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STUDY ON PHYTOCHEMICAL COMPOUNDS AND METALLIC NANO PARTICLES PRESENT IN LEAVES OF HIBISCUS ROSA-SINENSIS, PSIDIUM GUAJAVA AND CALOTROPIS GIGANTEA

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ABSTRACT

Phytochemistry deals with the study of phytochemicals which are present in natural vegetation like plants and it is used in medicine and therapeutics. In this reasearch conducted, three different medicinal plant samples were taken i.e., *Hibiscus rosa-sinensis, Psidium guajava* and *Calotropis gigantea.* These three plants samples extracts were obtained by using different solvents in a Soxhlet apparatus. Nutritional quantification and Phytochemical analysis was performed to identify the presence of carbohydrates, reducing sugars, proteins and phytoconstituent like polyphenols, alkaloids, flavonoids, tannins etc. Determination of Anti-inflammatory was carried out by protein denaturation method where percentage of more hemolysis was seen on *Hibiscus rosa-sinensis* by 91.91%. Anti-inflammatory activity detection done by Human Red Blood Cell(HRBC) method where percentage of protection was calculated and found out that *Psidium guajava* have highest anti-inflammatory activity by 81.6%. Determination of Antidiabetic was done by DNSA method where *Psidium guajava* was found to have highest percentage which is 19.8%. From Leaf samples metallic nanoparticles like copper, silver and iron were synthesized and was purified. Application of this nanoparticles towards antimicrobial activity was done against organisms like *E. coli, Pseudomonas*, *Bacillus* and *Staphylococcus aureus, Fusarium* and *Aspergillus* was used. *Hibiscus rosa-sinensis* shows highest antibacterial activity and antifungal activity compared to *Psidium guajava* and *Calotropis gigantea*. These studies suggests that *Hibiscus rosa-sinensis*, *Psidium guajava* and *Calotropis gigantea* have the potential to be used in future as medicine for various diseases.

KEYWORDS: Phytochemical properties, plant extract, anti-microbial properties, nanoparticles, *Hibiscusrosa-sinensis, Psidium guajava*and *Calotropis gigantean.*

1. INTRODUCTION

Nanotechnology is emerging as a swiftly rising box with its software in science and era for the aim of producing new fabrics at the nanoscale degree (Albrecht et al., 2006). Recently, biosynthetic methods using both organic microorganisms such as bacteria and fungus or vegetation extract have emerged as a simple and viable choice to extra advanced chemical artificial procedures to acquire nanomaterials. Different forms of nanomaterials like copper, gold and silver have arisen but silver nanoparticles have proved to be among the best because it has excellent antimicrobial efficacy towards bacteria, viruses and different eukaryotic microorganisms (Gong et al., 2007). Silver nanoparticles are taking up a major role within the box of nanotechnology and nanomedicine. An essential branch of biosynthesis of nanoparticles is the appliance of plant extract to the biosynthesis reaction. Plant extracts from reside Hibiscus, leaves of Guava, and Calotropis have served as green reactants in Ag NP synthesis (Gardea-Torresdey et al, 2003). In the prevailing investigation, we record the easy synthesis of silver nanoparticles by an environmentally friendly procedure involving the in-situ reduction of Ag by way of *Psidium guajava*, *Calotropis gigantea*, *Hibiscus rosa-sinensis* extracts and the evaluation in their antimicrobial activity against more than a few human pathogenicbacteria. Aim of the present study is to investigate the phytochemical components along with anti-diabetic property, anti inflammatory property present in Hibiscus r*osa-Sinensis*, *Calotropis gigantea*, and *Psidium guajava*, and also synthesis of metallic nano particles.

2. MATERIAL AND METHODS

2.1 Collectionand Processing Samples: Leaves of *Hibiscus rosa-sinensis*, *Psidium guajava* and *Calotropis gigantea* were collected from the local garden. The leaves were washed with tap water and followed by double distilled water to remove the dust particles adhering to it, later samples were kept in hot air oven for drying, on completion of drying the samples were crushed to a fine powder and used for extraction. The powdered sample of Hibiscus rosa-sinensis, Psidium guajava and Calotropis gigantean were extracted using soxhlet apparatus with suitable solvents, acetone for *Hibiscus rosa-sinensis* and *Psidium guajava*, ethanol for *Calotropis gigantean* were used.

2.2 Quantificationof Nutritional Elements

Solvent extracts of *Hibiscus rosa-sinensis, Psidium guajava and Calotropis* gigantean were screened for the presence of total carbohydrate, reducing sugars and protein by quantitative method. The presence of total carobohydrate and proteins was detected as described by karishma et al at 630nm and at 660nm respectively. The presence of reducing sugar was measured at 540nm using di-nitrosalycylic acid as described by Sadashivam et al.

2.3 Quantification of Phyto-Chemicals Components

2.3.1 Estimation of total phenolic compounds

Total phenolic content was assessed spectrophotometrically using gallic acid as standard at 760nm as described by Karishma et al. The concentration of phenols present in percentage was calculated from the standard graph

2.3.2 Estimation of Flavonoids

Flavonoids content present in *Hibiscus rosa-sinensis, Psidium guajava and Calotropis* was determined at 510nm using quercetin standard curve. (Karishma et al).

2.3.3 Estimation of Tannin

Tannic acid was used as standard to determine the presence of tannins in *Hibiscus rosa-sinensis, Psidium guajava and Calotropis*. Tannin content was determined spectrophotometrically at 700nm using Folin-Ciocalteu reagent, the percentage of tannin present was calculated from the tannic acid standard curve. (Karishma et al).

2.3.4 Estimation of Alkaloids

Percentage of alkaloids presents in *Hibiscus rosa-sinensis, Psidium guajava and Calotropis* were examined gravimetrically as described by Karishma et al. Dried leaf powder of *Hibiscus rosa-sinensis, Psidium guajava and Calotropis* were treated with 20% acetone for 4 hours, later the suspension was filtered and the volume was reduced to get concentrated sample, the concentrated sample was treated with ammonia solution to precipitate the alkaloids, and the alkaloid extract was filtered in pre-weighed filter paper, later the filter paper with alkaloid residue was dried at oven temperature set at 60°C for 30minutes, weight of filter paper after drying was recorded and the percentage of alkaloids was calculated using the below formula.

Percentage of alkaloid = (W2 –W1/WS)*100

Where,

W1= Weight of empty filter paper

W2=Weight of filter paper with alkaloid residue

Ws=Weight of sample

2.3.5 Determination of Anti-Inflammatory Activity

2.3.5.1 Protein Denaturation Method

The reaction mixture (5ml) consists of 0.2ml of egg albumin (from hen's egg), 2.8ml phosphate buffered saline (pH:6.4) and 2ml of plant extracts. Similar volume of double distilled water served as control.

Then the mixture were incubated at 37^0C in an incubator for 15minutes and heated at 70^0C for 5minutes and then cooled. After cooling absorbance was measured at 660nm.The percentage of protein denaturation in each sample was calculated by using the formula. (Banerjee S et al)

$$\%\text{Inhibition} = \frac{AC-AS}{AC} X100$$

Where, AC – Absorbance of control, AS- absorbance of sample

2.3.5.2 Human Red Blood Cells (HRBC) Membrane Stabilization Method

Anti- inflammatory property of the given sample was determined spectrophotometrically at 560nm, HRBC suspension was prepared from the blood of healthy individual, the blood sample collected was mixed with equal volume of aqueous alsever's solution containg 2% dextrose, 0.8%sodium citrate, 0.05% citric acid, 0.42% sodium chloride and isosaline, the mixture was centrifuged at 5000rpm for 10 minutes, the supernatant obtained after centrifugation is used as HRBC suspension for the test. The compound to be tested is treated with equal volume of HRBC suspension and incubated at 35°C for 30 minutes, later the test sample mixture is centrifuged at 3000rpm for 5 minutes; absorbance of haemoglobin content in the obtained supernatant is measured at 560nm. The absorbance of HRBC suspension is taken as control. The percentage of haemolysis and the percentage of protection is calculated using below formula to know the anti – inflammatory property. (Amin et al)

$$\text{Percentage of Heamolysis} = \frac{\text{Test Absorbance}}{\text{Control Absorbance}} X\ 100$$

Percentage of Protection = 100- Percentage of Haemolysis

2.3.6 Evaluation of Anti-diabetic property

Anti-diabetic property of the given sample was determined sepectrophotometrically at 540nm, blocking ability of a-amylase causative agent to release glucose by the hydrolysis of starch substrate is tested for the samples. The samples are treated with a- amylase for a period of time later the reaction mixture is treated with starch to check the efficiency of a- amylase to release glucose, the concentration of glucose liberated is determined at 540nm using 3,5 di-nitro salicylic acid and the percentage of anti-diabetic property was calculated from the below formula. Control was made in the absence of sample. (Sundar et al)

$$\text{Anti-diabetic property \%} = \frac{CA-SA}{CA} \times 100$$

Where, CA= Control absorbance, SA = Sample absorbance

2.4 SYNTHESIS OF METAL NANOPARTICLES

2.4.1 Processing of sample

The fresh leaves of *Hibiscus rosa-sinensis, Psidium guajava, Calotropis gigantean* were washed thoroughly with tap water followed by distilled water, later the leaves were chopped into small pieces, the leaves part were treated with sterile distilled water and stirred on magnetic stirrer for 1 hour to collect aqueous extract of plant material, the suspension was filtered thoroughly and the extract obtained was used for the synthesis of metallic nano particles.

2.4.2 Synthesis of Silver Nano particles

The precursor 0.1% silver nitrate was used for the synthesis of silver nano particles, 10ml of precursor was kept on magnetic stirrer with hot plate and heated until the temperature reached 60°C, on achieving the temperature plant extract filled in the burette was introduced with continuous stirring, change in color from colorless to brown color indicates the formation of silver nano particles the reaction was stopped at this stage, The synthesized nano particle was centrifuged at 5000rpm for 10minutes, the pellet obtained after centrifugation was washed with distilled water for 2- 3 times to remove un reacted compounds, later the nano particles were dissolved in ethanol and used for characterization.(Muhammad et al).

2.4.3 Synthesis of Iron Oxide Nano particles

For the synthesis of iron oxide nano particles from *Hibiscus rosa-sinensis, Psidium guajava, Calotropis gigantean* 0.1% ferrous sulphate was used as precursor, 10ml of precursor was kept on magnetic stirrer with hot plate and heated until the temperature reached 60°C, on achieving the temperature plant extract filled in the burette was introduced with continuous stirring, change in color from light brown to dark brown precipitate indicates the formation of iron oxide nano particles the reaction was stopped at this stage, The synthesized nano particle was centrifuged at 5000rpm for 10minutes, the pellet obtained after centrifugation was washed with distilled water for 2- 3 times to remove un reacted compounds, later the nano particles were dissolved in ethanol and used for characterization. (Saranya et al).

2.4.4 Synthesis of Copper Nano particles

The precursor 0.1% Copper sulphate was used for the synthesis of copper nano particles, 10ml of precursor was kept on magnetic stirrer with hot plate and heated until the temperature reached 60°C, on achieving the temperature plant extract filled in the burette was introduced with continuous stirring, change in color from blue to light brown indicates the formation of copper nano particles the reaction was stopped at this stage, The synthesized nano particle was centrifuged at 5000rpm for 10minutes, the pellet obtained after centrifugation was washed with distilled water for 2- 3 times to remove un reacted compounds, later the nano particles were dissolved in ethanol and used for characterization. (Ayona Jayadev et al)

2.5 Characterization Of Metal Nanoparticles

The synthesized metal nano particles were characterized to identify the maximum absorption peak using UV – visible spectrophotometer. All the metal nano particles absorbance was read from UV range 200nm to visible range 600nm. The maximum absorption peak achieved shows the characteristic of the metal nano particle.

2.8 Evaluation Of Anti-Microbial Proerty Present In Metal Nano-Particles

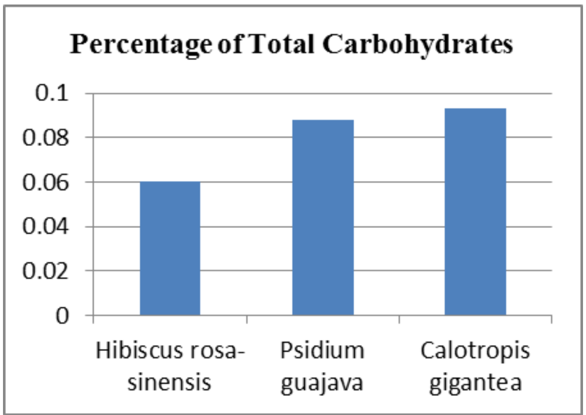
In vitro detection of anti-microbial property was examined for sample *Hibiscus rosa-sinensis, Psidium guajava, Calotropis gigantea*. Anti microbial property of samples against bacteria and fungi was investigated by the agar diffusion method. The samples were used for the determination of zone of inhibition or sensitivity, against bacterial strains *(Staphylococcus aureus, Pseudomonas sps, Bacillus sps* and *E.coli)* and fungal strains *(Fusarium sps* and *Aspergillus sps*, Anti-bacterial plates were incubated at 37°C for 24 hours where as anti-fungal plates were incubated at 30°C for 72 hours to observe zone of inhibition. (Behera et al).

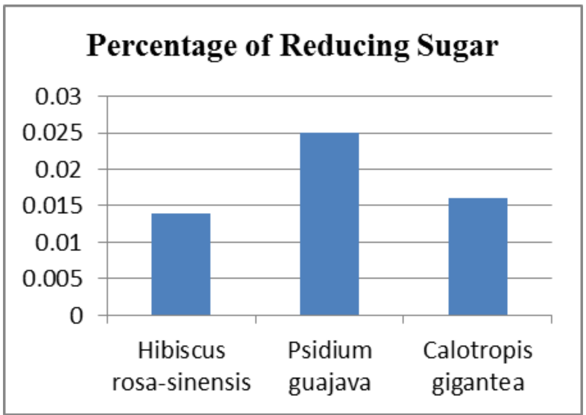
3. RESULT AND DISCUSSION

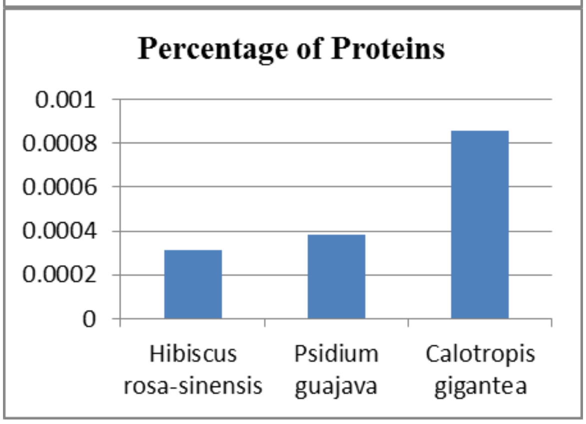
The present study was focused on the evaluation of phytochemical components present in *Hibiscus rosa-sinensis, Psidium guajava, Calotropis gigantean*. All the three plant varieties were screened for phytochemical elements such as total carbohydrates, reducing sugars, proteins, total phenol, alkaloids, tannin and flavonoids. Total carbohydrate and protein content in *Calotropis gigantean* was found be highest compared to *Hibiscus rosa-sinensis, Psidium guajava* whereas reducing sugars, tannins, flavonoids and alkaloids contents were more in *Psidium guajava* compared to *Hibiscus rosa-sinensis* and *Calotropis gigantean* Out of three plant varieties. Total phenols contents were more in *Hibiscus rosa-sinensis.* The plant varieties were screened for its anti-diabetic property and anti – inflammatory property through in-vitro tests. All the plant varieties showed the presence of anti-diabetic property and anti-inflammatory property. Among *Hibiscus rosa-sinensis, Psidium guajava, Calotropis gigantean, Psidium guajava* showed 19.8% anti- diabetic property and anti –inflammatory property 20.2% for protein denaturation method and 81.6% for HRBC stabilization method which was recorded highest compared to other two plant varieties. The results are tabulated in table 1 and the same is graphically represented. The study was also carried out on the synthesis of metal nano particles from *Hibiscus rosa-sinensis, Psidium guajava, Calotropis gigantean* and characterization of synthesised nano particles. Silver nitrate, copper sulphate and ferrous sulphate were used as precursor for the synthesis of silver nano particles, copper nano particles and iron oxide nano particles respectively. The synthesised nano particles were purified by following centrifugation. The purified nano particles were characterized by UV – visible spectrophotometer, the absorbance of the synthesised nano – particles were read from 200nm to 600nm wavelength to check maximum absorbance peak with surface Plasmon resonance (SPR). Silver nano particles UV – Vis Spectra showed SPR peak at 420nm, SPR peak at 540nm was observed for copper nano particles and 320nm SPR peak for Iron Oxide nano particles. The results were evidence of the findings of Muhammad et al, Ayona et al and Saranya et al. The synthesised nano particles were screened for its anti- microbial property against pathogens *Escherichia coli*, *Staphylococcus aureus*, *Bacillus sps*, *Pseudomonas sps*, *Fusarium sps* and *Aspergillus sps.* Results of anti-microbial property are tabulated in table: 2, 3 & 4. Silver, Copper and Iron oxide nano particles synthesized from *Hibiscus rosa-sinensis*, *Psidium guajava*, *Calotropis gigantean* exhibited anti – microbial property against all the test pathogen, highest zone of inhibition against the test pathogens were exhibited by silver nanoparticles. The results were evidence of the findings of Muhammad (2022), Ayona (2021) and Saranya (2017).

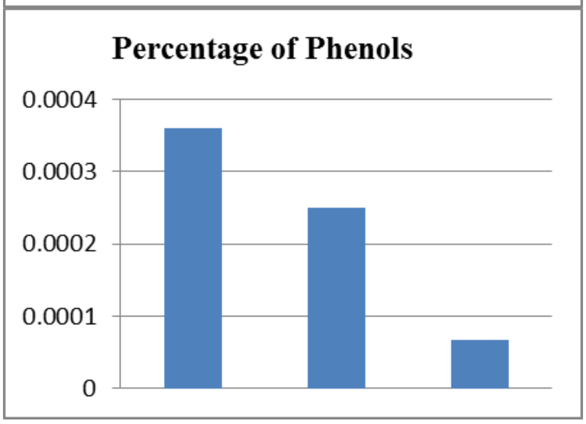
Table 1: Phytochemical contents of *Hibiscus rosa-sinensis, Psidium guajava, Calotropis gigantean.*

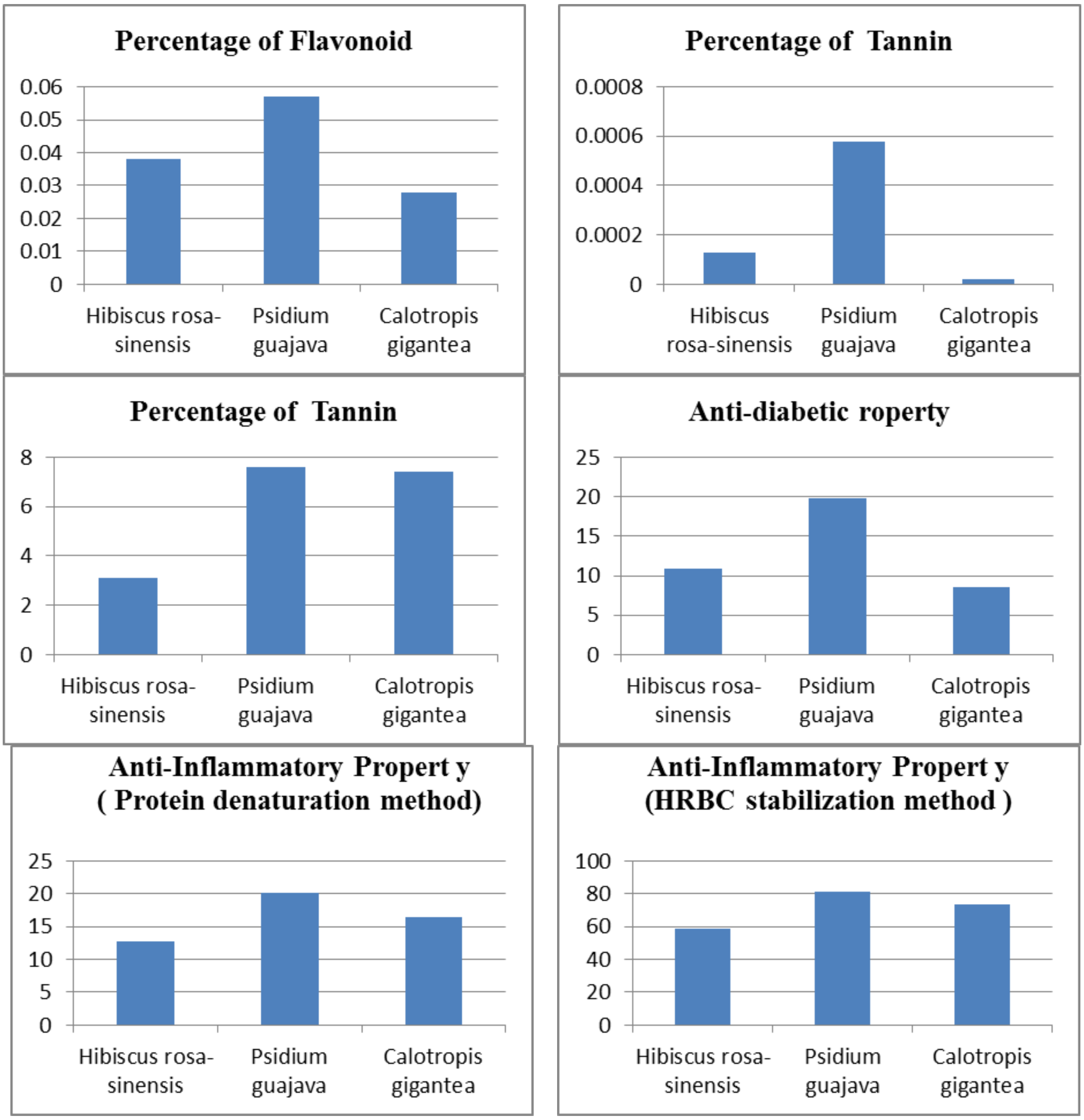
Phytochemical Contents	***Hibiscus rosa-sinensis***	***Psidium guajava***	***Calotropis gigantea***
Total Carbohydrates %	0.06	0.088	0.093
Reducing sugar %	0.014	0.025	0.016
Proteins %	0.00031	0.00038	0.00086
Total Phenols %	0.00036	0.00025	0.000068
Flavonoids %	0.038	0.057	0.028
Tannin %	0.00013	0.00058	0.00002
Alkaloids %	3.1	7.6	7.4
Anti-diabetic property %	10.9	19.8	8.6
Anti- inflammatory property % (Protein denaturation method)	12.8	20.2	16.5
Anti- inflammatory property % (HRBC stabilization method)	58.83	81.6	73.8











Graphical representation of Phytochemical contents present in *Hibiscus rosa-sinensis, Psidium guajava, Calotropis gigantea.*

Table 2: Anti-Microbial property of Silver nanoparticles.

Plant Material →	***Hibiscus rosa-sinensis***	***Psidium guajava***	***Calotropis gigantea***
Test Organism↓	**Zone of Inhibition in mm**		
Bacillus sps	02	02	02
Esherichia coli	09	05	06
Pseudomonas sps	14	04	06
Staphylococcus aureus	14	15	07
Fusarium sps	12	10	05
Aspergillus sps	08	07	07

Table 3: Anti-Microbial property of Copper nanoparticles.

Plant Material →	***Hibiscus rosa-sinensis***	***Psidium guajava***	***Calotropis gigantea***
Test Organism↓	**Zone of Inhibition in mm**		
Bacillus sps	02	02	02
Esherichia coli	01	04	03
Pseudomonas sps	10	08	06

Staphylococcus aureus	08	09	10
Fusarium sps	08	06	07
Aspergillus sps	11	06	11

Table 4: Anti-Microbial property of Iron Oxide nanoparticles.

Plant Material →	***Hibiscus rosa-sinensis***	***Psidium guajava***	***Calotropis gigantea***
Test Organism↓	**Zone of Inhibition in mm**		
Bacillus sps	02	02	02
Esherichia coli	04	03	07
Pseudomonas sps	06	03	05
Staphylococcus aureus	07	08	06
Fusarium sps	09	06	14
Aspergillus sps	06	03	05

4. CONCLUSION

The present study on *Hibiscus rosa-sinensis, Calotropis gigantea* and *Psidium guajava* proves that phytochemical contents are found abundance and also have the ability to produce metal nano particles such as silver, copper and iron oxide nano particles, the three plant varieties chosen are proven to have anti – diabetic property and anti-inflammatory property also. The synthesised metal nano particles showed UV characteristic property with a maximum SPR peak. The study was also focused on anti microbial property of the metal nano particles, the test bacterial pathogens *Escherichia coli, Staphylococcus aureus, Bacillus sps, Pseudomonas sps* and fungal pathogens *Fusarium sps and Aspergillus sps* were used.

Among the three nano particles, silver nano particles showed highest anti microbial activity against the test pathogens compared to copper and iron oxide nano particles. From the study we conclude that *Hibiscus rosa-sinensis, Calotropis gigantea* and *Psidium guajava* plant species have a rich medicinal properties and also the nano particles synthesised from the source has the same and equal medicinal properties.

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