International Journal of Health care and Biological Sciences

Content available at www.saap.org.in Online ISSN: 2582-7499

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In -vitro pharmacological evaluation of ethanolic and hydroalcholic extracts of ocimum kilimandscharicum linn.leaves

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Article History

Received on: 27-01-2023
Revised on: 19-02-2023
Accepted on: 10-03-2023

***Keywords*:** *Ocimum kilimandscharicum Linn*, Ethanolic extract, Hydro alcoholic extract, Anti-coagulation activity.

Abstract

According to the Ayurveda, plants have been used for the treatment of so many diseases. Herbal drugs are easily available and have fewer side effects. So, many people are attracted towards the herbal drugs. Ocimum kilimandscharicum is one of a few types of basil that is perennial. It is a well-known plant in the Indian traditional system of medicine. The genus of *Ocimum* belongs to the family Labiatae and one of the most popular culinary herbs known for its medicinal properties. In this present study ethanolic, hydro alcoholic extract of *Ocimum kilimandscharicum Linn* was studied for Anti-coagulation activity using *in-vitro* model. The study revealed that different concentrations of the extract exhibited significant Anti-coagulation activity in a dose dependent manner at a concentration of 400mg/ml respectively and well compared with standard drug. Thus, it could that due to the presence of chemical constituents present in the extracts have well prospective for the management of Coagulation. This Knowledge will be useful in finding more potent above activity from the natural resources for the clinical development of activities and therapeutics.

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DOI: https://doi.org/10.46795/ijhcbs.v4i1.424

Introduction

Health is universal human aspiration and more over a basic human need. The development of society, rich or poor, can be judged by the quality of its population's health, how fairly health is distributed across the social spectrum and the degree of protection provided from disadvantage due to ill-health. The right to the highest attainable level of health is enshrined in the charter of WHO and many international treaties (UN general comment 14, 2000) and no country or region should have to live with levels of ill-health that is unavoidable. The World Health Organization (WHO) defined "Health" in its broader sense as "a state of complete physical, mental, and social well-being (referred as the *health triangle*) and not merely the absence of disease or infirmity". The Ottawa charter claimed that health is not just a state, but also "a resource for everyday life, not the objective of living (WHO, 2006).

Cell being the structural and functional unit of all living organisms according to the tenets of 'Cell theory' by Theodor Schwann and Matthias Jakob Schleiden. A myriad of complex and diversified molecular reactions happens to perform every function like cell division, differentiation growth, reproduction and senescence etc. One type of biomolecule interacts with another which, in turn, affects another, and so on down the line, as cascade of molecular changes termed as biochemical pathways with tight, intrinsic control.

In many different and extremely complex ways, these pathways sometimes either over or under driven due to genomic and non- genomic influences causing the disorder and or disease. A mistake in one reaction might stop an important protein from being produced, lead to too much production or with aberrant structure or misfolding, can lead to chronic diseases the intensity of which may be mild to life threatening.

Drugs affect these pathways by interacting with certain molecules along the pathway, making them more active or less active, or changing their activity all together. The challenge of finding a potential new therapeutic entity to solve a complex disease is an incredible task of employing a combination of computational, experimental, translational, and clinical models that are just unbelievable in their depth and their complexity altogether with a high attrition rate. Thus, each success in the discovery and development of new medicines is built on many, many prior failures and recent advance in understanding human biology and disease are opening up exciting new possibilities for breakthrough medicines.

Preclinical safety testing of new drug candidates is a crucial step in pharmaceutical drug development and depends on a sequential series of *in- vitro, in- vivo* and *in- silico* tests before administration to humans. Currently, *in vivo* testing is a vital part of safety assessment, and is a regulatory requirement before a drug can progress into clinical trials. However, in recent years, many *in vitro* assays have been developed and validated for early- s t a g e screening aimed at filtering out molecules with a higher potential for toxicity and in some cases replacing or reducing the use of certain *in vivo* tests as an adherence to the 3Rs, a set of principles by ICH that outlines the replacement, reduction and refinement of the use of animals in research.

Broad-scale *in vitro* pharmacology profiling of new chemical entities is increasingly being used earlier in the drug discovery process to identify undesirable off-target activity profiles that could hinder or halt the development of candidate drugs and has recently become an essential tool to predict clinical adverse effects. The challenge for the pharmacologist will always be to correlate in vitro data with *In vivo* findings, bearing in mind the old saying: "*In- vitro simplicities, in - vivo veritas".*

Diabetes mellitus is a complex metabolic syndrome that results from a heterogeneous group of disorders characterized by chronic hyperglycemia, with disturbances of carbohydrate, fat and protein metabolism resulting from the improper insulin secretion and/ or inefficient insulin action and possibly by abnormally high amounts of other counter regulatory hormones such as growth hormone, sympathomimetic amines and corticosteroids [1].

This chronic hyperglycemia is associated with long term damage, dysfunction and failure of various organs (Multi Organ Dysfunction Syndrome) leading to diabetic retinopathy, nephropathy, neuropathy and macro vascular complications.

The historical accounts of Diabetes mellitus dates back to 2000 years and was written in the medical texts such as 'The Egyptian *Ebers Papyri'*, Greek Epidemics Book III of Hippocrates, and the Chinese 'Nei Chang' and also in ancient Indian treatise such as Ayurveda. Egyptian physicians described 'diabetes mellitus' as a disease associated with the 'passage of much urine'.

Evaluation of Enzyme Inhibitors in Drug Discovery is a valuable reference work that clearly addresses the need for medicinal chemists and pharmacologists to communicate effectively in the difficult and demanding world of drug discovery. Enzymes are considered by many in the pharmaceutical community to be the most attractive targets for small molecule drug intervention in human diseases. The attractiveness of enzymes as targets stems from their essential catalytic roles in many physiological processes that may be altered in disease states. The structural determinants of enzyme catalysis lend themselves well to inhibition by small molecular weight, drug-like molecules. As a result, there is a large and growing interest in the study of enzymes with the aim of identifying inhibitory molecules that may serve as the starting points for drug discovery and development efforts.

Recent surveys of the human genome suggest that the portion of the genome that encodes for disease-associated, "drug gable" targets is dominated by enzymes. It is therefore a virtual certainty that specific enzyme inhibition will remain a major focus of pharmaceutical research for the foreseeable future https://wheebox.com/sunpharmahttps://wheebox.com/sunpharmaA recent study suggested that the size of the human "drug gable genome" (i.e., human genes encoding proteins that are expected to contain functionally necessary binding pockets with appropriate structures for interactions with drug-like molecules) is more on the order of 3000 target proteins (i.e., about 10% of the genome), a significant portion of these being enzymes [2].

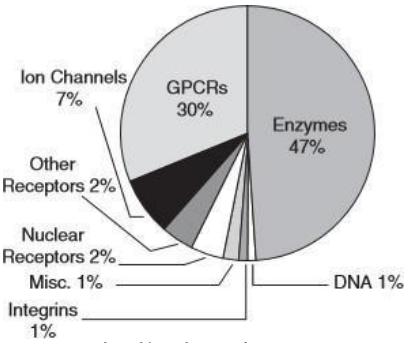

Fig no: 1.1 Distribution of enzymes as targets

Materials and Methods

Plant name: - *Ocimum kilimandscharicum Linn [3]*

Ocimum kilimandscharicum Linn plant

Botanical classification [4]

Kingdom Plantae Division Angiosperms Class Eudicots Sub class Asterids Order Lamiales

Family Lamiaceae

Genus *Ocimum*

Species *kilimandscharicum*

Vernacular names

Sanskrit Karpoorthulasi Malayalam Karpoorthulasi English Camphor basil Hindi kapurtulsi

Kannada karupuratulasi

Tamil karupuratulasi

***In vitro* Coagulation activity**

Collection of blood samples

The blood samples were obtained from normal individuals by using sterile syringes, withdrawn from vein of right arm of each individual and placed separately in containers containing tri-sodium citrate to prevent the clotting process [4]. Centrifugation (15 minutes at rate 3000 rpm) was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma (ppp) for prothrombin time test [5]. The obtained plasma sample of each individual were poured separately in plane containers using automatic pipette and stored at room temperature

Method

0.2 ml plasma, 0.1 ml of crude extract of different concentration and different volume of CaCl2 (25 mM) were added together in a clean fusion tube and incubated at 370C in water bath. For control experiment extract solution was replaced by same volume of 0.9% saline water. The clotting time was recorded with stopwatch by tilting the test tubes every 5 seconds. This time is called the prothrombin time [6].

Experimental Investigation

Phytochemical Screening of EEOK

Table 01: Phytochemical screening of EEOK

S. No	Phytochemical tests	Results
1	Amino acids Ninhydrin test	+
2	Carbohydrates: Molisch's test Barford's test Selivanoff's test Pentoses test	
3	Flavonoids: Shinoda test Alkaline reagent test Lead acetate test Ferric chloride test	+ + + +
4	Tannins: Ferric chloride test Chlorogenic test	+ +
5	Proteins: Warming test Trichloroacetic acid Biuret test	
6	Steroids: Libermann-burchard test salkowaski test	+ +
7	Glycosides: General test Born Tragers test Modified born Tragers test Hydroxy anthraquinones	+ + + +
8	Cardiac glycosides: Bal jet's test Libermann Burchard test Keller killiani test	+ + +
9	Reducing sugars: Benedicts test	+
10	Phenolic compounds: Ferric chloride test Lead acetate test	+ +
11	Alkaloids: Dragendorffs test Mayer's test	
	Tannic acid test	

(+) present, (-) absent

Table 02: Phytochemical Screening of Hydro Alcoholic Extract

S. No	**Phytochemical tests**	**Results**

1	Amino acids: Ninhydrin test	+
2	Carbohydrates: Molisch's test	+
3	saponins : froth formation test	+
4	Flavonoids: Shinoda test Alkaline reage test Lead acetate test Ferric chloride test	+ + +
5	Tannins: Ferric chloride test Chlorog test	+ +
6	Proteins: Warming test Trichloroacetic acid Biuret test	- + +
7	Glycosides: General test Borntrager's test Modified Born Tragers test Hydroxy anthraquinones	+ + + +
8	Steroids: Libermann-burchard test Salkowski test	+ +
9	Cardiac glycosides: Bal jet's test Liebermann Burchard test Keller killiani t	- + +
10	Reducing sugars: Benedicts test	+
11	Phenolic compounds: Ferric chloride test Lead acetate test	+ +
12	Alkaloids: Hager's test Tannic acid test	+ +

(+) present, (-) absent

Table 03: Total Phenolic and Total Flavonoid Content of EEOK and HAEOK

S. No	EXTRACTS	Total phenolic content (GAEmg/g of dry material)	Total flavonoid content (Qmg/g of dry material)
1	EEOK	103 .01 ±0.10	57.86 ± 0.124
2	HAEOK	91 .2 ± 0.05	46.78 ± 0.06

Results were expressed on Mean ± SEM (n=3)

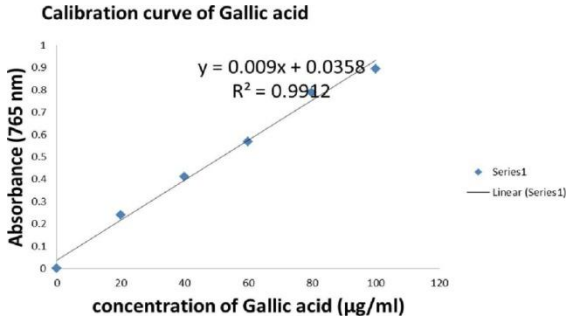

Figure 2 total phenolic content estimated by standard Gallic acid

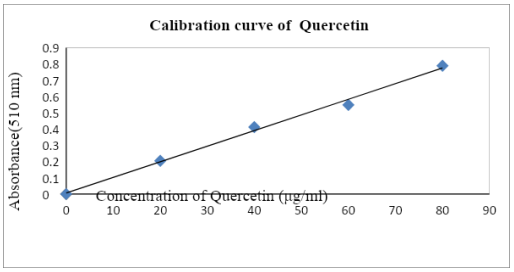

Figure 3 total flavonoid content estimated by standard Quercetin

Table 4.10 Determination of coagulation (Prothrombin time test) using EEOK.

S. No	Concentration (µg/ml)	extract	Amt of plasma	Amt of Extract	$CaCl_2$	Time
		Control EEO	0.2 ml	0.1 ml	0.3 ml	1:5 sec
1	200		0.2 ml	0.1 ml	0.3 ml	75 sec
2	400		0.2 ml	0.1 ml	0.3 ml	1:52 sec

Table 4.11 Determination of coagulation (Prothrombin time test) using HAEOK

S. No	Concentration (µg/ml	extract	Amt of plasma	Amt of Extract	$CaCl_2$	Time
1	200	HAEOK	0.2 ml	50 µl	0.5 ml	2:5 sec

2	400		0.2 ml	100 µl	0.5 ml	3:8 sec

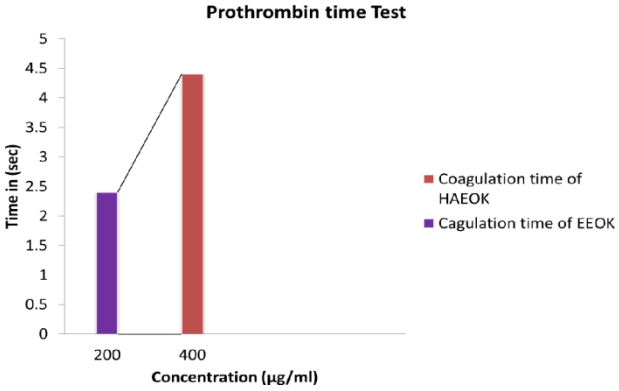

Figure 4 Prothrombin time

Discussion of Results

Preliminary phytochemical screening

The In the present study of *O. kilimandscharicum*, Preliminary phytochemical analysis – of Ethanol, hydro alcohol reveals the presence of pharmacologically active Ethanol soluble constituents, such as tannins, organic acids, amino acids, and steroids flavonoids, phenolic compounds, and mono-saccharide [7]. Phytochemical screening of *O. kilimandscharicum*, are shown in Table1 and Table 2 [8], the *Ocimum kilimandscharicum* plant leaves were investigated in preliminary phytochemical screening then I have done in some of the pharmacological activity.

in-vitro Anticoagulation assay Prothrombin time test

Anticoagulant from plant source must positively have a better safety margin and eliminate monitoring of therapy. Such anticoagulant may present with little or no side effects both for laboratory and clinical use [9]. It could be used in vivo (inside the body) or in vitro (outside the body). By using different extraction like ethanolic, hydro alcoholic extracts were subjected to anticoagulation activity and compared to tri sodium citrate [10]. The main aim of our present work was to evaluate anti-coagulation activity of plant extracts with that of standard [11]. The observed coagulation time shows quite good effect hydro alcoholic, ethanolic extract of *Ocimum kilimandscharicum* leaves [12]. It possesses a significant effect of a standard tri sodium citrate and makes a difference from control. Anti-coagulation assay we are taking concentration (200-400µg/ml) from the time of coagulation of EEOK is 75 sec to 1:52 sec, HAEOK is 2:5 sec to 3:8 sec and that of standard tri sodium citrate 1:5. Sec so it can be concluded as significant anti-coagulant agent [13].

Summary and Conclusion

Medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind. Over the last 2500 years, there have been very strong traditional systems of medicine such as Chinese, Ayurvedic, and the Unani, born and practiced, more in the eastern continent. These traditions are still flourishing, since approximately 80% of the people in the developing countries rely on these systems of medicine for their primary health care needs.

Folklore medicine claims that *Ocimum kilimandscharicum Linn*. has been implicated to treat a plethora of diseases and also forms part of our traditional culinary practices due to its spicy and aromatic flavor and it has been reported that green leafy culinary adjuncts contain bioactive secondary metabolites in pharmacologically active concentrations, which when consumed enough can elicit drug like effects by regulating key molecular pathways of disease mechanisms particularly, chronic and infectious diseases [14].

Anti-coagulation assay we are taking EEOK, HAEOK extracts concentration ranging from 200-400µg/ml showed concentration dependent from the time of coagulation of EEOK is 75 sec to 1:52 sec, HAEOK is 2:5 sec to 3:8 sec and that of standard tri sodium citrate 1:5. sec so it can be concluded as significant anti-coagulant agent.

The procedure involves the collection of herbs and processing steps for preparation of extract. The plant material of *Ocimum kilimandscharicum Linn* was collected from local market and then subjected to shade dried for 72 hrs. .The dried above products were then made into fine powder in order to suit for extraction process. Now the herbs were exposed to the extraction process by using ethanol as a solvent [15]. The extraction process was carried out by taking 70 gms of respective plant materials through continuous hot percolation method by the aid of Soxhlet apparatus. The whole extract of plant was collected in conical flasks, filtered and the solvents were evaporated to obtain a solid residue. Solvent selection plays an important role for the complete extraction of required constituents. After the extraction procedure, the extract was subjected to phytochemical screening and then to evaluate the anti-coagulation activity. In this study the ethanolic, hydroalcoholic extracts of the plant produce. Shows good Maximum inhibitory activity of the anti-coagulation, in dose dependent manner.

Conclusion

Historical accounts of certain medicinally relevant herbs reveal contain pharmacologically active substances in sufficiently high concentrations to have a drug like effect when consumed in reasonable quantity. The *in-vitro* anti-coagulant activities carried on the two different extracts viz, the ethanolic and hydroalcoholic extracts of *Ocimum kilimandscharicum*. The study revealed that the hydroalcoholic extract has promising results relative to ethanolic extract. However, these studies are not sufficient to claim and hence rigorous, stringent battery of pharmacological, phytochemical and bio analytical studies followed by observational studies in humans are to be carried to support folklore claim of the stated activities.

References

1. Garg D, Muley A, Khare N, Marar T. Comparative Analysis of Phytochemical Profile and Antioxidant Activity of some Indian Culinary Herbs. Research Journal of Pharmaceutical Biological and Chemical Sciences, 2012; 3(3):1-8.
2. Tulp M and Bohlin L. Unconventional natural sources for future drug discovery.3. Drug Discover, 2004; 9: 450–458.
3. Sato N. Roles of the acidic lipids sulfoquinovosyldiacylglycerol and phosphatidyl-glycerol in photosynthesis their specificity and evolution. J. PlantRes, 2004; 117: 495–505.
4. Willet WC. Diet and health what should we eat. Science, 1994; 264: 32-37.
5. EJ, Virtanen SM, Rasanen L, Tuomilehto J, Stengard J, Pekkanen J, Nissinen A, Kromhout
6. D. Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. Diabetes Care, 1995; (18): 1104–12.
7. Joy P P, Thomas J, Mathew S., Skaria BP. Indigenous less-known essential oils - A perspective.
8. PAFAI Journal, 1998; 20:13-20.
9. Srivatsava A. Areview on peppermint oil. Asian Journal of Pharmaceutical and ClinicalResearch, 2009; 2: 27-33.
10. Singh, A., Srinivasan, A.K., Chakrapani, L.N. and Kalaiselvi, P., 2019. LOX-1, the common therapeutic target in hypercholesterolemia: a new perspective of antiatherosclerotic action of aegeline. Oxidative medicine and cellular longevity, 2019.
11. Carlo, G.D., Mascolo, N., Izzo, A.A., Capasso, F. Flavonoids old and new aspects of a class of natural therapeutic drugs. Life Science, 1999; 65: 3-37.
12. Sumit Narwal. Review on Chemical Constituents & Pharmacological Action of Ocimum kilimandscharicum. Indo Global Journal of Pharmaceutical Sciences, 2011; 1(4): 287-293.
13. Gill Dolly. Ocimum kilimandscharicum a systematic review. Journal of Drug Delivery &Therapeutics, 2012; 2(3): 45-52.
14. Singh, A., Gowtham, S., Chakrapani, L.N., Ashokkumar, S., Kumar, S.K., Prema, V., Bhavani, R.D., Mohan, T. and Sathyamoorthy, Y.K., 2018. Aegeline vs Statin in the treatment of Hypercholesterolemia: A comprehensive study in rat model of liver steatosis. Functional Foods in Health and Disease, 8(1), pp.1-16.
15. Thayil Seema M. Methanol and Aqueous Extracts of Ocimum kilimandscharicum (Karpuratulasi) Inhibits HIV-1 Reverse Transcriptase in Vitro. International Journal of Pharmacognosy and Phytochemical Research, 2016; 8(7): 1099-1103.
16. Londhe A. M. In-Vitro Comparative Study of Antibacterial and Antifungal Activities A Case Study of Ocimum kilimandscharicum, Ocimum tenuiflorum and Ocimum gratissimum. International Journal of Pharmacognosy and Phytochemical Research, 2015; 7(1): 104-110.
17. R. S. A. Sorna Kumar. Formulation of simple syrup from Acorus calamus and Ocimum kilimandscharicum based on their antioxidant and antimicrobial activity. Scholars Research Library Der Pharmacia Letter, 2016; 8 (20): 75-78.
18. Sweet. Journal of Pharmacy Research, 2009; 2(4): 644-645.
19. Singh, A., Kumar, A. and Kalaiselvi, P., 2018. Aegeline, targets LOX1, the receptor for oxidized LDL to mitigate hypercholesterolemia: a new perspective in its anti-atherosclerotic action. Free Radical Biology and Medicine, 128, p.S41.
20. Thanigavelan Vembu. In vitro Anticoagulant

Activity of Siddha Sastric Formulation Linga Mathirai compared with Low Molecular Weight Heparin. Journal of Applied Pharmaceutical Science, 2016; Vol. 6 (06): pp 061-065.

21. Imadeldin M Taj Eldin. An In vitro anticoagulant effect of Fenugreek (Trigonella foenum-graecum) in blood samples of normal Sudanese individuals. Sudanese journal of paediatrics, 2011; Vol 13, Issue No. 2: pp 52-56.
22. Anslem O. Ajugwo. In Vitro Studies of Anticoagulation Activity of Pentaclethra Macrophylla. World Journal of Nutrition and Health, 2013; Vol. 1, No. 1: pp10-12.
23. Narjis Hadi Mansoor Al-Saadi. In vitro study of the anticoagulant activity of some plant extracts. Dian journal of applied research, 2013; Volume 3 Issue 7: 121-122.
24. Ojha S. N. In vitro and in vivo anticoagulant activity of imperata cylindrica a novel anticoagulant lead from natural origin. Phcog.Net | www.phcogj.com, January 2010; Vol 2 Issue 5: pp38-43.
25. Taj Eldin. An In vitro Anticoagulant Effect of Aqueous Extract of Ginger (Zingiber officinale) Rhizomes in Blood Samples of Normal Individuals. American Journal of Research Communication, 2016; Vol 4(1): pp113-121.
26. Ahmet Topala. The in vitro and in vivo effects of chlorpyrifosn acetylcholinesterase activity of rainbow trout brain. Journal of Applied Animal Research, 2015; Vol 44 No. 1: 243–247.
27. https://www.google.co.in/search?q=ocimumkilimandscharicum+plant+profile&tbm=isch&tbs=rimg:CXDjqy7yy7VaIjgIKVxtqhmVaxEebCWJ8byTo7
28. T. J. T. Josna, N. R. N. Rajesh, K. N. S. K. Naga Sindhura, K. H. R. K. Hema Ravali, N. U. J. N. Uma Jyothi, and V. N. G, "incidence and prevalence of various psychiatric disorders in psychiatric department of teaching based hospital ongole: a prospective observational study", Int J Indig Herb Drug, pp. 11-24, Aug. 2020.
29. https://en.wikipedia.org/wiki/Ocimum_kilimandscharicum
30. Sumit Narwal. Review on Chemical Constituents & Pharmacological Action of Ocimum kilimandscharicum. Indo Global Journal of Pharmaceutical Sciences, 2011; 1(4): 287-293.
31. Roli agrawal. Traditional Uses and Pharmacological Action of Ocimum Kilimandscharicum a Review. Journal of ultra-chemistry, 2017; Vol 13(6): 140-144.
32. Aiyegoro OA, OKoh AI. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longiifolium. DC, BMC Complementary and Alternative Medicine, 2010; pp.1-8.
33. Vasantha, V., L. jimmystevenjoshi, and G. Venkata Nagaraju. "PATTERN OF RESPIRATORY DISEASES REPORTING TO THE TERTIARY CARE HOSPITAL AT KAKINADA, ANDHRA PRADESH: PROSPECTIVE OBSERVATION STUDY". World Journal of Current Medical and Pharmaceutical Research, Vol. 1, no. 1, Jan. 2020, pp. 46-49, https://www.wjcmpr.com/index.php/journal/article/view/10.
34. Samuel Lallianrawna. Determination of total phenolic content total flavonoid content and total antioxidant capacity of Ageratina adenophora. Science Vision www.sciencevision.org, 2013; pp 150-156.
35. Nagaraju, G., & Pavan Kumar, G. (2018). AN EXTENDED REVIEW AND SUMMARY ON FACTS ABOUT ETIOLOGY EPIDEMIOLOGY CLINICAL DIAGNOSIS AND MANAGEMENT OF SCRUB TYPHUS INFECTION. Asian Journal of Pharmaceutical Research and Development, 6(5), 51-55. https://doi.org/https://doi.org/10.22270/ajprd.v6i5.404
36. Priyanka. evaluation of anti-oxidant activity of ethanolic root extract of albizia lebbeck benth Evaluation of antioxidant activity of ethanolic root extract of albizia lebbeck benth. International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS), 2013; 3(2): 93-101.
37. Amoolya Sree. In vitro Anti-Arthritic Activity of the Polyhedral Formulation –
38. Balapunarnavadi Choornam. Pharm. Sci. & Res, 2017; Vol. 9(8): pp 1281-1282.
39. Singh, A., 2022. Role of microbial metabolites in cardiovascular and human health. In

Microbiome, Immunity, Digestive Health and Nutrition (pp. 137-148). Academic Press.

40. Habibur Rahman. In-vitro Anti-inflammatory and Anti-arthritic Activity of Oryza sativa Var Joha Rice (An Aromatic Indigenous Rice of Assam). Am-Euras. J. Agric. & Environ. Sci, 2015; 15 (1): pp115-121.
41. T. J. T. Josna, S. R. SK. Rizwana, and G. G.V.Nagaraju, "CASE STUDY ON DEPRESSION", Int J Indig Herb Drug, pp. 1-3, Aug. 2019.
42. Prasanta Dey. Evaluation of in-vitro anticoagulant activity of Molineria recurpata leaf extract. J. Nat. Prod. Plant Resour, 2012; 2 (6): 685-688.
43. Konda RK. Brief description of Clinical Case study formats: a basic review. Journal of Case Studies and Case Reports. 2022 Apr 30:24-6.
44. Singh, A., 2022. Hyperlipidemia in cardiovascular health and digestion. In Nutrition and Functional Foods in Boosting Digestion, Metabolism and Immune Health (pp. 141-150). Academic Press.
45. Srikumar BN. assay of acetyl cholinesterase activity in the brain. National Institute of Mental Health and Neuro Sciences, 2004; 142-144.
46. Health and Neuro Sciences, 2004; 142-144.
47. https://www.medicalnewstoday.com/articles/7621.php
48. Roh, J., Hill, J.A., Singh, A.,Valero-Muñoz, M. and Sam, F., 2022. Heart failure with preserved ejection fraction: heterogeneous syndrome, diverse preclinical models. Circulation Research, 130(12), pp.1906-1925.
49. YL Chee, Coagulation, Royal College of Physicians of Edinburgh, 2014; 44:42–5
50. https://www.cancerclot.info/blood-clots-and-cancer/how-blood-clots-go-from-goo d-to-bad
51. Shiv Chandra Singh, A., Yu, A., Chang, B., Li, H., Rosenzweig, A. and Roh, J.D., 2021. Exercise Training Attenuates Activin Type II Receptor Signaling in the Aged Heart. Circulation, 144(Suppl_1), pp.A14259-A14259.
52. https://www.medicinenet.com/atrial_fibrillation_pictures_slideshow/article.htm#anticoagulation.
53. Boini, K.M., singh, A. and Koka, S.S., 2021. Gut Microbial Metabolite Trimethylamine N-oxide Enhances Endoplasmic Reticular Stress and Promotes Endothelial Dysfunction. Circulation, 144(Suppl_1), pp.A14071-A14071.
54. https://www.google.com/search?biw=1366&bih=613&tbm=isch&sa=1&ei=yjXUWoC7MIvevgTjpIfwAQ&q=neurodegenrative+causes&oq=neurodegenrative+causes
55. Pasula, S., S. Z. Humaira Hussaini, And N. Gv. "Case Study On Ifosmadide-Induced Cystitis". Asian Journal of Pharmaceutical and Clinical Research, vol. 13, no. 8, Aug. 2020, pp. 1-2, doi:10.22159/ajpcr.2020.v13i8.38040.
56. Lauren Reed-Guy. Brain Disorders. Health line, September 18, 2017; https://www.cancerclot.info/blood-clots-and-cancer/how-blood-clots-go-from-goo d-to-bad
57. ackwestin.com/resources/mcat-content/circulatory-system/coagulation-clotting-mechanism