Preparation and Characterization of Silver Nanoparticle of Calotropis Procera root extract for Anti-asthmatic potential.

Ashwini S Joshi*[1], Abhinit Mulay[2], Mohammad Asef Shaikh[3], Narendra Patre[4]

*[1]Department of pharmaceutics, Sri Venkateshwara College of Pharmacy, Madhapur, Hyderabad, Telangana, India-500019.

[2]Chemo India Formulations Pvt. Ltd., Lalgadi Malakpet, Hyderabad, Telangana, India-500101.

[3]School of Pharmacy, SRTM University, Nanded, Maharashtra, India-431606 .

[4]D.K. Patil Institute of Pharmacy, Loha, Nanded

ABSTRACT

Due to the less toxicity, cost effective and eco friendly nature plant derived nanoparticles has been increasingly gain the attention. In this study, prepared the silver nanoparticles using the extract of roots of Calotropis Procera medicinal plant used for the treatment of asthma. This method allowed the synthesis of silver nanoparticles, which was confirmed by FTIR, XRD and SEM. X-ray diffraction characterization of silver nanoparticles indicate that the structure of silver nanoparticles is face centred cubic structure of metallic silver. The topography and surface morphology of silver nanoparticles was examined by using Scanning Electron Microscopy (SEM) and the energy dispersive spectrum revealed that the presence of elemental silver in the sample. SEM images shows that most of the silver nanoparticles are predominantly spherical in shape with close compact arrangement with each other FTIR evidenced the presence of the functional group and where the reduction occurs and formation of silver nanoparticles which can be studied further to understand the chemical and molecular interaction which could be responsible for nanoparticle formation.

Keywords

Silver nanoparticles, XRD, Calotropis procera, Asthma

INTRODUCTION

Asthma is an inflammatory disease of respiratory system mainly in chronic form which is characterized by acute exacerbation of hacking, dyspnoea, and wheezing and chest tightness mainly at evening or in the early morning. Patients usually have decreased expiratory volume and airflow reduction. It also causes hyper-responsiveness of bronchi, irritation of respiratory track. These are non-specific to asthma. Bronchial Asthma is one of the parameters of Shwasa. From the different respiratory disorders Bronchial Asthma is most severe disease. The mortility and morbidity of it is increasing day by day in whole world. This continuous increase in number of asthmatic patients required much more scientific research on different drugs along with its treatment and mechanism of asthma. Now a day's asthma became most common disease in developing countries. The prevalence of asthma is mainly due to urbanization in these countries. The pharmacological evaluation is important to confirm pharmacological effectiveness, toxicity and potency of crude drug. Its increased morbidity, prevalence and death rates have perceived the developing seriousness of asthma in the community in the last 25 years. From1980 to 1987 the predominance rate of asthma in the United States expanded by 29 %. Asthma is also expanding in severity and is a main cause of mortality throughout the world [1].

Nanoparticles represent a particle with a nanometer size range 1-100nm. The nanoscale material has new, unique and excellent physical and chemical properties compared to its bulk structure, because of increase in the ratio of the surface area per volume of the particle [2]. Due to the extraordinary physicochemical properties, metabolic nanoparticles have been successfully applied in several fields, involving health care, synthetic biology and cellular transportation [3]. Silver nanoparticles have received particular attention due to their unique morphologies, stability and controlled geometry amongst several nanoparticles. The silver nanoparticles have been mostly used in the several electronic and sensing devices, data packing, coating materials and molecular switches. Other than this they have also used for diagnosis and treatment of several diseases. For various infectious diseases particularly silver nanoparticles posses excellent antimicrobial activities against various microorganisms which are known to be responsible [4].

Silver nanoparticles can be synthesized through various techniques, including the chemical reduction. The chemical method are used due to the easier and economical. This method is done by decreasing the silver salts by reducing agents, like sodium citrate or sodium borohydride [5]. Although, the use of chemical in the synthesis of silver nanoparticles results in the adsorption of toxic chemical reagents on the surface of material that's why it will have adverse and harmful effects on its application. That's why the use of environmentally friendly technique is desirable. Green synthesis approach for synthesizing the nanoparticles by using a natural products can be used to address the drawback by utilizing plants. The utilization of plants in the biosynthesis of the nanoparticles involve content of secondary metabolites as a reducing agents [6,7].

Plant Profile:

Calotropis Procera:

Figure No. 1 Calotropis Procera plant

Habitat: C. procera favours open habitat with little competition. The plant of this species grows in dry habitat where rainfall is limited to 150 to 1000 mm and also found in the area of excessive drained soil as much as 2000 mm of annual precipitation. It is also found in the common habitat of road-side, beachfront dunes, and widely disturbed in the urban areas. C. Procera is also found at the elevated areas up to 1,000 m. Because the plant is easy to propagate and manages and can grow under the xerophytic condition, sometimes it is also grown as an ornamental plant in dry or coastal areas [8,9].

Geographical Distribution: C. procera is inborn to Southern Asia and Indo-China to Malaysia, Macaronesia, West Africa North and East Africa, Madagascar, and Arabian Peninsula. The plant is naturalized in Australia, Central America, North, South America, and West Indies. The species is now accepted and culture in many countries such as Mexico, Central and South America, Pacific islands, Australia, and the Caribbean.

MATERIAL AND METHOD

Materials:

All the reagents purchased were of analytical grade and used without any further purification. Silver nitrate was purchased from Sigma -Aldrich with a ≥ 99.5% purity. Fresh roots of plant Calotropis Procera were collected from Swami Ramanand Teertha Marathwada university campus, dried and authenticated from the science college Nanded. Distilled water used for preparing aqueous solutions all over the experiments.

Method:

Soxhlet extraction method: Soxhlet extraction is an exhaustive extraction technique widely applied to analytes that are sufficiently thermally stable. The extraction solvent is continuously cycled though the matrix, by boiling and condensation, with the sample being collected in the hot solvent. It ensures that all the analyte extracted from the plant.

Why ethanol used as solvent?

From the literature survey, it reveals that ethanol as solvent for extraction is good because phytochemical extracted with the ethanol show good result for anti-asthmatic activity and less toxic. Most of the polar compounds are easily eluted by these solvents which is bioactive responsible for their activity .some non-polar groups may also be dissolved in these solvents. easy to evaporate the elutant for quick & easy availability. That most of the bioactive compounds are soluble in these type of solvents and it is also helps us to purify the compounds for further analysis.

Method of preparation of silver nanoparticle

Nanoparticle offer targeted delivery of drugs, enhancing bioavailability, sustaining drug or gene effect in target tissues, and enhancing the stability. There are 3 methods of preparation of silver nanoparticle as follows:

1. Physical method
2. Chemical method
3. Biological method

Chemical reduction method involves the reduction of $AgNO_3$ in aqueous solution by an effective reducing agent in the presence of appropriate stabiliser, which is necessary in shielding the growth of silver particles through aggregation. During the formation of silver nanoparticles by the chemical reduction method, some of the parameters like the particle size and aggregation state of silver nanoparticles are affected by initial $AgNO_3$ concentration, reducing agent, $AgNO_3$ molar ratios and stabiliser concentrations. So many methods are suggested for the chemical synthesis of silver nanoparticle formation; chemical reduction method, polyol method and radiolytic process have been developed for the synthesis of silver nanoparticles. The best and most easy method of yielding nanoparticles without aggregation, high yield and low preparation cost is chemical reduction method.

Experimental Work

Preparation of Plant Extract:

The plant of Calotropis Procera is collected from Swami Ramanand teerth Marathwada university campus, dried and authenticated from the science college Nanded. The root of Calotropis Procera is dried in presence of sun light and root is grind into the powder form. The powder of Calotropis Procera root is weighed and 400gm powder is taken for the extraction in 1000 ml ethanol. Extraction of Calotropis Procera root powder is done with the Soxhlet apparatus at 40-60 degree Celsius for 48 hours. 9 gm extract is obtained.

Formation of silver nanoparticle of Calotropis Procera Extract:

Weigh 13.5 g of polyvinyl pyrrolidone were dissolved in 75ml ethylene glycol. 50ml of this solution were transferred to a beaker and agitated with a magnetic stirrer before adding 0.30g of silver nitrate. And also add Calotropis Procera extract into the solution. Upon the addition of silver nitrate, the pale yellow solution of ethylene glycol polyvinyl pyrrolidone slowly changed to pale brown indicating the formation of silver nanoparticle. The dissolution of silver nitrate is slow and lasted for about 15min. The reaction was allowed to proceed at room temperature 27℃ ±1℃ for 67 hours.

Fig. No. 2: After centrifugation of nanoparticle **Fig. No. 3:** After drying nanoparticle

Characterization of Silver Nanoparticle of Calotropis Procera Extract: For the evaluation of silver nanoparticle of Calotropis Procera extract was characterized by FTIR, XRD and FESEM.

The Calotropis Procera root extract was subjected to FT-IR analysis before and after preparation of silver nanoparticles to understand and identify functional groups formed during nanoparticle preparation. FTIR measurements were carried out to identify the possible biomolecules in the Calotropis Procera root extract responsible for the reduction of ions and also the capping agents responsible for the stability of the biogenic nanoparticle solution. The XRD is characterized for the understanding of crystalline or amorphous nature of the molecule. The FESEM is characterized for the understanding of size of the particle present in the silver nano-particle of extract.

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy (FTIR): The FTIR spectra of Calotropis Procera root extract was taken in the range of 400-4000 cm-1 in which functional group is identified. Peaks appearing in the spectrum were assigned to various groups and bond in accordance with their respective wave number.

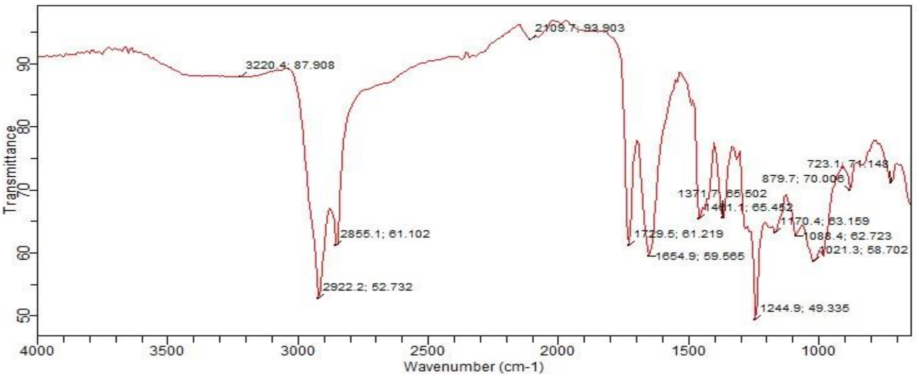


Fig 4: FTIR Spectra of silver nanoparticle of Calotropis Procera root extract

Table No. 1: Principle peaks of silver nanoparticle of Calotropis Procera root extract

Functional group	**Range**	**Silver nanoparticle of extract**
C-H stretching	2850-2960	2922.2
C=O stretching	1680-1760	1729.5
C=N stretching	1630-1755	1654.9
C-H bending	1400-1580	1411.1
C-O bending	1110-1275	1244.9
P-OH stretching	Approx. 1090	1021.3
Ester	Approx. 750	723.1

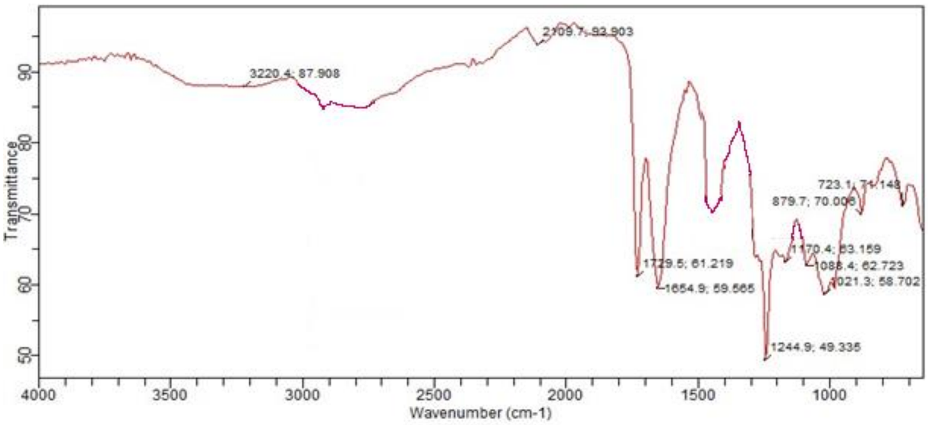


Fig.5: FTIR Spectra of Calotropis Procera root extract

Table No. 2: Principle peaks of Calotropis Procera root extract

Functional group	**Range**	**Extract of Calotropis Procera root**
C=O stretching	1680-1760	1729.5
C=N stretching	1630-1755	1654.9

C-O bending	1110-1275	1244.9
P-OH stretching	Approx. 1090	1021.3
Ester	Approx. 750	723.1

X-ray diffraction Studies (XRD): The nanoparticle of Calotropis Procera root extract is characterized by the X-ray diffraction studies for the information of to know about the crystalline and amorphous nature of the substance. The X-ray diffractogram are shown in figure 6. XRD pattern of nanoparticle of Calotropis Procera root extract shows characteristic diffraction and intense peak at 27, 33, 46.6, 56 & 58 indicating crystalline nature of nanoparticles of Calotropis Procera root extract.

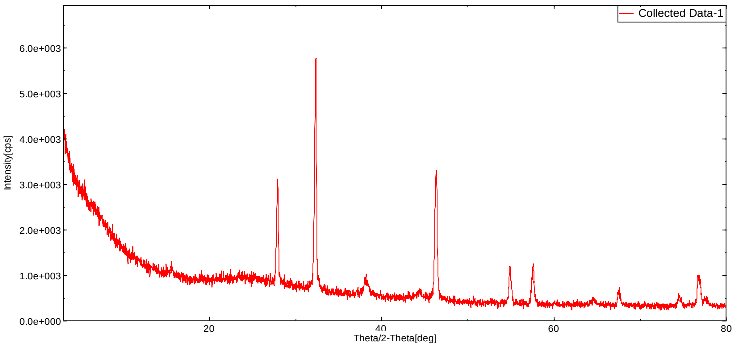
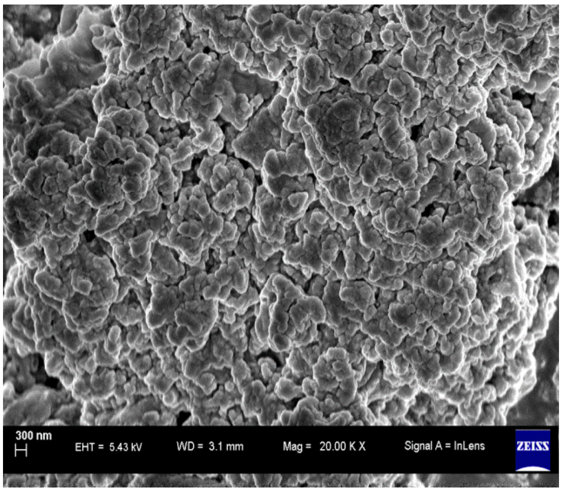


Fig. 6: XRD Spectra of Calotropis Procera root extract

Scanning Electron Microscopy (SEM): The studies of Calotropis Procera root extract shows the nanoparticles size with the magnification at 20.00 KX & 100.00 KX are examined by ZEISS instrument. In this area, one can notice the presence of nanoparticles of sizes within 100nm & 300nm respectively. SEM images shows that most of the silver nanoparticles are predominantly spherical in shape with close compact arrangement with each other. The SEM images shows that nanoparticles may direct contact within the aggregate during this aggregation the smaller nanoparticles become larger.



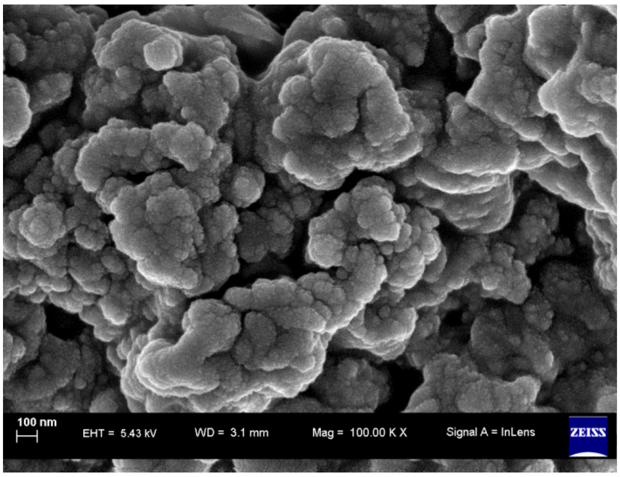


Fig 7: SEM images of Calotropis Procera root extract and Nanoparticles

DISCUSSION

FTIR measurements were carried out to identify the possible biomolecules in the Calotropis Procera root extract responsible for the reduction of ions and also the capping agents responsible for the stability of the biogenic nanoparticle solution. The FT-IR spectra of Calotropis Procera root extract showed peak at 1729.5(C=O stretching), 1654.9 (C=N stretching), 1244.9 (C-O bending), 1021.3 (P-OH stretching) and 723.1 (ester). But FT-IR spectra of Calotropis Procera root mediated with silver nanoparticle shows peaks at 2922.2 (C-H stretching), at1729.5(C=O stretching), 1654.9 (C=N stretching), 1411.1 (C-H bending) 1244.9 (C-O bending), 1021.3 (P-OH stretching) and 723.1 (ester). Absence of peak, corresponding to C-H stretching & bending, shows that reduction goes through these groups. According to literature may be C-H group in the flavonoid, glycoside is responsible for the reduction of silver particle. By doing XRD we know the nature of substance that is crystalline due to this solubility of substance known and spatial arrangement of particle identified which would be helpful for further process. In the scanning electron microscope (SEM), we identified the size of nanoparticle, shape of the nanoparticle and we understand that nanoparticle is formed or not.

CONCLUSION

Calotropis Procera root extract was found suitable for the preparation of silver nanoparticles. The reduction of Silver ions resulted in the formation of stable nanoparticles with spherical shape and of 100 or 300 nm in size. The Spectroscopic characterizations using SEM, and XRD were useful in proving the formation of nanoparticles and also in confirming their size, shape, and nature of nanoparticle. FTIR evidenced the presence of the functional group and where the reduction occurs & formation of silver nanoparticles. which can be studied further to understand the chemical and molecular interaction which could be responsible for nanoparticle formation. Thus it can be further be applied in various biomedical and biotechnological fields and their properties and applications can be further explored.

REFERENCES

1) Martin B. L., Craig T., Caruana M, B., (1997). Comprehensive management of asthma. Drugs Today, 33: 149-160.
2) Cushing B., Kolessnichenko V. Recent advances in the liquid phase synthesis of inorganic nanoparticles Chemical review. 2004;104(9): 3893-3946.
3) Shailesh L. Patwekar, Karna B. Khavane, Padmavati R. Chainpure, Santosh A.Payghan Shivraj S. Shivpuje. A review on different preparation methods used for development of curcumin nanoparticles. International Journal of Creative Research Thoughts. 2021, 9(1); 4088-4101.
4) Burda C., Chen X., Narayanan R. Chemistry and properties of nanocrystals of different shapes. Chem. Rev. 2005;105:1025-1102.
5) Khan A., Rashid R., Zahra A. Gold nanoparticles : Synthesis and applications in drug delivery. Trop. J. Pharm. Res. 2014;13:1169-1177.
6) Zielinska A., Skwarek E., Zaleska A. Preparation of silver nanoparticles with controlled particle size. Procedia chemistry. 2009;1(2): 1560-1566.
7) Singh A., Jain M., Upadhyay K., Khandelwal N., Verma D. Green synthesis of silver nanoparticles using Argenone Mexicana leaf extract and their characterization. Digest journal of nanomaterials and biostructures.2010;6(1):483-489.
8) Solomon S., Bahadory V., Jeyarajasingam A. Synthesis and study of silver nanoparticles . Journal of chemical education.2007;84(2):322-325.
9) Peter J. B., Chung C. P., Fan K., (2003). Inflammatory mediators of asthma: An update. The American Society for Pharmacology & Experimental Therapeutics, 50(4): 517-596.
10) Rang H. P., Ritter J. M., Dale M. M., (2003), Pharmacology. 6th edn. Churchill Livingston Publication London, 342-343.

11) Bethesda, Nhlbi, (1997), National Asthma Education and Prevention programme Expert Panel Report, 2, Guidelines for the Diagnosis and Management of Asthma, NTH Publication 97, 4051.
12) Graig C. R., Wilkins, Williams, L., Stitzel, R. E., (2004). Drug used in asthma. In: ‘Modern pharmacology with clinical applications’. 6th edition, The New England Journal Medicine, 458-469.
13) Busse, W. W., Lemanske R. F., (2001). Jr: Asthma. The New England Journal Medicine, 336-350.
14) P. R. Chainpure, S. L. Patwekar, S. S. Shivpuje,Sheetal Rathod. A study of carica papaya concerning it's ancient and Traditional uses - recent advances and modern Applications for improving the milk secretion in Lactating womens. International Journal of Research. 2019, VIII(II); 1851-1861.
15) Robinson D. S., (2004). The role of the mast cell in asthma: induction of airway hyper responsiveness by interaction with smooth muscle. Journal of Allergy and Clinical Immunology, 114(1): 58–65.
16) Aletta, D. K., Mirjam, K., Hanneke, P. M., Andrys, C. D., Frank, A. M., Anneke, H. H., Redegeld, F. P. N. (2002). Key role for mast cells in nonatopic asthma. The Journal Immunology, 169: 2044-53.
17) Vinay K., Abbas L., Abul K., (1999). In: Robins and Croton (Ed.) Pathologic Basis of Disease. Elsevier India Pvt. Ltd., New Delhi, 6 th edition. 712-720.
18) Bousquet, J., Busse, W. W., Jeffery, P. K., (2000). Asthma: From bronchoconstriction to airway inflammation remodeling. American Jr. Respiratory Critical Care Medicine, 161: 1720-1745.
19) Wanner A., O’Riordan T. G., Salathe M., (1996). Mucosiliary clearances in the airway. American Journal of Respiratory Critical Care. Medicine 154: 1868-1902.
20) Sur S., Kephart G. M., Crotly T. B., (1993). Sudden-onset fatal asthma: A distinct entity with few eosinophils and relatively more neutrophils in the airway sub mucosa. American Review Respiratory Disease, 148: 713-719.
21) Judith B., (2002). The role of mast cells in the Pathophysiology of asthma. The New England Journal of Medicine, 1742-43
22) Takei M., Koichi E., Ueno M., (1992). Role of intracellular Ca+ 2 on Histamine release from rat peritoneal mast cells. Pharmacology Science, 81(6): 518-20.

23) Kulkarni. S. K., (1976). Biological Distribution of Histamine Receptors. Indian Journal Pharmacology, 9(3): 157-59.
24) Monica RP Rao, Swati Taktode, Shivraj Sangappa Shivpuje, Shilpa Jagtap. Optimization of Transmucosal Buccal Delivery of Losartan Potassium using Factorial Design. Indian Journal of Pharmaceutical Education and Research. 2016, 50(2); S132-S139.
25) Barar F.S.K., (2003). In; Essential of Pharmacotherapeutics, S. Chand & Company Ltd New Delhi, 3 rd edn. 544-52.
26) Nagarajan, R., Naik S. R., (1995). Recent development on anti-asthmatic agents- An overview. Indian Drugs, 33(7): 30.
27) Narendra Patre, Shailesh Patwekar, Suresh Dhage , Shivraj Shivpuje. Formulation & Evaluation Of Piroxicam Bionanocomposite For Enhancement of Bioavailability. European Journal of Molecular & Clinical Medicine 2020, 07(11); 9362-9376.
28) Mali R G and Dhake A S, 2011. A review on Herbal Antiasthmatics, Oriental Pharmacy and Experimental Medicine, 11:77-90.
29) Monica Rp Rao, Shivraj Shivpuje, Rohit Godbole, Chaitanya Shirsath. Design And Evaluation Of Sustained Release Matrix Tablets Using Sintering Technique. International Journal of Pharmacy and Pharmaceutical Sciences. 2016, 8(2); 115-121.
30) Taur D J and Patil R Y, 2011. Some medicinal plants with antiasthmatic potential: a current status. Asian Pacific Journal of Biomedicine, 413-418.
31) S Patwekar, S Gattani, R Giri, A Bade, B Sangewar, V Raut , Review on nanoparticles used in cosmetics and dermal products, World J. Pharm. Pharm. Sci 3, 1407-1421.
32) Gaurav parihar and neelam balekar, (2016) a review on calotropis procera: a phytochemical and pharmacological review,ips academy college of pharmacy, TJPS , 40 (3): 115-131.
33) Pravin P. Karle , Shashikant C. Dhawale, Vijay V. Navghare and Shivraj S. Shivpuje. Optimization of extraction conditions and evaluation of Manilkara zapota (L.) P. Royen fruit peel extract for in vitro α-glucosidase enzyme inhibition and free radical scavenging potential. Future Journal of Pharmaceutical Sciences (2021) 7:151.
34) P. R. Chainpure, S. L. Patwekar, S. S. Shivpuje, Zeba Ashfaq Sheikh. Formulation and evaluation of Garciniacambogiaand Commiphoramukul Herbal tablets used for Anti-Obesity. International Journal of Engineering, Science and Mathematics. 2019, 8(4);180-195.