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Estimation of Phytochemical Analysis and Anti-Inflammatory Activity of Fresh Extract of Parmotrema Perlatum and Vitex Negundo

Ms. K. Neelima[1], Asst. Prof. Dr. G. Sony[2], Dr. Y. Sabitha [3]
[1, 2]*Department of Biochemistry, St.Francis College for Women, Hyderabad*
[3] *Research Head, Ciencia Life sciences, Hyderabad*

Abstract: ***Lichens are known for their extraordinary secondary metabolites and thrombolytic activity. Parmotrema perlatum is commonly called Canary moss or Mangalore spices. This lichen is used as spices in the food in the Indian tradition. The use of P. perlatum in medicine is based on the fact that they contain unique and varied biologically active substances, as natural Anti-oxidant, Anti-microbial, and Anti-coagulant. Since they are natural anti-biotics, their metabolites exert a wide variety of biological actions including anti-mycotic, antiviral, anti-inflammatory, analgesic, anti-pyretic, anti-proliferative, and cytotoxic effects, they are considered as potential drugs. They contain a variety of secondary metabolites flavonoids, terpenoids, saponins . The lichens can be used as active ingredients for the preparation of drugs for their broad range of activity. The results obtained as a range of phytochemicals like terpenoids, flavonoids, glycosides, carohydrates, proteins were detected in the leaf extracts. Fresh leaves of Parmotrema perlatum and Vitex negundo Aqueous, Chloroform, and Methanol extracts were prepared and used to find out the anti-inflammatory activity of Parmotrema perlatum and Vitex negundo by In Vitro anti-inflammatory method. The study showed significant results.***

Keywords: Anti-inflammatory, Phytochemicals, P. perlatum, Vitex negundo

I. INTRODUCTION

A. Anti-Inflammatory Activity

Inflammation is a severe response by living tissue to any kind of injury. There can be four primary indicators of inflammation: pain, redness, heat or warmness and swelling. When there is injury to any part of the human body, the arterioles in the encircling tissue dilate. This gives a raised blood circulation towards the area (redness). Inflammation is either acute or chronic inflammation. Acute inflammation may be an initial response of the body to harmful stimuli. In chronic inflammation, the inflammatory response is out of proportion resulting in damage to the body.

Steroidal and non-steroidal anti-inflammatory drugs (SAIDs and NASIDs, respectively) are currently the most widely used drugs in the treatment of acute inflammatory disorders, despite theirrenal and gastric negative secondary effects. These drugs block COX-1 and COX-2 enzyme activity. COX enzymes assist with prostaglandin production. NSAIDs, steroidal anti-inflammatory drugs are being used till now, As a result long term uses of these drugs cause adverse side effects and damage the human biological system such as liver, gastrointestinal tract, etc. As a result of adverse side effects, like gastric lesions, cardiovascular, renal failure and gastrointestinal damage.

Now there is a need for the new safe, potent, non-toxic or less toxic anti-inflammatory drug. Plant medicines are of great importance in primary healthcare in many developing countries. According to the World Health Organisation (WHO) still about 80% of the world population rely mainly on plant-based drugs.

In Ayurveda, Siddha, and Unani, utilising a large number of medicinal plants were used for the treatment of human diseases. Plants have the ability to synthesise a wide variety of phytochemical compounds as secondary metabolites. Many of the phytochemicals have been used to effectively treat the various ailments for mankind.

The World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species. Most of the medicinal plant parts are used as medicines and they possess varied medicinal properties. Plants have a great potential for producing new medicines and used in traditional medicine to treat chronic and even infectious diseases. In the present review an attempt has been made to investigate the anti-inflammatory activity of some medicinal plants. Indian spices have many medicinal properties.

B. Medicinal Plants

The term “medicinal plant” includes various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses. The word “herb” has been derived from the Latin word, “herba” and an old French word “herbe”. Nowadays, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term “herb” was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities.

Plants have been used for medicinal purposes long before the prehistoric period. Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exists that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while others developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically.

Traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.

Among ancient civilisations, India has been known to be a rich repository of medicinal plants. The forest in India is the principal repository of a large number of medicinal and aromatic plants, whichare largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practised in India.

Medicinal plants are considered as a rich resource of ingredients which can be used in drug development either pharmacopoeial, non- pharmacopoeial or synthetic drugs. Apart from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as an important source of nutrition and as a result of thatthey are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric etc. Some plants and their derivatives are considered as important sources for active ingredients which are used in aspirin and toothpaste etc.

Apart from the medicinal uses, herbs are also used in natural dye, pest control, food, perfume, tea and so on. In many countries different kinds of medicinal plants/ herbs are used to keep ants, flies, mice and flee away from homes and offices. Nowadays medicinal herbs are important sources for pharmaceutical manufacturing.

C. Parmotrema Perlatum

Parmotrema perlatum, commonly known as black stone flower, is a species of lichen used as spice in India. Typically used in meat dishes like nahari (Papaya), Bombay biryani, Goat meat stews, it is also used in vegetarian dishes. It is one of the ingredients in East Indian Bottle Masala, used for cooking Meats, fish and vegetables [1]

According to Ayurveda, Stone Flower is useful in reducing the risk of as well as removing Mutrashmari (renal calculi) or kidney stones by increasing the production of urine due to its diuretic property. Stone Flower powder is very effective in promoting wound healing as it possesses antibacterial and anti-inflammatory properties.

Although there are no side effects of the consumption of Stone Flower, it might aggravate some problems like cough and cold in people with weak immunity or who regularly suffer from these ailments due to its Sita (cold potency) nature.

Fig 1. Parmotrema perlatum (Black stone flower)

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Table :1 Parmotrema Perlatum classification

Kingdom:	Fungi
Division:	Ascomycota
Class:	Lecanoromycetes
Order:	Lecanorales
Family:	Parmeliaceae
Genus:	Parmotrema
Species:	P. perlatum

The Black Stone Flower with the botanical name Parmotrema Perlatum belongs to the genus Foliose lichen. Lichen is a classified species between fungi and photosynthetic partners, and very rarely they include algae's.

They thrive as invasive plants throughout the southern and northern hemispheres of the earth with moderate temperatures. The black stone flower with the Sanskrit word "Shaileyam" is used as a spice in various parts of the world, including India.

These fungi are known by many names like Dagad Phool, Patthar ke Phool, Kallu Hoovu, and Raathi Pootha. The soft brown and black lichen, widely known in Tamil as Kalpaasi, can onlygrow in a certain altitude above sea level or have the characteristics of expecting very lowtemperatures.

The fungus that grows on rocks, stones, and trees is recognized as one of the most unusual spices inIndian review. A rare type of flower that is admired by all, it does not have a distinctive scent although it does provide a mild soil and woody scent depending on the growing environment.

These fungi, which are widely used in India, are found in large numbers in the Ooty and Kodaikanal hills of Tamil Nadu, where they have a very light fluffy texture.

Black Stone Flower or Kalpasi, which has intense properties and unique traits than any other spice used in the world and is compatible with all types of food, and still it dominates all traditional Chettinad products.

In addition, this edible lichen is also widely used in Hyderabad and Marathi cuisine. As already mentioned these black-purple flowers do not have their own flavour although they add a mysterious flavor to whatever food they are added to.

Lichens are symbiotic organisms which are composed of fungi and algae. Lichens have been used in various fields, especially as a source of natural drugs in the pharmaceutical industry and food supplement. Lichens are effective in the treatment of bronchitis, tuberculosis, and haemorrhoids. Lichens are important traditional medicines in many different cultures. This information has been made available to us from the contributions of hundreds of traditional knowledge holders incommunities across the world. It is our responsibility to respect and value the knowledge that has been given to us.

Lichens produce a wide range of organic compounds that can be divided into two groups, called primary metabolites and secondary metabolites. Primary metabolites are proteins, lipids, carbohydrates, and other organic compounds that are essential to the lichen's metabolism and structure. Some of these metabolites are produced by the lichen's fungal partner and algal or cyano bacterial partners.

Secondary metabolites are produced by the fungus alone and secretedonto the surface of hyphae either in amorphous forms or as crystals. If these substances are only found in lichens, then they are called lichen substances.

D. Vitex Negundo

Vitex negundo, commonly known as the Chinese chaste tree,[2] five-leaved chaste tree, or horseshoe vitex, or nisinda is a large aromatic shrub with quadrangular, densely whitish, tomentose branchlets.

It is widely used in folk medicine, particularly in South and Southeast Asia.[2]

Fig 2. Vitex negundo (Five-leaved chaste tree)

Table 2: Classification of Vitex negundo

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Lamiales
Family:	Lamiaceae
Genus:	Vitex
Species:	V. negundo

Vitex negundo (VN), commonly known as "chaste tree", is an ethnobotanically important plant withenormous medicinal properties. Different species of Vitex vary in chemical composition, thus producing different phytochemicals. Several bioactive compounds have been extracted from leaves, seeds, roots in the form of volatile oils, flavonoids, lignin, iridoids, terpenes, and steroids. These bioactive compounds exhibit anti-inflammatory, antioxidant, anti-diabetic, anticancer, antimicrobial.[2] VN is typically known for its role in the modulation of cellular events likeapoptosis, cell cycle, motility of sperms, polycystic ovary disease, and menstrual cycle. It is found throughout the greater part of India, ascending to an altitude of 1,500m. in theouter Himalayas. It is known under a variety of names in different languages.
Latin name: vitex negundo Linn. (Verbenaceae) .
Indian name: Sambhalu, Nirgundi, Sephali, Panjgusht (fanjangusht).English Names: Five-Leaved Chaste Tree, Monk's Pepper.

E. Phytochemicals

Phytochemicals are chemicals of plant origin. Phytochemicals (from Greek phyto, meaning"plant")are chemicals produced by plants through primary or secondary metabolism.

They Generally have biological activity in the plant host and play a role in plant growth or defence against competitors, pathogens, or predators. They are found in fruits, vegetables, grains, beans, and other plants. Some of these phytochemicals are believed to protect cells from damage that could lead to cancer. Phytochemicals such as secondary metabolites and antioxidants have important medicinal properties. Studies have shown that high intakes of fruits and vegetables are correlated with lower risks of chronic disease and obesity, partly because of these phytochemicals, also called phytonutrients[3].These plant-derived chemical compounds play important preventive activities mainly anti-inflammatory, anti-diabetic, anti-aging, anti-microbial, anti-parasitic, anti-depressant, anti-cancer ,anti-oxidant, and wound healing [4,5,6,7,8,9].

F. Terpenoids

Terpenoids diverse class of naturally occurring organic chemicals derivedfrom the 5-carbon compound isoprene, and the isoprene polymers called terpenes. and these terpenoids are also called as isoprenoids.

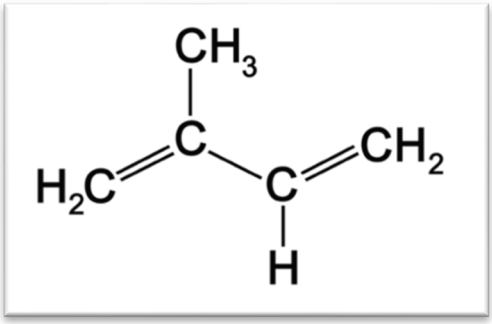


Fig 3. Structure of Terpenoids

Terpenoids are the largest class of plant secondary metabolites, representing about 60% of known natural products.Terpenoids contain many medicinal properties like anticancer, antimicrobial, antifungal, antiviral, anti-hyperglycemic, analgesic, anti-inflammatory, and antiparasitic[8] .They alsoenhances skin penetration, prevent inflammatory diseases. Terpenoids are divided into monoterpenes, sesquiterpenes, diterpenes, sesterpenes, and triterpenes depending on its carbonunits .

G. Flavonoids

Flavonoids are the compounds found naturally in many plants and they arepolyphenolic secondary metabolites .General structure of flavonoids have 15 carbon skeletons which consist of two phenyl rings and a heterocyclic ring.

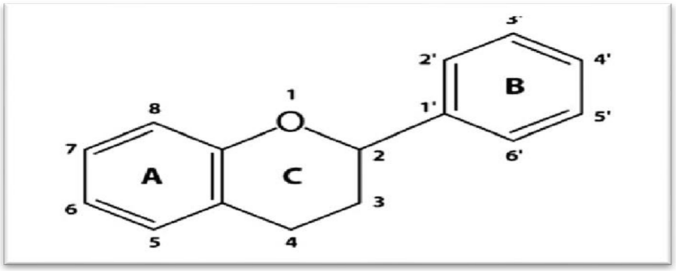


Fig 4. Structure of Flavonoids

Flavonoids are the largest group of phytonutrients, with more than 6,000 types. Quercetin and kaempferol are best known flavonoids . flavonoids are powerful antioxidants with anti- inflammatory and immune system benefits. The flavonoids can be divided into six major subtypes,which include chalcones, flavones, isoflavonoids, flavanones, anthoxanthins and anthocyanins.

H. Glycoside

Glycoside, any of a wide variety of naturally occurring substances in which a carbohydrate portion,consisting of one or more sugars or a uronic acid (i.e., a sugar acid), is combined with a hydroxy compound. The hydroxy compound, usually a non-sugar entity (aglycon), such as aderivative of phenol or an alcohol, may also be another carbohydrate, as in cellulose, glycogen, or starch, which consist of many glucose units.

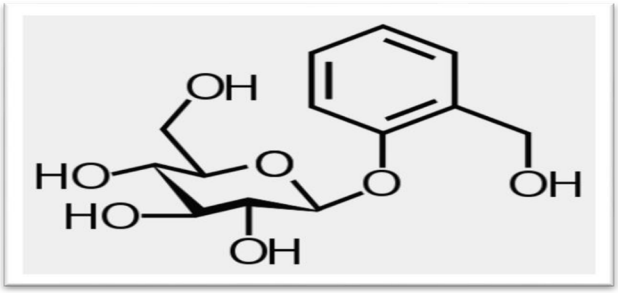


Fig 5 Structure of Glycoside

I. Anthraquinone

Anthraquinone, also called anthracenedione or dioxoanthracene, is an aromatic organic compound with formula $C_{14}H_8O_2$. Isomers include various quinone derivatives. The term anthraquinone, however refers to the isomer, 9,10-anthraquinone (IUPAC: 9,10-dioxoanthracene)wherein the keto groups are located on the central ring. It is a building block of many dyes and is used in bleaching pulp for papermaking. It is a yellow, highly crystalline solid,
poorly soluble in water but soluble in hot organic solvents. It is almost completely insoluble in ethanol near room temperature but 2.25 g will dissolve in 100 g of boiling ethanol. It is found innature as the rare mineral hoelite.

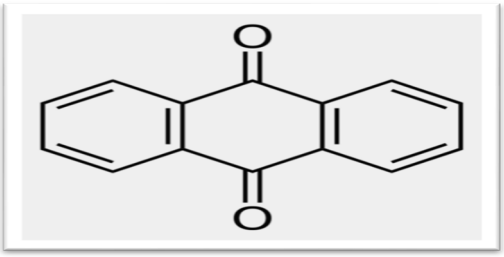


Fig 6. Structure of Anthraquinone

II. MATERIALS AND METHODS

A. Collection Of Sample

Lichens *of Parmotrema perlatum* were collected randomly from supermarket in secunderabad. After collecting, they were dried thoroughly. After complete drying, the flower are made into powder for further analysis.
Vitex negundo was Collected from kavirivaripallem village, Prakasham district, Andhra Pradesh. After gathering the leaves, they are cleaned thoroughly. The primary objective of drying is to remove excess water from leaves so they can stored. After complete drying, the leaves should be made into powder for further analysis.
These dry leaves were collected, cleaned, washed thoroughly and were made into fine powder afterrying.

Fig 7. Dried Parmotrema perlatum

Fig 8. Dried Vitex negundo

In this experiment three different extracts of *Parmotrema perlatum and Vitex negundo* tested for their anti-inflammatory activity. They are Methanol Extract, Aqueous Extract,Chloroform Extract. The samples for the membrane stabilization assay (Haemolysis assay), protein denaturation, and proteinase assay were prepared by using the procedure with these three extracts.

B. In-Vitro Anti-Inflammatory Activity

1) *Membrane Stabilization Assay* [10] ,[11]*:* Preparation of haemoglobin rich red blood cells suspension: The blood was collected from healthy human volunteers who has not taken any NSAIDs (Non-steroidal anti-inflammatory drugs) for two weeks prior to the experiment and transferred to the centrifuge tubes. It was centrifuged at 3000rpm for 10 min and were washed three times with equal volume of saline. The volume of the blood was measured and reconstructed with 10% V/V suspension with normal saline.
2) *Heat Induced Haemolysis Assay:* The reaction mixture 2ml consisted of 1ml of test sample of different concentration (100,200,300,400,500µl/ml) and 1ml of 10% hRBCs suspension. instead of test sample only saline was added to the control test tube. Aspirin (100µg/ ml) was used as standard drug.All the test tube containing reaction mixture was incubated in water bath at 56□ C for 30 minutes. After the incubation,it was cooled for 5min.Then the reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant was taken at 560 nm using UV- Vis spectrophotometer .The experiment was performed in triplicates and percentage inhibition of haemolysis was calculated as follows**;**

$$\text{Percentage Inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100$$

3) *Hypotonicity Induced Haemolysis Assay:* The reaction mixture 3ml consisted of 0.5ml of different concentration (100,200,300,400,500µl/ml) and 2ml of hyposaline, 0.5ml hRBCs suspension, in control test tube instead of test sample 0.5ml ofphosphate buffer was added. Diclofenac sodium (100µg/ml) was used as standard drug. All the test tubewere incubated at 37˚C for 30 minutes and centrifuged at 3000 rpm for 10 min. The absorbance of supernatant was taken at 560nm using UV-Vis spectrophotometer. The percentage inhibition of haemolysis was calculated as follows;

$$\text{Percentage Inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100$$

C. Protein Denaturation

1) *Inhibition of Albumin Denaturation:* The equal amount of 3ml reaction mixture (0.2ml egg albumin + 2.8ml phosphate buffer saline) was added to different concentration of test sample (100,200,300,400,500µg/ml) and make up the 2 ml of distilled water. In control test tube instead of test sample, PBS was added. Aspirin (100µg/ml) was used as standard drug. All the test tube were incubated at 37˚C for 15 minutes and incubated at 70˚C for 10 minutes. The samples were cooled for 5 minutes and absorbance was noted at 660nm using UV-Visspectrophotometer The percentage inhibition of albumin denaturation was calculated as follows;

$$\text{Percentage Inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100$$

2) *Inhibition of BSA denaturation:* The equal amount of 450µl BSA (1mg/ml) was added in different concentration (100,200,300,400,500µl/ml) of test samples and made up into 1ml of distilled water. All the rest of steps were followed according to the albumin denaturation.

Proteinase inhibition assay:

The test followed according to the modified method ofThe reaction mixture (2ml) containing 6µl trypsin, 1ml (20mM) tris HCl and along with different concentration of test samples (100,200,300,400,500µg/ml). The mixture was incubated at 37 oC for 5 min after that 1 ml of 0.8%(w/v) casein was added. The mixture was incubated again for 20min, 2ml (70%) perchloric acid was added to arrest the reaction.

Cloudy suspension was observed, it was centrifuged for 5 min and the supernatant was read at 210 nm using UV-Vis spectrophotometer. Control was run as same procedure but instead of test sample PBS was suspended.
The percentage of inhibition of anti-proteinase was calculated as follows;

$$\text{Percentage Inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100$$

D. Phytochemical Qualitative Analysis

The plant extracts of methanol, chloroform and aqueous solutions were assessed for the existence of the phytochemical analysis by using the following standard methods:

1) *Test for Anthraquinones:* 10 ml of benzene was added in 6 g of the Ephedra powder sample in a conical flask and soaked for 10 minutes and then filtered. Further 10 ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 seconds and pink, violet, or red colour indicated the presence of anthraquinones in the ammonia phase.
2) *Test for Tannins: 10* ml of bromine water was added to the 0.5 g aqueous extract. Decoloration of bromine water showed the presence of tannins.
3) *Test for Saponins:* 5.0 ml of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously
4) *Tests for Flavonoids*

Shinoda Test:

Pieces of magnesium ribbon and Hcl concentrated were mixed with aqueous crude plant extarct after few minutes and pink colour showed the presence of flavonoid. Alkaline Reagent Test. 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow color was produced, which became colorless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

5) *Tests for Glycosides*

Liebermann's Test:

We added 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous plant crude extract. The mixture was then cooled and we added H2SO4 concentrated. Green color showed the entity of aglycone,steroidal part of glycosides.

6) *Test for Terpenoids :* 2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water path and then boiled with 3 ml of H2SO4 concentrated. A grey color formed which showed the entity of terpenoids.
7) *Test* for Carbohydrates: Add 2-3 drops of α-naphthol solution to 2 ml of the test solution. Very gently pipette 1ml conc. H2SO4 along the side if the test tube so that the two distinct layers are formed. Carefully observe any color change at the junction two layers. Appearance of purpose color indicates the presence of carbohydrates in the sample preparation or the test solution.
8) *Test for Proteins*

Biuret Test: Take a small quantity of the dispersion of the sample in a test tube and add 2 ml of NaOH solution into it. Now add 4-5 drops of 1% CuSO4 solution and warm the mixture for about 5 minutes. Appearance of Bluish violet colour indicates the presence of proteins.

III. RESULT & DISSCUSSION QUALITATIVE ANALYSIS

Table 3. Phytochemical analysis of methanol, aqueous and chloroform extracts

S.NO	Secondary metabolites	PP methanol	PP aqueous	PP chloroform	VN methanol	VN aqueous
1.	Tannins	-	-	-	-	-
2.	Saponins	-	-	-	+	-
3.	Flavonoids	+	+	+	-	-
4.	Terpenoids	+	+	+	-	+
5.	Glycoside (salkowski test)	+	+	+	-	-
6.	Carbohydrate	+	+	+	-	-
7.	Protein	+	+	+	-	-
8.	Anthraquinones	-	-	-	+	+

Premortema perlatum AND Vitex negundo

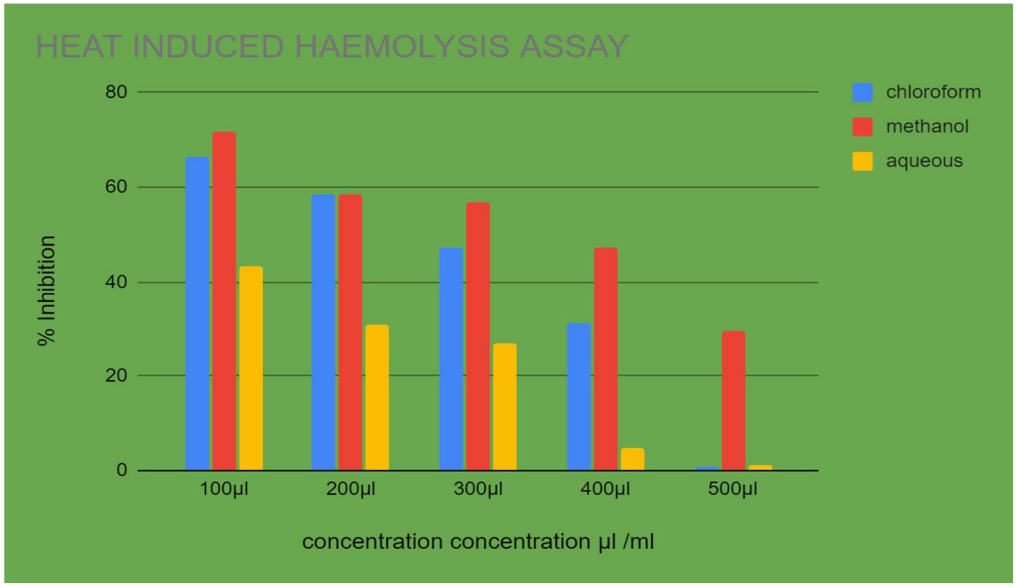
A. In-Vitro Anti-Inflammatory Activity

1) Membrane Stabilization Assay: The HRBC membrane stabilization is one of the techniques followed to study the anti-inflammatory activity. Erythrocyte's membrane is closely resemblance of lysosomal membrane. The stabilization of the lysosomal membrane depends on the sample and the concentrate. The enzyme released during response results in various disorder especially with chronic and acute inflammation. The role of drug administrated showed the inhibition and stabilization of membrane.

2) Heat Induced Haemolysis Assay

Table 4. Heat induced haemolysis assay of Parmotrema perlatum

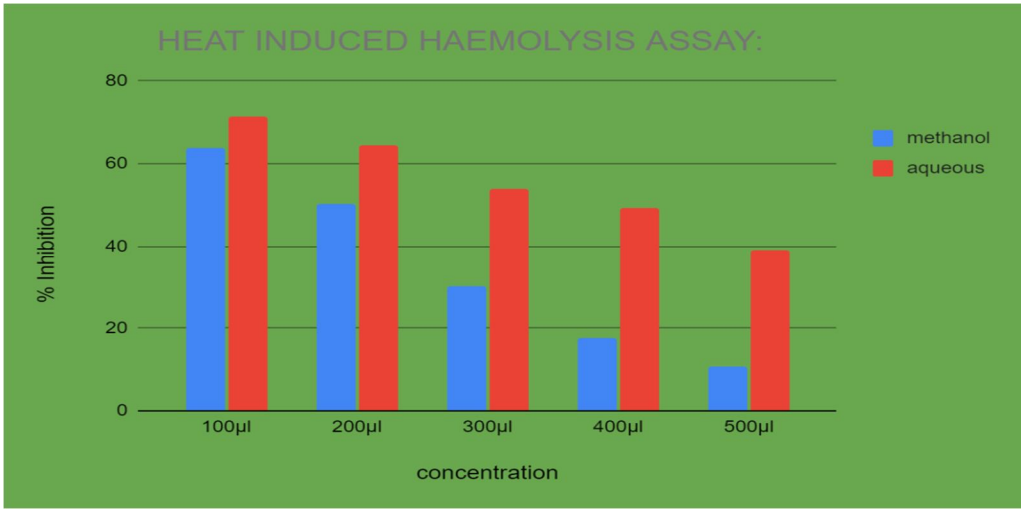
STONE FLOWER			
Concentration (µl/ml)	Chloroform % INHIBITION	Methanol % INHIBITION	Aqueous % INHIBITION
100µl	66.366	71.85	43.386
200µl	52.852	58.51	30.687
300µl	47.447	57.03	26.984
400µl	31.231	52.59	4.761
500µl	0.9009	29.62	1.058



Graph 1. Heat induced haemolysis assay of Parmotrema perlatum

Table 5. Heat induced haemolysis assay of Vitex negundo

NEGUNDO		
Concentration (µl/ml)	Methanol % INHIBITION	Aqueous % INHIBITION
100µl	78.5035	82.0223
200µl	72.1909	66.0691
300µl	65.1685	54.2755
400µl	33.9887	41.8604
500µl	20.5673	25.5617



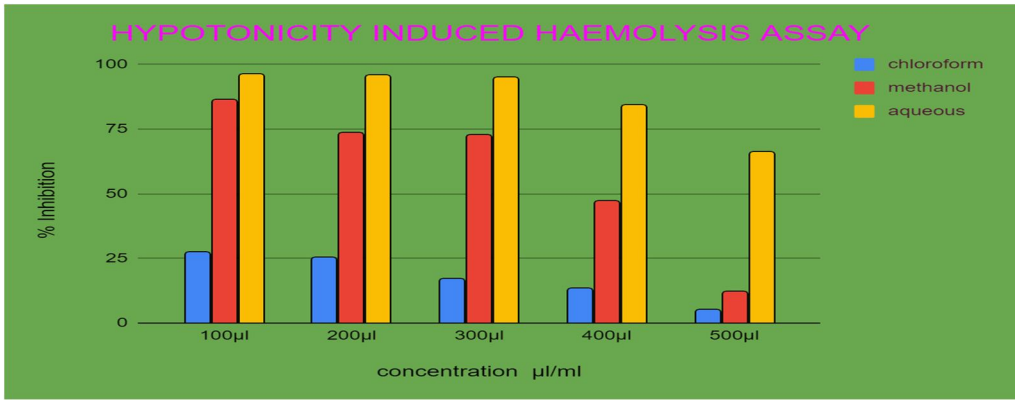
Graph 2. Heat induced haemolysis assay of Vitex negundo

The tested samples were analyzed to study the inhibiting reaction against the membrane lysis. Parmotrema perlatum and Vitex negundo showed the maximum protection at the concentrated inhibition. From the results observed all the samples showed a good significant protection of erythrocyte membrane from damage.

3) Hypotonicity Induced Haemolysis Assay

Table 6. Hypotonicity induced haemolysis of Parmotrema perlatum

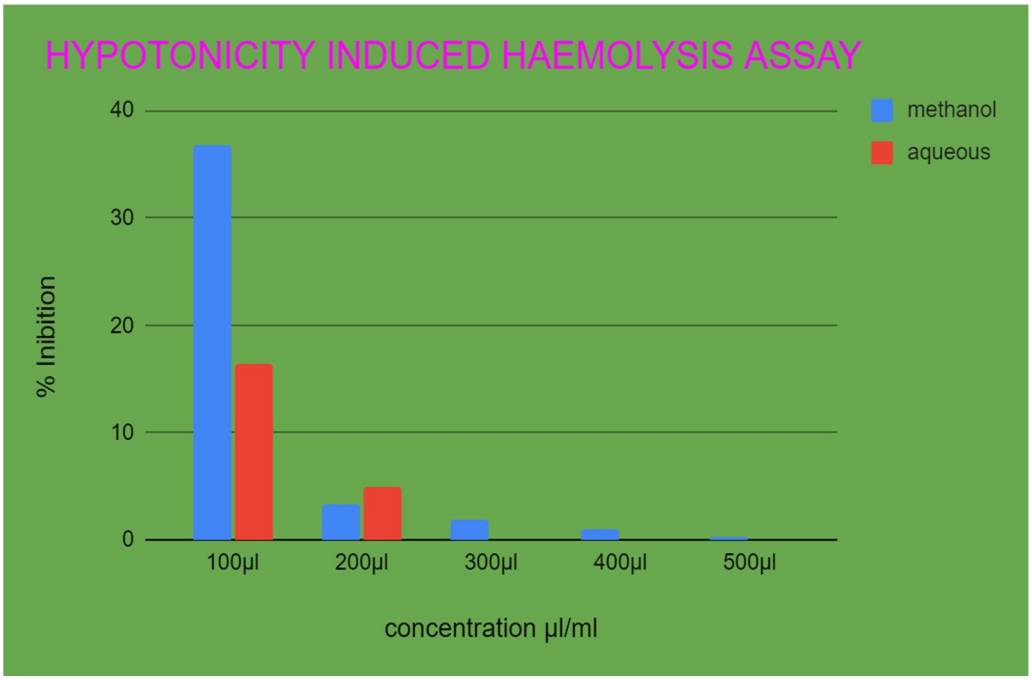
STONE FLOWER			
Concentration (µl/ml)	Chloroform % INHIBITION	Methanol % INHIBITION	Aqueous % INHIBITION
100µl	27.479	86.379	96.415
200µl	25.209	73.476	95.937
300µl	17.204	72.759	95.101
400µl	13.381	47.192	84.229
500µl	5.0179	11.947	66.069



Graph 3. Hypotonicity induced haemolysis of Parmotrema perlatum

Table. 7 Hypotonicity induced haemolysis of Vitex negundo

NEGUNDO		
Concentration (µl/ml)	Methanol % INHIBITION	Aqueous % INHIBITION
100µl	36.7693	16.4718
200µl	3.4006	4.8883
300µl	1.9128	0
400µl	1.0626	0
500µl	0.2125	0



Graph 4. Hypotonicity induced haemolysis of Vitex negundo

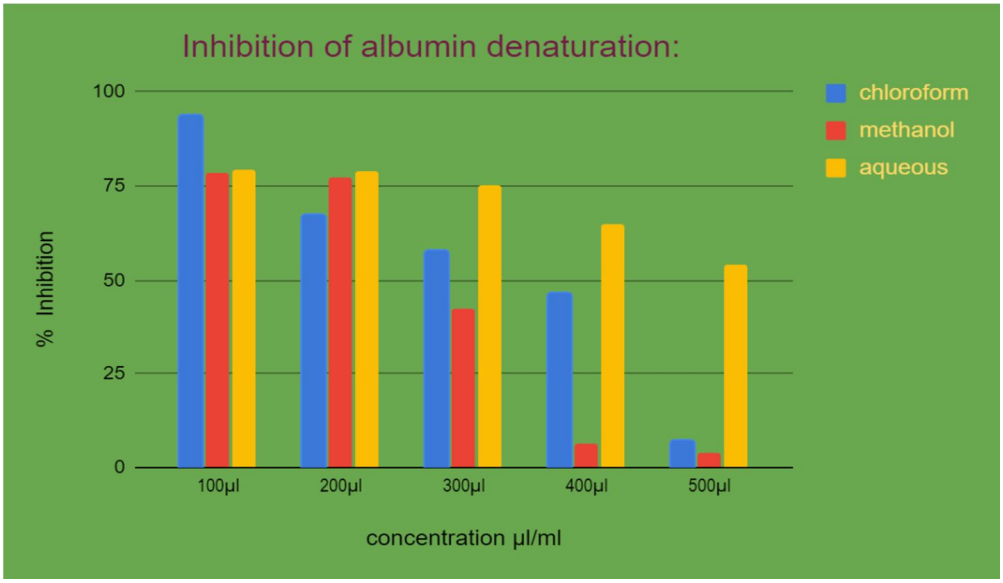
The inhibition of hypotonicity induced HRBC membrane lysis and the stabilization of the membrane by tested sample was taken as the measure of anti-inflammatory activity. The percentage of inhibiting lysis of membrane depends on tested sample.

4) Protein Denaturation

a) Inhibition Of Albumin Denaturation

Table 8. Inhibition of albumin denaturation of Parmotrema perlatum

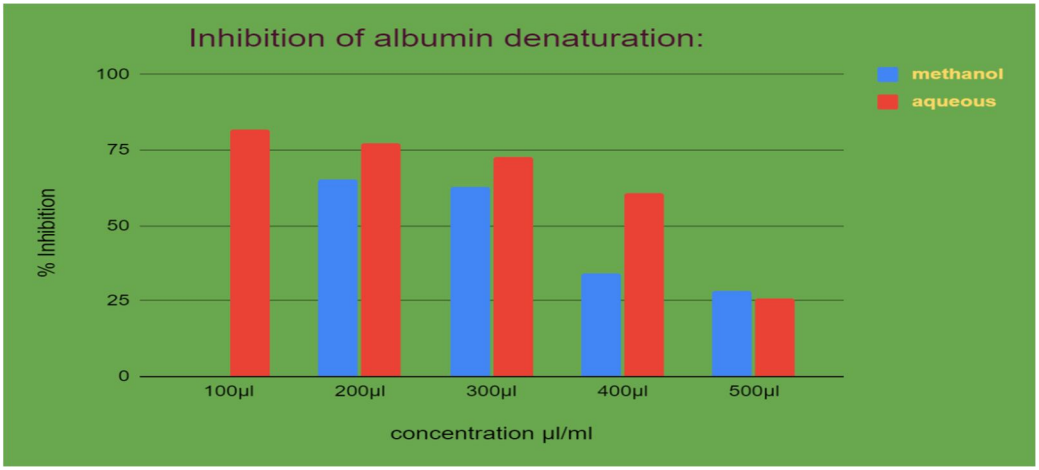
STONE FLOWER			
Concentration (µl/ml)	Chloroform% INHIBITION	Methanol % INHIBITION	Aqueous % INHIBITION
100µl	93.8241	78.5035	79.4536
200µl	67.5771	77.4346	78.8598
300µl	57.9572	42.2802	75.2968
400µl	46.5558	6.1757	65.0831
500µl	7.0071	3.9192	54.2755



Graph 5. Inhibition of albumin denaturation of Parmotrema perlatum

Table 9. Inhibition of albumin denaturation of Vitex negundo

NEGUNDO		
Concentration (µl/ml)	Methanol % INHIBITION	Aqueous % INHIBITION
100µl	72.1909	82.0223
200µl	65.1685	77.2471
300µl	62.9213	72.7527
400µl	33.9887	60.9550
500µl	28.0898	25.5617

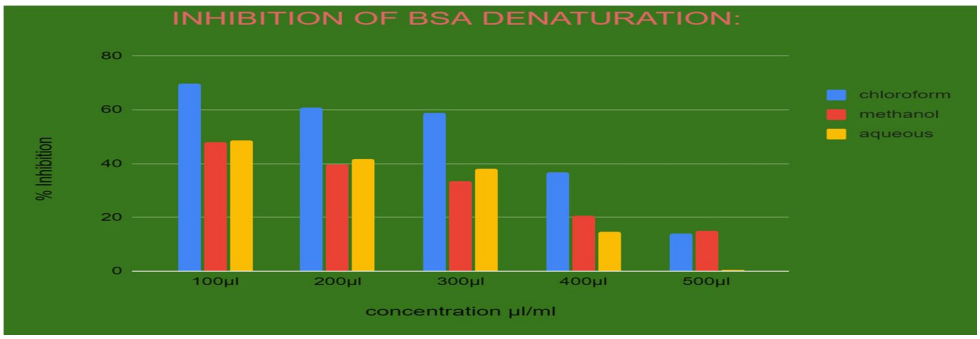


Graph 6. Inhibition of albumin denaturation of Vitex negundo

b) Inhibition Of Bsa Denaturation

Table 10. Inhibition of BSA denaturation of Parmotrema perlatum

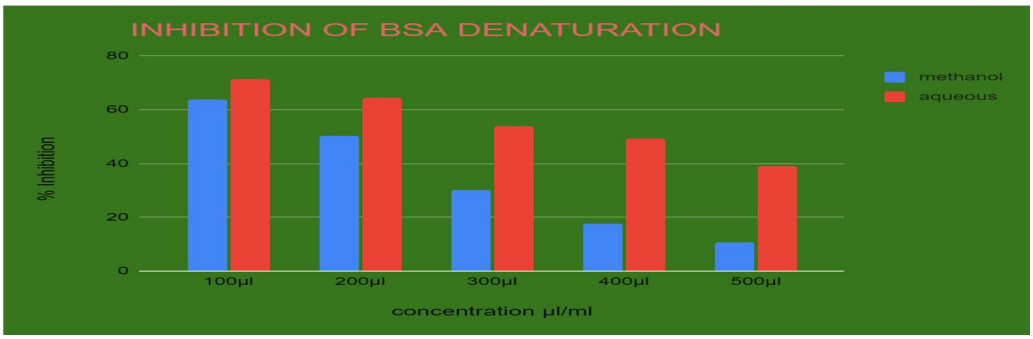
STONE FLOWER			
Concentration (µl/ml)	Chloroform % INHIBITION	Methanol % INHIBITION	Aqueous % INHIBITION
100µl	69.6057	47.9229	48.5049
200µl	60.7883	39.8176	41.8604
300µl	58.8174	33.4346	38.2059
400µl	36.7219	20.5673	14.6179
500µl	13.9004	14.9949	0.3322



Graph 7. Inhibition of BSA denaturation of Parmotrema perlatum

Table 11. Inhibition of BSA denaturation of Vitex negundo

NEGUNDO		
Concentration (µl/ml)	Methanol % INHIBITION	Aqueous % INHIBITION
100µl	63.6611	71.2643
200µl	50.2732	64.6232
300µl	30.0546	54.0229
400µl	17.7595	49.2975
500µl	10.6557	38.9527



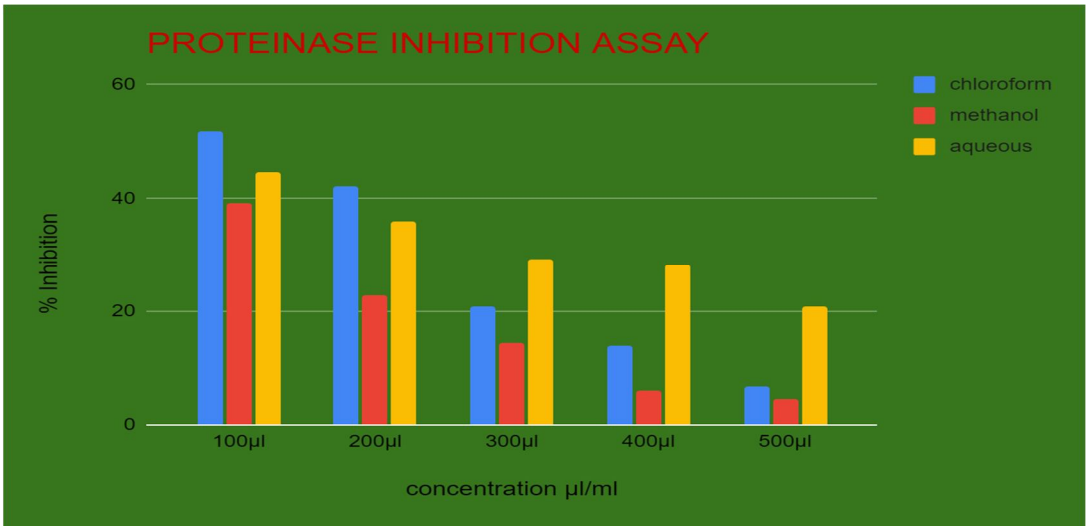
Graph 8. Inhibition of BSA denaturation of Vitex negundo

Protein denaturation is technique in which the protein loses their original form by the external stress or any other chemical compounds. It is one of main cause during the inflammation process. the anti-inflammatory mechanism, different concentration of test sample along withstandard were checked for the ability of inhibiting protein denaturation.

5) Proteinase Inhibition Assay

Table 12. Proteinase inhibition assay of Parmotrema perlatum

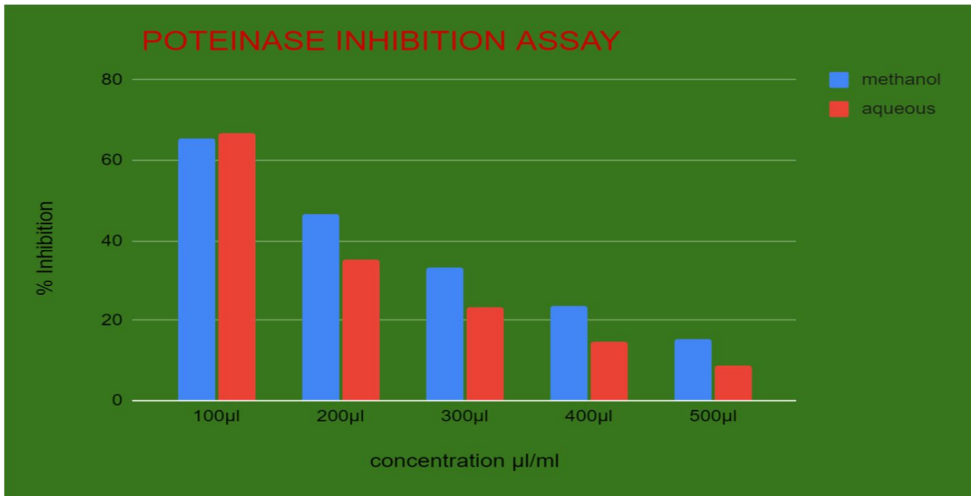
STONE FLOWER			
Concentration (µl/ml)	Chloroform % INHIBITION	Methanol % INHIBITION	Aqueous % INHIBITION
100µl	51.6986	39.2816	44.5182
200µl	42.2451	22.7479	35.8803
300µl	20.8271	14.5381	29.2358
400µl	13.8847	5.9863	28.2392
500µl	6.7946	4.4469	20.9302



Graph 9. Proteinase inhibition assay of Parmotrema perlatum

Table 13. Proteinase inhibition assay of Vitex negundo

NEGUNDO		
Concentration (µl/ml)	Methanol % INHIBITION	Aqueous % INHIBITION
100µl	65.6307	66.8756
200µl	46.6739	35.1097
300µl	33.1515	23.0929
400µl	23.5550	14.7335
500µl	15.2671	8.8819



Graph 10. Proteinase inhibition assay of Vitex negundo

Serine proteinase is one of major enzyme released from neutrophils and plays a vital role in the development of tissue damage during inflammation response and significant protection was given by proteinase inhibitors. Samples such as standard diclofenac sodium exhibited significant antiproteinase activity at different concentrations as illustrated in Samples showed maximuminhibition.

IV. DISSCUSSION

Phytochemicals are non-nutritive plant chemicals possessing varying degrees of disease- preventive properties. They are invaluable sources of raw materials for both traditional and orthodox medicine. Phytochemicals may display their health protective effects in diverse ways.

HRBC method was selected for the in vitro evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes[12]. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. Invitro anti-inflammatory activity of methanol extract of Enicostemma axillare was also reported [13]

Egg albumin method provides a cheap alternative method of testing the anti-inflammatory activity of herbal medicine using denaturation technique and this method should be validated by conducting further studies. The plant of Parmotrema perlatum and Vitexnegundo was found to be anti-inflammatory in nature.

V. CONCLUSION

The Parmotrema perlatum and Vitex negundo are rich in nutrients and contain many phytochemicals with strong potential for use as food supplements. The results obtained are in agreement with these assertions as a range of phytochemicals viz; terpenoids, flavonoids, glycosides were detected in the leaf extracts. All these phytochemicals were detected in all three aqueous, chloroform and methanolic extracts. Invitro anti-inflammatory activity was evaluated using albumin denaturation assay, proteinase inhibitory activity, membranestabilization, at different concentrations. This study gives the idea that the compound of the plant Parmotrema perlatum and Vitex negundo can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as cancer, neurological disorder, ageing and inflammation.

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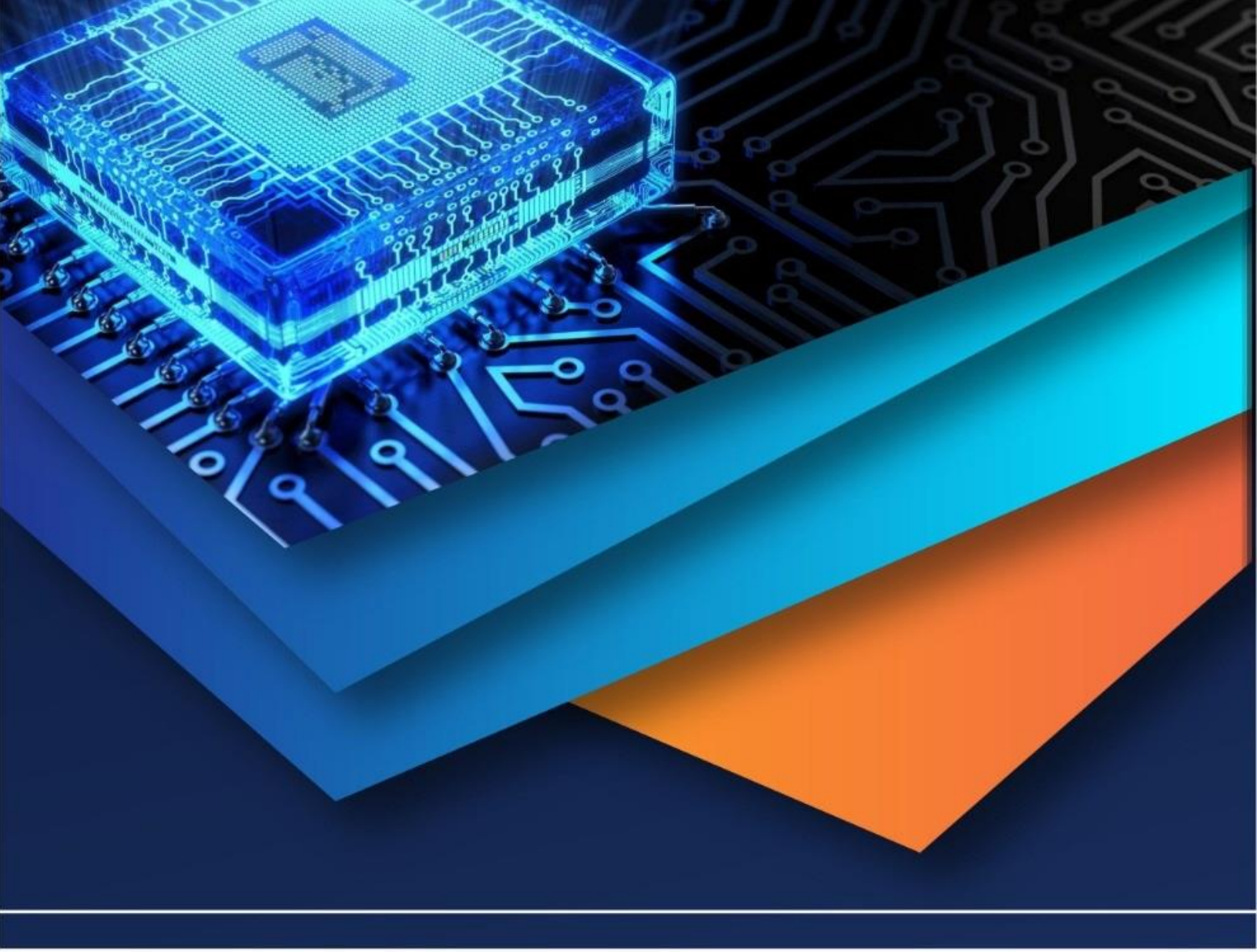

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