Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 3 Year 2024 Page 670-678

Antibacterial Activity And Cytotoxicity Of *Strobilanthes Heyneanus* Leaf

Eldhose M.J[1], Hasbiya K.V[2]*, Jashira A.K[3], Najma K.P[4], Anju K[6], Mohammed junaid K[7],

[1,2*,3,4,5,6,7]*Department of pharmacology, Karuna college of pharmacy, Thirumittacode*

***Corresponding Author: ,** Hasbiya K.V*
[7]*Department of pharmacology, Karuna college of pharmacy,Thirumittacode*

Abstract

The investigation of herbal plants for their antibacterial activity and cytotoxicity has gained considerable interest in recent years due to the emergence of antibiotic resistance and the need for safer alternatives to conventional drugs. Bacterial resistance to antibiotics is a growing concern globally, contributing to over 41% of infectious diseases. Herbal extract is found to be more efficient safer, and better quality drugs with improved anti-bacterial activities. The present study was aimed to evaluate the antibacterial activity and cytotoxicity of methanolic extracts of "*Strobilanthes heyneanus*" leaf against different gram positive (Staphylococcus aureus & Bacillus cereus) and gram negative (Escherichia coli, Pseudomonas aeruginosa, proteus mirabilis) reference bacterial strains. This study would provide valuable insights into its therapeutic potential, contributing to the development of novel herbal medicines and pharmaceutical formulations for the treatment of bacterial infections and cancer. The results indicated that the methanolic extracts of leaves have potential antibacterial, antioxidant and anticancer activity. Further studies on isolation and chemical structure determination of active compounds from these extracts are necessary for their utilization to treat infections caused by pathogenic and often multi drug resistant bacteria.

KEYWORDS: Strobilanthes heyneanus, Antibacterial, Cytotoxicity, Thin layer chromatography, DLA Ascites

1.INTRODUCTION

Strobilanthes heyneanus belongs to the family Acanthaceae that contains many species with potential for diverse medicinal uses. It is also called 'Karun kurinji' and is commonly found in the South-West regions of India. The species are commonly used in rheumatic complaints, sprain of the ankle, and hernia [1].

Bacteria, as microscopic single-celled organisms, inhabit virtually every environment on Earth, from the depths of the ocean to the soil beneath our feet. While some bacteria are beneficial, aiding in processes like digestion and nutrient cycling, others can cause diseases in humans, animals, and plants. One of the significant challenges posed by bacteria is their ability to develop resistance to antibiotics, the primary agents used to combat bacterial infections. This resistance can arise through genetic mutations or the acquisition of resistance genes from other bacteria. As a result, bacterial infections that were once easily treatable with antibacterial agents are becoming more difficult to manage, leading to increased morbidity, mortality, and healthcare costs [2,3].Antibacterial activity refers to the ability of substances, such as antibiotics, to inhibit the growth or kill bacteria. However, as bacteria develop resistance to antibiotics, researchers are continually seeking new antimicrobial agents or alternative approaches to combat bacterial infections effectively. We aimed to perform antibacterial activity on various gram positive and gram negative organisms [4,5].

BACTERIA	**COMMON RESISTANCE TO**
Staphylococcus aureus	Beta lactam antibiotics, macrolides, fluoroquinolones
Enterococcus spp.	Ampicillin, aminoglycosides
Enterobacteriacea	Cephalosporins, fluoroquinolones, aminoglycosides
Acinetobacter	Ceftazidime, aminoglycosides, carbapenems

The present study was aimed to evaluate the antibacterial activity and cytotoxicity of methanolic extracts of "*Strobilanthes heyneanus*" against different gram positive (Staphylococcus aureus & Bacillus cereus) and gram negative (Escherichia coli, Pseudomonas aeruginosa, proteus mirabilis) reference bacterial strains. [6-13]. India has often been referred to as the medicinal garden of the world. The clinical use of plants described in Indian Vedas for curing different disease. [14-20]. For the large proportions of world's population medicinal plants continue to show a dominant role in the health care system and this is mainly true in developing countries, where herbal medicine has continuous history of long use. Studying medicinal plants helps to understand plant toxicity and protect human and animal from natural poisons [21-27].Cytotoxicity assays are invaluable tool in various field, including drug development, cancer research, environmental health and toxicology. The significance lies with their ability to assess the potential risk and benefits of chemical compound contributing to advances in health care, environmental protection, and regulatory science[28-31]..The primary objective includes Extraction of leaves of *Strobilanthes heyneanus,* Preliminary phytochemical screening ,TLC (Thin layer chromatography) , MIC(minimum inhibitory constant) , Cytotoxicity and In-vitro antioxidant .Further development of this drug can leads to develop a drug with low resistance to antibiotics, low cost with high efficacy.

2MATERIALS AND METHODOLOGY

2.1 COLLECTION OF PLANT MATERIAL

2.2 EXTRACTION: The Air dried powder was extracted with methanol by cold maceration. After the repeated extraction the extract was separated by filtration. The filtrate was pooled and dried.

2.3 PHYTOCHEMICAL SCREENING: It includes the following steps:

- Preliminary phytochemical screening
- Thin layer chromatographic analysis

2.3.1 PRELIMINARY PHYTOCHEMICAL SCREENING

Phytosterols:

The extract is dissolved in acetic anhydride and added concentrated sulphuric acid to sides of the test tube. An array change shows the presence of phytosterols.

Terpenoids:

1 ml of extract 2 ml of chloroform and concentrated sulphuric to the test tube. Yellow to brick red color indicates presence of terpenoid

flavonoids:

The extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

2.3.2 THIN LAYER CHROMATOGRAPHIC STUDIES

Thin layer chromatographic studies were conducted on the crude extracts using different mobile phases. The stationary phase used was silica gel slurry prepared by mixing 30 g silica gel G and 100 ml water. Prepared activated plates were then used for the studies [32].

2.4 QUANTITATIVE ANTIBACTERIAL ACTIVITY ASSAY BY MINIMUM INHIBITORY CONCENTRATION (MIC)

2.4.1 TEST ORGANISM

The antibacterial potency of extract was evaluated using five bacterial strains. Two strains of gram positive (Staphylococcus aureus & Bacillus cereus) and three strains of gram negative (Escherichia coli, pseudomonas aeruginosa, Proteus mirabilis) bacteria.

2.4.2 MINIMUM INHIBITORY CONCENTRATION (MIC)

Minimum inhibitory concentration is defined as the lowest concentration of the antimicrobial agent that inhibits microbial growth after 24 hours of incubation. Agar plates are inoculated with a standardized inoculum of the test microorganism. 8 mm in diameter wells containing the test compound are punched into the agar. After the incubation antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured [33].

2.5 CYTOTOXIC ACTIVITY

The in-vitro Cytotoxic Activity was carried out by using the test compound was studied for short term in vitro Cytotoxicity using Dalton's Lymphoma Ascites(DLA). The tumor cells aspirated mice were washed. Cell viability was determined by trypan blue exclusion method. Viable cells suspension (1*106 cells in 0.1 ml) was added to tubes containing various concentrations of the test compounds. Control tube contained only cell suspension. These assay mixtures were incubated for 3 hours at 37 C. Further cell suspension loaded on a haemocytometer after mixed trypan blue. The numbers of stained and unstained cells were counted separately [34].

$$\% \text{ Cytotoxicity} = \frac{\text{NO. of dead cells}}{\text{NO. Of lived cells} + \text{NO. of dead cells}} * 100$$

2.6 ANTI-OXIDANT ACTIVITY

The anti-oxidant potential was evaluated by using different in-vitro Anti-oxidant tests.

Methods:

1. DPPH scavenging activity
2. Hydrogen peroxide scavenging (H2O2) assay

2.6.1-DPPH Scavenging activity:

Plant extract (0.3ml) prepared in 95% methanol at various concentrations was mixed with freshly prepared methanolic solution containing DPPH radicals (0.004% w/v, 2.7 ml). Shaken vigorously and stand 60 min in dark, anti-stable absorption values are obtained. The absorbance of the solution was taken as 230nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using following equation.

$$\% \text{ scavenged} = \frac{\text{Absorbance of Control-Absorbance of sample}}{\text{Absorbance of control}} * 100$$

Plant extract (1 ml) prepared in distilled water at various concentrations was mixed with 0.6 ml of 4 mm. H2O2 solution prepared in phosphate buffer (0.1 M, PH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230nm. Ascorbic acid was used as positive control compound [35]. The percentage of inhibition was calculated following equation;

$$\% \text{ scavenged} = \frac{\text{Absorbance of Control-Absorbance of sample}}{\text{Absorbance of control}} * 100$$

3. RESULT AND DISCUSSION

3.1 EXTRACTIVE VALUE

The dried powder of *strobilanthus heyneanus* was extracted with methanol (600ml) yielded2.5g of total methanolic extract. percentage yield of the total methanolic extract was found to be **2.5/20 *100 = *12.5 % w/w***

3.2 PRELIMINARY PHYTOCHEMICAL SCREENING

Methanolic extract was subjected for qualitative chemical analysis for the identification of various phyto constituents like alkaloids, glycosides, phenolics, flavonoids, carbohydrates, proteins, amino acids, terpenoids, sterols and saponins. The test shows positive result for alkaloids(Wagner's), carbohydrates(molishs), saponins(foam),phytosterol(liberman burchads), terpenoids(salkowski),fixed oils (spot)phenolics(ferric chloride),flavonoids(alkaline agent).

3.3 THIN LAYER CHROMOTAGROPHY:

The TLC analysis of methanolic extract with various mobile phase was carried out and the result obtained are tabulated below**:**

Phytochemical	Solvent system	Confirmatory test	No. of spots	Rf value
Alkaloids	Ethyl acetate: chloroform: water(5:3:1)	Mayer's reagent spray	1	0.92
Flavonoid's	N-butanol: ethyl acetate: water (5:10:15)	3% boric acid + 10% oxalic acid spray	1	0.91

3.4 MINIMUM INHIBITORY CONCENTRATION:

The plant species were investigated to evaluate their antibacterial activity against both gram positive (Staphylococcus aureus & Bacillus cereus) and gram negative (E. coli, Pseudomonas aeruginosa & Proteus mirabilis) bacterial strains and recorded.

Bacillus zone

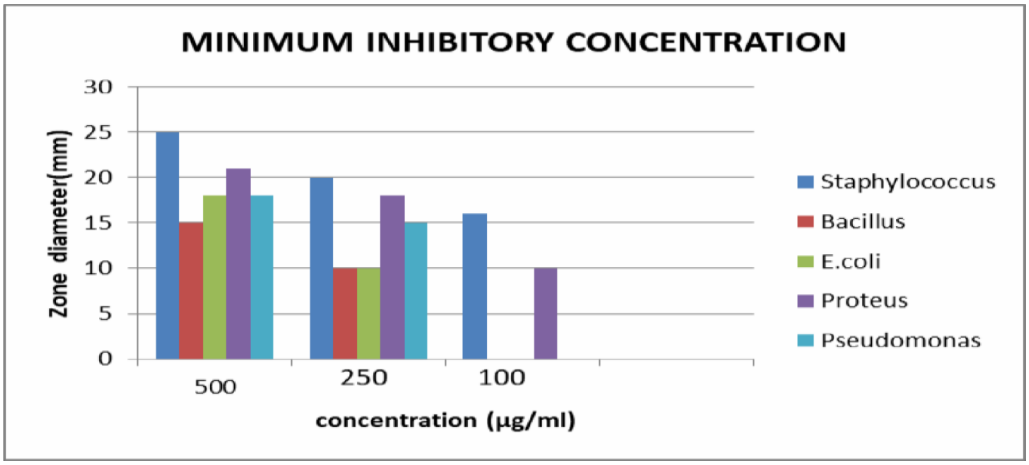
Escherichia zone

Proteus zone

Pseudomonas zone

Staphylococcus zone

Concentration (µg/ml)	Staphylococcus zone diameter(mm)	Bacillus zone diameter (mm)	Escherichia zone diameter (mm)	Proteus zone diameter (mm)	Pseudomonas zone diameter (mm)
500	25	50	18	21	18
250	20	10	10	18	15
100	16	-	-	10	-
50	-	-	-	-	-



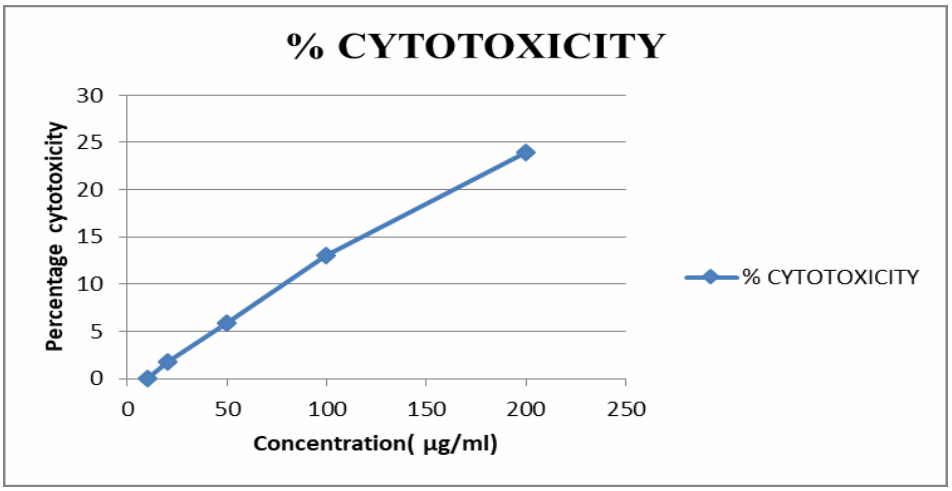
The results revealed that the plant extract were potentiallyeffective in suppressing the growth of both gram positive and gram negative bacteria. The inhibitory effect of *Strobilanthes heyneanus* leaf extract started at 100µg/ml with inhibition zone of 16 and 10 mm against Staphylococcus aureus and Proteus mirabilis. The

concentration 500µg/ml the extract shows inhibitory effect to all gram positive and gram negative bacteria with inhibition zone of 25,15,18,21, 18 mm against Staphylococcus Aureus Bacillus cereus, E. coli, Proteus mirabilis and Pseudomonas aeruginosa respectively.

3.5 CYTOTOXICITY STUDY

Anti-cancer evaluation using in-vitro DALTONSLYMPHOMA ASCITES method (DLA method) and the % cytotoxicity of various concentration of plant was recorded.

Drug concentration (microgram/ml)	%Cell death
10	0
20	1.8 ± 0.1
50	5.9±0.7
100	13±1
200	24±1.1

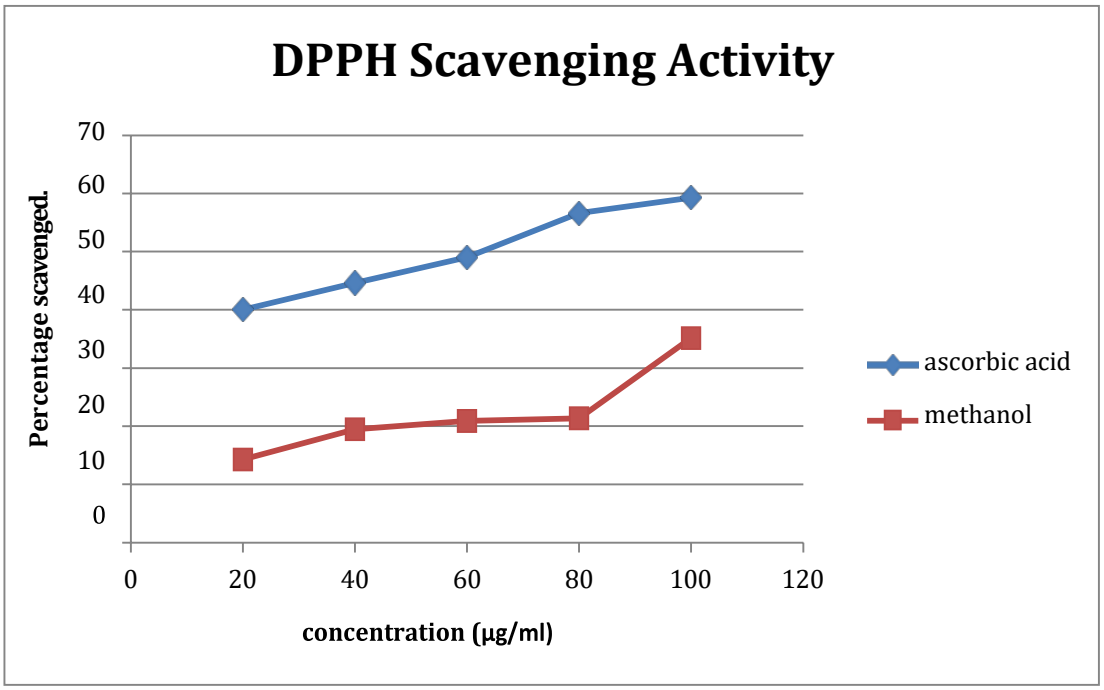


The result shows that the plant extract has significant cytotoxic activity. According to this at the concentration of 20µg/ml, the extract shows mild cytotoxic activity. At the concentration 200µg/ml, the extract showing significant cytotoxic action, were the extract showing highest % cell death of 24± 1.1.

3.6 ANTI OXIDANT ACTIVITY

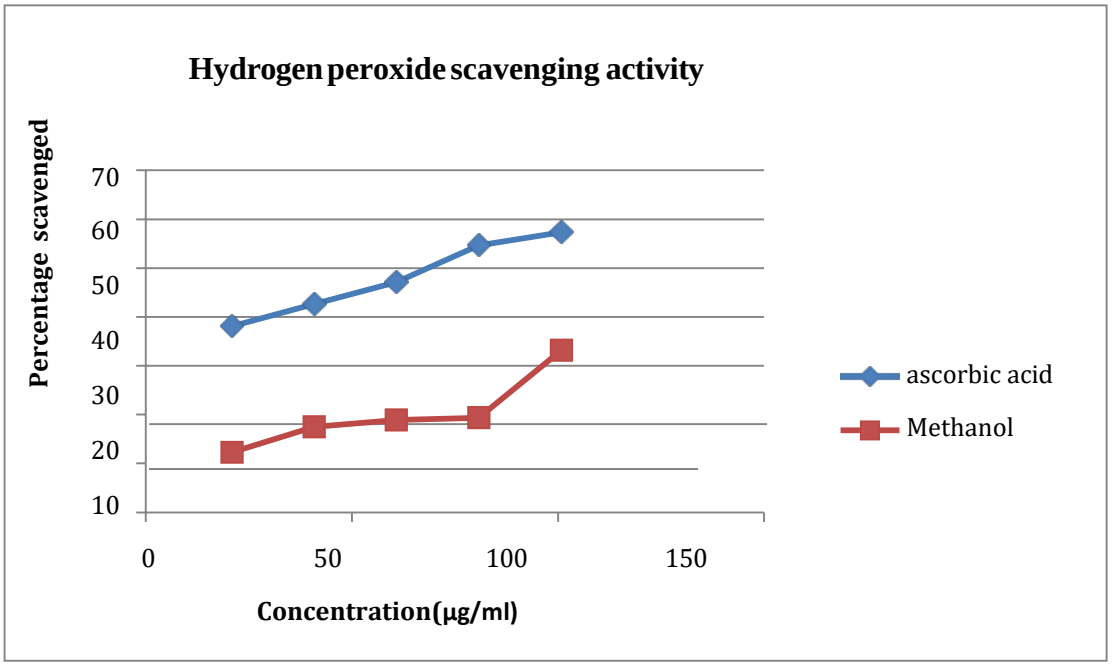
3.6.1 DPPH scavenging activity

SL. NO	SAMPLE	CONCENTRATION (µg/ml)	ABSORBANCE (at 230nm)	% SCAVENGING
1	CONTROL	-	0.21	-
2	ASCORBIC ACID	20	0.152	39.52
		40	0.135	46.39
		60	0.125	50.82
		80	0.112	55.92
		100	0.1	62.09
3	METHANO LIC EXTRACT	20	0.221	12.46
		40	0.198	21.29
		60	0.189	25.62
		80	0.182	27.89
		100	0.258	29.02



HYDROGEN PEROXIDE SCAVENGING ACTIVITY

SL. NO	SAMPLE	CONCENTRATION (µg/ml)	ABSORBANCE (at 230nm)	% SCAVENGING
1	CONTROL	-	0.21	-
2	ASCORBIC ACID	20	0.152	39.
		40	0.135	46.
		60	0.125	50.
		80	0.112	55.
		100	0.1	62.
		20	0.221	12.
		40	0.198	21.
3	METHANO LIC EXTRACT	60	0.189	25.62
		80	0.182	27.89
		100	0.258	29.02



The result shows that the extract has significant antioxidant activity by comparing with% scavenging activity of ascorbic acid. Phenolic and other flavonoid compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidases. The phytochemical study clearly demonstrated that the plant *Strobilanthus heyneanus* is a rich source of phenolics and flavonoids. Therefore, the presence of these compounds in the plant extracts has exhibited the antioxidant activity

4. CONCLUSION

The study concludes that the methanolic extract of *strobilanthes heyneanus* leaves exhibit promising antibacterial, antioxidant, and anti-cancer activities. The extract demonstrated effective inhibition of both gram-negative and positive bacteria, indicating its potential as a novel antibacterial agent. Additionally, the extract displayed significant antioxidant activity, attributed to its rich composition of flavonoids and phenolics, which could be beneficial in treating diseases mediated by free radicals. Moreover, the extract showed notable cytotoxic activity against cancer cells, suggesting its potential as an anticancer agent. Further research is recommended to isolate and identify the active compounds for utilization in combating infections caused by multidrug-resistant bacteria with low cost and much effective.

5. REFERENCES

1. Vijayakumar Sundaram , Selvaraju Sadhasivam, Sivaraj Chandrasekaran, Raaman Nanjian3 and Arjun Pandian.Strobilanthes heyneanus root extract as a potential source for antioxidant and antimicrobial activity: Future Journal of Pharmaceutical Sciences (2021) 7:91
2. Geo F Brooks, Karen C Carroll, Janet S Butel , Stephen A. Jawetz, Melnick&Adelberg's Medical Microbiology: Sultan Qaboos University Medical Journal.2007;7(3):273-275.
3. Neil H. Mendelson.Bacterial Growth and Division: Genes, Structures, Forces, and Clocks:American Society for Microbiology.1982;vol.46,No.3,341-375.
4. BorrisR.P. Natural Products Research: Perspectus From a Major P harmaceutical company: Journal of Ethnopharmacology .1996;51;29-38.
5. Brantner A, Grein E. Antibacterial Activity of Plant Extracts Used Externally in Traditional Medicine, Journal of Ethnopharmacology,1994;44:35-40.
6. Andreea Irina , Barzic and Silvia Loan, Antibacterial Drugs from Basic: Concepts to Complex Thersapeutic Mechanism of Polymr System.
7. Ryo Nomura , HidemasaNakaminami, Kotaro Takasao, ShunaMuramatsu, Yuji Kato,
8. Norihisa Noguchi : Journal of Global Antimicrobial Resistance , Elsevier.2003
9. Stuart B Levy &Bonnie MarshallAntibacterial resistance worldwide: Causes, Challenges and responses 2004;Volume 10.
10. Jocelyn Y. Ang, Elias Ezike&Basim I. Asmar Antibacterial resistance:The Indian Journal of Pediatrics 2004;71,229-239.
11. Ursula Theuretzbacher Global antibacterial resistance : Journal of Global Antimicrobial Resistance 2013;vol 1:63-69.
12. Gislene GF Nascimento, Juliana Locatelli, Paulo C Freitas, Giuliana L Siver Antibacterial activity of plant extract and phytochemicals on antibiotic resistant bacteria.Braz.J.Microbiol. 2000;31:247-256.
13. Sharma Rashmi, Kapoor Bhuvneshwar Antibacterial resistance : Current problems and Possible Solutions : Indian Journal of Medical Science 2005; 59, 120-129.
14. K Valarmathy et. Al., M.Gokulakrishnan, M.SalmaKausar, Dr. Kusum Paul A Study of Antimicrobial Activity of Ethanolic Extracts of Various Plant Leaves Against Selected Microbial Species: International Journal of Pharma Sciences and Research(IJPSR) 2010;Vol.1(18),293-295.
15. Prakash , P. and Gupta, N. Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with anote on Eugenol and its Pharmacological Actions : A Review. *Indian Journal of Physiology and Pharmacology,* 2005*;***49,** 125-131.
16. Fansworth, N.R. The role of Ethno Pharmacology in Drug Development. Ciba Foundation Symposium 154.Bioactive compounds from Plants.John Wiley & Sons, BaffinsLane,Chichester (England), 1990;2-21.
17. Newman,D.J., Crag, G.M. and Snader, K.M. The influence of Natural Products upon Drug Discovery .*Natural Product Reports,* 2000; vol:17, 215-234.
18. Kumar N, Wani ZA, Dhyani S. Ethnobotanical study of the plants used by the local people of Gulmarg and its allied areas, Jammu & Kashmir, India.International Journal of Current Research in Bioscience and

plant biology. 2015;2(9):16-23
19. Bernhoft A. A brief review on bioactive compounds in plants, In:Bioactive compound in plants –benefits and risks for man and animals, Oslo:The Norwegian Academy of Science and letters,2010,11-17.
20. WHO,(1998).Regulatory situation of herbal medicines.A world wide review.Pp 1-5. Geneva, Switzerland.
21. Rafieain-Kopaie M, Baradaran A. Plants antioxidants: From laboratory to clinic. Nephropathology 2013; 2 (2): 152-3.
22. Sharafati R, Sherafati F, Rafieian-kopaeiM.Biological characterization of iranian walnut (Juglansregia) leaves.Turk J Biol 2011;35:635-9.
23. Shirzad H, Taji F, Pourgheysari B, Raisi S, Rafieian-kopaeiM.Comparison of antitumor activities of heated and raw garlic extracts on fibrosarcoma in mice.JBabolUniv Med Sci 2012;14:77-83.
24. Kazemi S, Asgary S, Moshtaghian J, Rafieian M, Adelnia A, Shamsi F, Liver- Protective effects of hydroalcoholic extract of allium hirtifoliumbosis.In rats with alloxan – induced diabetes mellitus.ARYAAtheroscler 2010;6:11-5.
25. KhosarvI-BoroujeniH,Mohammadifard N, Sarrafzadegan N, Sajjadi F, Maghroun M, Khosravi A, et al.Potato consumption and cardiovascular disease risk factors among Iranian Population . Int J Food SciNutr 2012;63:913-20.
26. Shirzad H, ShahraniM, Rafieian-kopaeiM.Comparison of morphine and tramadol effects on phagocytes in vivo.Intimmunopharmacol 2009;9:968-70.
27. Rafieian-kopaie M, BaradaranA.Teucriumpolium and kidney.J Ren InjPrev 2013; 2:3- 4.
28. Heiderian E, Rafieian – kopaei M, Ashrafi K. The effect of hydroalcoholic extract of Allium latifolium on the liver phosphatidate phosphatase and serum lipid profile in hyperlipidemicrats.JBabolUniv Med Sci 2013;15:37-46.
29. Davidson P.M. , Taylor M.T.Chemical Preservatives and Natural Antimicrobial compounds, in Food Microbiology;Fundamentals and Frontiers, eds Doyle P,BeuchatL.R,Montville T.J,Editoirs.2007;(713-734).
30. Sharma rashmi, K Bhuvneshwar; Antibacterial Resistance: Current Problems and Possible Solutions:Indian Journal of Medical Science;2005;59;120-129.
31. HuehZan Chong et al.BMC Complement AlternMed.In Vitro Cytotoxicity of Strobilanthes Crispus Ethanol Extract on Hormone Dependent Human Brest Adenocarcinoma MCF-7 Cell:U.S National Library of Medicine.
32. Ishaku Leo Elisha, Francien S. Botha, and Jacobus NicolaasEloff. The antibacterial activity of extracts of nine plant species with good activity against Escherichia coli against five other bacteria and cytotoxicity of extracts:BMC Complement Altern Med.2017;17:133.
33. ShahlaSahraei,Zaynab Mohkami, Farhad Gaolsani, Fereshteh Javadian, SaeideSaeidi and GelarehSohilBaigi. Antibacterial activity of five medicinal plant extracts against some human bacteria:European Journal of Experimental Biology.2014;4(3):194-196.
34. Nazia Rashid, Han A Koh, Hilda C Baca, Zhaoliang Li, Susan Malecha,OyewaleAbidoye, Anthony Masaquel . Clinical impact of chemotherapy-related adverse events in patients with metastatic breast cancer in an integrated health care system:Journal of managed care &speciality pharmacy 2015; 21 (10):863-871b.
35. K.O.Subbulekshmi, S.E.Godwin,A.AbdulVahab. Phytochemical And In-Vitro Antioxidant Activity of Ethanolic Extract of Strobilan thes Barbatus NeesLeaves:Asian Journal of Pharmaceutical Research and Development.2015:13-20.
36. Pradeep Kumar Samal.Antidiabetic And Antioxidant Activity of StrobilanthesAsperrimus In Rats:Journal of Global Trends in Pharmaceutical Sciences.2013;Vol 4,Issue 2,1067-1072.
37. RubaniShahni and P J Handique.Antibacterial properties of leaf extracts of Strobilanthescusia (Nees) Kuntze, arare ethno-medicinal plant of Manipur,India:International Journal of Pharm Tech Research.2013;vol.5,No.3,1281-1285.
38. **[**Maria Cineola Fernandes, Krishnan Sellapan. Phytochemical Analysis of Strobilanthus Blume Species From Northern Western Ghats of India:International Journal of Pharmaceutical Research .2016;Vol 8,Issue 3.
39. V.S. Shende, S.D. Jadhav, N.H. Aloorkar, A.S. Kulkarni and SV Suryavanshi.Pharmacognistic and Phytochemical Evaluation of Strobilan thes Sessilis Nees. Leaves: International Journal of Pharmacognosy.2015;Vol.2(6): 310-314.
40. Mathew George,Lincy Joseph and Sony s.etal.A review on Pharmacological and Biological Activities of StrobilanthesCiliatusNees:World Journal of Pharmacy and Pharmaceutical Sciences.2017;504-512.