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Evaluation of Phytochemicals and Antioxidant Activity in Medicinal Herbs

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Abstract: ***As the presence of excess free radicals in body can cause damage to the system and may lead to several diseases like Alzheimer's, Parkinson's, Cancer, Atherosclerosis and many more. Free radicals should be neutralized or eliminated out of the body. Apart from the antioxidants present in biological system natural antioxidants available from the diet help in the removal of free radicals from the body. Therefore, the antioxidants present in fruits, vegetables and medicinal herbs can be used to scavenge free radicals and treat the disorders due to oxidative stress. Five different medicinal herbs were collected locally to quantitate phytochemicals, antioxidant activity and inhibition of lipid, protein and sugar oxidation. Among the different samples, highest Phenolics, flavonoid and antioxidant activity was seen in aqueous extract of Ocimum basilicum. This study demonstrates that it can be used in nutraceuticals and can be a potential source of natural antioxidants to combat the free radical mediated diseases.***
Keywords: Antioxidant, Free radicals, Medicinal herbs, Ocimum basilicum, phytochemicals

I. INTRODUCTION

Free radicals are well documented for playing a dual role in our body as both deleterious and beneficial species. Low/moderate concentration of free radicals are involved in normal physiological functions but excess production of free radicals or decrease in antioxidant level leads to oxidative stress. Most common radical derivatives of oxygen include superoxide radical anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), lipid peroxyl ($LO^{\cdot}$), lipid alkoxyl ($LOO^{\cdot}$) and lipid peroxide (LOOH) as well as non-radical derivatives such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are collectively known as ROS. Hydroxyl radical is the most reactive of them.

These free radicals are produced mainly from two important sources, cellular metabolism and environmental sources. Fortunately, free radical formation is controlled by compounds known as antioxidants. A proper balance between prooxidants and antioxidants is necessary for proper functioning of a cell.

Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Antioxidants are capable of stabilizing, or deactivating free radicals before the latter attack cells and biological targets.

They are therefore critical for maintaining optimal cellular and systemic health and wellbeing [1]. Antioxidants may be endogenous inbuilt within the organisms or exogenous in the diet. When endogenous antioxidant concentration becomes low when compared to increase free radicals, it results in oxidative stress. Therefore, to neutralize these excess free radicals, exogenous antioxidants are beneficial. These antioxidants may protect against direct physical alterations induced by radical attack on membrane lipids, DNA and proteins. It has been established that lipid, protein and sugar oxidation is associated with ageing, oxidative stress, and a number of diseases [2,3,4,5].

Protein carbonylation is an irreversible protein modification, associated with crucial alterations in their functional and structural integrity, contributing to cellular dysfunction and tissue damage [6,7]. The primary objective of this work is to explore the primary, secondary metabolites and antioxidant activities in medicinal plants which are locally available. In view of this and the present understanding about ROS induced multiple diseases, screening of the antioxidant activity and inhibition of protein oxidation, lipid oxidation and nucleic acid oxidation was carried out for the aqueous extracts of Centella asiatica, Ocimum basilicum, Ocimum sanctum, Leucas aspera and Plectranthus amboinicus.

II. MATERIALS AND METHODS

The locally available medicinal herbs such Centella asiatica, Ocimum basilicum, Ocimum sanctum, Leucas aspera and Plectranthus amboinicus were obtained locally. Unless otherwise stated all chemicals used in these experiments were of analytical grade, obtained either from Merck or Sigma chemicals or SRL chemicals.

A. Preparation of Extract

The leaves obtained from plant was cleaned and washed with distilled water. 1g of sample was taken and ground with 10ml of distilled water using pestle and mortar and centrifuged at 10,000xg for 10 minutes at 4°C in a cold centrifuge and filtered through Whatmann No.1 filter paper. The aqueous extracts were evaporated to drynesss. The yield was calculated and expressed in percentage. The residue obtained was dissolved in required amount of water and was stored at 4^0C for performing various activities.

B. Determination of total phenolics by Folin-Ciocalteau assay

The concentration of total phenolics in all the extracts was determined by the Folin-Ciocalteau assay. It involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium [8]. In this study Gallic acid was used as spectrophotometric standard. The phenolic contents of the extracts were determined from calibration curve and were expressed in mg of Gallic acid equivalents/ g sample.

C. Estimation of Total Flavonoids

Aluminium chloride colorimetric method [9] was used for flavonoids determination. The absorbance of the reaction mixture was measured at 420 nm with UV visible spectrophotometer. Quercetin was used as standard for the calibration curve. The concentration of flavonoid was expressed in terms of mg/g of sample.

D. Antioxidant Activity by DPPH method

Antioxidant activity of all the leaf extracts was determined using 2,2-Diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) [10]. Reduction of DPPH by an antioxidant or by a radical species result in a loss of absorption at 517nm. Thus, the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. Percentage of radical scavenging activity was calculated for all the extracts. A linear curve was generated by plotting percentage of radical scavenging activity versus concentration of extracts.

E. Determination of Reducing Power

Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form Potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm.The reducing power of all the extracts was evaluated according to the method of Oyaizu [11]. The absorbance was then measured at 700 nm against blank sample.

F. Antioxidant Activity by TBA Method

Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen, which can be detected spectrophotometrically at 532nm as per Halliwell and Gutteridge [12]. The egg yolk was used for the study of invitro lipid per oxidation. The percentage of anti- lipid per oxidative activity (%ALP) is calculated for all extracts.

G. Inhibition of Protein Oxidation

Protein oxidation can lead to the introduction of carbonyl groups into amino acid residues of the protein. The derivatization of this functional group with carbonyl-specific reagents provides a method to detect and quantify protein carbonylation. The most common method to analyze protein carbonylation is the reaction with 2,4- dinitrophenylhydrazine (DNPH). The resulting hydrazones are quantified spectrophotometrically. The effect of all five aqueous extracts on protein oxidation was studied by using the method of Yang et al. [13]. The percentage of inhibition of protein oxidation by extracts was determined.

H. Inhibition of Deoxyribose Oxidation

The extent of deoxyribose degradation was tested by the TBA method as described by Halliwell et al. [14]. All the five extracts were screened for the inhibition of deoxyribose sugar oxidation. Extracts were incubated with deoxyribose in phosphate buffer (pH 7.4), $FeCl_3$, EDTA and H_2O_2.

The hydroxyl radical generated attacks the deoxyribose and results in a series of reactions that lead to the formation of MDA. The absorbance of the mixture was read spectrophotometrically at 532 nm. The percentage of inhibition of deoxyribose sugar degradation was determined for all extracts.

III. RESULTS AND DISCUSSION

Free radicals are highly reactive and possess a lone pair of electron with a very short half-life and high tendency to pull out an electron. Free radicals withdraw an electron from molecules or atoms resulting in the production of free radicals in a series of oxidizing reactions leading to altered structure and function of Protein, lipid and sugars causing damage to the system. The defense mechanism for combating these free radicals are antioxidants. Antioxidants are compounds which acts as inhibitors of the oxidative process. Free radicals are produced endogenously within the organism either from cellular metabolism or environmental sources. The free radicals are also beneficial components in the body. The imbalance between free radicals and antioxidant results in oxidative stress. Therefore, to neutralize these excess free radicals exogenous antioxidants are beneficial. These antioxidants protect radical attack on membrane lipids, proteins and DNA.

The present study was aimed to evaluate the locally available medicinal herbs for total phenolics and flavonoid contents, efficacy in free radical scavenging, inhibition of lipid peroxidation, protein oxidation and DNA damage protection activities using in vitro models. Phytochemicals are secondary metabolites of plants which are non-nutritive chemicals that have protective or disease preventive properties. Centella asiatica (CA), Ocimum basilium (OB), Ocimum sanctum (OS), Leucas aspera (LA), Plectranthus amboinicus (PA) are the plants used in the treatment of various diseases. Therefore, our aim was to identify the important phytochemicals and antioxidant activity in the leaves which promotes the therapeutic application. The 1g of fresh leaves obtained from all five sources was used for aqueous extraction. After extraction the extracts were centrifuged at 10,000 rpm for 10mins and the supernatant obtained was evaporated to dryness. The residue obtained was used for detection of phenolics, flavonoids and antioxidant activity by DPPH method, reducing power activity, inhibition of lipid peroxidation by TBA method, inhibition of protein oxidation and deoxy ribose degradation activity.

Polyphenols are known to inhibit free radicals generated in the cellular system when they are obtained through the diet. Polyphenols are believed to prevent conversion of substances into carcinogens and inhibit mutations. Total Phenolics was estimated for all the five extracts at 100 µg concentration as shown in Table 1 and Figure 1. Out of the 5 extracts, Ocimum basilium extract with 554.8 mg gallic acid equivalent/g of extract showed maximum amount of phenolics followed by the PA, LA, OS and CA extracts with 539, 260.4, 57 and 12.32 mg gallic acid equivalent/g of extract. The functionality of these compounds is exhibited through their action as an inhibitor or an activator for a large variety of mammalian enzyme systems and as scavenger of free oxygen radicals. These results gives a reason for the activity of these plants as antioxidant and how these plants extracts enable to scavenge the free radicals.

Flavonoids are found in a wide variety of fruits and vegetables. They act as antioxidants. Resveratrol, quercetin, hesperidin, tangerin, myricetin, kaempferol and apigenin are some flavonoids. They act as anticarcinogen, anti-allergen, anti-inflammatory and reduce the risk of heart diseases, cancer and urinary tract infection. The total flavonoid content of all the extracts was determined by the Aluminum chloride colorimetric method.

The results are depicted in Table 1 and figure 2. The flavonoid content was found to be maximum in Ocimum basilium extract with 490 mg/g of extract followed LA, OS, PA and CA with 421.6, 119.7, 114, and 23.2 mg quercetin equivalent/g of extract. Flavonoids are a class of polyphenol compounds acting as ROS scavenger and metal chelators.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Table 1 and figure 3 shows the reducing power activity of extracts at 100 µg concentration using the potassium ferricyanide reduction method. At 100 µg concentration, the Centella asiatica, Ocimum basilium, Ocimum sanctum, Leucas aspera, Plectranthus amboinicus extracts showed absorbances of 0.0014, 0.047, 0.043, 0.005 and 0.0021 respectively at 700 nm. Thus, the highest reducing activity was observed in Ocimum basilium extract.

The reducing power activity is due to the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom.

The natural antioxidants are easily absorbable in human system. To meet the antioxidant requirement and to prevent degenerative diseases, the diet should include foods rich in antioxidants. Therefore, Free radical scavenging potentials of all the leaf extracts at 100 µg concentrations were tested by the DPPH method and the results are shown in Table 1 and figure 4. CA, OB, OS, LA and PA showed 62%, 79.43%, 68%, 65% and 62.5% free radical scavenging activity respectively according to the DPPH method. The highest radical scavenging activity was found in Ocimum basilium. DPPH is a stable radical that has been used to evaluate the antioxidant activity of extracts. Antioxidant reacts with DPPH, which is a stable free radical, and converts it to α, α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The activity of extracts is attributed to their hydrogen donating ability. Increasing the number of hydroxyl or catechol groups increases radical scavenging activity.

Lipids and proteins are more susceptible to oxidative damage. Antioxidant activity of the leaf extracts was performed using thiobarbituric (TBA). The results of the effect of various leaf extracts at 100 µg concentration to prevent lipid peroxidation are shown in Table 1 and figure 5. Maximum inhibition of lipid peroxidation was observed with the aqueous extract of Ocimum basilium. The antioxidant activity of this extract directly correlates with the total phenolics content. Increased formation of lipid peroxides and aldehydes has been observed in atherosclerosis, ischemia-reperfusion, heart failure, Alzheimer's disease, rheumatic arthritis, cancer, and other immunological disorders (15). Therefore, decreasing the formation of lipid peroxidation products or scavenging them chemically could be beneficial in limiting the deleterious effects of ROS in various pathological conditions.

A number of age-related diseases have been shown to be associated with elevated levels of oxidatively modified proteins (16). The assay of carbonyl groups in proteins provides a convenient technique for detecting and quantifying oxidative modification of proteins. 2,4-dinitrophenylhyrdazine (DNPH) reacts with protein carbonyls to produce hydrazones. Hydrazones can be detected spectrophotometrically at an absorbance of 370nm. The results of protein oxidation depicted in Table 1 and Figure 6 shows maximum inhibition of protein oxidation by Ocimum basilium with 89.4% followed by OS, CA, LA and PA with 83.6%, 68.6%, 64.5% and 55.2% respectively. Oxidative stress is able to degrade lipids and carbohydrates to highly reactive intermediates, which eventually attack proteins at various functional sites (17). The deoxy ribose degradation inhibition studies was carried out for all extracts at 100ug concentration. The results are shown in the Table 1and Figure 7. Ocimum basilium showed maximum inhibition and PA minimum inhibition with 89.4% and 13.8%.

IV. CONCLUSION

Therefore, the medicinal herb Ocimum basilium with high concentration of phenolics, flavonoids and high antioxidant activity possess property of inhibition of free radical mediated destruction of biomolecules. Further the purification and isolation of the active ingredient in aqueous Ocimum basilium extract is to be studied for the therapeutic value.

V. ACKNOWLEDGEMENT

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Table 1

	Centella asiatica	Ocimum basilium	Ocimum sanctum	Leucas aspera	Plectranthus amboinicus
Phenolics	12.32 mg	554.8 mg	57mg	260.4mg	539 mg
Flavonoids	23.2mg	490 mg	119.7mg	421.6mg	114 mg
Reducing power assay	0.0014 OD	0.047OD	0.043O.D	0.005 OD	0.0021 OD
DPPH	62%	79.43%	68%	65%	62.5%
TBA	38%	72%	65%	33%	42%
Protien oxidation	68.6%	89.4%	83.6%	64.5%	55.2%
Deoxy ribose oxidation	51.02%	89.4%	68%	23.4%	13.8%

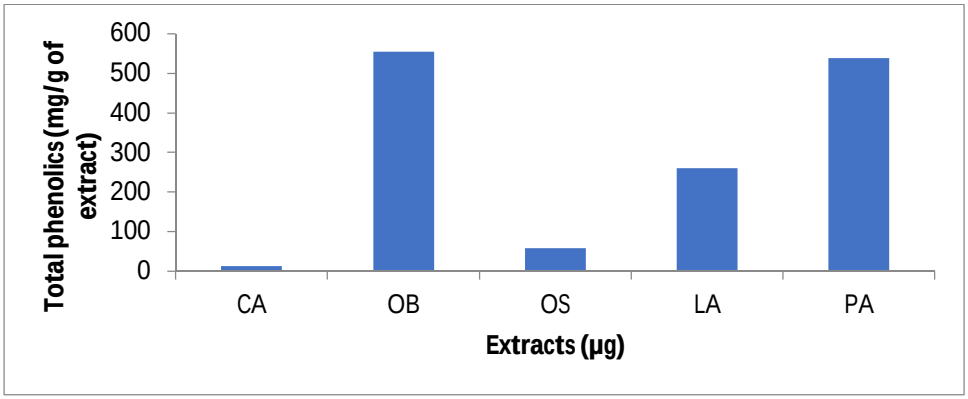

Figure 1: Total phenolics in leaf extracts of different medicinal plants.
Centella asiatica (CA), Ocimum basilium (OB), Ocimum sanctum (OS), Leucas aspera (LA), Plectranthus amboinicus (PA).

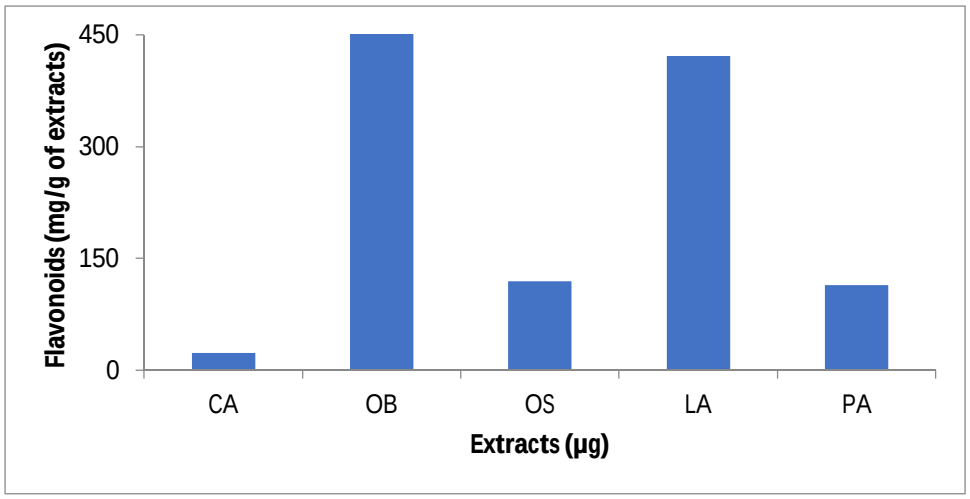

Figure 2: Total flavonoids in leaf extracts of different medicinal plants.
Centella asiatica (CA), Ocimum basilium (OB), Ocimum sanctum (OS), Leucas aspera (LA), Plectranthus amboinicus (PA).

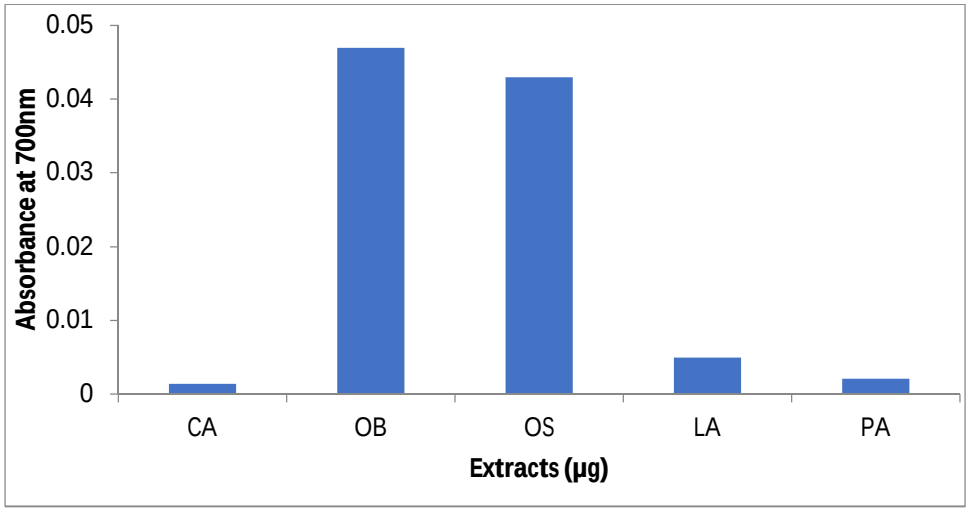

Figure 3: Reducing power activity in leaf extracts of different medicinal plants.
Centella asiatica (CA), Ocimum basilium (OB), Ocimum sanctum (OS), Leucas aspera (LA), Plectranthus amboinicus (PA).

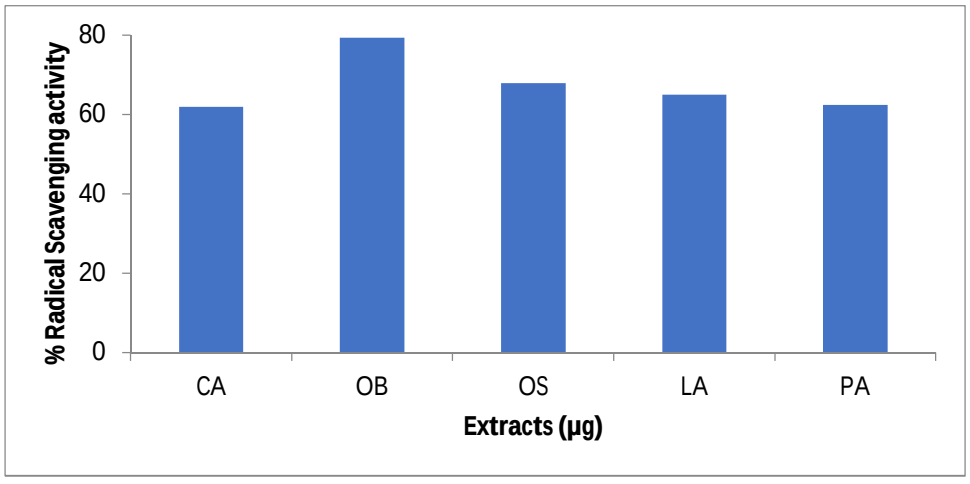

Figure 4: Percentage of Radical scavenging activity in leaf extracts of different medicinal plants.
Centella asiatica (CA), Ocimum basilium (OB), Ocimum sanctum (OS), Leucas aspera (LA), Plectranthus amboinicus (PA).

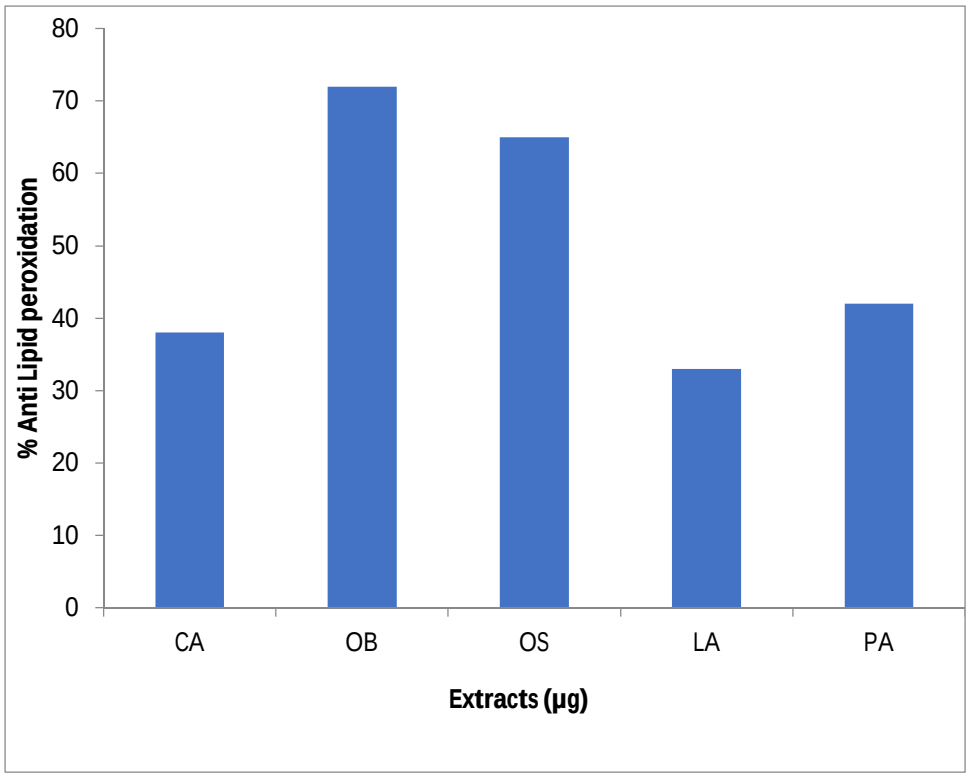

Figure 5: Percentage inhibition of lipid peroxidation in leaf extracts of different medicinal plants. Centella asiatica (CA), Ocimum basilium (OB), Ocimum sanctum (OS), Leucas aspera (LA), Plectranthus amboinicus (PA).

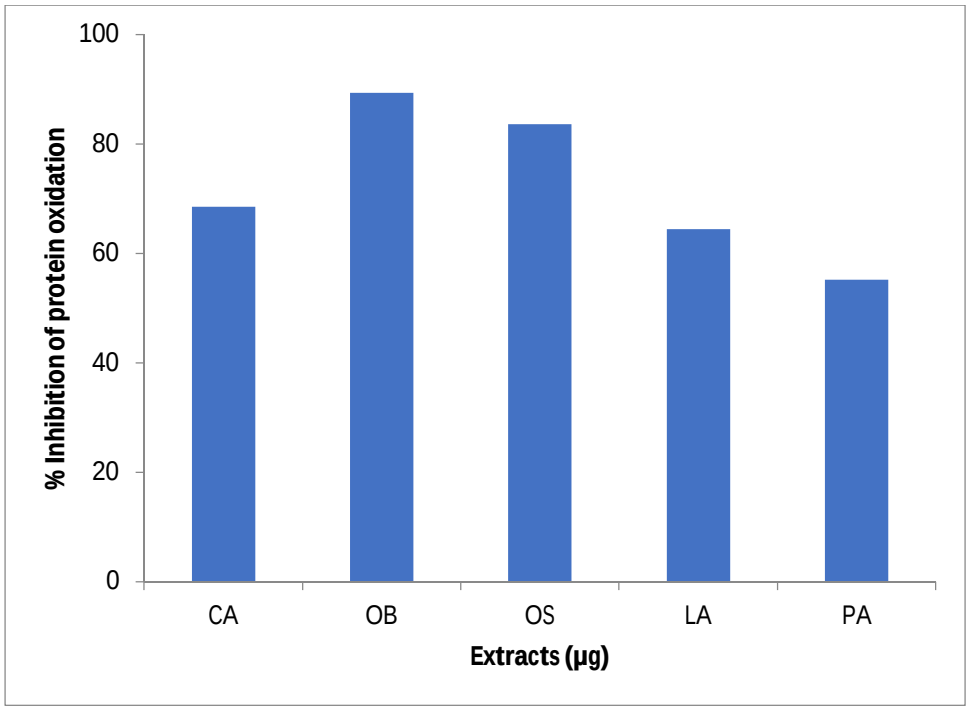

Figure 6: Percentage inhibition of protein oxidation in leaf extracts of different medicinal plants. Centella asiatica (CA), Ocimum basilium (OB), Ocimum sanctum (OS), Leucas aspera (LA), Plectranthus amboinicus (PA).

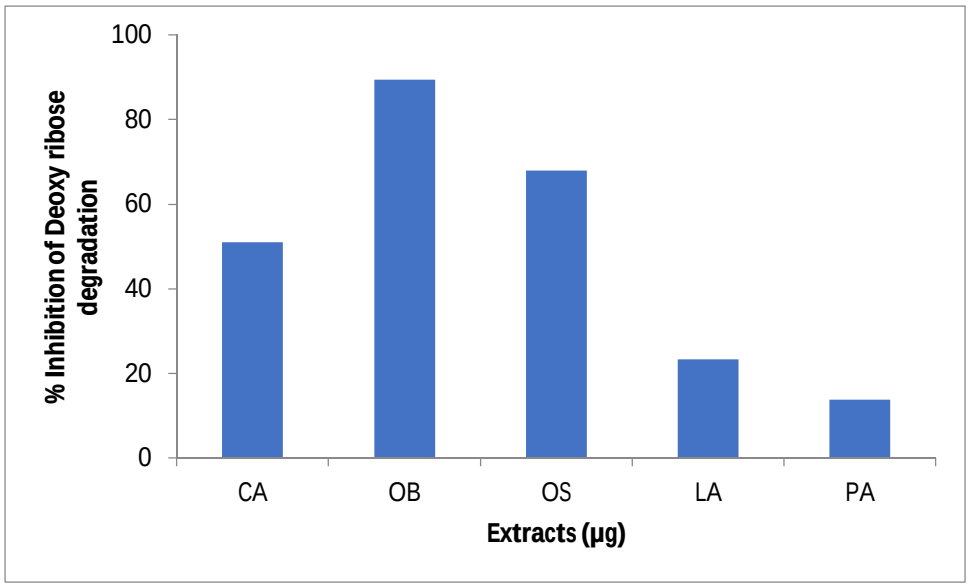

Figure 7: Percentage inhibition of deoxy ribose degradation in leaf extracts of different medicinal plants. Centella asiatica (CA), Ocimum basilium (OB), Ocimum sanctum (OS), Leucas aspera (LA), Plectranthus amboinicus (PA).

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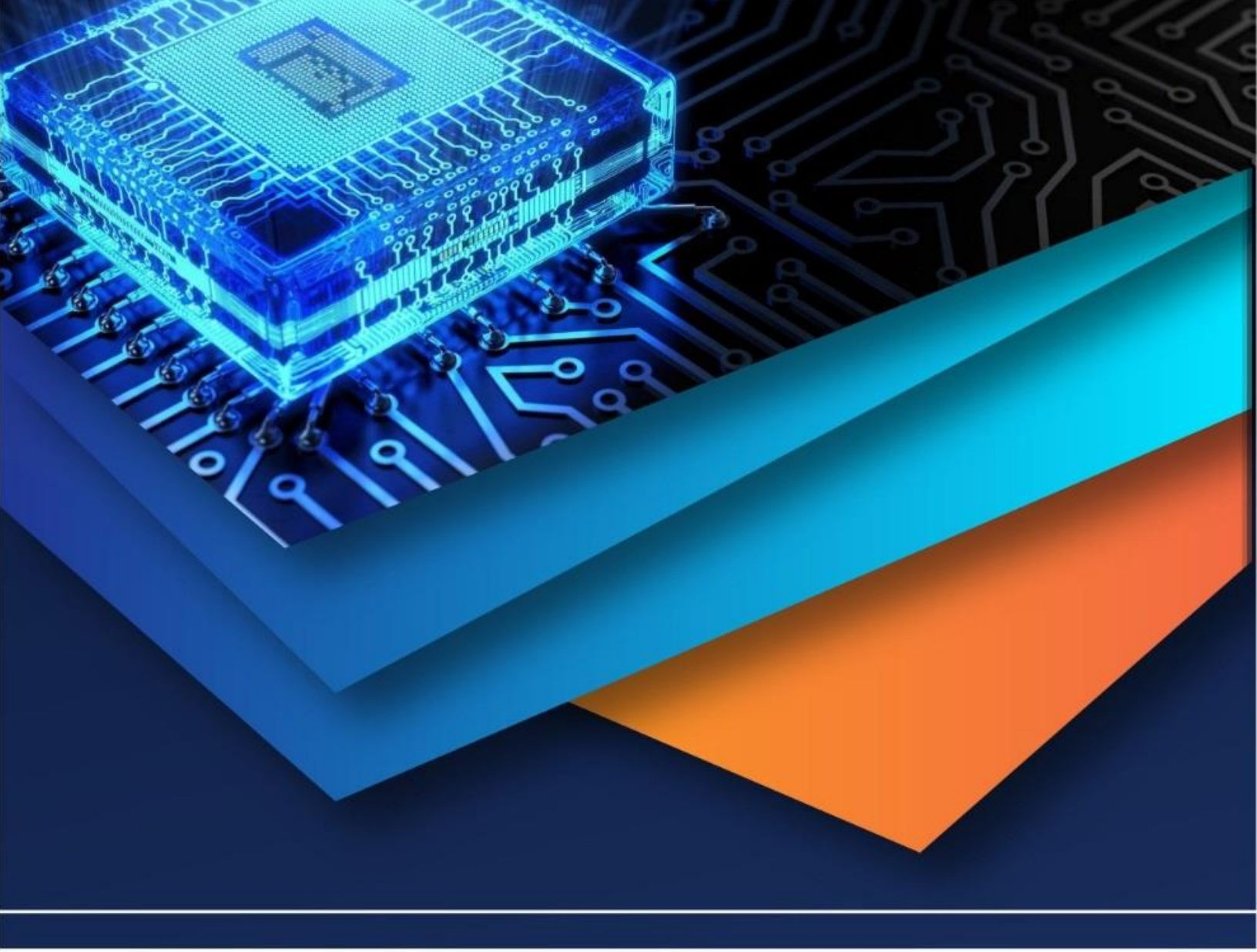

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