Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 1984-1990
Received: 01-05-2018
Accepted: 05-06-2018

S Suresh
Department of Chemistry, Vetri Vinayaha College of Engineering & Technology, Trichy, Tamil Nadu, India

G Pradheesh
Department of Chemistry, SNS College of Technology, Coimbatore, Tamil Nadu, India

Vincent Alex Ramani
Department of Chemistry, Natural products chemistry lab, St. Joseph' College, Trichy, Tamil Nadu, India

Correspondence
S Suresh
Department of Chemistry, Vetri Vinayaha College of Engineering & Technology, Trichy, Tamil Nadu, India

Biosynthesis and characterization of CuO, MgO and Ag_2O nanoparticles, anti inflammatory activity and phytochemical screening of the ethanolic extract of the medicinal plant *Pavetta indica* Linn

S Suresh, G Pradheesh and Vincent Alex Ramani

Abstract
In this study, the Copper oxide, Megnesium oxide and Silver oxide nanoparticles are biosynthesized by a novel, green chemistry procedure using the leaf extract of the medicinal plant *Pavetta indica* Linn. The presences of various phytochemical constituents were identified through screening of ethanolic extract of plant *Pavetta indica* Linn. The biosynthesis of CuO, MgO and Ag_2O nanoparticles were accomplished by the literature reported standard method. The nanoparticles were subjected to the studies – X-ray Diffraction, Scanning Electron Microscopy and Particle Size Analysis. The plant extract showed a significant anti inflammatory activity on denaturation of egg albumin.

Keywords: *Pavetta indica* Linn, nanoparticles, biosynthesis, anti inflammatory activity

Introduction
Nanotechnology is a multidisciplinary field undergoing phenomenal development. The nanometer-sized particles assume novel structural, optical and electronic properties that are not attainable by individual molecules as bulk solids [1]. The characters of metal and metal oxide nanoparticles have been of great interest due to their distinctive features such as catalytic activity, optical, magnetic and electrical properties. Various viable methodologies have been designed for the synthesis of nanoparticles having size-specific dependent properties. The concept of green chemistry has provided a lead towards the green-synthesis of materials that are not harmful to environment and human health [2]. Plant extracts have enzymes (particularly hydrogenases and reductases) and phytochemicals such as terpenoids, flavonoids, phenols and dihydric phenols and many such compounds which may react with metal salts leading to metal oxide nanoparticles synthesis. *Pavetta indica* Linn. [3, 4] (Tamil: Kattu thirani, Panna pavadai, Sirukonnai, Pavattai) is a shrub or small tree belongs to the family of Rubiaceace. The leaves very variable elliptic – oblong to elliptic – lanceolate and obovate – oblong, glossy – green flowers are white. The roots are said to possess purgative, aperient, diuretic and tonic properties and are prescribed in visceral obstructions, jaundice, headache, urinary diseases and dropsical affections. The phytochemical investigation [5], chemical composition of essential oils [6] and physio – phytochemical screening [7] had been reported in this plant. The plant was studied anti – inflammatory potential [8], analgestic [9], antimicrobial [10], antipyretic activities [11]. The present study involves the green-chemistry approach for the synthesis of CuO, MgO and Ag_2O nanoparticles from the plant extract - *Pavetta indica* Linn. It also focuses on the phytochemical screening, anti inflammatory activity of the crude extract and the morphological characterization of the synthesized nanoparticles.

Materials and Methods
The leaves of *Pavetta indica* Linn. were collected from Narthamalai region (Near Pudukkottai District) from the month of July at 11.00 am. They were identified and authenticated by Dr. S. Soosairaj (SJCBOT 2474), Assistant Professor, Department of Botany and with Rapinet Herbarium, St. Joseph college (Autonomous) Tiruchirappalli -620002, Tamil Nadu, India.

Sample preparation
The leaves of *Pavetta indica* Linn. were shade dried and powdered well. About 20g of the plant leaves were soaked in 100 mL of ethanol. It was left for 24 hours in order to extract the phytoconstituents- alkaloids, carbohydrate, tannins, steroidal glycosides, steroids, flavanoids,

acids and others. The extract was filtered using Whatmann No.1 filter paper to remove the residues.

Phytochemical Screening [12, 13]

The phytochemical screening of the leaf extract was carried out applying the standard methods and tests as prescribed by J B Harbone [14]. Hence, the presence or absence of various phytoconstituents was determined. The experimental procedures and the results are given in Table No -1.

Table 1: Details of Phytochemical Screening of the ethanolic extract of *Pavetta indica* Linn.

S. No	Name of the Test	Experimental Procedure	Phytoconstituent
1	a) Mayer's test	0.5 mL of extract was treated with Mayer's reagent (pottassiomercuric iodide solution) to gave cream colored precipitate.	Alkaloids
	b) Dragondraff test	0.5 mL of extract was treated with Dragendroff's reagent (potassium bismith iodide). Formation of orange or orange red precipitate was obseved.	Alkaloids
	c) Wagner's test	0.5 mL of extract was treated with Wager's reagent (solution of iodine with KI) and it gave an brown or reddish brown precipitate.	Alkaloids
2	a) Molish's test	0.5mL of extract was treated with 1 mL of alpha napthol and Con. H_2SO_4, which gave purple coloation.	Carbohydrates
	b) Fehling's test	0.5 mL of extract to which equal quantity of Fehling's solution A & B were added. The content was heating to give brick red precipitate was obtained.	Carbohydrates
3	Foam test	Dilute 1mL of alcohol in 0.5 mL of extract. The mixture was diluted to 20 mL of distilled water. It was shaken well for 15min. The formation of foam was observed.	Saponins
4	Lead acetate test	0.5 mL of alcoholic or aqueous extracts was treated with lead acetate solution. White precipitate was observed.	Tannins
5	Ferric chloride test	0.5 mL of alcoholic extracts was treated with 2 drops of neutral ferric chloride. brownish green coloration was observed	Pseudo Tannin (Condensed tannin)
6	Ammonia test	0.5mL of extract was treated with aqueous ammonia solution. It was exposed to air which gradually develops a green color.	Chlorogenic acid
7	NaOH test	0.5 mL of extract was treated with aqueous sodium hydroxide solution formation of blue violet coloration	Anthocyanin
8	Libermann's Burchard test	0.5 mL of extract was dissolved in 2mL chloroform. The mixture was treated with acetic acid, acetic anhydride and conc. H_2SO_4 gave bluish green coloration.	Steroidal glycosides
9	H_2SO_4 test	0.5 mL of extract was treated with 80% H_2SO_4, gave deep yellow color.	Saponins glycosides
10	Ammonia test	0.5 mL of extract was mixed 2mL of ammonia. The mixture was observed under UV and visible lights - formation of fluorescence.	Flavonoids
11	Shinoda's test	0.5 mL of alcoholic extract was treated with magnesium foil and conc. HCl. It gave intense cherry red coloration	Flavones
12	NaOH test	0.5 mL of alcoholic extract was treated with 10% Sodium hydroxide solution, yellow coloration was observed.	Coumarins
13	Salkowaski test	0.5 mL of extract was dissolved in 1mL of chloroform. The mixture was treated with Conc. H_2SO_4. It gave red coloration.	Steroids

Synthesis of Nanoparticles [15 - 17]

The nanoparticles of metal oxide were synthesized using the metal nitrate salt and the plant extract of *Pavetta indica* Linn. Exactly 0.1M solution of the metal nitrate was mixed with 10 mL of the plant extract, through magnetic stirring at a temperature range 100 – 120 °C for about 24 hours. The metal ions might form complex with phytoconstituents of the plant extract. The progress of the complexation reaction was observed through the characteristics colour change. The complex formed was ultra centrifuged at 5000 -10000 rpm for 20 minutes. The residual complex was dried in air oven at 140 °C. The complex was calcinated in muffle furnace at 450°C for about 3 hours. The nanoparticles formed were taken further studies. The details of the synthesis of nanoparticle are given here.

Table 2: Details of the favorable condition used in the Synthesis of Nanoparticles

S. No	Metal Salt	Plant Extract	Magnetic stirring and Complex formation	Ultra Centrifugation	Oven drying	Calcination
1	Copper Nitrate $Cu(NO_3)_2.3H_2O$	Ethanolic extract of *Pavetta indica* Linn.	stirring for 24 h at 100 – 120 °C deep blue to brick red complex	5000 – 10000 rpm	140 °C for 8h	450°C
2	Magnesium Nitrate $Mg(NO_3)_2.3H_2O$	Ethanolic extract of *Pavetta indica* Linn.	Stirring for 24 h at 100 – 120 °C colourless complex	5000 – 10000 rpm	140 °C for 8h	450°C
3	Silver Nitrate $AgNO_3$	Ethanolic extract of *Pavetta indica* Linn.	Stirring for 24 h at 100 – 120 °C colourless complex	5000 – 10000 rpm	140 °C for 8h	450°C

Characterization studies on the Nanoparticles

Powder X-Ray Diffractometry (XRD) was performed with a Shimadu, Japan instrument at a scanning rate of 10 per minute in the theta – 2 theta range of 10 to 80, with Cu Ka radiation (k = 1.5406 A˚) and a nickel filter. Particle Size Analysis was measured using the Malvern Instruments, UK (Zeta Sizer Nano ZS90). The surface morphology of the nanoparticles was studied by scanning electron microscopy employing the instrument JEOL, Japan (JSM 6390) along with EDAX using INCA Energy 250 LN_2 Closed.

Anti-inflammatory Activity

The sample solution were prepared in five different concentrations – 100, 200, 300, 400 and 500 µg/mL. Exactly 2 mL of each sample solution was mixed with 2.8 mL of phosphate buffered saline of pH 6.4 and 0.2 mL of egg

albumin (from fresh hen's egg). Each mixture was incubated at (37±1) °C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 5 min. After cooling, the absorbance was measured at 660 nm taking water as the blank. A set of five different concentrations of diclofenac sodium standard solutions (100, 200, 300, 400 and 500 µg/mL) was prepared as the reference drug. Absorbance was measured of each of the standard solution was done by the same procedure. Each of the absorbance values was compared control and percentage inhibition was calculated for the protein denaturation process using the formula given here.

$$\% \text{ Inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Statistical analysis

Tests were carried out in triplicate for three separate experiments. The amount of sample required to inhibit free radicals concentration by 50% (Inhibition Concentration) IC_{50}, was graphically determined by a Linear Regression method using Ms - Windows based graphed Instat, version-3 software. The results were represented graphically by indicating mean and Standard deviation values.

Results and Discussion

Phytochemical screening

The results of the phytochemical screening of the plant *Pavetta indica* Linn. It was analysed qualitatively for the phytochemically active compounds and the results are given in the Table No-3. The ethanolic and methanolic extracts of the leaves of *Pavetta indica* Linn. Showed the presence of phytochemically active compounds such as alkaloids, carbohydrate, tannins, steroidal glycosides, steroids, flavonoids. The following metabolites were analysed to be absent in the ethanolic and methanolic extracts - saponins, sapanin glycosides, cumarin, anthocyanin and flavones. The details are given in the Table No - 3.

Table 3: Details of Phytochemical Screening of the extracts of *Pavetta indica* Linn.

S. No	Phytochemical Constituents	Name of the Test	Methanol Extract	Ethanol Extract
1	Alkaloids	Mayer's test Dragondraff test Wagner test	+ + +	+ + +
2	Carbohydrates	Molish test Fehling test Benedicts test	+ - -	+ + -
3	Saponins	Foam test	-	-
4	Tannins	Lead Acetate test	+	+
5	Pseudo tannins	Ferric chloride.	Condensed Tannin	Condensed Tannin
6	Chlorogenic acid	Ammonia test	+	+
7	Anthocyanin	NaOH test	-	-
8	Steroidal Glycosides	Liebermann's Burchard test	+	+
9	Saponins glycosides	H_2SO_4 test	-	-
10	Flavonoids	Ammonia test	+	+
11	Flavones	Shinoda's test	-	-
12	Coumarin	Sodium chloride test	-	-
13	Anthracene glycoside	Ammonia test	-	-
14	Steroids	Salkowaski test	+	+

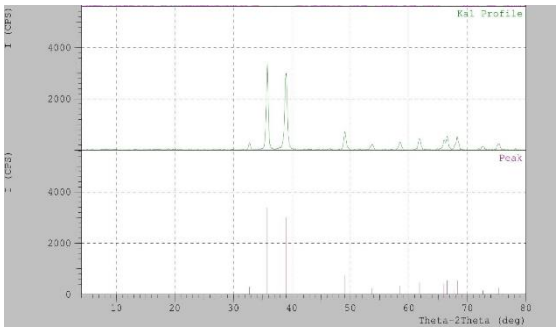
Note: + = Present - = Absent

Nanoparticle Characterization [18 - 20]

X- Ray Diffractometer was used to study the powered sample for confirming the presence of CuO, MgO and Ag_2O for obtaining its structure. The peaks of '2θ value' of synthesized nanoparticles are good agreement with corresponding pure CuO, MgO and Ag_2O. The peaks observed in the graph are found to be similar with the literature as reported by Samira Bagheri *et. al* [21]. The average size of the particles was determined by applying the Debye-Scherrer's formula.

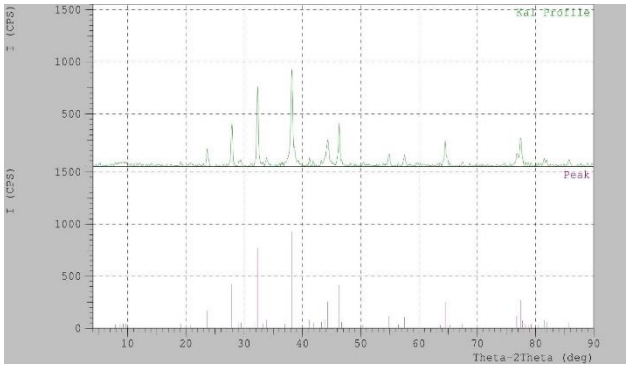
Table 4: X- Ray Diffraction studies of 2Θ - Values of Nanoparticle

Sl. No	Nano Oxides	2Θ - Values of Nano particle		Inference
		Theoretical Values	Experimental Values	
1	CuO	35.6	35.77	The values are in good agreement with earlier reports [22,23]
		39.0	38.98	
		49.7	49.06	
2	MgO	37.10	36.97	
		43.10	42.93	
		62.10	62.29	
3	Ag_2O	38.45	38.17	
		46.35	44.31	
		64.75	64.51	
		78.05	77.45	



(a) Graph of 2 Θ values of CuO

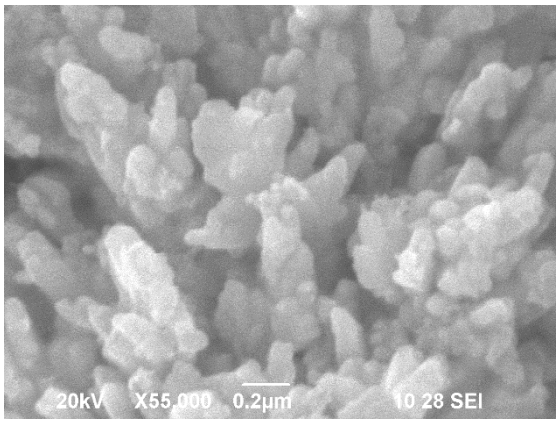
(b) Graph of 2 Θ values of MgO



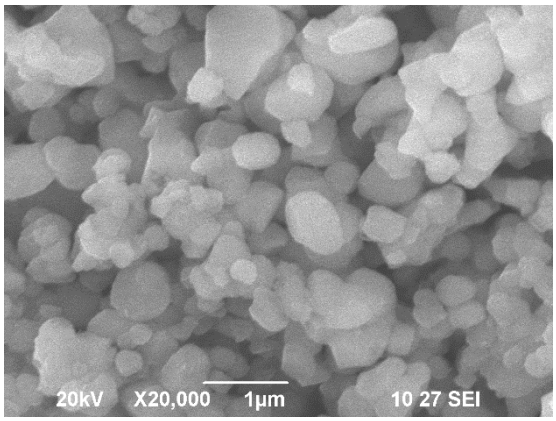
(c) Graph of 2 Θ values of Ag_2O

Fig 1: X-Ray Diffractometry spectrum of Nanoparticles

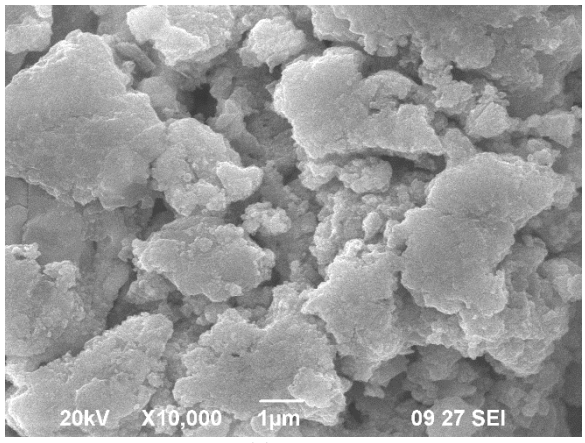
The Scanning Electron Microscopy analysis was used to determine the structure of the reaction product (CuO, MgO and Ag_2O) that were formed. Thin films of the sample were prepared on a carbon-coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. The individual Copper oxide nanoparticles were observed to exhibit the elongated hexagon shape existing in several aggregates. The Magnesium oxide nanoparticles were found to have the regular hexagonal morphology. The Silver oxide nanoparticles were identified to be in distorted square shape.



(a) CuO



(b) MgO

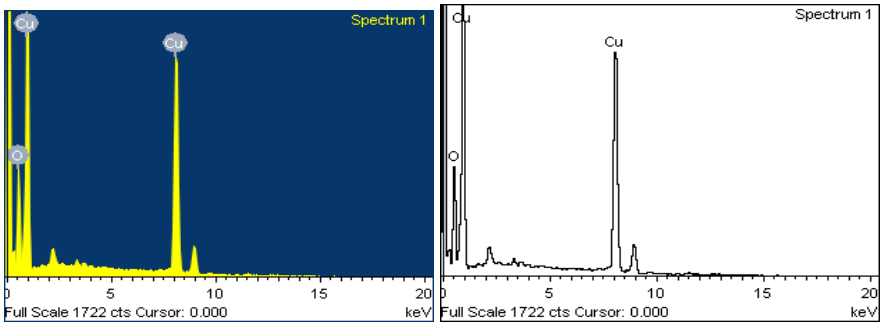


(c) Ag_2O

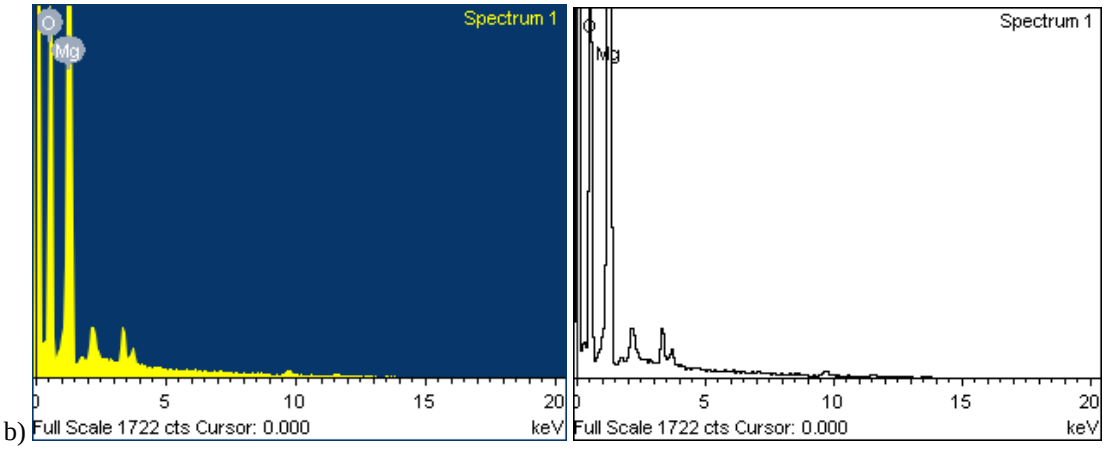
Fig 2: Scanning electron microscopy images of nanoparticles

EDAX results in Figure No – 3 further confirmed the formation of the CuO, MgO and Ag_2O nanoparticles, indicating the appropriate signals for Copper, Magnesium, Silver and Oxygen. (In the respective images).

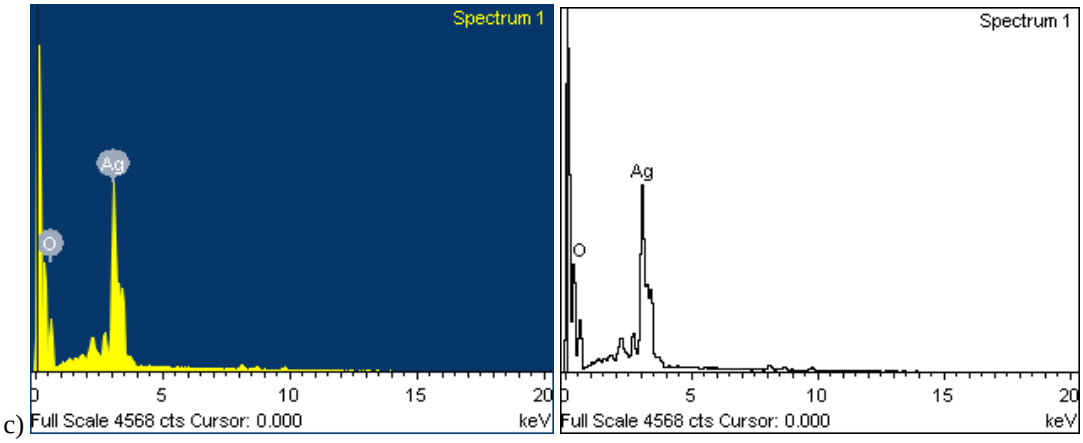
a)



(a) CuO



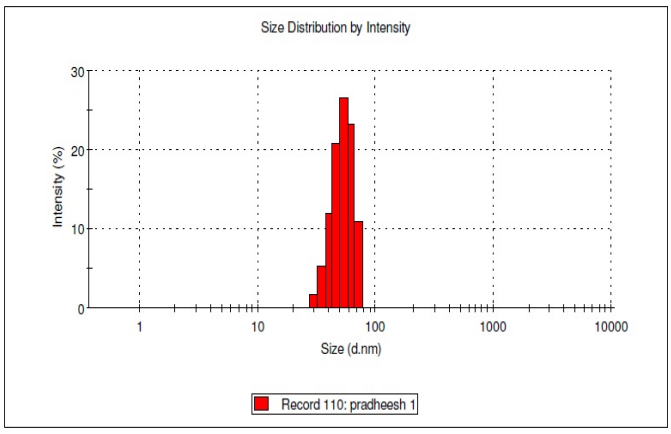
b) (b) MgO



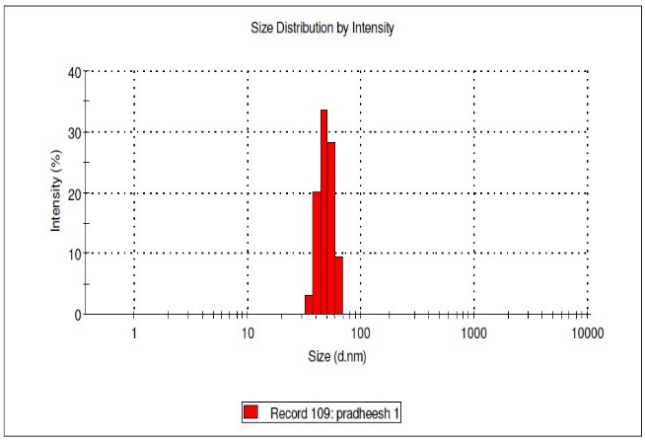
c) (c) Ag_2O

Fig 3: EDAX Images of Nanoparticles

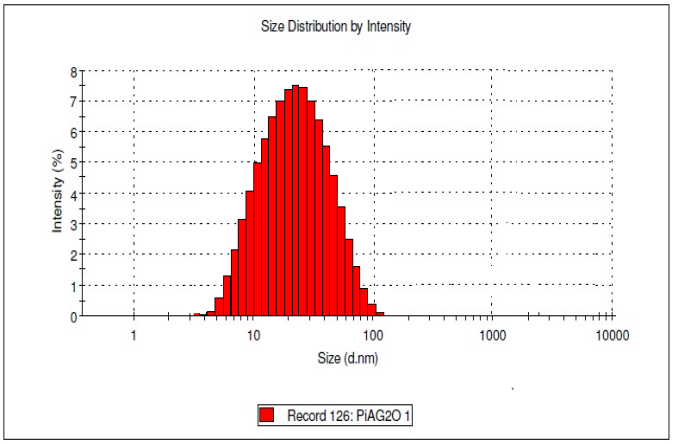
The average size of the nanoparticles and the statistical distribution of the size are determined using the particle size analyzer. The results are shown in Figure No - 4. The particle size of Copper oxide nanoparticles is found to be in 78 nm and the width is 68.32 nm. The Magnesium oxide nanoparticle has to be size of 81 nm of width 25.08 nm. Silver oxide nanoparticle size was to be identified as 49.8 nm of width 169.2 nm.



(a) CuO



(b) MgO



(c) Ag_2O

Fig 4: Particle Size Analyzer Images of Nanoparticles

Anti-inflammatory activity [24, 25]

The inhibitory effect of different concentrations of extract on protein denaturation is shown in Table No - 5 (Figure No – 5). The inhibition on denaturation of egg albumin was found to increase proportionally with increase in concentration of the plant extract from 100 to 500 □g mL^{-1}. The *in-vitro* anti-inflammatory activity of the extract in the chosen concentrations was comparable to the diclofenacsodium, a reference drug used in the concentrations ranging from 100 to 500 µg/mL. A remarkable difference in the inhibition of protein denaturation was observed for the concentrations of 400 and 500 µg/mL of the extract. The IC_{50} values of ethanolic extract of *Pavetta indica* Linn. and the standard - diclofenac sodium were determined to be 304.60 and 267.63 500 µg/ mL, respectively.

Table 5: *In vitro* Anti Inflammatory Activity of Egg albumin (Mean ± SD)

Test Solution	Concentrations (µg / mL)	Optical Density	% inhibition	IC $_{50}$ - Values
Control	-	2.368	-	-
Standard - Diclofenac sodium	100	1.86±19	21.37±1.98	267.63
	200	1.50±86	36.45±2.37	
	300	1.04±34	55.94±3.47	
	400	0.48±66	79.45±4.65	
	500	0.36±82	84.45±6.84	
Ethanolic Extract of the Plant *Pavetta indica* Linn.	100	1.850±1.81	21.87±1.53	304.60
	200	1.521±2.31	35.76±2.50	
	300	1.181±3.91	50.12±3.50	
	400	0.885±4.83	62.40±4.36	
	500	0.550±5.67	76.77±5.37	

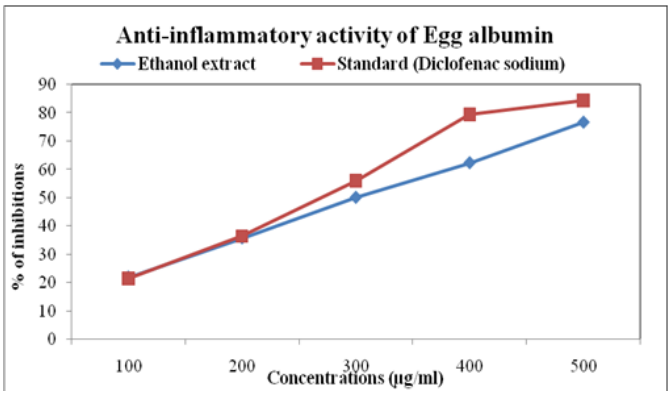


Fig 5: *In vitro* Anti-inflammatory activity of Egg albumin

Conclusion

The results of the phytochemical screening revealed that the ethanolic extract of *Pavetta indica* Linn. was found to have the phytoconstituents - alkaloids, carbohydrate, tannins, steroidal glycosides, steroids and flavonoids. The significant anti-inflammatory activity of the extract was attributed to the presence of the observed phytochemicals. The synthesized elongated hexagon shaped Copper oxide, regular hexagonal shaped Magnesium oxide and distorted square shaped Silver oxide nanoparticles were confirmed to exist in acceptable statistical distribution.

Acknowledgement

We acknowledge the Management, Principal and Mrs. S. Vasantha Gowri, Head, Department of General Engineering, Vetri Vinayaha College of Engineering and Technology for their constant encouragement in doing this part of my research work. We place on record once sincere acknowledgement to Rev. Fr. Dr M. Arockiasamy Xavier SJ, Principal, St. Joseph's College (Autonomous) for permitting us to do laboratory facilities in the Department of Chemistry, Tiruchirappalli - 620002.

References

1. Anitha Siromani, Kiruba Daniel, Garima Singhal, Riju Bhavesh, Kunalkasaraiya, Ashish Rajanan Sharma, *et al*. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. Journal of Nanoparticle Research. 2011; (1):2981-2988.
2. Patete JM, Peng X, Koenigsmann C, Xu Y, Karn B, Wong SS. Green Chemistry. 2011; (13):482.
3. The Wealth of India. A dictionary of Indian raw materials and industrials products, Raw material. Publications and information Directorate, CSIR, New Delhi. 1951; (2):282-283.
4. Bur Kill HM. The Useful Plants of western tropical. 1985; (4).
5. Ramamoorthy J, Venkatramanan S, Meena R, Devi AP, Satkar Prasad. Phytochemical investigation of *Pavetta indica* Linn. Indian Journal of Chemical Science. 2011; (9):397-402.
6. Prasad K, Moulekhi K, Bisht G. Chemical composition of the essential oil of *Pavetta indica* Linn._L leaves. Research Journal of Phytochemistry**.** 2011; (5):66-69.
7. Ramamoorthy J, *et. al*. Physio – Phytochemical screening and Diuretic activity of leaves of *Pavetta indica* Linn.

Journal of Pharmaceutical Science and Research. 2010; 2:506-512.
8. Subhash C, Mandal *et al*. Evaluation of anti – inflammatory potential of *Pavetta indica* Linn. leaf extract (family: Rubiaceae) in rats. Phytotherapy Research. 2008; (17):817-820.
9. Golwala DK, *et al*. Analgestic activity of Ethanolic leaf Extract of *Pavetta indica* Linn. International Journal of Pharmaceutical Science and Drug Research. 2009; (1):119-120.
10. Vinod Kumar Gupta, Charanjeet Kaur, Aritra Sim Lai, Amit Roy. Antimicrobial activity of *Pavetta indica* Linn. Leaves. Journal of Applied Pharmaceutical Science. 2013; (3):78-82.
11. Lakshmi S, Mohana *et al*. Antipyretic Activity of *Pavetta indica* Linn. (Rubiaceae) Leaf Extract. Selected issues in ethnopharmacology / Division of Pharmacognosy and Phytochemistry. Department of Pharmaceutical Technology. Jadavapur University, Kolkata, India, 2009.
12. Evans WC, Trease and Evans. Pharmacology. 15th Edition, Reed Elsevier India Pvt Ltd., New Delhi, 2006.
13. Harbone JB. A Guide to Modern Techniques of Plant Analysis. 3rd Edition, Kluwer Academic Publisher U.S.A, 1998.
14. Harbone JB, Phytochemical Methods. 2nd Edition, 1928.
15. Vasudev D, Kulkarni, Pramod S, Kulkarni. Green Synthesis of Copper Nanoparticles using *Ocinum Sanctum* leaf extract. International Journal of Chemical Studies. 2013; (1):1-4.
16. Suresh J, Yuvakkumar R, Sundarajan R, Hong SI. Green Synthesis of Magnesium oxide nanoparticles using the plant extract *Nephelium lappaceum* L. Advanced Material Research. 2014; (9):141-144.
17. Ahmed S, Ullah S, Ahmad M, Swami BL, Green synthesis of Silver nanoparticles using *Azadirachta indica* aqueous leaf extract. Journal of Radiation Research and Applied Science. 2015; (9):1-7.
18. Sayedeh Fatemeh Shaffiey, Maryam Shapoori, Abbas Bozorgnia, Mohammad Ahmadi.Synthesis and evaluation of bactericidal properties of CuO nanoparticles against *Aeromonas hydrophila***.** Nanomedicine Journal. 2014**; (**1):198-204.
19. Arul Dhas, Paul Raj, and Gedanken. Synthesis, Characterization and properties of metallic Copper nanoparticles. Chemistry of Materials. 1998; (10):1446-1452.
20. DR. Baer DJ, Gaspar, Nachimuthu P. Application of Surface chemical analysis tools for characterization of nanoparticles. Analytical and Bio analytical chemistry. 2010; (396):983-1002.
21. Samira Bagheri, Chandrappa KG, Sharifah Bee Abd Hamid Facile. Synthesis of nano-sized ZnO by direct precipitation method, Der Pharma Chemica. 2013; (5):265-270.
22. Ponnaiah Tamilselvi, Arunagiri Yelilarasi, Muthusamy Hema and Ramasamy Anbarasan. Synthesis of hierarchical structured MgO by sol-gel method. NANO BULLETIN. 2013; (2):130106.
23. Kumari Jyoti, Mamta Baunthiyal, Ajeet Singh. Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. leaves and their synergistic effects with antibiotics. Journal of Radiation Research and Applied Sciences. 2016; (9):217-227.
24. Sangita Chandra, Priyanka Chatterjee, Protapaditya Dey and Sanjibn Bhattacharya. Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacific Journal of Tropical Biomedicine. 2012; S178-S180.
25. Williams LAD, O. Connar A, Latore L, Dennis O, Ringer S, Whittaker JA. The *in vitro* Anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process, West Indian Medical Journal. 2008; (57):327-331.