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Formulation and Evaluation of Herbal Cream for Arthritis

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Abstract: ***Inflammation is the symptoms of many diseases like rheumatoid arthritis and osteoarthritis. It is the cause of pain in joints, swelling and stiffness. Various analgesic and Non Steroidal Anti- inflammatory Drugs (NSAID) such as aspirin, diclofenac, ibuprofen etc. this may leads to severe side effects. Therefore the present study was aim to formulate herbal cream to treat arthritis. The herbal cream contains methanol extract of Vitex negundo and Solanum trilobatum and mucilage of Abelmoschus esculentus. Various samples were extracted and phytochemicals were qualitatively analyzed. Antibacterial activity from the tested plants can be useful in warding of infectious diseases against human pathogens (Staphyllococcus aureus). The presences of different bioactive compounds in plants are responsible for the antibacterial activity. The formulation of cream was prepared by two phases oily phase and aqueous phase. The ingredients used in the preparation of cream have unique role as a moisturizer, emulsifier, pH adjuster, hardening agent and smoothing agent. The physico chemical properties were studied which shows satisfactory results for color, odor, washability, spreadability and others. So, an herbal cream is non-toxic, safe, and effective. Further, determination of exact molecular level pharmacological investigations can be carried out to confirm its efficacy and mechanism of action.***

Keywords: Vitex negundo, Solanum trilobatum, Abelmoschus esculentus, Phytochemicals, Antibacterial activity, Herbal Cream.

I. INTRODUCTION

Arthritis is an inflammatory disease that causes pain in joints, stiffness and swelling and decreased range of motion [1]. It is a chronic, inflammatory, systemic autoimmune disorder. Cartilage is associated with protection of the joint pain and allows it to move smoothly. Arthritis involves the breakdown of cartilage and causing decreased amount of cartilage, it results the bones rub together causing pain, swelling and stiffness [2]. It is caused by T lymphocytes that release cellular toxins (cytokines) and degradable enzymes. The role of inflammatory cytokines such as TNF-α, interleukin (IL)-1b, IL-6 and chemokines; inflammatory enzymes such as cyclooxygenase (COX)-2, 5-lipoxygenase (5-LOX) and Matrix Metallo Proteinase (MMP)-9 and adhesion molecules in the pathogenesis of arthritis (Dinesh Khanna *et al.* 2019). Septic arthritis is a cause of bacterial infection of the joint space causes pain in knee, followed by the hip, shoulder, and elbow. The major organism involved in cause of septic arthritis is *Staphylococcus aureus* a gram positive bacteria and some bacteria like *Nesseria gonorrhoeae*, *Nocardia asteroids* [3].

In India 15% of people are affected by arthritis i.e. more than 180 million people. The World Health Organization (WHO) classified over 100 rheumatologic disorders [4]. Hormonal changes are also cause of increased level of rheumatoid arthritis. The risk increases with age it commonly develops between ages 40 to 60, and it also cause at any age [5]. The survey says that the levels of rheumatoid factor are higher in women than in men. In 2030, more than 25% of world population will be affected by one type of arthritis due to life style changes [6]. Various analgesic and non steroidal drugs are used to treat arthritis. Analgesics like acetaminophen and Non Steroidal Anti- inflammatory Drugs (NSAID) such as aspirin or ibuprofen [7]. These drugs are target to suppress the prostaglandins through inhibition of the cyclo-oxygenase enzymes. But these causes side effects like stomach ulcers, gastrointestinal bleeding and also damage the kidneys, livers and others. Moreover, some of these drugs are quite expensive [8]. The awareness of side effects by chemical medicines is grown among the people, so the requirement of natural medicines is become high [9].

Natural products have been studied extensively in multiple different ailments such as cancer, infectious diseases and autoimmunity. The mechanism of action of natural products is a high priority, is also emphasized by National Center for Complementary and Integrative Health (NCCIH) [8]. Since ancient time in India, herbal medicines have been the basis of treatment and cure for various diseases physiological conditions in traditional methods practiced such as Ayurveda, Unani, and Siddha. Medicinal plants have bioactive compounds which are used to cure various human diseases and also play an important role in healing. They have antifungal, antibacterial and anti-inflammation activities. Many infectious agents such as fungi and parasites may harm the plants. The presence of secondary metabolites in plants will protect against the cause of infectious agents. Phytochemicals are naturally occurring in the different parts of medicinal plants like leaves, flowers, fruits, vegetables and roots that have defense mechanism to protect from various diseases [10].

The phytochemical analysis of the plants is very important for the production of new drugs and has great interest in pharmaceutical companies Some of the plants are used in traditional medicine to treat arthritis such as *Callicarpa macrophylla, Cardiospermum halicacabum Linn, Jatropha curcas Linn, Lantana camara Linn, Pongamia pinnata Linn,* etc [11].

The present study is aimed to formulate and evaluate herbal cream by using different plant samples like leaves of *Vitex negundo,* fruits of *Solanum trilobatum and* mucilage of *Abelmoschus esculentus* vegetables. Preliminary phytochemical studies and anti bacterial activity of these extracts were studied.

Vitex negundo belongs to the family verbenaceae, is an aromatic shrub is commonly called as nirgundi or nochi is found throughout the India. It is an erect, 2 – 5 m in height, slender tree with quadrangular branch lets. The leaves have 5 leaflets in a palmately arrangement, which are lanceolate. The bluish purple flowers are numerous. The fruit is succulent, black when ripe, rounded and about 4mm in diameter [12]. Plant extracts of *Vitex negundo* possess different activities like anti-microbial, anti inflammatory, anti-oxidant, anti- pyretic and others. It is also used as a larvicidal, mosquito repellent and insecticidal [13]. Caryophyllene, ledol, phytol are some of the important phytochemicals to cure inflammation.[30]

Fig:1 *Vitex negundo* leaves

Solanum trilobatum Linn. belongs to the family Solanaceae, is a thorny shrub widely distributed in Bengal, Uttar Pradesh, Southern India and Srilanka in most places and it is commonly called as Thoothuvalai, Climbing Brinjal [14]. It is prickly diffuse, green perennial herb, woody at the base, 2–3 m height. The plant having much branched spiny scan dent shrubs. Leaves are deltoid or triangular, irregularly lobed. Flowers are purplish-blue, in cymes. Berry is globose, red or scarlet [15]. The extracts of *S. trilobatum* fruit in rats significantly inhibited the paw edema induced by carrageenan [16]. Solasodine is one of the steroidal glycolalkaloid compound present in the plant plays an important role to generate inflammation. The extract obtained from ethanol and methanol at usage at different doses as proved the anti-inflammatory property in the test animals [17].

Fig:2 *Solanum trilobatum* fruits

The *Abelmoschus esculentus* (Okra, bendi, kopiarab, bhindi) belongs to the family Malvaceae was first found in Former Abyssinia (Ethiopia) and is spread across a number of tropical countries, including Africa, India, South and North America, Northeastern Brazil [18]. This plant has been used to treat various disorders, amongst which inflammatory conditions [19]. Mucilage is soluble hydrophilic polysaccharides and polymers of carbohydrates with branched structures.

It consists of polyuronides and galactouronides that chemically resemble the pectic compounds, galactose, arabinose, glucose, mannose, xylose and various uronic acids are the most frequently observed component [20]. Okra is rich in Polysaccharides, Vitamins, calcium, potassium and other mineral matters. The extract of *A. esculentus* vegetable, also known as okra gum, has been efficiently used as a lubricant and to stabilize foams and suspensions [21].

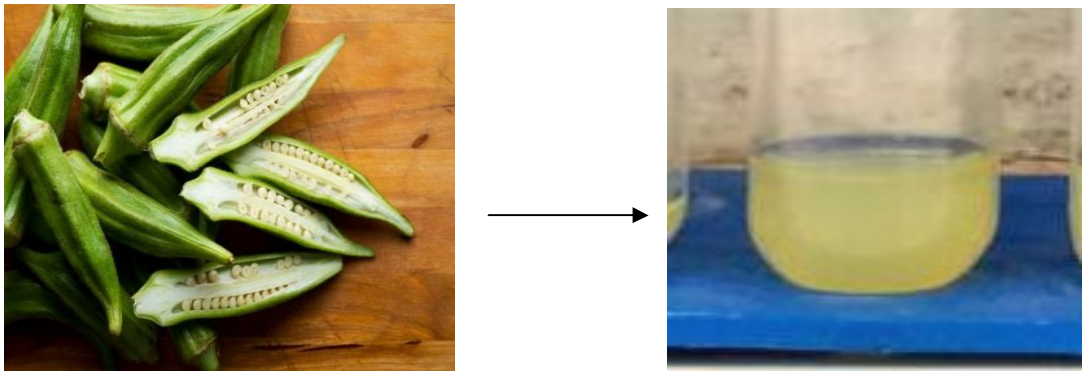

Fig: 3 *Abelmoschus esculentus* mucilage

II. METHODOLOGY

A. Collection of Plant Material and Authentication

Leaves of *Vitex negundo* were collected from the local area of Madurai, fruits of *Solanum trilobatum* were collected from local area of Coimbatore and vegetables of *Abelmoschus esculentus* were purchased from the market of Coimbatore. These plant samples were submitted to Botanical Survey of India TNAU and authenticated.

B. Preparation of Plant Extracts

1) *Soxhlet Extraction of Vitex negundo Leaves:* The leaves were cleaned and dried under sunlight for 10 days. The dried leaves were powdered and successfully extracted with the methanol for 24 hours. The extract was air dried at room temperature and sample was collected.
2) *Soxhlet Extraction of Solanum trilobatum fruit:* Fruit of *Solanum trilobatum* was dried under sunlight for 10days and it was powdered. The coarse powder of dried fruit of *Solanum trilobatum* was extracted in Soxhlet apparatus with methanol for 24 hr. The extract was air dried at room temperature and sample was collected
3) *Extraction of mucilage from vegetable of Abelmoschus esculentus:* The 1kg of *Abelmoschus esculentus* vegetables are cleaned and dried under sunlight for 15 days. Then it was cut into small pieces and weighed in a beaker containing distilled water. It was heated in a heating mantle with continuous stirring at 60°C for 1hr. Then it was filtered using muslin cloth and mucilage was collected.

C. Phytochemical Analysis: [22, 23]

Phytochemical analysis were performed qualitatively to identify the chemical composition of different sample extracts employed by precipitation and coloration reactions to assess the major secondary metabolites like alkaloids, glycosides, steroids, tannins and terpenoides. To identify the chemical constituents of different sample extracts by standard procedures have been followed. The extract was qualitatively tested for the presence of chemical constituents using the following reagents and chemicals.

1) *Test for Alkaloids (Wagner's test):* 1.5% hydrochloric acid was added in 1ml of extract and few drops of wagner's reagent were added to it. Appearance of yellow/ brown precipitate indicates the presence of alkaloids.
2) *Test for Flavonoids:* 5 mL of dilute ammonia solution were added to a portion of the extract followed by addition of concentrated H_2SO_4. Formation of a yellow colouration in the extract indicates the presence of flavonoids. The yellow coloration disappears after some time.
3) *Test for Proteins (Biuret test):* To the extract, 1% of Sodium hydroxide and 2-3 drops of copper sulphate solution were added. Presence of red violet color indicated the presence of proteins.
4) *Test for Tannins:* To 1ml of extract, 2ml of 5% ferric chloride were added and the presence of dark blue indicates a positive tannin test.
5) *Test for Steroids (Salkowski Test):* Few drops of chloroform and sulphuric acid have been added to the extract which gives the bluish red to cherry colour in chloroform indicates the presence of steroids.

6) *Test for Saponins (Foam test):* A few ml of extract was taken in a test tube and shaken vigorously with a small amount of water. If a stable, characteristic froth was obtained, saponins were present.
7) *Test for Anthrocyanin:* 1ml of extract was treated with 2N sodium hydroxide then heated. Formation of bluish green color indicated the presence of anthrocyanins.
8) *Test for Glycosides:* Add 2 ml of acetic acid and 2ml of chloroform with the plant extract. The mixtures were then cooled and add concentrated H_2SO_4. The appearance of green color indicates the presence of glycosides.
9) *Test for Phenols:* Add 3 to 4 drops of 5% Ferric chloride solution has been added to the extracts which forms the bluish black colours precipitate, indicating the presence of phenols.
10) *Test for Coumarins:* To the extract 10% NaOH was added, and chloroform was added. Yellow colour formation indicates the presence of coumarins.
11) *Test for Terpenoids:* 2 ml of Chloroform and 3ml of concentrated Sulphuric acid was added to 5ml of extract which forms a monolayer of reddish brown coloration interface which is positive to terpenoids.

D. Anti Bacterial Activity

The antibacterial activity of the extract was assessed using well diffusion. Agar plates were prepared first using agar nutrient medium. It was then poured into a petridish. A suspension of the microorganisms (*Staphylococcus aureus)* was uniformly swabbed on agar plates using sterile cotton swabs. The mucilage and methanolic extract of samples were done by well diffusion method. The plates were inverted and incubated at 37°C for 24 hr. The antibacterial activity was evaluated by measuring the diameter of the resulting zone of inhibition against the tested microorganisms in centimeters. [24]

E. Formulation Of Cream Base

The cream base was prepared by the addition of aqueous phase to the oily phase with agitation. The oily phases contain Cetostearyl alcohol (4gm), liquid paraffin oil (1ml), Bee wax (3gm) and Stearic acid (3.5 gm) was heated up to 80°C. At the same time aqueous phase consists of distilled water (q.s), Glycerine (3ml), Sodium benzoate (0.2gm) and Triethanolamine (1ml) was heated to the same temperature. The aqueous phase and oily phase were mixed at same temperature. [9, 25]

F. Formulation Of Cream Base With Samples

The cream were prepared by mixing accurate weighed extracts of mucilage from *Abelmoschus esculentus* vegetable, air dried methanol extracts of *Vitex negundo* leaves and fruit of *Solanum trilobatum* were put in mortar and add the cream base bit by bit and then cream was crushed until homogenize and finally transferred in a suitable container.

Table:1

Components	Role
Cetostearyl alcohol	Surfactant
Liquid paraffin oil	Moisturizer
Bee wax	Thickener, Demulcent
Stearic acid	Coolant, Stabilizer
Glycerine	Emollients, protects skin from infections
Triethanolamine	pH balancer
Distilled water	Vehicle
Sodium benzoate	Preservative
Vitex negundo leaves	Active ingredient, Anti inflammatory property, anti bacterial activity
Solanum trilobatum fruit	Active ingredient, Anti inflammatory, Anti bacterial property
Abelmoschus esculentus mucilage	Active ingredient, Lubricating agent, moisturizer, and anti inflammatory property

G. Evaluation of Cream

1) Spreadability: The spreadability can be determined by placing a 2gm of cream between two glass slides and it was compressed to uniform thickness by placing a definite weight for definite volume. Spreadability was then calculated by using formula,

$$\text{Spreadability} = m \times l/t$$

Where,

m = Weight tide to upper slide
l = length moved on the glass slide
t = time taken.

2) Extrudability: The formulated cream were filled into the tube and sealed. The tubes were pressed to extrude the material and extrudability of the formulation was checked.

3) Homogeneity: The formulation was tested for the homogeneity by visual appearance and by touch.

4) Appearance: The appearance of the cream was judged by its color, roughness and graded.

5) Color and Odour: The color and odour of cream can be examined visually.

6) Solubility: The solubility test should be carried out in boiling water, ether and chloroform.

7) Washability: Cream was applied in skin and the ease extend of washing with water was carried out.

8) LOD Test: Loss on drying is a moisture test it can be done by placing a cream in petri dish on water bath at 105°C temperature.

9) Stability study: Stability study was carried out in various temperature conditions to check the stability of the cream.

10) Skin Irritancy Test: Skin irritancy test was carried out by applying small amount in the skin of human and the effect was observed.

III. EXPERIMENTAL RESULTS

A. Phytochemical Analysis

The phytochemical analysis of methanolic extract of *Vitex negundo* leaves, fruits of *Solanum trilobatum* and mucilage of *Abelmoschus esculentus* were carried out and it shows the presence of different bioactive compounds. Bioactive compounds are two types primary and secondary metabolites. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain glycosides, steroids, tannins terpenoids and alkaloids. Presence of these phytochemicals was analyzed by the qualitative test which showed the following results. (Table: 1) This is supporteby various researchers who have also elucidated that some phytochemicals present in the extracts [26,27].

Table 2: Phytochemical analysis

Phytochemical tests	Vitex negundo (leaves) (methanolic extract)	Solanum trilobatum (fruit) (methanolic extract)	Abelmoschus esculentus Mucilage (Water extract)
Alkaloids	–	+	+
Flavonoids	+	+	–
Saponins	–	–	+
Phenols	+	+	+
Proteins	+	+	+
Glycosides	+	+	–
Coumarins	–	–	+
Steroids	+	+	+
Terpenoids	+	+	+
Anthrocyanin	–	–	–
Tannins	–	+	+

B. In vitro Antibacterial activity

Antimicrobial activity is the ability of substance to inhibit or kill bacterial diseases. Different types of antibiotics are used in the treatment of various diseases [28]. The different plants parts like leaves, barks, mucilage, seeds, roots, fruits and flowers are used in preparation of herbal drugs. These plant extracts play the vital role to fight against bacteria like human pathogens. In the present investigation, the antibacterial activity of mucilage from *Abelmoschus esculentus* and methanolic extract *Vitex negundo* leaves and fruits of *Solanum trilobatum* fruits were performed against human pathogenic bacteria (*Staphylococcus aureus*).

C. In vitro Antibacterial activity

Fig:4 Methanol extract of *Vitex negundo*

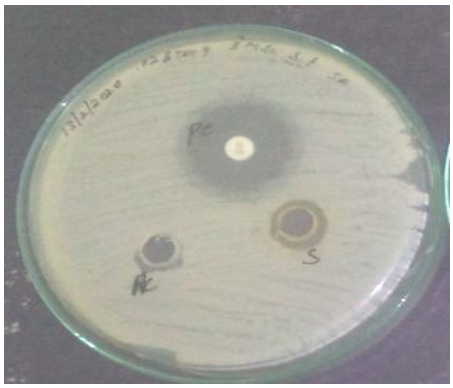

Fig:5 Methanol extract of *Solanum trilobatum*

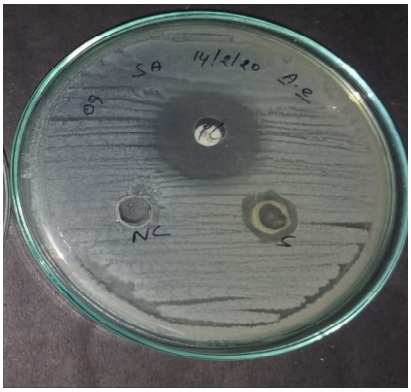

Fig:6 Mucilage of *A.esculentus*

Table: 3

S.no	Samples	Microrganism	Minimum inhibitory Concentration (cm)		
			Positive control	Negative control	Sample
1	*Vitex negundo* (100%)	*Staphylococcus aureus*	1.2	0.1	0.7
2	*Solanum trilobatum* (100%)	*Staphylococcus aureus*	1.2	0.1	0.5
3	*Abelmoschus esculentus* (100%)	*Staphylococcus aureus*	1.2	-	0.3

Positive control: Gentamycin
Negative control: 1,2- Methanol; 3- water

D. Formulation of Cream

The herbal cream was formulated by using methanolic extract of *Vitex negundo* leaves, fruits of *Solanum trilobatum* and mucilage of *Abelmoschus esculentus*. The extracts were prepared by simple method using soxhlet apparatus and methanol has a solvent to obtain a good yield of extracts and there was no harm to the chemical constituents and their activity. The *Abelmoschus esculentus* mucilage was extracted by simple maceration process using distilled water, it serves as the polymer and binder to the formulated cream and it also plays the vital role to cure inflammation. The cream was formulated by mixture of two phases oily phase and aqueous phase. The literature reported that the mucilage of *Abelmoschus esculentus* is used as a natural polymer, binding agent and humidifying properties in medicine [18].

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Fig: 7 Formulated cream

E. Evaluation of Cream

The formulated cream was evaluated to check the efficacy of cream. The pH of the cream is an important parameter because drop in pH value during storage of creams in an indication of bacterial growth. According to Bureau of Indian Standards (BSI) the skin cream should have pH 4.0 to 9.0 [29]. The stability study was carried out to check the stability of cream. It is evident from the results that cream formulation is stable in different temperatures (2°C, 25°C, 37°C). The visual appearance and odour of cream formulation was checked at the time of preparation and after several weeks. There was found no significant difference in visual appearance and odour. The skin irritancy test was carried out to check the irritancy, itching or other allergies. There was found to be no irritation, itching or any other allergies after applying to the skin of humans. The formulated cream was subjected to solubility test. The cream found to be soluble in boiling water, chloroform and ether. The pH of formulated cream is in the range of 5.6. Extrudability is a useful empirical test to measure the force required to extrude the material from tube, the desired cream can be extrude. The formulated cream showed good extrudability. So the herbal cream formulation was successfully passed all the parameters such as good consistency, good spreadability, homogeneity, pH, non greasy. The evaluation of cream was showed in the Table 3.

Table: 3 Evaluation of cream

Physiochemical parameters	Observation
Color	Pale green
Odour	Characteristic
Consistency	Smooth
Washability	Good
pH	5.6
Spreadability (seconds)	7
Solublility	Soluble in boiling water, chloroform and ether
Extrudability	Good
Skin irritancy	Non irritant
Stability study (2°C, 25°C, 37°C)	Stable
Loss of drying	30%

IV. CONCLUSION

From above discussion it is concluded that the prepared formulation showed good results. The formulated herbal cream is free from paraben, non-toxic, safe and effective which is highly acceptable. From this study, it improves the utilization of herbal extracts in small scale as well as large scale production. Further, determination of exact molecular level pharmacological investigations can be carried out to confirm its efficacy and mechanism of action.

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VI. CONFLICT OF INTEREST

There is no conflict of interest.

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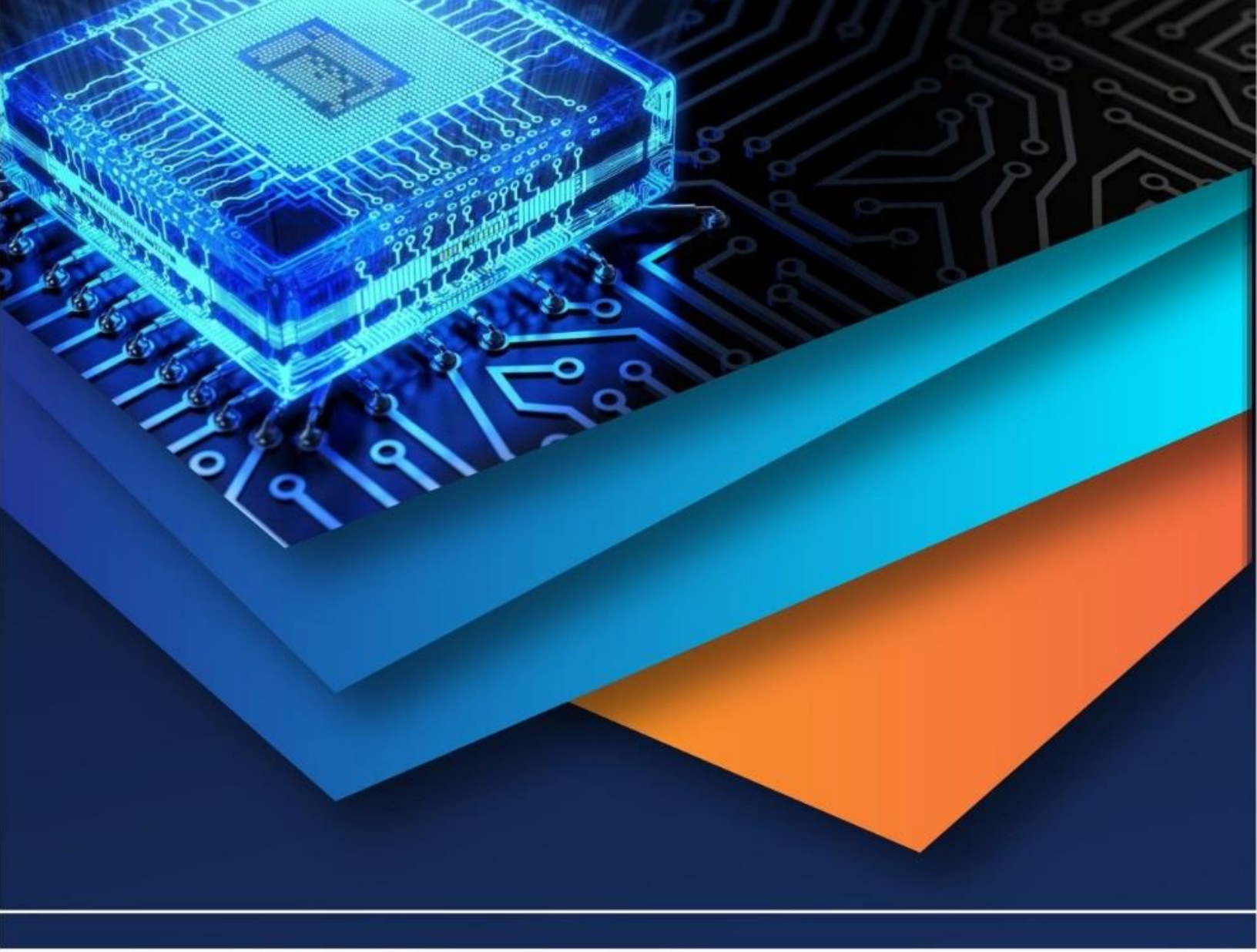

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