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Green Synthesis of Magnesium Nanoparticles from *Tribulus terrestris* and In-vitro Optimization of Anti-Oxidant and AntiUrolithiatic Activity

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Abstract: ***The green synthesis of Magnesium Nanoparticles is mostly used in Research purpose because of its capability for environment friendly development. In common, the nanoparticle productions by various chemical processes are not ecofriendly. The synthesis of Magnesium Nanoparticles using Tribulus terrestris extract was carried out. The whole plant Tribulus terrestris extract was investigated for its antiurolithiatic property. The fruits of Tribulus terrestris have long been used to treat Urolithiasis and various urinary diseases. The antiurolithiatic activity of Tribulus terrestris significantly reduces the level of protein, oxalate, glycosamoglycan and citrate in patient's 24hrs urine sample. But there is no change in urine volume and phosphate level in the sample's serum. This work defines the treatment of kidney stones by Magnesium Nanoparticles of Tribulus terrestris. The nanoparticles are identified by using Fourier transform infra red spectroscopy (FTIR), Scanning electron microscopy (SEM), X-ray diffraction (XRD), UV visible spectroscopy. The antiurolithiatic activity showed better percentage of inhibition for the synthesis of Magnesium Nanoparticles by using Tribulus terrestris extract.***
Keywords: Tribulus terrestris, **Magnesium Nanoparticles, In-vitro Antiurolithiatic activity, Kidney stones treatment.**

I. INTRODUCTION

The crystal stone formation within the kidney is called Kidney stone disease. In human health, it is a raising urological disorder [1]. There are different types of kidney stones, they are Calcium stones, Struvite stones, Uric acid stones, Cystine stones, and Drug induced stones [1]. The medicinal plants are always playing an important role as a source for drug lead compound [2]. Around the world, the *Tribulus terrestris* is used to treat various types of kidney diseases in both the Chinese and Indian medicine system. The different parts of *Tribulus terrestris* contains a various chemical constituent which are medicinally important, such as Alkaloids, Flavonoids, Steroidal saponins and Flavonoidal glycosides. It also has antiurolithiatic, antidiabetic, antimicrobial, anti-inflammatory and anticarcinogenic activity [4]. The particles with size of 1 to 100 nanometers are called Nanoparticles. The Nanoparticles are made up of metal , carbon , organic matter or metal oxides[5]. The Nanoparticle synthesis can be done by biologically or chemically. There are different types of nanoparticles such as Copper, Silver, Gold, Magnesium, Alloy, and Magnetic. Nanomedicine plays an important role in improvement of the treatment and diagnosis of human diseases [6].

Magnesium Nanoparticles has spherical black high surface area and its size is 20-60nm in diameter and range is 30-70m^2/g. Due to the novel properties of MagnesiumNanoparticles have received intense attention in recent years [8]. The key application of magnesium Nanoparticles are Automobile and Airplants coatings, nanowires, plastic and nanofibers, hydrogen storage textiles [7]. Magnesium is an identified inhibitor of the formation of calcium oxalate crystals in the urine and was planned for prophylactic treatment in renal stone disease as early as the 17th and 18th centuries [9].

In urine, the stone forming constituents reduction and their low kitention reduces the crystallizing salts solubility which could contribute to the antiurolithiatic property of the extract [10].

Fig.1: *Tribulus terrestris* Plant.

II. MATERIALS AND METHODS

High purity Merck grade chemicals were used. Magnesium sulphate and Magnesium Chloride were used. The plant *Tribulus terrestris* was collected from kamarajar road, Coimbatore, Tamilnadu, during the month of April 2022.

A. *Preparation of Plant Extract*

After the collection of plant, the plant was washed, shade dried at room temperature and then grinded. Add 100ml of distilled water with grinded plant and boil at 100^{o}C for 10mins. Then the obtained extract was stored in refrigerator for further use.

B. *Preparation of Precursor Solution*

Magnesium chloride and Magnesium sulphate precursor solutions were prepared in molar concentrations. 0.4g of Magnesium sulphate and 2.0 g of magnesium chloride were dissolved in 100ml of distilled water. This solution is used for the green synthesis of Magnesium sulphate and magnesium chloride nanoparticles.

C. *Synthesis of Magnesium Nanoparticles*

Magnesium Sulphate and Magnesium chloride nanoparticles were synthesized by a method described by Sharma *et al* (2017). 10 ml of *Tribulus terrestris* extract transferred into 30 ml of magnesium sulphate and magnesium chloride solution. This solution was incubated for 24 to 48 hours at room temperature. Then the appearance of brownish colloidal and dark brown colour indicating the formation of nanoparticles. Then the nanoparticle sample was characterized by Fourier transform infra red spectroscopy (FTIR), Scanning electron microscopy (SEM), X-ray diffraction (XRD), UV visible spectroscopy.

D. *Phytochemical Analysis*

Some qualitative analyzes for the presence of bioactive components were performed using standard tests, like test for alkaloids (Wagner test), phenol (ferric chloride test), reducing sugars (Fehling test) saponins (foam test), flavonoids, phytosterols (Salkowski test), amino acids and proteins (ninhydrin Test), tannin, glycosides.

III. ANTIOXIDANT ACTIVITY

A. *DPPH Assay*

The free radical scavenging activity of methanolic plant extract was determined using 2,2- Diphenyl-1-picrylhydrazyl (DPPH) DPPH free radical scavenging activity was measured Following the procedure described by (Braca et al., 2001). Aliquot 3 mL of 0.004% DPPH solution in Methanol and 0.5 to 2.5 µl of plant extract/ascorbic acid at various concentrations were mixed. The mixture was stirred and allowed to come to normal temperature for 30 min. Discoloration was noted by measuring absorbance at 517 nm. A control was prepared using 0.1 ml of each vehicle Instead of mushroom extract/ascorbic acid. The extent of inhibition of DPPH radicals by the extract/compound was determined by comparing the absorbance values of the control and experimental tubes

B. *FRAP Assay*

Antioxidant capacity of mushroom extract samples was estimated according to the method described By Bennie and Strain (1996) and modified by Pulido et al. (2000). FRAP Reagent (900 µl), freshly prepared and incubated at 37ºC, was washed with 90 µL of distilled water and 30 µL of test sample or acetone (for the Reagent blank). The test samples and reagent blank were incubated in a water bath at 37°C for 30 minutes. The FRAP reagent contained 2.5 mL of 20 mmol/L TPTZ solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L FeCl2.6H2O and 25 ml 0.3 mol/l acetate buffer, pH 3.6 (Bennie and Strain, 1996). At the end of the incubation over the period absorbance values were immediately recorded at 593 nm using a spectrophotometer. The known An Fe (II) concentration in the range between 100 and 2000 µmol/l (FeSO4.7H2O) was used to produce the Calibration curve. EC1 was Calculated as the concentration of antioxidant that corresponds to an increase in absorbance in the FRAP assay. The theoretical extinction value of a Fe (II) solution with a concentration of 1 mmol/l, determined with the Corresponding regression

A. *Nitric Oxide (NO) Scavenging Assay:*

The NO scavenging activity (Garratt DC., 1964) of the sample was determined by adding 400 µl of 100 mM sodium Nitroprusside, 100 µL PBS (pH – 7.4) and 100 µL plant extract of different concentration. This mixture of reaction was kept for incubation at 25°C for 150 minutes. To 0.5 ml of the above solution add 0.5 ml of semolina Reagent was added (0.1 mL sulfanilic acid and 200 µL naphthyl ethylene diamine dichloride (0.1%) w/v).This was incubated for 30 minutes at room temperature and finally an absorbance at 540 is observed Nm. All reactions were performed in triplicate

IV. ANTIUROLITHIATIC ACTIVITY

A. Spectrophotometric estimation of calcium oxalate method

Group I: 1ml of calcium oxalate (1mg/ml) + 1ml of distilled water

GroupII: 1ml of calcium oxalate (1mg/ml) + 1ml of Cystone solution (400mg/ml)

Group III: 1ml of calcium oxalate (1mg/ml) + 1ml MgNp (1 mg/ml)

All the three groups were packed together in egg semi permeable membrane which was tied with thread at one end and were suspended in a conical flask containing 150 ml 0.1 M Tris buffer each. At another end of thread tied by a stick placed on the mouth of conical flask and covered with aluminum foil. All groups were kept in an incubator, pre C for 4 hours, kept for three days. The entire content of each group was heated to 37°C and was removed from semi permeable membrane and was transferred into test tube l of 0.02Mμ individually. 4ml of 1N H_2SO_4 and 60-80 $KMnO_4$ were added and kept aside for 2 hours. After 2 hours, Colour change was observed from dark pink to colourless. Against 620nm, Change of colour intensity was measured spectrophotometrically.

V. RESULTS AND DISCUSSION

A. Characterization

1) FTIR

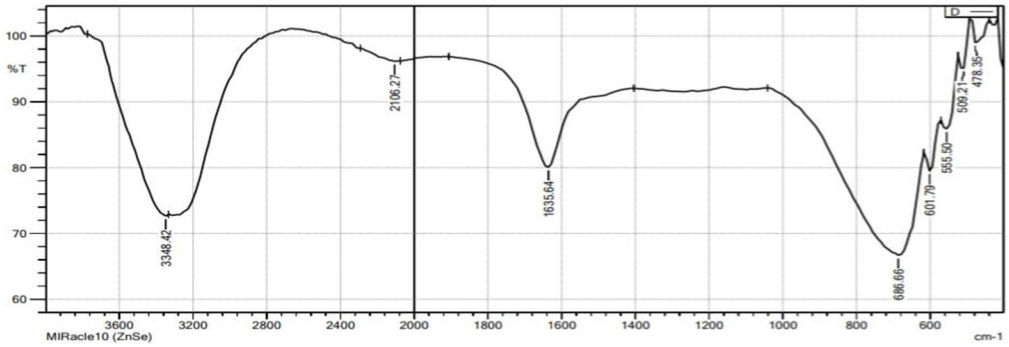

Fig.2:FTIR Characterization of synthesized Magnesium Nanoparticles

2) XRD

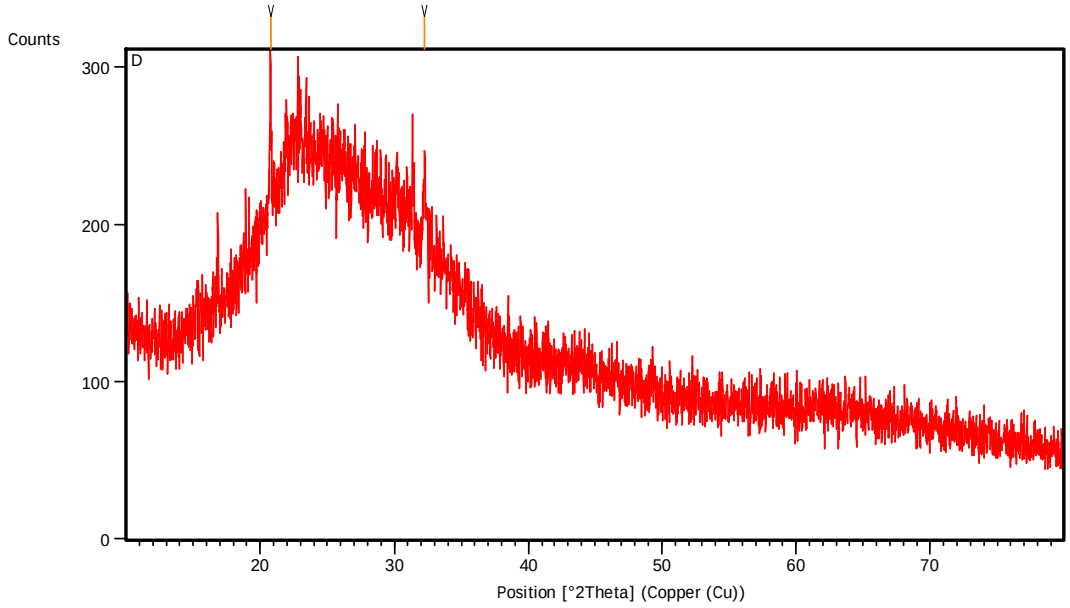

Fig.3: XRD Characterization of synthesized Magnesium Nanoparticles

3) UV-VIS Spectrum

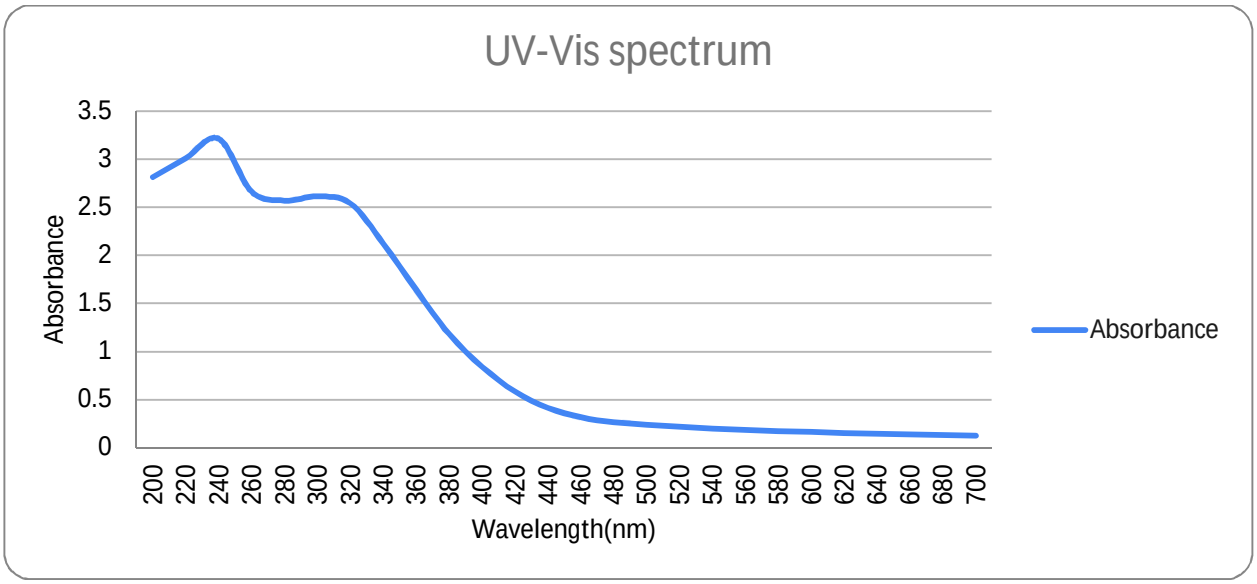

Fig.4: UV- VIS Spectrum Characterization of synthesized Magnesium Nanoparticles

4) SEM

SEM images of nanoparticles at different magnification, which clearly exhibit the nanoparticles like morphology indicate well uniform particles with narrow size distribution lies in the range 6.79 mm of 500 nm and magnification range is 30 kx. Particles seem to have an irregular shape with chemical homogeneity with uniform morphology due to the presence of interparticle surface connectivity.

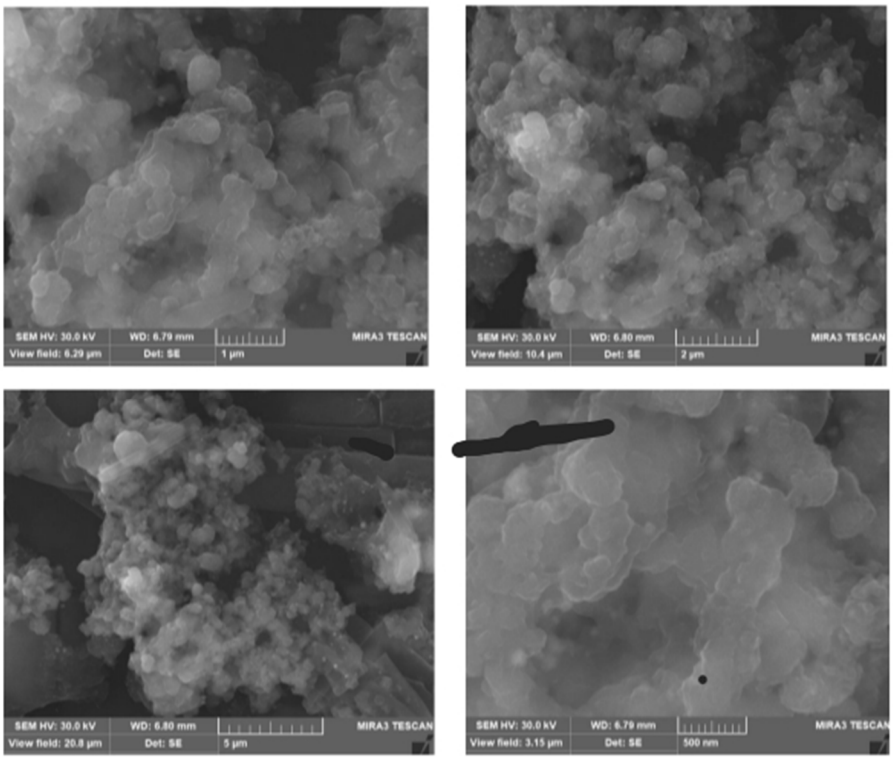

Fig.5: SEM Characterization of synthesized Magnesium Nanoparticles

B. Phytochemical Analysis

The qualitative analysis of bioactive compounds were tested using standard test

BIO ACTIVE COMPOUND	APPEARANCE	RESULTS
Alkaloids	Yellowish orange	+++
Flavanoids	Dark yellow	+++
Phenol	Reddish brown	+++
Aminoacids & Proteins	Yellowish white	++
Tannins	Reddish Brown	+++
Glycosides	Pale yellow	++
Phytosteroids	Yellowish green	++
Reducing sugar	Dark blue with precipitation	+++
Steroids	Greenish Yellow	+
Saponin	Foam formed	+++

Table.1: Phytochemical analysis

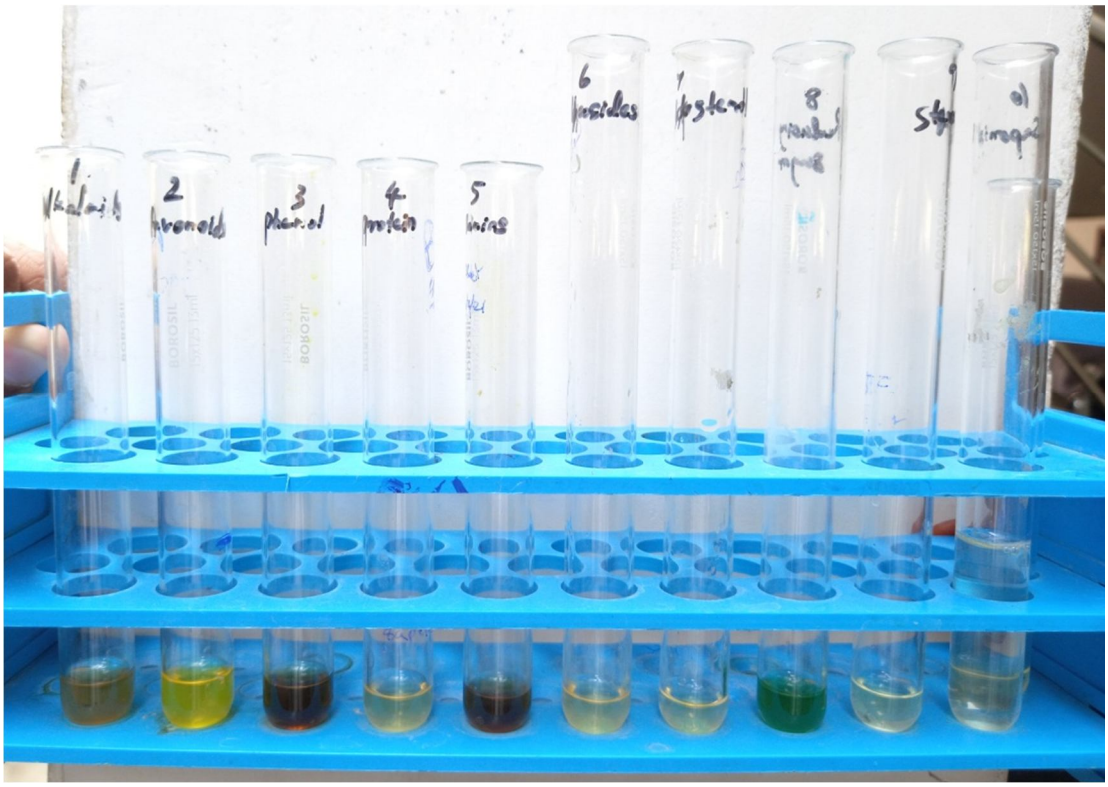

Fig.6: Phytochemical Analysis

C. Antioxidant Activity

1) DPPH ASSAY: Decolorization of DPPH was determined by measuring the absorbance at 518 nm. A control was prepared using 1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of DPPH radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes.

Concentration	Standard(Ascorbic acid)	MgNp of Tt
5 µl	15 %	34%
10 µl	38 %	52%
15 µl	56 %	61%
20 µl	73 %	70%
25 µl	86 %	83%

Table.2: DPPH Assay

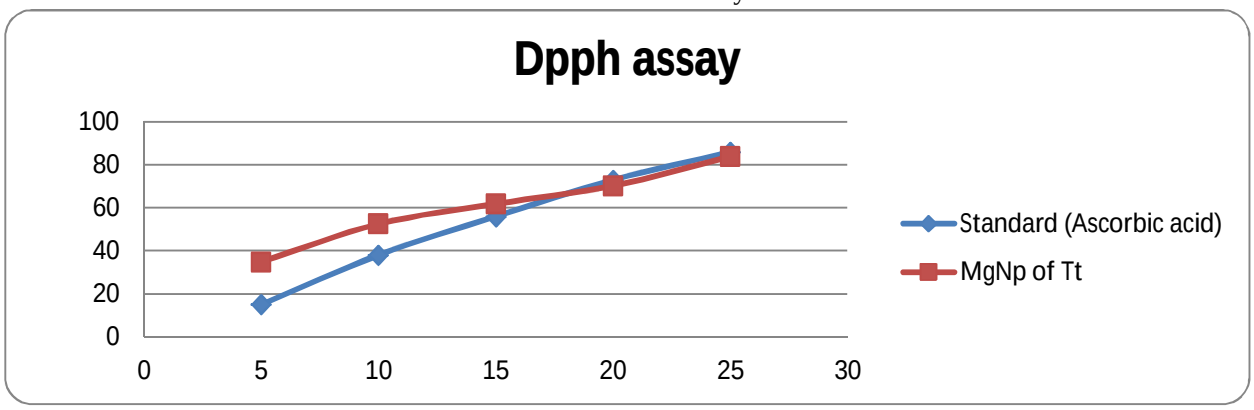

Fig.7: DPPH Assay

2) FRAP ASSAY: The concentration of antioxidant has a ferric- TPTZ reducing ability equivalent to that of 1 mmol/l FeSO4.7H2O. EC1 *was* calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/l concentration of Fe (II) solution determined using the corresponding regression equation.

Concentration	FRAP Standard	FRAP Sample
5 µl	33 %	27%
10 µl	44 %	44%
15 µl	52 %	56%
20 µl	71 %	61%
25 µl	83 %	74%

Table.3:FRAP Assay

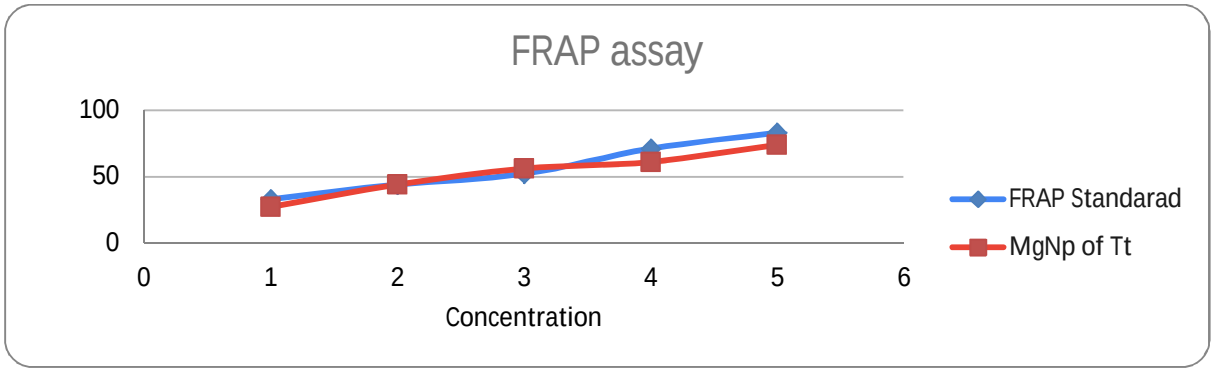

Fig.8: FRAP Assay

3) *NITRIC OXIDE (NO) SCAVENGING ASSAY:* The antioxidant activity of plant extract was evaluated by nitric oxide scavenging activity using different concentrations. Finally absorbance of nanoparticle is observed at 540 nm by UV spectroscopy. All the reactions were performed in triplicates.

Concentration	Standard(Ascorbic acid)	Sample
5 µl	30 %	26%
10 µl	46 %	41%
15 µl	58 %	58%
20 µl	73 %	72%
25 µl	81 %	86%

Table.4: Nitric oxide scavenging Assay

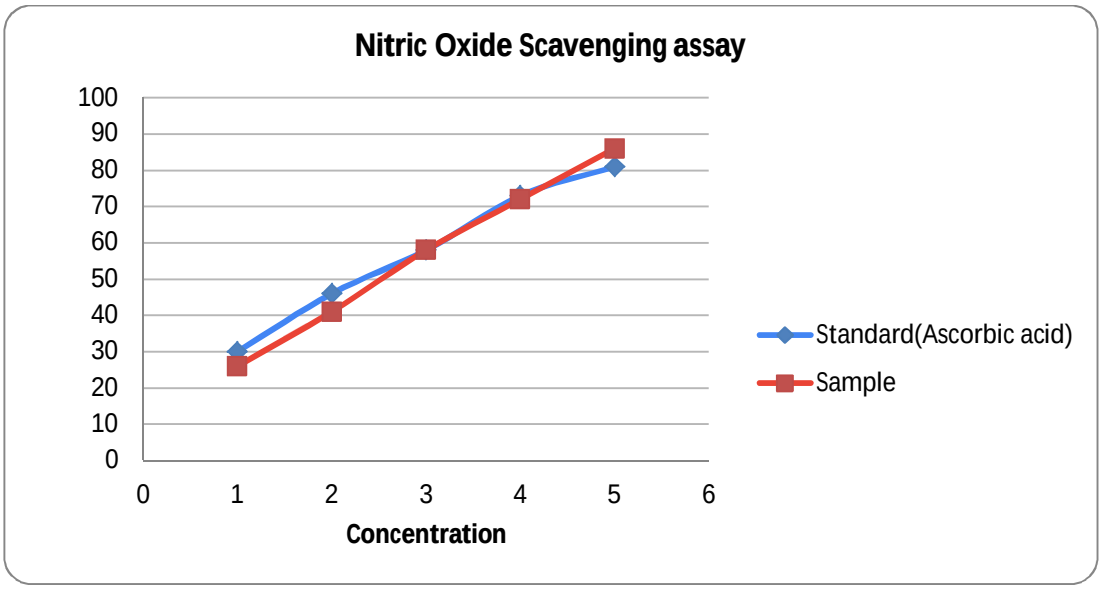

Fig.9: Nitric Oxide Scavenging Assay

D. Antiurolithiatic Activity

The Nanoparticle extract has greater capability to dissolve calcium oxalate as foremost element for stone forming in urinary tract. Lower percentage indicates a lot of efficiency in dissolution of calcium oxalate crystals.

Groups	Weight of calcium reduced	Dissolution %
Group I	-	-
Group II	0.076	58 %
Group III	0.054	49 %

Table.5: Anti-Urolithiatic Activity

VI. CONCLUSION

Based on the work, we consider that the *Tribulus terrestris* is a game changer and has a promising role in the future. In addition, Our findings suggests the possibility of using *Tribulus terrestris* as a therapeutic agent to treat urinary tract infection and further characterization of its active compound(s) could lead to a new candidate drug for patients with Urolithiasis.

This plant is used in ethno medical practice to treat urinary problems and kidney stones. The study provided fundamental scientific evidence for the traditional use of the plant in the prevention and treatment of Urolithiasis. Therefore, this plant may well be a possible source of recent drug molecules with anti-urolithiatic activity.

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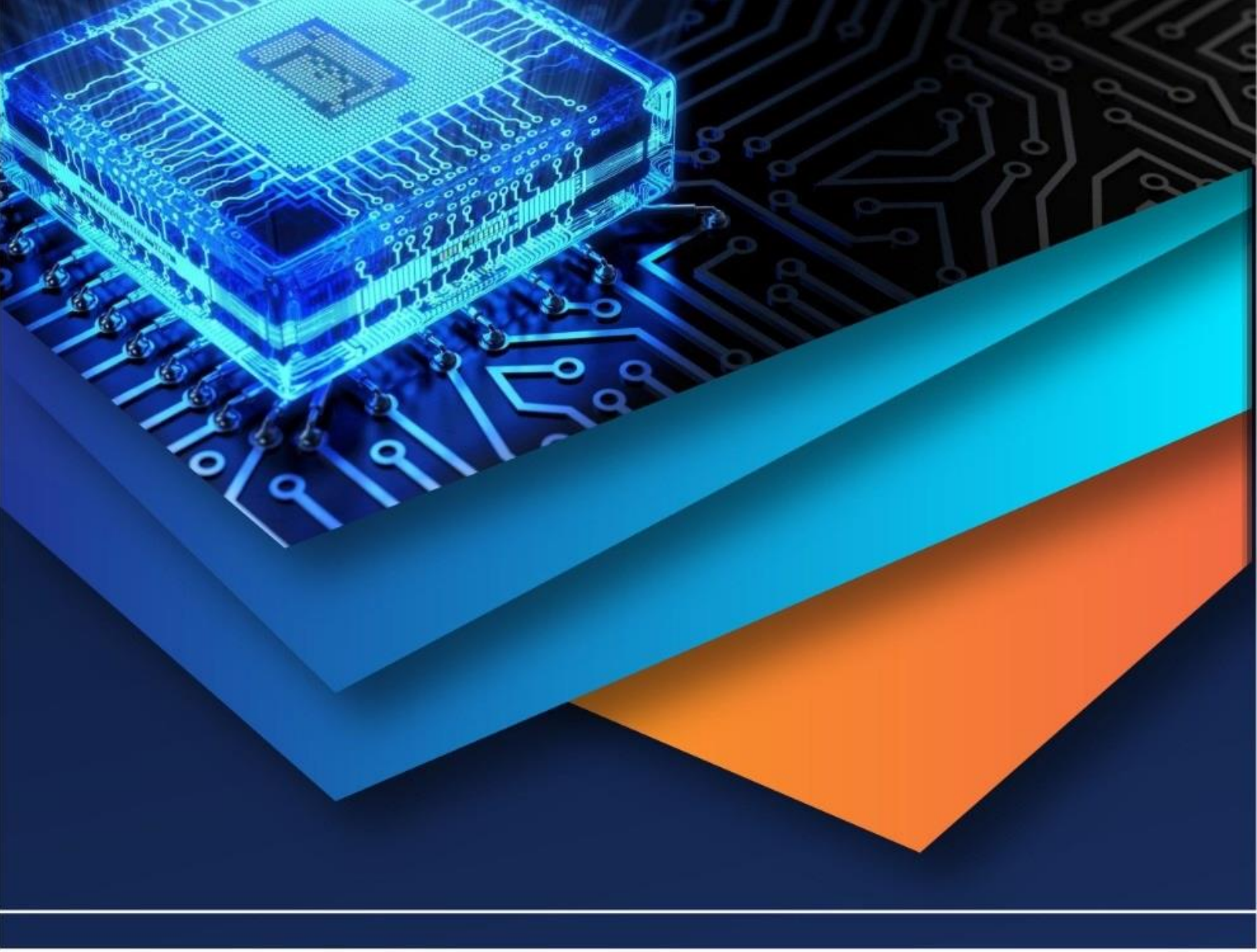

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