India
8	Propylene glycol	Medlise Chemicals ,Kannur
9	Methyl paraben	Burgoyne Burbidges &Co. Mumbai,India
10	Propyl paraben	Burgoyne Burbidges &Co. Mumbai,India
11	Disodium EDTA	Medlise Chemicals ,Kannur
12	Triethanolamine	Medlise Chemicals ,Kannur

Collection of plants

The fresh plant part of *Cynodon dactylon* were collected from local vendors in kasaragod district, Kerala, India in the month of November 2018. The plant material were identified and authenticated by by Dr.A. Rajagopalan, Professor, Department Of Horticulture, College Of Agriculture, Padannakad, Kasargod, Kerala.

Determination of Physicochemical parameters

1. Ash values

a. Total ash: About 2 g of powdered leaves of powdered drug was accurately weighed and transferred to silica crucible which are previously weighed. It is then heated at a temperature of about 500-600° C in a muffle furnace until it is carbon free. The silica crucibles were the taken out from the muffle furnace and placed in a descicator for cooling purpose. It is then weighed and percentage of total ash with reference to the air-dried drug.

$$\% \text{ Total Ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

b. Acid –insoluble ash: Total ash obtained were taken in to a beaker and are boiled for about 5 minute in 25ml of dilute sulfuric acid (1M HCL). It is then filtered through a whatman

filterpaper no.41 and residues were washed with hot water until it is free from chlorides. The residue containing filter papers were then placed in different silica crucibles and ignited in muffle furnace at 550±20° C for 1 hour. It is then cooled and percentage of acid insoluble ash was calculated with reference to the air- dried drug.

$$\%\text{Acid insoluble ash value} = \frac{\text{Weight of total ash} - \text{Weight of acid insoluble ash}}{\text{Weight of crude of drug taken}} \times 100$$

C. Water soluble Ash value: Total ash obtained from were taken into a beakers and are boiled for about 5 minute in 25ml of water. It is then filtered through Whatman filter paper no.41 and the residues werre washed with hot water until it was free from chlorides. The residue containing filter papers were then placed in different silica crucibles and ignited in muffle furnace at a temperature 450° C to constant weight. It was then cooled and percentage of water soluble ash was calculated with reference to the air dried drug.

$$\%\text{ Acid soluble ash value} = \frac{\text{Weight of total ash} - \text{Weight of acid soluble ash}}{\text{weight of crudedrug taken}} \times 100$$

2. Determination of moisture content

Loss on drying method

Accurately 3g powder was weighed and transferred into a china dish. It is then heated at 105°C in an an oven until a constant weight was obtained. Then the percentage of moisture content of the sample was calculated with reference to the air dried drugs.

3. Extractive value

a. Water soluble Extractive value

Accurately weighed about 5g of air dried powdered plant material were taken in 100 ml of chloroform water in a closed flask for 24 hours. During the first 6 hours it was shaken frequently and filtered. 25ml of the filtrate were evaporate to dryness in a flat-bottomed shallow petridish and dried at 105°C and weighed. Then the percentage water soluble extractive values were calculated with reference to the air-dried drugs.

b. Alcohol soluble extractive value

Accurately weighed about 5g of air dried powdered plant material were taken in a closed flask and 100 ml of 95% ethanol was added into it. During the first 6 hours it was shaken frequently and filtered. 25ml of the filtrate were evaporate to dryness in a flat-bottomed

shallow petridish and dried at 105°C and weighed. Then the percentage ethanol soluble extractive values were calculated with reference to the air-dried drugs.

Extraction of plant materials

The extraction was carried out by soxhlation method using soxhlet apparatus. The collected plant materials are shade dried then coarsely powdered. Required amount of plant material was packed uniformly into a thimble and extracted with 300 ml of solvent which is taken in the round bottom flask and placed in the heating element.

The process was continued till the solvent in siphon tube of the apparatus became colourless. The crude extract was filtered and concentrated under vacuum and controlled temperature. The extract was stored in refrigerator at 4 °c until further use.

Drug –Excipient compatability study by FT-IR

The FT-IR study were carried to determine the occurance of any interaction of drug with the excipient used for the preparation of liposomal gel. Fourier-transform infrared spectra were obtained by using JASCO FT-IR 4700 L spectrometer.

FT-IR Samples are,

Sample A: Ethanolic extract of drug

Sample B: Drug sample +Soyalecithin+Cholesterol+ Carbapol 934

Preparation of drug loaded liposome

By using two by three factorial design about 9 formula were constructed , F1-F9

Formula of developed liposome

Table 2:

Sl.no	Weight of drug extract(mg)	Cholesterol(mg)	Soyalecithin(mg)
1	500	100	100
2	500	100	200
3	500	200	100
4	500	200	200
5	500	150	100
6	500	150	200
7	500	150	150
8	500	100	150
9	500	200	150

Preparation of Liposome

soyalecithin(F1-F9) are accurately weighed and then dissolved in 15 ml mixture of Here the drug, chloroform chloroform:methanol(2:1) ratio. The mixture is sonicated for 5 min, then vortexed in RBF to remove the solvent about 30 min The lipid layer is then hydrated with 10 ml of phosphate buffer at 60^0c for 1 hr. The dispersion is kept undisturbed at room temperature for complrte swelling of lipid film.

Formulation of liposomal gel

The optimised liposomal formulation was used for the preparation of 1% carbopol gel. The required measure of carbopol 934 (1% W/W) was gradually sprinkled into a 500ml distilled water with consistent mixing utilizing mechanical stirrer (at the base speed to prevent the entrapment of air) . After complete homogenization of the carbopol polymer with distilled water the prepared liposome was introduced into the above blend gradually with nonstop mixing pursued by expansion of 10 % propylene glycol. Required measure of additives were taken in a container and isdissolved by warming it over a water bath and is then added to the above blend. The above mixture is nutralized by triethanolamine with constant blending for adjusting the skin pH (6.8-7) and to get a gel at required consistency.

Evaluation of liposomes

a. Vesicle size of liposome

Liposomal suspension was subjected to ultrasonic irradiation for 30 min.The sample was cooled down and kept in fridge at 4^0c for one day prior to the test.The particle size is determined by optical microscopy. The liposomal suspension evaluated for the particle size in suitable dilution. Diameter of the particle is observed under oil immersion lense.

b. Vesicle shape of liposome

The shape and morphological characters were obtained by SEM photographs of the optimized niosomes. The formulations were placed into circular aluminium stubs using double adhesive carbon tape and coated with gold in HITACHI ION SPUTTER E-1010 vacuum evaporator, it was observed in HITACHI SU6600 FE SEM (field emission scanning electron microscope) having acceleration voltage of 10.0kv and magnification of 60.0k-100.0k .

Evaluation of liposomal gel

a. Physical examination: The hebal liposomal gel was prepared and evaluated for colour, odour, transparency.

b. Spreadability: Inoder to determine the spreadality the gel formulation are sandwitched between two glass plate. A weight of 5gm allowed to rest on the upper glass platefor 5 min.The increase in diameter due to spreadability of the formulation was not increase in diameter due to spreadability of the formulation was noted.The spreadability is expressed in terms of time in second taken by two slides to slip off from the formulation under the application of certain load.

c. pH: pH was determined by digital pH meter at room temperature. About 2.5 gm of the gel dispersed in 25 ml distilled water and stored for 2 hr.

d. Viscosity: Viscosity of the formulation was determined by Brookfield Viscometer at 6 rpm.

e. Homogeneity: The formulations were tested for the homogeneity by visual appearance.

f. Extrudability: It measure the force required for the gel to extrude out from the tube. The prepared gel was filled into a collapsible tube and it was sealed and the weight of the tube was recorded. Placed a 500 g weight on the tube and the amount of gel hat extruded out was collected and weighed. Then, the percentage of gel extruded was calculated.

g. washability: The product is applied in the hand and observed under the running water.

RESULTS AND DISCUSSION

Plant collection and authentication

The plants *Cynodon dactylon* (Linn) were collected from Kasaragod district, Kerala, in the month of November 2018 and collected plant materials were authenticated by Dr.A. Rajagopalan, Professor, Dept. of Hortriculture, Padannakad, Kasaragod, Kerala.

Physico-chemical parameters

After the collection of plant materials, they were shade dried and powdered coarsely in an electronic blender and stored in air-tight containers until further use. Physico-chemical parameters of the plant were tabulated in Table no.8. Parameters such as ash value, extractive values and moisture contents were estimated.

Table 3: Physico-chemical parameter of *Cynodon dactylon.*

Test	*Cynodon dactylon*
Total Ash (%w/w)	6.8%(NMT 7%)
Acid insoluble Ash (%w/w)	2.8%(NMT 3%)
Water soluble Ash (%w/w)	6.2%(NLT 6%)
Water soluble extractive value (% w/w)	5.67% (NLT 5%)
Alcohol soluble extractive value (%w/w)	9%(NLT 1%)
Moisture content(%w/w)	10.05%

Total ash value was found to be 6.8% ,Acid insoluble ash - 2.8%, water soluble ash - 6.2%, water soluble extractive value - 5.67%, alcohol soluble extractive value - 9%, moisture content 10.05%.These values were found to be with in the limits.

Extraction of plant materials

The extraction of dried leaves of *Cynodon dactylon* (Linn) were carried out by using soxhlet extraction process by using ethanol as the solvent. The extract obtained were collected and concentrated**.** The concentrated extract was then weighed and kept in a desiccator which was previously filled with fused calcium chloride until it was used for the preparation of liposomal gel.

Fig. 10: Soxhlet extraction.

Preformulation study

Drug-Excipient Compatibility Studies by FT-IR

The interaction study was carried out to ascertain any kind of chemical interaction of drug with the excipients used in the preparation of gel formulation. The FT-IR spectra were

obtained by using JASCO FT-IR 4700 L spectrophotometer. The FT-IR results obtained are shown below.

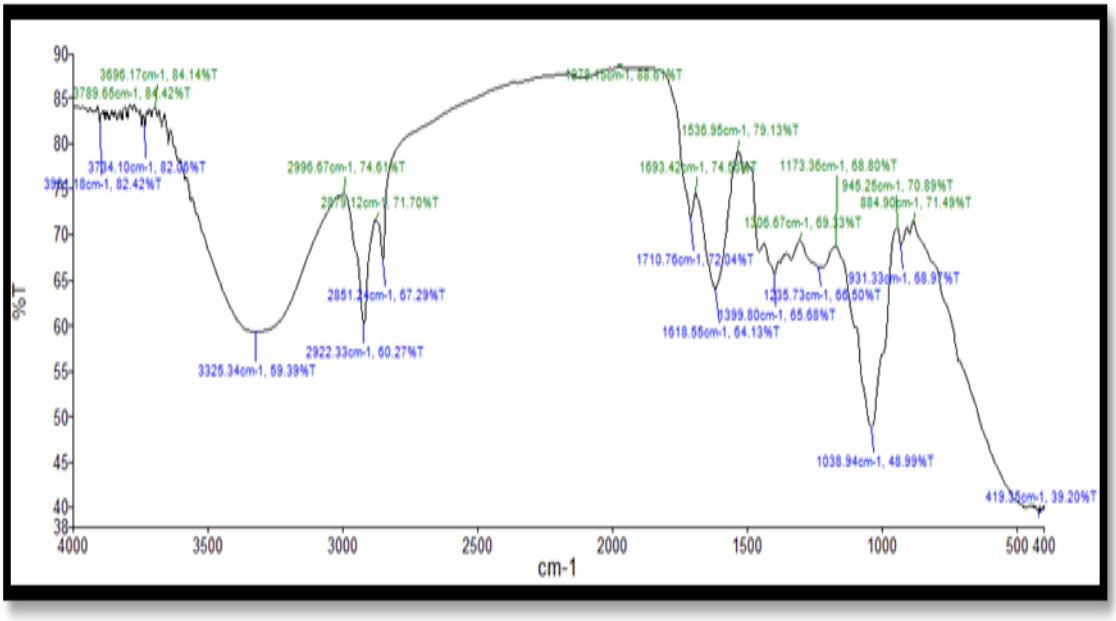

Fig. 11: FT-IR Spectrum of *Cynodon dactylon* (Sample A).

Sample A: 3901.8 cm^{-1} (O-H stretching,H bond),3325.34 cm^{-1} (C-H stretching),2922.33 (C-H stretching,C-H bond),1038.94 cm^{-1} (C-O stretching)

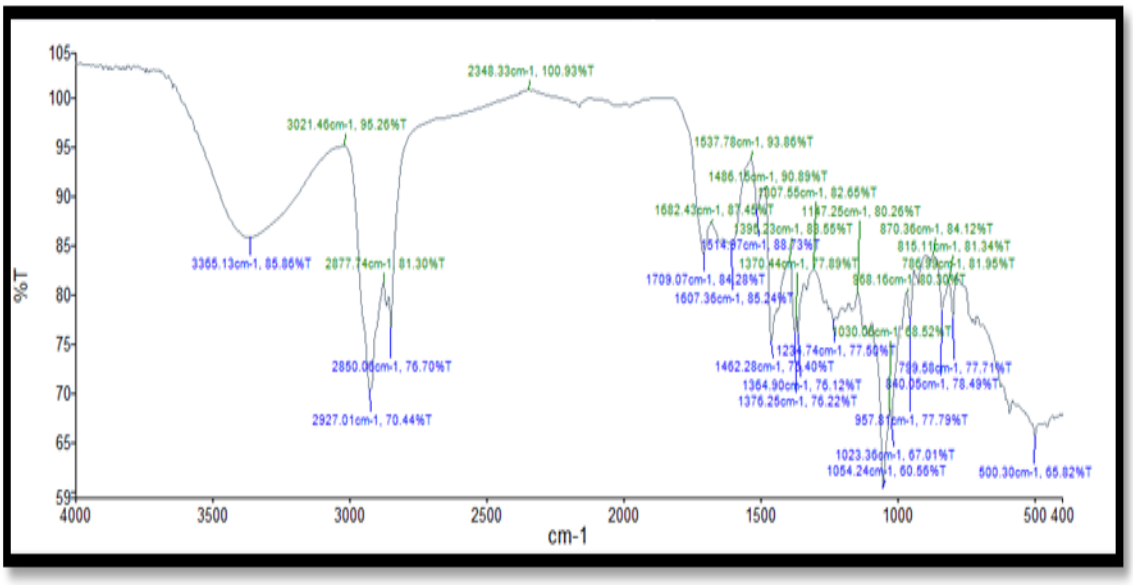

Fig. 12: Drug+Soyalecithin+Carbapol 934+Cholesterol(Sample B).

Sample B: 3365.61 cm^{-1} (O-H Stretching, H bond)2927.08 cm^{-1}(C-H stretching),2850.13cm^{-1}(C=O Stertching), 1054.28cm^{-} (C-O stretching),1709.56 cm (C=O stretching).

Evaluation of Liposome

Vesicle size of liposome

Table 4: Vesicle size of liposome.

Sl. No.	Formulation code	Mean particle size ± SD (µm)
1	F1	3.2±0.01
2	F2	2.4±0.05
3	F3	5.4±0.01
4	F4	5.3±0.04
5	F5	8.6±0.02
6	F6	7.8±0.05
7	F7	5.7±0.06
8	F8	5.3±0.02
9	F9	5.8±0.05

Values are expressed in mean ± SD (n=3)

Vesicle shape of liposome

The vesicle shape of formulated liposome (F2) was determined by SEM.

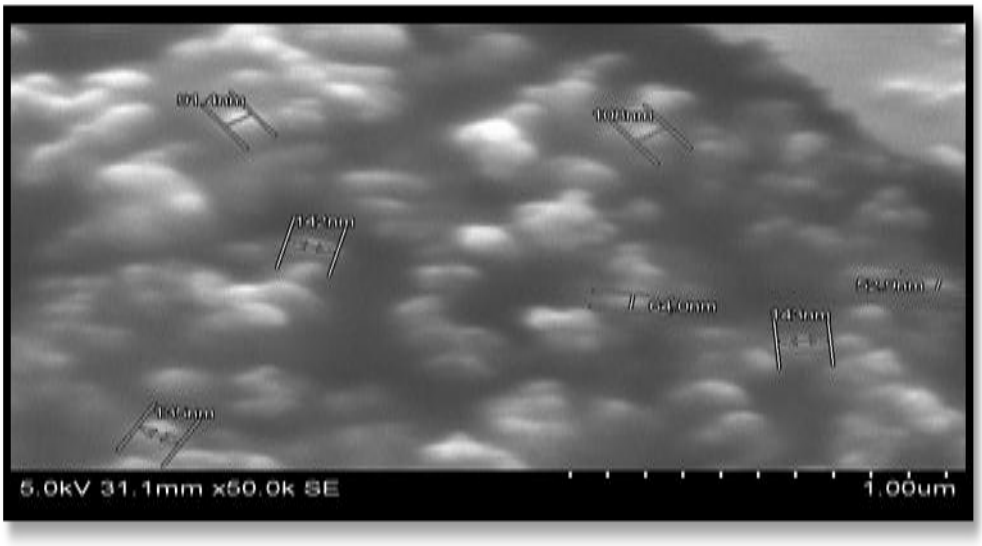

Fig. 16: SEM photographof formulated liposome (F2).

Optimization of liposome

Based on the vesicle size, vesicle shape and invitro antioxidant activity F2 was selected as the optimized formulation. F2 showed comparatively smaller vesicle size, shape and are suitable for liposomal gel preparation.

Formulation of liposomal gel

The optimized formula F2 was converted to gel by using carbopol 934.

Evaluation of liposomal gel

Physiochemical evaluation

Table 5: Physiochemical evaluation of gel.

Parameters	Observation
Color	Pale Yellow
Odor	Characteristic
Appearance	Clear and translucent

Homogeneity

The optimized gel was tested for homogeneity by visual inspection. The liposomal gel possesses uniform distribution without any aggregates.

Extrudability studies

Extrudability of optimized liposomal gel was performed and about 80% of the gel was extruded from the collapsible aluminium tube. This indicated that the extrudability character of the liposomal gel (F2) was good.

Washability

The optimized gel was applied on the skin and was removed easily by washing with tap water.

pH

The pH of the optimized formulation was carried out by digital pH meter. Triplicate measurement is taken and are represented in the table.

Table 6: pH of the liposomal gel.

Formulation code	Samples			Average pH ± SD (n=3)
	1	2	3	
F2	5.71	5.73	5.70	5.71± 0.015

Values are expressed in mean ± SD (n=3)

Spreadability

The term spredability is used to calculate extent of area to which the gel readily spreads on application to the skin. The spredability of liposomal formulation was found to be 11.65±0.02 indicates better spredability. The value obtained was given in the table.

Table 7: Spreadability of liposomal gel.

Formulation code	Trials			Average spredability(gcm/sec)
	1	2	3	
F2	11.67	11.65	11.63	11.65± 0.02

Values were expressed in mean ± SD (n=3)

Viscosity

The viscosity of the formulation indicate the consistency. It is determined by Brookfield viscometer, Three measurement were taken . From this average pH is then calculated. it given in the table.

Table 8: Viscosity of viscosity of liposomal gel.

Formulation code	Trials			Viscosity (cps)
	1	2	3	
F3	11348	11345	11347	11346±1.52

Values were expressed in mean ± SD (n=3)

Swelling index

Table 9: swelling index.

Time(hr)	Swelling index (%)
1	14.64 ± 0.04
2	19.75± 0.03
3	28.43± 0.1
4	39.42± 0.02
5	43.42 ± 0.02
6	52.33± 0.02
7	58.12 ± 0.01
8	60.72 ± 0.15
9	63.03± 0.01
10	65.28± 0.01

Values were expressed in mean

CONCLUSION

There are many conventional medication are available in the market, but it will have many side effect. Under this circumstance, the herbal medicine are very important. The main aim of this project work is to develop topical herbal liposomal gel. The plant *Cynodon dactylon* were selected. Ethanolic extract of this plant were incorporated into Novel drug delivery system such as liposome. Preformulation study were carried out inorder to develop liposomal gel. Optimization of the liposomal dispersion is done on the basis of vesicle size and antioxidant

study. From this F2 was selected as best. Liposomal gel was prepared and all the evaluation were carried out.

ACKNOWLEDGEMENT

First of all, I thank God for giving me the strength to keep going, facing the trial and breaking the hurdles for this accomplishment.

I am immensely indebted to **Kerala University Of Health Sciences, Trissur**, for providing me a platform to do my post-graduation under them.

I sincerely thankful to all faculties and friends at **Malik Deenar College Of Pharmacy,** for providing facilities for finishing my work.

REFERENCES

1. Yogeshwer Shukla. Herbal medicine: Current status and the future. *Asian pacific Journal of cancer prevention*, 2019; 4(1): 281-288.
2. Kalyani Pathak and Ratna Jyoti. Herbal medicine: A rational approach in health care system. *International Journal of Herbal Medicine*, 2011; 4(3): 109-128.
3. Singab A.N and Eldahshan.O. Medicinal Importance of Herbs & Spices. *Med aromat plants*, 2015; 4(4): 1-12.
4. Bassam Abdul Rasool Hassan. Medicinal Plants (Importance and Uses). *Pharmaceutica Analytica Acta*, 2013; 3(10).
5. Refaz Ahmad Dar, Mohd Shahnawaz and Parvaiz Hassan Qazi. General overview of medicinal plants: A review. *The Journal of Phytopharmacology*, 2017; 6(6): 349-351.
6. Abayomi Sofowora and Adedeji Onayade. The Role and Place of Medicinal Plants in the Strategies for Disease Prevention. *Asian Journal Of Traditional, Complimentary, Alternative medicine*, 2013; 10(5): 210–229.
7. Elizabeth Opara and Magali Chohan. Culinary Herbs and Spices: Their Bioactive Properties, the Contribution of Polyphenols and the Challenges in Deducing Their True Health Benefits. *International Journal of Molecular Sciences*, 2014; 15(1): 19183-19202.
8. Davyson de L. Moreira, Sabrina Schaaf Teixeira and Maria Helena D. Monteir. Traditional use and safety of herbal medicines. *Brazilian Journal of pharmacognosy*, 2014; 24(1): 248-257.
9. Yogeshwer Shukla. Herbal medicine: Current status and the future. *Asian pacific Journal of cancer prevention*, 2019; 4(1): 281-288.

10. Kalyani Pathak and Ratna Jyoti. Herbal medicine: A rational approach in health care system. *International Journal of Herbal Medicine,* 2011; 4(3): 109-128.
11. Singab A.N and Eldahshan.O. Medicinal Importance of Herbs & Spices. *Med aromat plants,* 2015; 4(4): 1-12.
12. Bassam Abdul Rasool Hassan. Medicinal Plants (Importance and Uses). *Pharmaceutica Analytica Acta,* 2013; 3(10).
13. Refaz Ahmad Dar, Mohd Shahnawaz and Parvaiz Hassan Qazi. General overview of medicinal plants: A review. *The Journal of Phytopharmacology,* 2017; 6(6): 349-351.
14. Abayomi Sofowora and Adedeji Onayade. The Role and Place of Medicinal Plants in the Strategies for Disease Prevention. *Asian Journal Of Traditional, Complimentary, Alternative medicine,* 2013; 10(5): 210–229.
15. Elizabeth Opara and Magali Chohan. Culinary Herbs and Spices: Their Bioactive Properties, the Contribution of Polyphenols and the Challenges in Deducing Their True Health Benefits. *International Journal of Molecular Sciences,* 2014; 15(1): 19183-19202.
16. Davyson de L. Moreira, Sabrina Schaaf Teixeira and Maria Helena D. Monteir. Traditional use and safety of herbal medicines. *Brazilian Journal of pharmacognosy,* 2014; 24(1): 248-257.