iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 5 **Issue:** VI **Month of publication:** June 2017

DOI:

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Volume 5 Issue VI, June 2017
ISSN: 2321-9653
International Journal for Research in Applied Science & Engineering Technology (IJRASET)

Analysis the Phytoconstituents and Anti-Inflammatory Potentials of Indian Medicinal Plant Anethum Graveolens L

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***Abstract:** Unlike modern allopathic drugs which are single active components that target one specific pathway, herbal medicines work in a way that depends on an orchestral approach. The use of herbal medicines becoming popular due to toxicity and side-effects of allopathic medicines. Medicinal plants play an important role in the development of potent therapeutic agents. The present study is to analysis the phytoconstituents and anti-inflammatory potentials of Indian medicinal plant Anethum graveolens L. This is followed with sequential extraction using Hexane, Ethyl acetate and Methanol then the extracts were qualitatively analysed for the phytoconstituents and also to undrstand the anti-inflammatory potentials by HRBC assay.*
Key words-Anethum graveolens L, phytoconstituents, methanol, anti-inflammatory and HRBC assay.

I. INTRODUCTION

A. *Herbal medicine*

Medicinal plants represent a rich source of antimicrobial agents. Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the local flora for antibacterial and antifungal activity. For thousand years ago, the medical knowledge of the Indian subcontinent was termed as Ayurveda. It remains an important system of medicine and drug therapy in India. Plant alkaloids are the primary active ingredients of Ayurvedic drugs. Today the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. Only a certain percentage of plants are used in traditional medicines. A plant contains a multitude of different molecules that act synergistically on targeted elements of the complex cellular pathway [9]. Medicinal plants have been source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions [4].

B. *Anethum graveolens l.*

Anethum graveolens L. (dill) has been used in ayurvedic medicines since ancient times and it is a popular herb widely used as a spice and also yields essential oil. It is an aromatic and annual herb of apiaceae family. The Ayurvedic uses of dill seeds are carminative, stomachic and diuretic. There are various volatile components of dill seeds and herb; carvone being the predominant odorant of dill seed and α-phellandrene, limonene, dill ether, myristicin are the most important odorants of dill herb. Other compounds isolated from seeds are coumarins, flavonoids, phenolic acids and steroids.

It is used in Unani medicine in colic, digestive problem and also in gripe water [6]. *Anethum graveolens* L. is used in the preparations of more than 56 ayurvedic preparations, which include Dasmoolarishtam, Dhanwanthararishtam, Mrithasanjeevani, Saraswatharishtam, Gugguluthiktaquatham, Maharasnadi kashayam, Dhanwantharam quatham and so on [8]. *Anethum graveolens* L. (dill) believed to be the native of South-west Asia or South-east Europe [2].

Anethum seeds are used as a spice and its fresh and dried leaves called dill weed are used as condiment and tea. The aromatic herb is commonly used for flavoring and seasoning of various foods such as pickles, salads, sauces and soups [5]. Fresh or dried leaves are used for boiled or fried meats and fish, in sandwiches and fish sauces. It is used in perfumery to aromatize detergents and soaps and as a substitute for caraway oil [7].

Anethum is used as a preservative as it inhibits the growth of several bacteria like Staphylococcus, Streptococcus, Escherichia coli and Pseudomonas. Compounds of dill when added to insecticides have increased the effectiveness of insecticides. Essential oil of A. graveolens L. is used as repellent and toxic to growing larvae and adults of *Tribolium castaneum*, wheat flour insect pest. In doses of 60 minims, anethole is a fairly potent vermicide for hookworm [3].

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Anethum grows up to 90 cm tall, with slender stems and alternate leaves finally divided three or four times into pinnate sections slightly broader than similar leaves of fennel. The yellow flower develops into umbels [10]. The seeds are not true seeds. They are the halves of very small, dry fruits called schizocarps. Dill fruits are oval, compressed, winged about one-tenth inch wide, with three longitudinal ridges on the back and three dark lines or oil cells (vittae) between them and two on the flat surface. The taste of the fruits somewhat resembles caraway. The seeds are smaller, flatter and lighter than caraway and have a pleasant aromatic odor. Qualitative phytochemical analysis of the crude powder of plant parts collected was determined. The phytochemical screening of plant showed that leaves, stems and roots were rich in tannins, terpenoids, cardiac glycosides and flavonoids [1].

II. MATERIALS AND METHODS

A. *Sample Collection And Preparation*

The disease free, fresh leaves of *Anethum graveolens* L. was collected from the herbal garden, Biozone Research Technologies Pvt. Ltd., Chennai. Seeds were obtained from a commercial vendor. The seeds were powdered and stored. Leaves were washed thoroughly and stored at 4^{o}C.

B. *Sequential Extraction*

The seed powder was mixed with solvent Hexane in the ratio 1:3 and left overnight for cold percolation of secondary metabolites. The mixture was then filtered using whatmann filter paper and the filtrate was collected. The residue was then mixed with ethyl acetate solvent and left overnight. This process was repeated for methanol solvent. The filtrates were then dried under pressure and stored at 4^{o}C till use.

C. *Phytochemical Tests*

1) *Test for carbohydrates:* To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.
2) *Test for tannins:* To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.
3) *Test for saponins:* To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.
4) *Test for flavonoids:* To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.
5) *Test for alkaloids:* To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.
6) *Test for Quinone's:* To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of Quinone's.
7) *Test for glycosides:* To 2ml of plant extract, 3ml of choloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.
8) Test for cardiac glycosides: To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.
9) *Test for terpenoids:* To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.
10) *Test for phenols:* To 1ml of the extract, a few drops of Phenol Ciocalteau's reagent was added followed by few drops of 15% Sodium carbonate solution. Formation of blue or green color indicates presence of phenols.
11) *Test for coumarins:* To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins
12) *Steroids and phytosteroids:* To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids
13) *Phlobatannins:* To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins
14) *Anthraquinones:* To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate

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Volume 5 Issue VI, June 2017
ISSN: 2321-9653
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indicates the presence of anthraquinone

15) *Hypotonicity induced human red blood cell (HRBC) membrane stabilization method:*1.0 mL of test sample of different concentrations (50 – 200 µg) in 1 ml of 0.2 M phosphate buffer and 0.5 mL of 10% HRBC suspension, 0.5 ml of 0.25 % hyposaline were incubated at 370C for 30 min and centrifuged at 3,000 rpm for 20 min and the haemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac was used as standard and a control was prepared by distilled water instead of hypo saline to produce 100 % hemolysis without samples. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the following formula:
% of Hemolysis = (Optical density of test sample / Optical density of control) X 100 % Protection = 100 – [(Optical density of test sample / Optical density of control) X 100]

III. RESULTS AND DISCUSSION

A. *Qualitative Analysis*

The qualitative analysis of phytoconstituents in the hexane plant extracts showed the presence of carbohydrates, tannins, saponins, cardiac glycosides while flavonoids, alkaloids, quinones, glycosides, terpenoids, phenols, coumarins, steroids and phytosteroids, phlobatannins and anthraquinones were found to be absent.

Further, carbohydrates, tannins, flavonoids, alkaloids, quinones and phenols were found to be present in the ethyl acetate extract while saponins, glycosides, cardiac glycosides, terpenoids, coumarins, and anthraquinones were not present. Finally, Methanol extract of the seeds showed the presence of carbohydrates, flavonoids, alkaloids and phenols while the other phytoconstituents were found to be absent. The result were shown in the table 1. The colour of phytochemicals compound were shown in Fig. 1- Fig. 3.

B. *Qualitative Analysis*

Table1: Show the presence of phytochemical compound

S.no	Phytochemical tests	Leaf sample		
		Hexane extract	Ethyl acetate extract	Methanol extract
1	Carbohydrates test	+	+	+
2	Tannins test	+	+	-
3	Saponins test	+	-	-
4	Flavonoids test	-	+	+
5	Alkaloid test	-	+	+
6	Quinones test	-	+	-
7	Glycosides test	-	-	-
8	Cardiac glycosides test	+	-	-
9	Terpenoids test	-	-	-
10	Phenols test	-	+	+
11	Coumarins test	-	-	-
12	Steroids &phytosteroids	-	-	-
13	Phlobatannins test	-	-	-
14	Anthraquinones test	-	-	-

+ Present , - Absent

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Volume 5 Issue VI, June 2017
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C. HEXANE EXTRACT

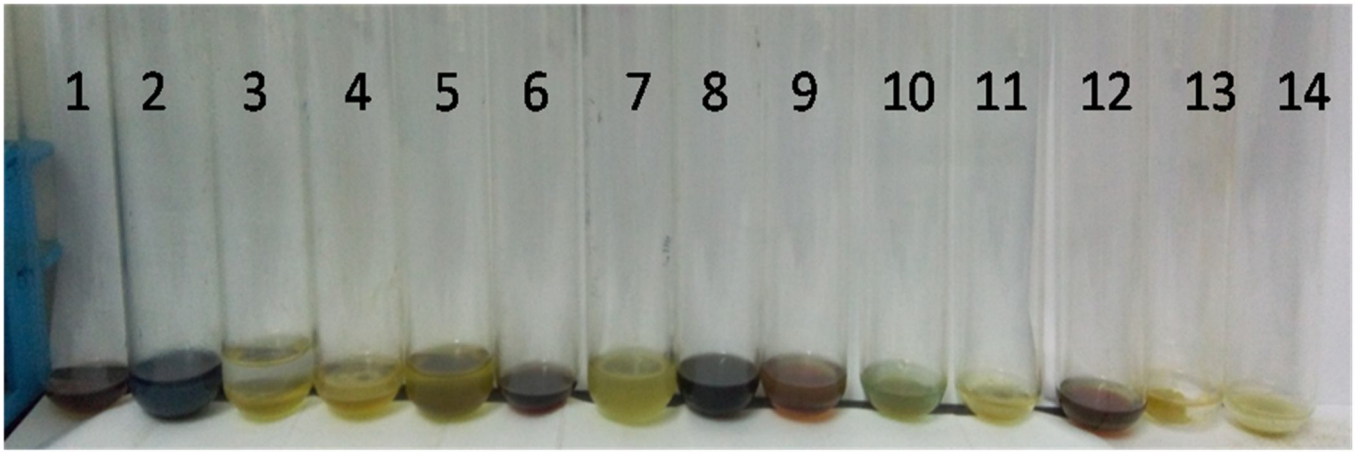

Fig. 1 Colour indicate the presence of carbohydrate, tannins, saponins and cardiac glycosides in hexane extract.

D. ETHYL ACETATE EXTRACT

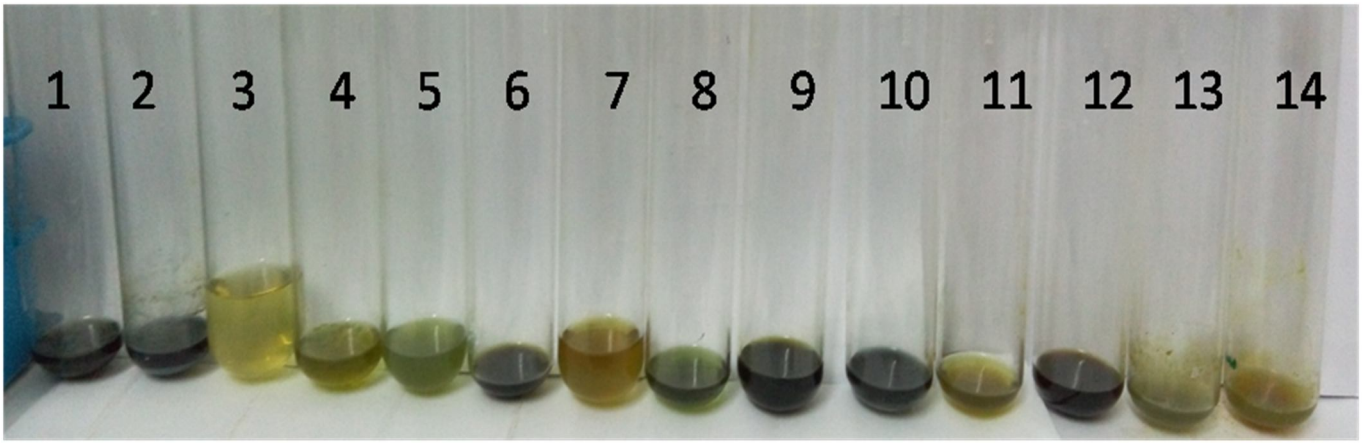

Fig. 2 Colour indicate the presence of carbohydrate, tannins, flavonoids, alkaloid, quinones and phenols in ethyl acetate extract.

E. METHANOL EXTRACT

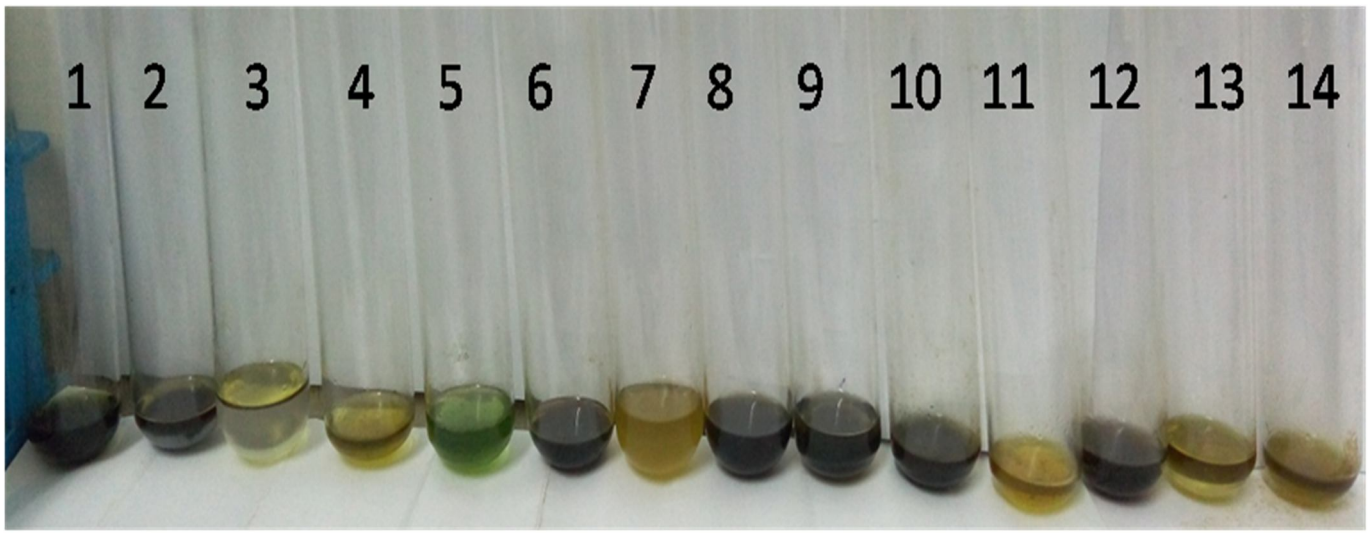

Fig. 3 Colour indicate the presence of carbohydrate, flavonoids, alkaloid and phenols in methanol extract.

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Volume 5 Issue VI, June 2017
ISSN: 2321-9653
International Journal for Research in Applied Science & Engineering Technology (IJRASET)

F. HRBC method

Hypotonicity induced human red blood cell (HRBC) membrane stabilization. The plant extracts displayed significant anti-inflammatory property when analysed using the HRBC method. The percentage of haemolysis was found to be 36% in the methanol extract of A.graveolens seeds. The result were plot in the graph as shown in Fig. 4 and Fig. 5.

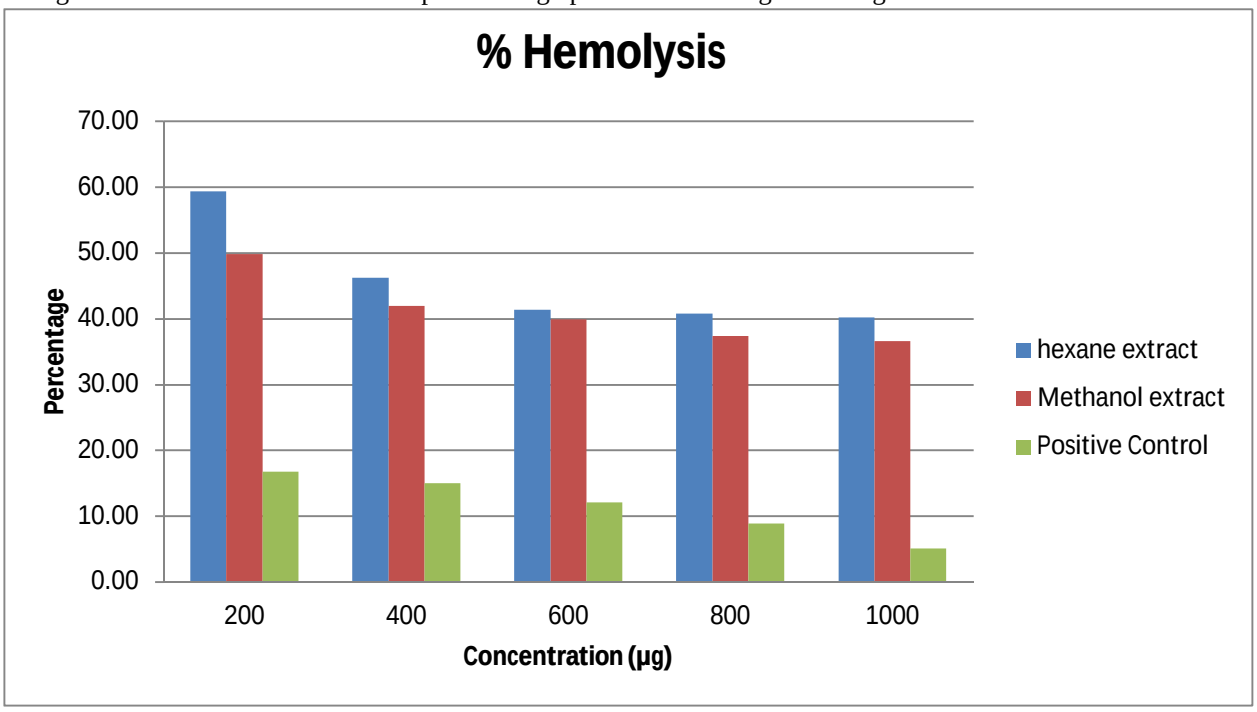

Fig. 4 showing percentage protection activities of Samples and Positive control

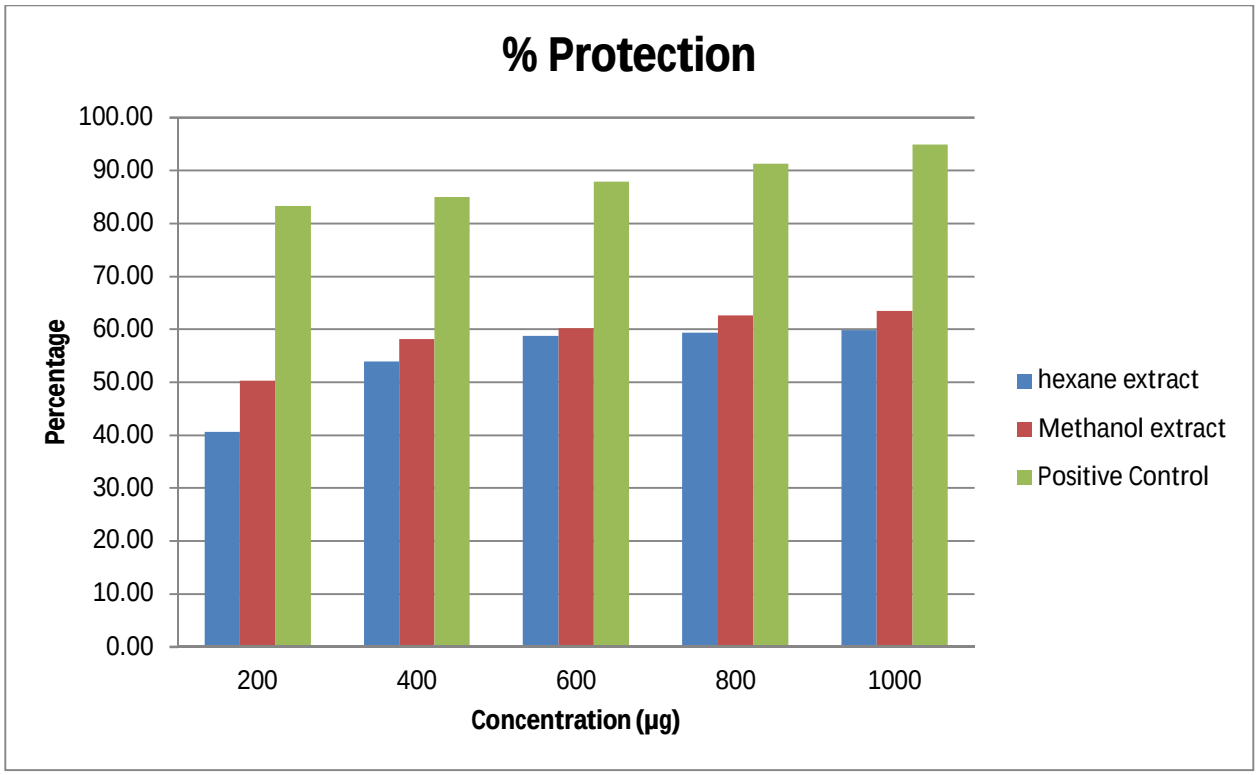

Fig. 5 showing percentage protection activities of Samples and Positive control

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IV. CONCLUSION

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. Inflammation is currently treated by NSAIDs. Unfortunately these drugs cause increased risk of blood clot resulting in heart attacks and strokes. Therefore, the developments of potent antiinflammatory drugs from the natural products are now under considerations. Natural products are rich source for discovery of new drugs because of their chemical diversity. A natural product from medicinal plants plays a major role to cure many diseases associated with inflammation. The conventional drug available in the market to treat inflammation produces various side-effects. Due to these side-effects, there is need for the search of newer drugs with less or no side-effects. There are hundreds of phytoconstituents reported to have many pharmacological activities although most of these reports are of academic interest and very few find entry in clinical trials.

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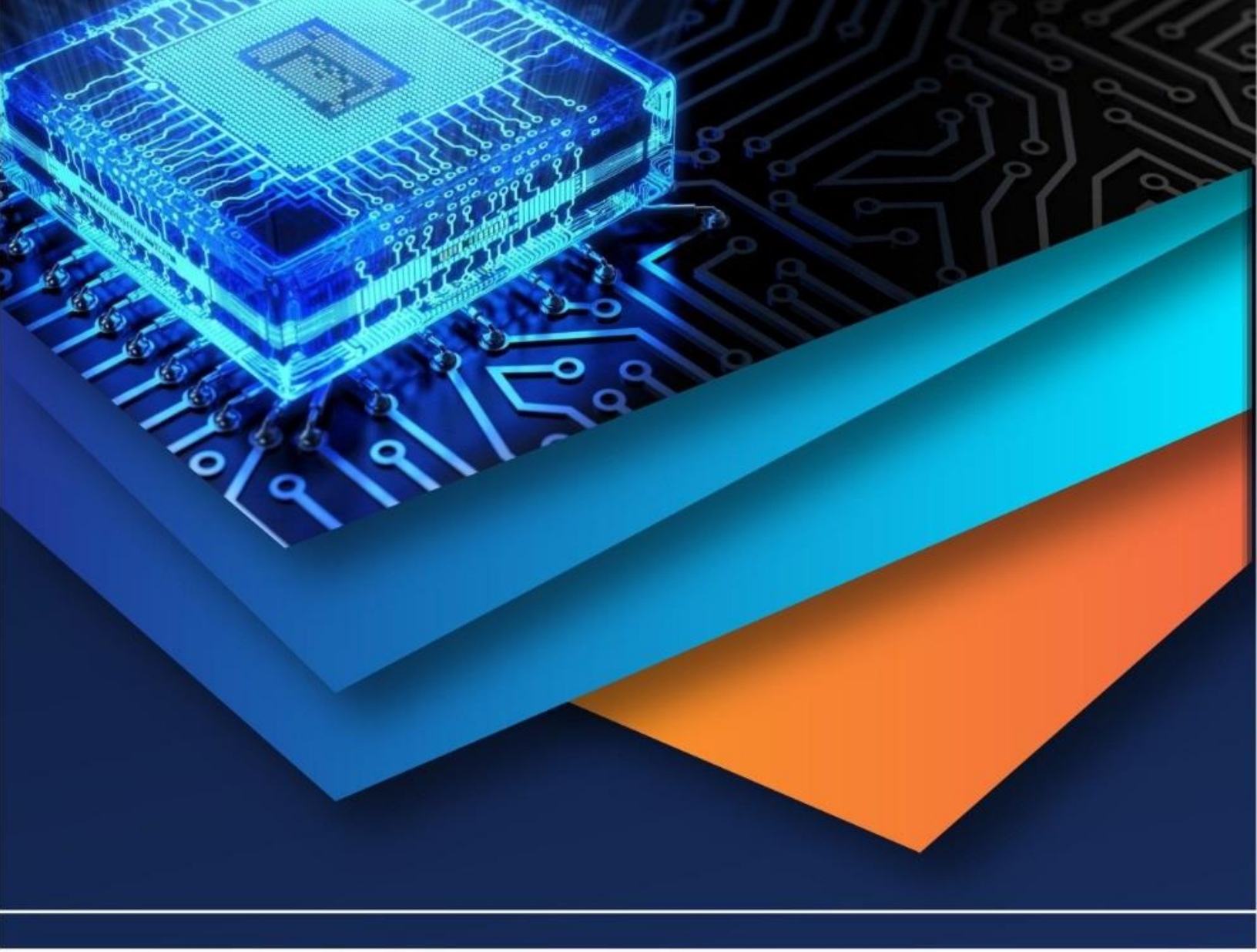

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