iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 13 **Issue:** I **Month of publication:** January 2025

DOI: https://doi.org/10.22214/ijraset.2025.66358

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue I Jan 2025- Available at www.ijraset.com

Extraction and Standardization of BioJoin Trademark Product for Joint Health and Muscle Strength

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Abstract: Boswellic acids are naturally present in gum extrudes of Boswellia serrate tree. Boswellic acids has promising activity on joint pain and muscle strength.

The study aims at developing a more potential product "BioJoin™", combining Boswellia serrata and Acacia catechu, which provides bone health, immunity support, enhanced mobility and promote joint health and pain relief. Boswellic acid, Catechins, Polyphenols enhanced with required minerals were combined to increase the efficacy of the product and designed into powder form and tablet form for consumption. The Acacia catechu extract was tested with HPLC for the catechin content and found to be not less than 40% and boswellic acid in Boswellia serrata extract was found to be not less than 65% by HPLC. Final Biojoin product was tested for total polyphenol content and was found to contain not less than 15% polyphenols which could provide benefit to the bone health and strength

Bone health is important at every age and stage of life. The skeleton is our body's storage bank for several minerals that are necessary for our bodies to function. If we do not have enough minerals in our diets to keep our bodies functioning, this causes our bones to grow weaker and leads to inflammatory responses. Loss of bone strength can lead to several bone disorders in which bones become very fragile and more likely to break. Polyphenols, a class of natural antioxidants, is found in Acacia catechu which belongs to the Fabaceae family. Catechins in Acacia catechu have shown anti-inflammatory effects by modulating inflammatory pathways. These compounds may inhibit the production of pro-inflammatory cytokines and enzymes such as COX-2 (cyclooxygenase-2), 5-LOX (5-lipoxygenase), and TNF-alpha (tumor necrosis factor-alpha), which play a role in chronic inflammation. As such, Acacia catechu extract can potentially alleviate symptoms in inflammatory conditions like arthritis, rheumatoid arthritis, and gout.

Keywords: BioJoin™, Boswellia serrata, Acacia catechu, catechins, boswellic acids, Polyphenols

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The study aims at developing a more potential product "BioJoin™", combining Boswellia serrata and Acacia catechu, which provides bone health, immunity support, enhanced mobility and promotes joint health and pain relief. Boswellic acid, Catechins, Polyphenols enhanced with required minerals were combined to increase the efficacy of the product and designed into powder form and tablet form for consumption. The Acacia catechu extract was tested with HPLC for the catechin content and found to be not less than 40% and boswellic acid in Boswellia serrata extract was found to be not less than 65% by HPLC. Final Biojoin product was tested for total polyphenol content and was found to contain not less than 15% polyphenols which could provide benefit to the bone health and strength

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I. INTRODUCTION

Bone health is the state of the bones in the body which includes their density, strength, and ability to support the body and internal organs. Maintaining strong and healthy bones is essential for overall health and mobility, especially as a person ages. Several factors influence bone health, including genetics, nutrition, physical activity, and lifestyle choices. Managing Bone Health involves a proactive approach that combines proper nutrition, physical activity, lifestyle choices, and regular health monitoring to ensure bones remain strong and healthy throughout life. Bone health is especially important as age increases, as bone density tends to decrease over time, increasing the risk of conditions such as osteoporosis, fractures, and other bone-related disorders. A plant extract product combining *Acacia catechu* and *Boswellia serrata* could offer a variety of potential health benefits, as both of these natural substances are known for their medicinal properties. Here's an overview of each plant and how their combination might work:

A. Acacia catechu (Black Catechu, Khair)

Acacia catechu now known as *Senegalia catechu* is a deciduous, thorny tree which grows up to 15 m in height, commonly found in India and Southeast Asia. Its heartwood is often used in traditional medicine, and it contains active compounds such as polyphenols (catechins), tannins, and flavonoids. Numerous researchers have studied the pharmacological properties of extracts made from different parts of *A. catechu* (involving heartwood, bark, leaves, seeds, and pods) in recent years [7,8,9,10,11,12].*A.catechu* contain very high amounts of flavonoids, tannins, and phenolic compounds, especially catechin/epicatechin, epigallocatechin, taxifolin, procyanidin, quercetin, taxifolin, etc. These active compounds such as catechin or epicatechin perform significant functions as ananti-inflammatory and antioxidant agent; likewise, tannins are found to be responsible for astringent action ions in the human body and have been considered as having good potential for curing wounds in the human body [12]. More specifically, catechin is abundant in this plant and serves as an antioxidant and antimicrobial agent [13,14]. A. catechu bark extract was explored and it was declared that *Acacia Catechu* Bark shows considerable anti-inflammatory activity. The ethanolic solvent form of Acacia Bark exhibits an inhibitory activity when examined at 200-1000 µg/ml by inhibiting denaturation of protein and comparing its effect with diclofenac sodium as a standard drug. Denaturation of protein produced autoantigen in rheumatoid arthritis[15].

B. Boswellia serrata (Frankincense)

Boswellia serrata is a tree native to India, and its gum (often referred to as frankincense) has been used for centuries in traditional medicine. The several active compounds in Boswellia are present, which have anti- inflammatory, analgesic, and antioxidant properties. Studies have been carried out by researchers and have noted that Boswellic acid is the active ingredient in *Boswellia serrata*, and several research indicate that 3-O- Acetyl-11-keto-beta-boswellic acid (AKBA) is a boswellic acid with strong pharmacological activity.

AKBA has been found to have powerful inhibitory effects on 5-lipoxygenase. Minor adverse side effects include nausea, headache, abdominal pain, diarrhoea, fever, and general weaknes were noticed. Systematic review of studies showed large and clinically important treatment effects with SMD -1.61 (95% CI
−2.10, −1.13) for pain relief.[5] But it must be noted the quality of evidence is low. Another systematic review and meta-analysis of 545 participants showed the potential for Boswellia to relieve pain and function (VAS pain weighted mean difference (WMD) -8.33 (95% CI -11.19, −5.46)); WOMAC pain WMD -14.22 (95% CI -22.34, −6.09) along with improvements in stiffness and joint function.[6] Based on current evidence, the recommended duration of treatment is around 4 weeks with at least 100–250 mg daily of Boswellia. [4]

C. Combining effects of Boswellia serrata and Acacia catechu

Combined effects of Boswellia and Acacia extracts for bone health could be beneficial due to the complementary properties of each plant that target various factors related to bone maintenance, joint function, and inflammation.

The synergy of these two plant extracts makes them a powerful combination for supporting overall bone health and preventing or managing bone-related issues.

D. Anti-Inflammatory Benefits for Joint and Bone Health

Chronic inflammation is a major contributor to bone and joint issues, especially in conditions like osteoarthritis, rheumatoid arthritis, and other degenerative bone diseases. Both ***Boswellia serrata*** and ***Acacia catechu*** have potent anti-inflammatory properties that can help address this inflammation.

1) *Boswellia Serrata*: The active compounds in Boswellia, particularly boswellic acids, are well-known for their powerful anti-inflammatory effects. These compounds can inhibit the production of pro-inflammatory enzymes like 5- lipoxygenase, which play a key role in the inflammatory process. By reducing inflammation, Boswellia can help protect the cartilage and bone tissue from further degradation, improve joint mobility, and reduce pain associated with conditions like arthritis.
2) *Acacia Catechu*: *Acacia catechu* also possesses anti-inflammatory effects due to its catechins and tannins. While it may not be as well-researched for bone health specifically, its ability to reduce inflammation could help reduce joint pain and discomfort related to bone diseases. This could make it a useful addition to a formula aimed at reducing inflammatory processes in the body.

E. Improved Joint Function and Mobility

Inflammatory diseases such as arthritis lead to the breakdown of cartilage and the eventual damage of the bone tissues in the joints. By targeting inflammation, both *Boswellia serrata* and *Acacia catechu* can help slow down or prevent this process.

1) *Boswellia serrata* has shown positive effects on the improvement of joint mobility, especially in people with osteoarthritis and rheumatoid arthritis. It helps protect against cartilage destruction and stimulates the production of synovial fluid, which lubricates the joints and keeps the bones in optimal contact. This helps reduce stiffness and pain, improving overall joint function.
2) *Acacia catechu* may aid in improving circulation and blood flow to the joints, supporting their nutrition and helping to prevent joint stiffness.

F. Bone Health and Osteoporosis Prevention

Both *Boswellia serrata* and *Acacia catechu* can offer indirect support for bone health by addressing underlying inflammation and promoting the healing of tissues.

1) *Boswellia serrata* has been shown to support bone regeneration and remodelling by influencing the NF-kB signalling pathway (a pathway associated with inflammation and immune responses). This pathway also plays a role in bone metabolism, and Boswellia's effect on it could help maintain bone density and prevent bone loss, which is critical in osteoporosis.
2) *Acacia catechu's* antioxidant properties may help in reducing oxidative stress, which is a known factor in the deterioration of bones and joints. Oxidative stress can lead to bone resorption and osteoporosis, so by reducing oxidative damage, *Acacia catechu* may help preserve bone density and strength.

G. Pain Relief and Analgesic Properties

Chronic pain, particularly from arthritis or bone-related issues, can significantly affect quality of life. Both Boswellia and *Acacia catechu* have analgesic (pain-relieving) properties.

1) *Boswellia serrata* is commonly used as a natural alternative to NSAIDs (non-steroidal anti-inflammatory drugs) for pain relief. Its ability to reduce the production of inflammatory molecules can help decrease the pain associated with inflammation in the bones and joints.
2) *Acacia catechu* also has mild analgesic properties, often used in traditional medicine to relieve pain and swelling. This can complement Boswellia's effects, offering additional pain management benefits.

H. Antioxidant Support for Bone Health

The balance between oxidative stress and antioxidants is crucial for maintaining bone health. Chronic oxidative stress can lead to bone resorption (the breakdown of bone tissue), contributing to conditions like osteoporosis.

1) *Boswellia serrata* contains natural antioxidants like **boswellic acids**, which help to neutralize free radicals and protect bone cells from oxidative damage. This may play a role in protecting bone cells (osteoblasts) and cartilage cells (chondrocytes) from oxidative damage.
2) *Acacia catechu* also contains powerful antioxidants (such as **flavonoids** and **catechins**) that can help combat oxidative damage. By supporting antioxidant defences in the body, *Acacia catechu* can work synergistically with Boswellia to protect bone and joint tissues.

I. Supporting Healthy Bone Remodelling

Bone remodelling is a continuous process where old bone tissue is replaced with new bone. This process is critical to maintaining bone density and strength throughout life. The combination of Boswellia and *Acacia catechu* could support this process:

1) *Boswellia serrata* has been shown to positively influence bone formation and inhibit excessive bone resorption, supporting the balance between bone resorption and formation that is vital for maintaining bone strength.
2) *Acacia catechu*, while not as directly linked to bone remodelling in research, has beneficial effects on tissue repair, wound healing, and may help maintain the integrity of bone tissue through its anti-inflammatory and antioxidant actions.

II. METHODOLOGY/EXPERIMENTAL DETAILS

A. Extraction of Boswellia serrata

Dirt and dust particles were removed from the *Boswellia serrata* exudates. It was washed and sun dried and kept for extraction with ethanol at 60-65°C for six hours. The process was repeated thrice. The ethanol layer was separated during each process and all the ethanol layers were combined and filtered through a Nutche filter(5µ). This filter was concentrated at 60-65°C, ~ 630mm of Hg for 7-10 hours. KOH solution was added to the ethanol extract paste and pH was adjusted t0 5 with HCl. After discarding the precipitate, pH was again adjusted to 2 with HCl. The precipitate was again filtered through Nutche filter(5µ). *Boswellia serrata* crude extract was obtained. This crude extract was then washed with water for two time, dried at 55ºC for 10 hours, >600mmHg, pulverized and sieved through 40 mesh. This was then passed through Magnetic separator (11500 Gauss to obtain the final *Boswellia serrata* extract.

B. Extraction of Acacia Catechu

Raw material of *Acacia Catechu* stem was cleaned thoroughly and kept for extraction using mix of ethanol and water. The filteration was done by passing through the Nutche filter(5µ).). This filter was concentrated at 60-65°C, ~ 630mm of Hg for 7- 10 hours. The concentrate was further subjected to drying, powdering and blending to obtain the final *Acacia catechu* extract.

C. Procedure for Preparing BioJoin™

The Boswellia extract containing 65% boswellic acid , Acacia extract 40% catechins and other excipients were all cleared of the foreign particles. All the material were blended together in required proportion. The Blended material was then passed through the sieve to get uniform fine flowing powder. This material was further added with the binders and dry mixing was carried out. Further wet mixing was carried out at required temperature and punched in the machine to form the tablets.

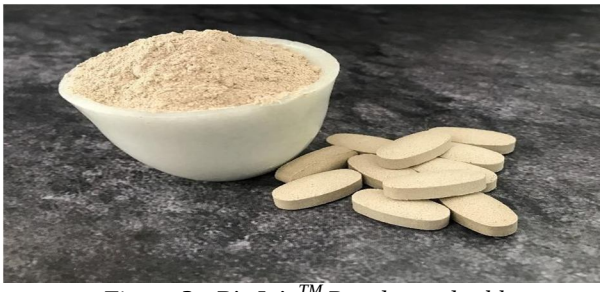

Figure 3 : *BioJoin™ Powder and tablets*

D. HPLC Analysis of Catechin

Mobile Phase-A consists of mixture of 750ml of Double distilled water, 0.75 ml of formic acid and 250ml of HPLC grade Acetonitrile. Mobile Phase-B consists of 900ml of double distilled water and 0.9ml of formic acid and 100ml of HPLC grade Acetonitrile. The Total Run time of the analysis is 25 minutes and wavelength of detection of Catechin is 280 nm. Total flow rate is 1.5ml/min, with injection volume of 20 µl.

Standard preparation: 5mg of working standard dissolved in 10ml methanol with sonication for 5 mins.

Sample preparation: 5mg of sample dissolved in 10ml methanol with gentle heating and sonication for 5 mins. Before injection sample was passed through a filter of 0.45µm pore size.

E. HPLC Analysis of Boswellic acid

A Shimadzu P-series HPLC with auto-sampler was operated via Lab solutions software to run the test. The machine includes a UV detector as well as PDA (Photo-Diode array) detector with a 5 µm C-18, shim-pack column of dimensions 1.6-mm x 25-cm. The Gradient mobile phase consisted of mixture of acetonitrile, water and glacial acetic acid (900:100:0.1). The Total Run time of the analysis is 45 minutes and wavelength of detection of Boswellic acid is at 210 nm. Total flow rate is 1.0ml/min, with injection volume of 20 µl.

F. Polyphenol Analysis of BioJoin™ Reagents

1) 10.75% Sodium Carbonate Solution: Accurately weigh 10.75gm of sodium carbonate in 100ml Volumetric flask make up the to 100ml with water.
2) Folin-Ciocalteu reagent: Accurately take 5ml of FCR and make up the volume to 10ml with water.
3) Gallic Acid 99% Standard

G. Sample Preparation

Weigh 25mg of sample in 50 ml volumetric flask and make up the volume with water to make stock solution. 2ml from the sample stock solution in 50 ml flask and add 5 ml of FCR solution and wait for 1 min.. Complete the volume with 10.75% of sodium carbonate solution and keep it standing for 1 Hr. Take the absorbance at 760nm after 1 hour. using the given blank solution and using gallic acid as the standard.

H. Calculation

Polyphenol $conte$: $$\frac{Abs\square\square rbance\ of\ sample \times Weight\ of\ Working\ Std \times Purity\ of\ working\ std}{Absorbance\ of\ Working\ Std \times Weight\ of\ sample}$$

III.RESULTS AND DISCUSSION

After extraction of *Acacia catechu* and *Boswellia serrata*, standard parameters were tested and results were noted as mentioned in Table 1 & Table 2 to check the physical parameters and chemical content before further taking them for production of BioJoin™.

TABLE I
RESULT FOR *ACACIA CATECHU* 40% EXTRACT

SR. NO.	TEST PARAMETER	RESULT
1.	Appearance	Off white to light brown color* powder
2.	Odour & Taste	Characteristic odor and taste
3.	Loss on drying	Not more than 7.0%w/w
4.	Untapped bulk density	Between 0.30 to 0.60g/ml
5.	Tapped bulk density	Between 0.40 to 0.80g/ml
6.	Particle size	100% passing through 40 mesh
7.	Content of Catechins by HPLC	Not less than 40.0% w/w

TABLE II
RESULT FOR *BOSWELLIA SERRATA* 65% EXTRACT

SR. NO.	TEST PARAMETER	RESULT
1.	Appearance	Creamish yellow colour powder
2.	Odour & Taste	Characteristic odor
3.	Loss on drying	Not more than 5.0% w/w
4.	Untapped bulk density	Between 0.30 to 0.60g/ml
5.	Tapped bulk density	Between 0.40 to 0.80g/ml
6.	Particle size	NLT 100% passing through 40 mesh
7.	Content of Boswellic acid by HPLC	Not less than 65.0% w/w

RESULT FOR BioJoin™

SR. NO.	TEST PARAMETER	RESULT
1.	Appearance	Off white to white colour powder
2.	Odour & Taste	Characteristic odor
3.	Loss on drying	Not more than 5.0% w/w
4.	Untapped bulk density	Between 0.30 to 0.50g/ml
5.	Tapped bulk density	Between 0.40 to 0.80g/ml
6.	Particle size	NLT 80% passing through 80 mesh
7.	Content of Polyphenol by UV	Not less than 15.0% w/w

The Final product Bio Join™ after final production was tested for the contents of polyphenol by UV which is one of the main ingredients for improvement of the bone health. Polyphenol content was found to be more than 15% which is useful for bone health. The synergy of both *Acacia Catechu* and *Boswellia serrata* are expected to provide an anti-inflammatory effect and provide relief with osteoarthritis and joint function.

IV. CONCLUSIONS

Bio Join™ has been developed by Bio-Med Ingredients and it contains active constituents which are known to biologically act as anti-inflammatory, effective to Improve Joint Function and Mobility, Bone Health and Osteoporosis Prevention, Pain Relief and Analgesic Properties, Antioxidant Support for Bone Health, Supporting Healthy Bone Remodelling.

This product is used to promote bone health and immune support, enhance mobility and provide pain relief in case of inflammation or arthritis. Although several secondary metabolites have been identified from A. catechu, the molecules, catechin, epicatechin, and quercetin, are the principal contributor to therapeutical properties. Nowadays, plant-based secondary metabolites are extensively used in the management of various infectious diseases and achieved clinical benefits in the health care system. . As suggested by several clinical trials Catechin may inhibit the activities of cyclooxygenase-2 (COX-2), lipoxygenase (5-LOX), and platelet phospholipase A2. It may also suppress the migration of T-cells and inhibit the production of nitric oxide (NO) and prostaglandin E2. Combination of *Acacia catechu* and *Boswellia serrata* provides with active ingredients like polyphenols, tannins, flavonoids boswellic acid, AKBA which put together provide a synergetic effect on the inflammatory response, anti arthritic effects, reduce bone and cartilage degradation by inhibiting catabolic proinflammatory pathways, potentially preventing joint cartilage damage. These metabolites together also address the cascade of effects like COX 1 & 2 enzyme activity, 5-LOX enzyme activity , Oxidative stress , Reactive Oxygen Species (ROS), Free radical production, Messenger compounds, including prostaglandins which may be caused by failure in joint health.

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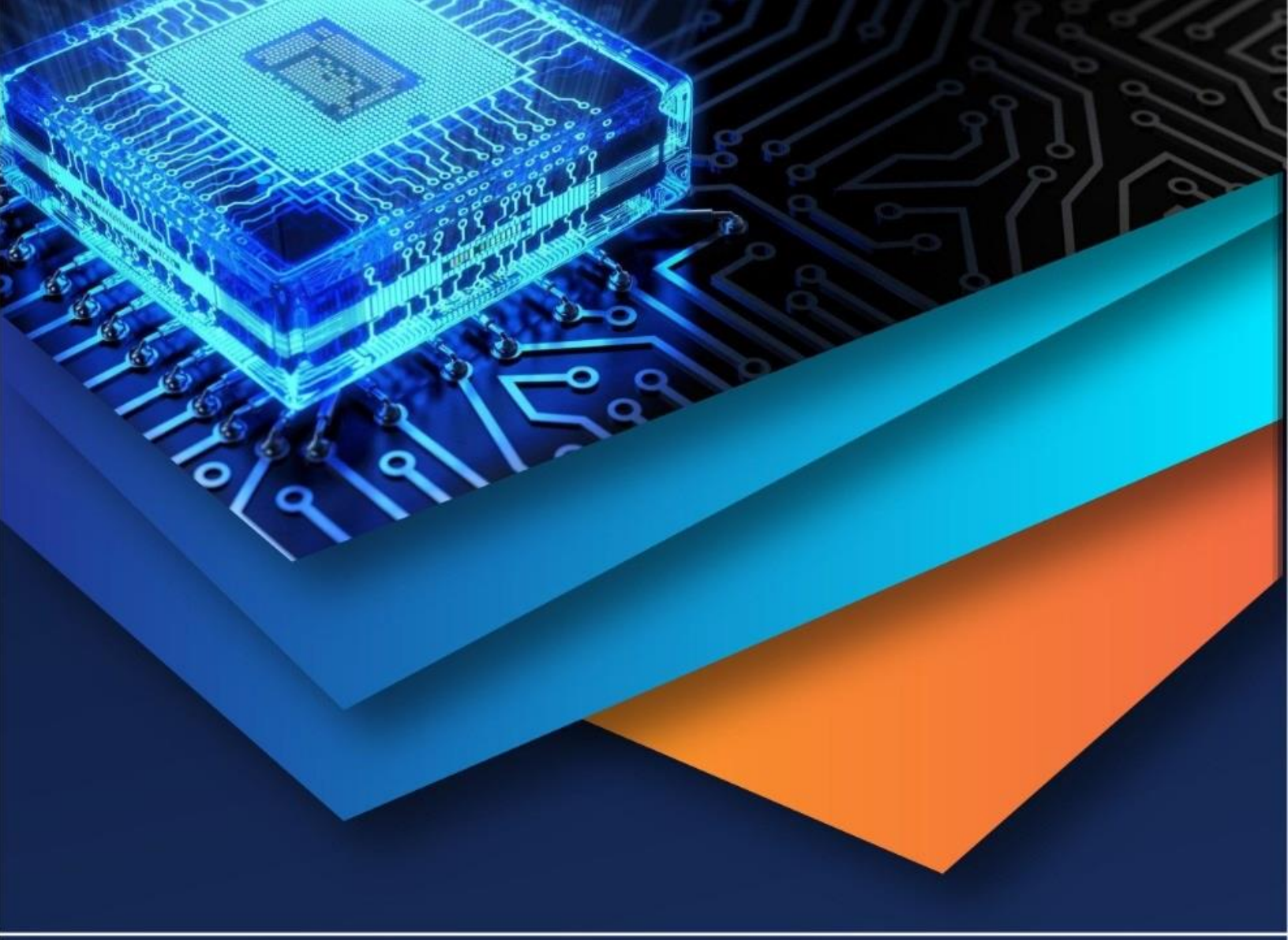

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