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Research Article

GREEN SYNTHESIS OF IRON NANOPARTICLES FROM USED GREEN TEA LEAVES

Pradhipa S and Priya R Iyer

Department of Biotechnology Women's Christian College, College Road, Chennai-600006

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ABSTRACT

The *Camellia sinensis* (green tea leaves) are discarded and considered as waste once it's prepared. Hence, the used green tea leaves can act as an adsorbent for the removal of heavy metals and other applications. The extracts were obtained by cold and soxhlet methods using ethanol and aqueous solvents. These were qualitatively and quantitatively analyzed which showed the presence of alkaloids, phenols, glycosides, tannins, flavonoids, steroids, terpenoids, carbohydrates, proteins and saponins. The nanoparticles were synthesized using 0.01M of ferric chloride at varying ratios and were characterized using UV – spectroscopy, SEM + EDAX, XRD & FTIR. The UV – Spectra peaks showed absorption at 200 – 300nm. The antibacterial activity was performed using *E.coli, P.aeruginosa, B.subtilis, S.aureus &S.pyogenes* and the antifungal activity using *P.chrysogenum, C.albicans & A.niger.* The maximum zone of inhibition against *P.aeruginosa* for Soxhlet Tea ethanol (STE) and *C.albicans* for Cold Extract Tea ethanol (CETE) was measured as 25mm and 10mm. Several applications were carried out such as anticoagulation & thrombolytic activity showed positive results, 67.5% of antioxidant activity was seen in CETW (Cold Extract Tea water), total antidiabetic activity was higher in STE, CETE has shown 36.64% of anti inflammatory activity, 77.7% of water hardness was removed using CETW extract, STE showed result for larvicidal activity, 76.33% of heavy metals were removed by STE and these were found to be non – toxic to the vero cells.

INTRODUCTION

Camellia sinensis are commonly called as green tea leaves which belongs to the Thaeceae family *(Tea family).* The green tea leaves contains alkaloids (caffeine, theobromine, theophylline, etc.), flavonoids (has anti-inflammatory, antioxidant, anti microbial effects),polyphenols (catechins of gallocatechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (EGCG) and flavonoids) which ends the chain reaction of the free radicals acting as an antioxidant, etc. These catechins exhibits anti hypercholesterolemia activity, maintains elasticity of the skin, strengthens the capillaries etc.

Nanotechnology refers to any substances in the nanometric range (i.e. between 1-100nm).There are mainly two approaches for the synthesis of nanoparticles such as, bottom-up approach (these are chemically synthesized by assembling the smaller building blocks into a larger structure under controlled reaction parameters) and top-down approach (the atoms and molecules are removed from any bulk material to obtain the desired nanoparticles. These are synthesized by lithographic technique and mechanical methods).These approaches requires highly reactive and toxic reducing agents (sodium borohydrate, hydrazine hydrate) causing detrimental impacts to the environment. Thus, the field of green nanotechnology came into existence. These are produced using any plant sources, organisms (such as bacteria, actinomycetes, fungi, yeast etc.) thus making it eco-friendly, more economically reliable with increased surface area, and are easy to scale up.

The iron nanoparticles (FeNPs) has been used for this study as the iron metal is non-toxic, most abundant, easier production, eco-friendly and cheaper. In this paper, several applications of the used green tea leaves – FeNPs (iron nanoparticles) synthesized has been carried out such as anticoagulants / blood thinners (helps in the prevention of blood clots, and used for treating pulmonary embolism, strokes, deep vein thrombosis etc.), thrombolytic agents are clot busters, converting plasminogen into plasmin which breaks down the fibrinogen and fibrin, dissolving the clot.Antioxidant activity prevents the cellular damage due to the over production of reactive oxygen causing cancer, inflammation etc. Heavy metals are those metals which has high density. Some of these in larger amounts can be toxic [such as chromium (Cr), mercury (Hg), thallium (TI) etc.]and cause adverse health effects. Water hardness refers to the amount of calcium and magnesium salts dissolved in the water. The hardness in the water can lead to health

Corresponding author:* **Pradhipa S
Department of Biotechnology Women's Christian College, College Road, Chennai-600006

effects (malformations of central nervous system; diabetes – due to the higher levels of magnesium, since all the kinases, ATP- related enzymes and channels regulating the insulin action are dependent on magnesium etc.) The typical water softening process uses ion-exchange unit and regeneration process. This requires chemicals such as resin, brine etc., proper maintenance of water softeners and costs are the major limitations compared to the natural adsorbents. In this paper, these problems are being tackled using the green synthesis.

MATERIALS AND METHODOLOGY

Collection and Preparation of the Plant Materials

The used green tea leaves were collected & prepared for the extraction (*S.E Abah et al- 2011*)[17].

Methods of Extraction

Two methods of extraction used in this study are soxhlet and cold extraction using ethanol and aqueous solvents (*S.E Abah et al- 2011*) [17].

Phytochemical Analysis

Qualitative Analysis

The extracts prepared were qualitatively analyzed to check the presence of the phytoconstituents using the standard procedure (*S.A Emmanuel et al - 2014*) [16].

Quantitative Analysis

Determination of Total Alkaloids

The total alkaloid content was estimated according to *Vijay D Tambe et al* (2014) method [21].

Determination of Total Phenols

The total phenolic content was determined according to Folin's ciocalteau assay method (*Vijay D Tambe et al – 2014*) [21].The standard used for calibration was gallic acid.

Determination of Total Glycosides content

The cyanogenic glycoside content was estimated using alkaline picrate method *(Nur Faezah Omar - 2012)[13]*.

Determination of Tannins

The total tannin content was determined by Folin's phenol method (Vijay D Tambe et al – 2014) [21].

Determination of Flavonoids content

The total flavonoid content was performed by aluminium chloride method (Vijay D Tambe et al – 2014) [21].

Estimation of Steroids

The steroid content was estimated by using potassium hexacyanoferrate (G.Krisgnaveni et al method - 2014)[4].

Determination of Total Terpenoids

The total terpenoids content was determined by ammonium molybdate method (Dharmalingam Subha et al - 2015)[2].

Estimation of Carbohydrates

The total carbohydrate content was measured using phenol – sulphuric acid method (Neeru Agrawal et al – 2015)[12].

Estimation of Proteins

Total protein content was measured by Bradford's method (Marion M. Bradford – 1976) [10].

Determination of Saponins Content

The total saponins content was determined by vanillin – sulphuric acid method (Raj kumar Tiwari et al - 2016)[15].

Synthesis of Nanoparticles

The prepared extracts were used for the synthesis of iron nanoparticles (Gottimukkala KSV et al - 2017)[6].

Characterization

The synthesized nanoparticles were characterized by using UV-Visible spectroscopy, FTIR, SEM & XRD (Lebogang Katata et al – 2017)[9].

Applications

Antibacterial & Antifungal activity

The antibacterial and antifungal activity was performed based on the standard procedure
(Lebogang Katata et al – 2017)[9].

Anticoagulation assay

The anticoagulation assay was performed according to Kartheek Chegu et al (2018)[8].

Thrombolytic activity

Thrombolytic activity was assessed according to Sikandar Khan Sherwani *et al* (2013)[18].

Antioxidant activity

The antioxidant activity was evaluated using DPPH method (Shalini et al - 2014) [3].

The inhibition percentage was calculated by the formula –
Inhibition (%) = [(Acontrol – Atest) / Acontrol]* 100
Where, Acontrol = ascorbic acid; Atest = absorbance of the samples.

Anti Diabetic Assay

The anti diabetic activity was measured by DNSA method (M.N. Wickramaratne et al - 2016)[11].

Anti Inflammatory test

The anti inflammatory activity was performed according to G. Leelaprakash et al (2011)[5].

Water Hardness Removal

The removal of water hardness activity was performed by the standard method for measuring the water hardness (Suleyman A. Muyibi et al - 1994)[19]. The percentage of removal was calculated by (C_i = initial hardness (untreated), C_e = final hardness)% of removal = $C_i – C_e / C_i * 100$

Larvicidal test

The larvicidal test was performed according to C. Kamaraj et al (2011)[1].

Removal of Heavy Metals

The removal of heavy metals was performed by Jeyaseelan et al (2016)[7].

Anticancer Activity

The anticancer activity of the samples on VERO and HEP2 cell lines were determined by MTT assay to assess the cytotoxicity (P. Senthilraja et al – 2015)[14].% cell viability was calculated using

% of viability = Treated– Blank / Control – Blank * 100

RESULTS AND DISCUSSION

Collection of Plant Materials

Figure 1 Dried Green Tea Leaves

The used tea leaves were collected and powdered well. These were used for the extraction process.

Extraction

The soxhlet method of extraction was carried out at 50ºc for 6 hours and cold extraction at 4ºc for 72 hours. These were analyzed for the presence of phytochemicals**.** Similar procedure was performed using varied weight and concentrations at 40ºc and soxhlet was carried at 80ºc for 8 hours [17].

Figure 2 Soxhlet Extraction

Figure 3 Cold Extraction

Phytochemical Analysis

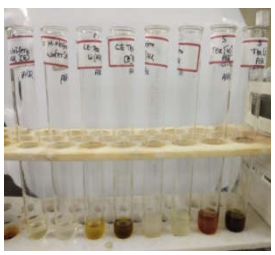


Figure 4 Alkaloids

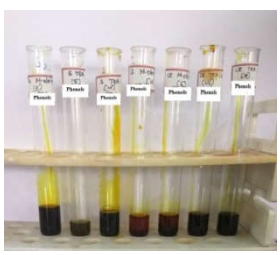


Figure 5 Phenols

Figure 6 Glycosides

Figure 7 Tannins

Figure 8 Flavonoids

Figure 9 Steroids

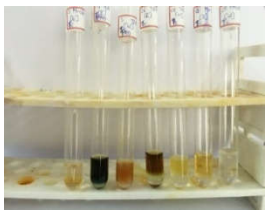


Figure 10 Terpenoids

Figure 11 Carbohydrates

Figure 12 Proteins

Figure 13 Saponins

These figures 4– 13 depict the presence of the phytochemicals in the extract such as alkaloids[21], phenols[21], glycosides[13], tannins[21], flavonoids[21], steroids[4], terpenoids[2], carbohydrates[12], proteins[10] and saponins[15]. This is a preliminary analysis which is essential for quantitative estimation of the phytochemicals.

Table 1 Qualitative Analysis of the extracts

Phytochemical Constituents	Tests	Samples (Solvent extracts)			
		CETW	CETE	STW	STE
Alkaloids	Mayer's test	$+^{ve}$	$++^{ve}$	$++^{ve}$	$++^{ve}$
Phenols	$FeCl_3$ test	$++^{ve}$	$++^{ve}$	$++^{ve}$	$++^{ve}$
Glycosides	Conc. H_2SO_4 test	$++^{ve}$	$++^{ve}$	$++^{ve}$	$++^{ve}$
Tannins	Alcoholic $FeCl_3$ test	$++^{ve}$	$++^{ve}$	$++^{ve}$	$++^{ve}$
Flavonoids	10% Lead acetate test	$++^{ve}$	$++^{ve}$	$++^{ve}$	$++^{ve}$
Steroids	Conc. H_2SO_4 test	$+++^{ve}$	$+++^{ve}$	$++^{ve}$	$+++^{ve}$
Terpenoids	Salkowski test	$+^{ve}$	$++^{ve}$	$++^{ve}$	$+++^{ve}$
Carbohydrates	Iodine test	$+^{ve}$	$++^{ve}$	$+^{ve}$	$++^{ve}$
Proteins	Biuret's test	$+^{ve}$	$+^{ve}$	$+^{ve}$	$+^{ve}$
Saponins	Foam test	$+^{ve}$	$+^{ve}$	$++^{ve}$	$+^{ve}$

CETW – indicates Cold Extraction Tea in Water, **CETE** – Cold Extraction Tea in Ethanol, **STW** – Soxhlet Tea in Water, **STE** – Soxhlet Tea in ethanol; $+^{ve}$= Slightly present, $++^{ve}$=Moderately present, $+++^{ve}$= Highly present.

Table 1shows that Soxhlet extraction gave better results when compared to the cold extraction and ethanol was an effective solvent to extract the phytochemical constituents due to its highly polar nature than aqueous.

Table 2 Quantitative Analysis of the *C.sinensis* extracts (mg/ml)

Solvent extracts	Tannins	Alkaloids	Proteins	Phenols	Carbohydrates	Steroids	Flavonoids
U1 (CETW)	1.2	28.38	0.3	5.4	0.576	7.8	35.4
U2 (CETE)	1.8	61.92	1.236	4.2	0.552	10.44	24.6
U3 (STW)	3.75	155.4	2	16.25	0.65	13.25	28.75
U4 (STE)	13.75	145.04	2.125	5	1.05	10.25	66.25

Solvent extracts	Glycosides	Terpenoids	Saponins
Tea extract	0.028	0.1488	3.264

Table 2 summarizes the results of the quantitative analysis of various phytochemicals. The total protein content was found to be higher in STE (2.125mg/ml) and lower in CETW (0.3mg/ml). Total carbohydrate content was 1.05mg/ml for STE (ethanolic extract).

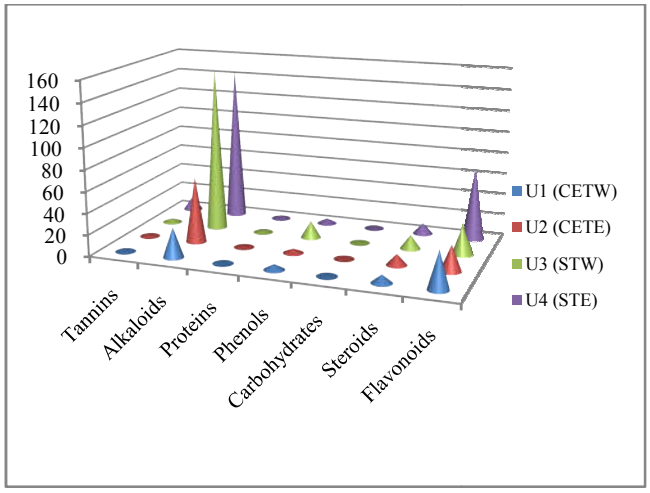


Figure 14 Quantitative analysis of the phytoconstituents

The total tannins content was higher in STE (13.75 mg/ml) and lower in CETW (1.2mg/ml). STW has shown higher result (155.4mg/ml) and CETW with lower (28.38mg/ml) for alkaloids. STW has recorded higher content (16.25mg/ml) and CETE with lower content (4.2mg/ml) for total phenol. STW has recorded higher content (13.25mg/ml) for steroids than the aqueous extract. STE has recorded highest (66.25mg/ml) and CETE has the lowest (24.6mg/ml) for the total flavonoids content. The earlier workers of *C.sinensis* leaf extracts have estimated for alkaloids, glycosides, tannins, flavonoids, steroids, terpenoids, carbohydrates and saponins only using various solvents such as ethanol, methanol and aqueous. The total phenolic (0.7mg/g) and flavonoid (14mg/g) content was found to be lower than the present investigation[20].

Synthesis of Nanoparticles

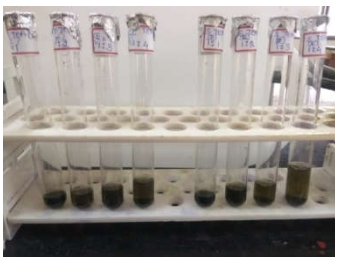


Figure 15 Synthesis of iron nanoparticles (0.01M of $FeCl_3$ in used *C.sinensis* leaf extracts)

The iron nanoparticles were synthesized at varying concentrations (1:1 – 1:4). The colour change from faint yellow to reddish brown and black shows the formation of iron nanoparticles. The earlier worker synthesized the iron nanoparticles only in 1:1 proportion [6].

Characterizationof Nanoparticles

UV-Visible spectroscopy

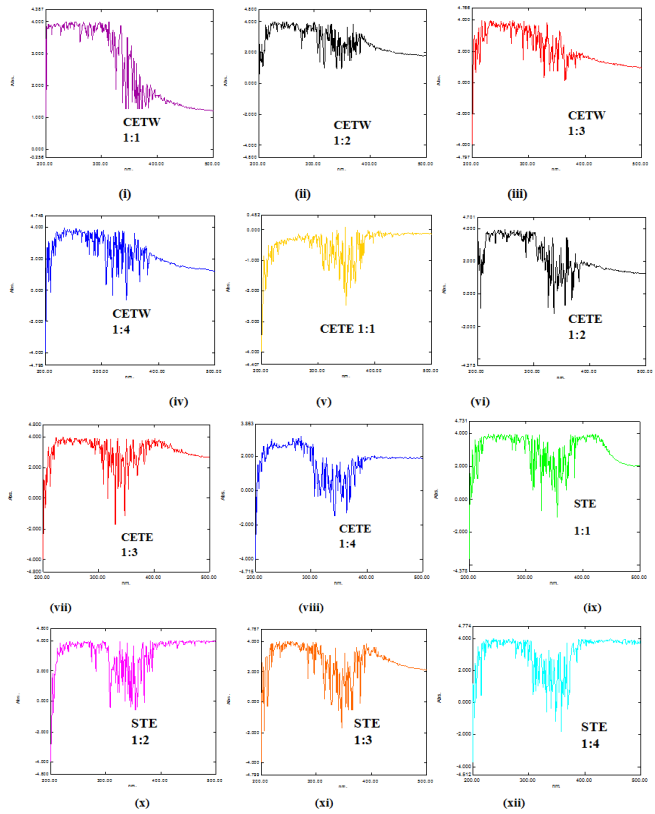


Figure 16 UV – Visible absorption peaks of C.sinensis FeNPs

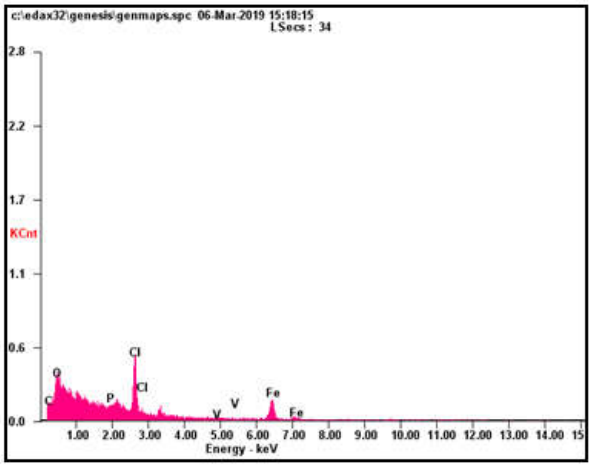
This figure 16 shows the UV spectra of CETW FeNPs. This was measured in the range of 200 – 500nm. The absorption spectrometer works in the range of 200nm (near ultraviolet) to 800nm (very near infrared).

Table 3 UV – Visible spectra measurements

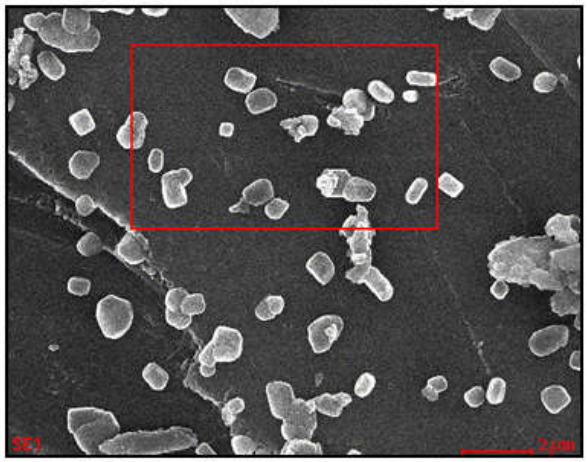
Sample (Extract-FeNPs)	Concentrations	Wavelength(nm)	Absorbance
U1 (CETW)	1:1	473nm	1.277
		413nm	1.610
		297nm	4.00
	1:2	-	-
	1:3	482nm	1.050
		233nm	3.969
	1:4	386nm	2.404
U2 (CETE)	1:1	-	-
	1:2	249nm	3.944
	1:3	430nm	3.562
		236nm	4.000
	1:4	282nm	3.148
U3 (STW)	1:1	-	-
	1:2	349nm	3.293
		302nm	3.901
	1:3	346nm	2.433
		267nm	3.198
	1:4	-	-
U4 (STE)	1:1	416nm	3.970
	1:2	418nm	4.000
	1:3	391nm	3.837
		233nm	3.988
	1:4	389nm	3.972

Table 3 describes that CETW (297nm and 233nm), CETE (249nm, 236nm & 282nm), STW (267nm) and STE (233nm) has lesser wavelengths. Hence amongst these, few were chosen for the further applications. The earlier work was performed with *M.oleifera* seeds and leaves FeNPs. The presence of the nanoparticles was confirmed by the formation of new peak at 240nm.[9]

Scanning– Electron Microscope



(a)



(b)

Figure 17 (a) EDX peak (b) SEM image of *C.sinensis* FeNPs

Figure 17 (a) represents the composition of the elements present in CETW FeNPs (1:1). EDX (Energy Dispersive X-ray Spectroscopy) results obtained shows the presence of contaminants such as carbon of 40.01% (might be obtained by the use of carbon tape for spreading the powdered samples) and oxygen of 39.33% along with other constituents such as iron (10.14%), chloride (09.57%), phosphorus and vanadium (00.78% & 00.17%). The SEM image obtained clearly shows the different structural orientation confirming the formation of the nanoparticles. In the earlier work performed using *C.sinensis* leaves FeNPs, it had an average diameter of about 116nm [6].

FTIR

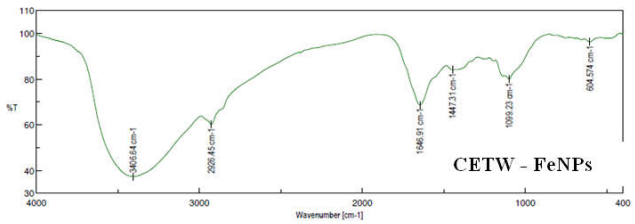


Figure 18 FTIR spectra of CETW – FeNPs

Figure 18 displays the stretching at 3406.64 cm^{-1} for OH stretching, 2926.45 cm^{-1} for C-H stretching, 1646.91 cm^{-1} for C=C stretching, 1447.31 cm^{-1} for C-H bending, 1099.23 cm^{-1} for C-F stretching and the formation of band at 604.574 cm^{1}confirms the presence of the FeNPs. The earlier worker performed with *M.oleifera* FeNPs showinga band at 567cm^{1}confirming the formation of FeNPs.[9]

XRD

Figure 19 depicts that noise was observed which can be due to fluorescence of an element in the sample thus enhancing the background etc. The earlier work in *M.oleifera* seeds and leaves FeNPs showed peak around 2θ of 45° on MOS – FeNPs[9].

Applications

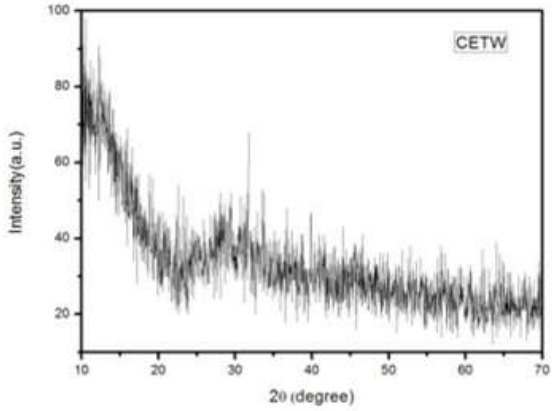
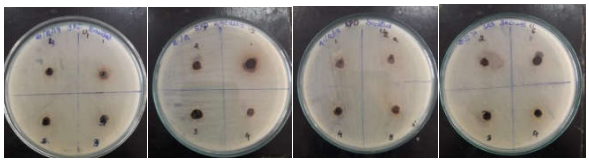


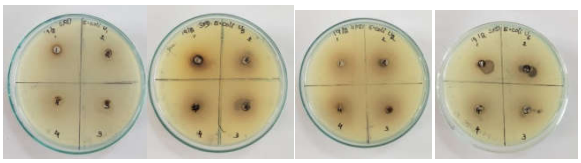
Figure 19 XRD pattern for CETW – FeNPs

Antibacterial Activity



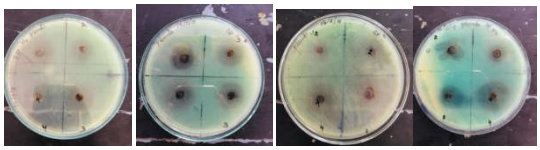
(a)CETW (b) CETE (c) STW (d) STE

(i)*Bacillus subtilis*



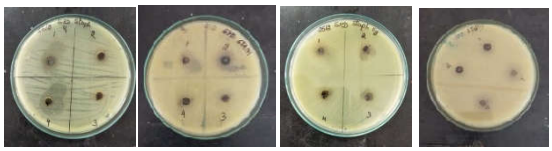
(a)CETW (b) CETE (c) STW (d) STE

(ii) *Escherichia coli*



(a)CETW (b) CETE (c) STW (d) STE

(iii) *Pseudomonas Aeruginosa*



(a)CETW (b) CETE (c) STW (d) STE

(iv) *Staphylococcus Aureus*

(a)CETW (b) CETE (c) STW (d) STE

(v) *Streptococcus pyogenes*

Figure 20 Antibacterial activity

(c)

Figure 21: Anti fungal activity (a)*Candida albicans,* (b) *Penicillium chrysogenum,*(c) *Aspergillus niger*

Table 5 Zone of inhibition for Anti- fungal activity

ORGANISMS	ZONE OF INHIBITION (mm)			
	U1 (1:1) - CETW	U1(1:3) CETW	U5 (1:2) CETE	U6 (1:3) STE
P. chrysogenum	Negative	Negative	Negative	Negative
C.albicans	Negative	10mm	10mm	7mm
A. niger	Negative	Negative	Negative	Negative

Figure 21 and table 5 clearly shows that *P.chrysogenum* and *A.niger* were resistant to these samples – FeNPs. CETW (1:3) and CETE (1:2) showed higher zones (10mm) for *C.albicans*. The earlier worker performed only with *C.albicans* for *Anchomanes difformis* leaf extracts [17].

Anticoagulation Assay

Figure 22 shows that all the tea FeNPs (i.e. U1, U5 & U6) showed positive results within few seconds.The earlier worker performed using *Allium sativum, Zingiber officinale.* [8]

Table 4 Zone of inhibition (in mm) of *C.sinensis* FeNPs

Sl.No.	Organisms	Concentration (in ratios) Zone of inhibition (in mm)															
		CETW				CETE				STW				STE			
		1:1	1:2	1:3	1:4	1:1	1:2	1:3	1:4	1:1	1:2	1:3	1:4	1:1	1:2	1:3	1:4
1	*B. subtilis*	0.05	-	-	-	10	0.06	10	0.08	-	-	-	-	12	10	10	-
2	*Escherichia coli*	0.06	12	13	13	12	13	18	18	16	17	18	19	10	14	15	16
3	*Pseudomonas aeruginosa*	5	15	19	17	17	18	18	20	12	16	20	17	13	19	16	25
4	*Staphylococcus aureus*	15	-	-	18	12	13	11	10	15	20	-	22	10	10	12	13
5	*Streptococcus pyogenes*	8	11	-	-	7	6	6	-	-	-	7	11	10	17	15	18

This shows that the STE FeNPs 1:1 (12mm) showed higher zones for *B. subtilis*. Aqueous extract of tea (STW - 19mm) at 1:4 showed susceptibility for *E.coli*. STE (25mm) at 1:4 showed the maximum activity for *P. aeruginosa*. STW (22mm) at 1:4 showed maximum zone for *S. aureus*. *S. pyogenes* was more susceptible to STE (18mm) at 1:4. Comparatively all the extracts – FeNPs showed higher zone of inhibition for *P. aeruginosa*.The earlier worker performed with *M.oleifera* FeNPs and different antibiotic discs against *E.coli*[9].

Anti Fungal Activity

(a)

(b)

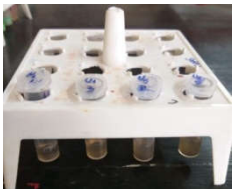


Figure 22 U1 (1:1, 1:3), U5 (1:2) and U6 (1:3)

Thrombolytic Activity

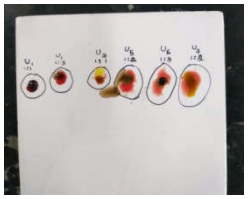


Figure 23 Anti thrombolytic activity

Figure 23 shows that all the tea extracts FeNPs (i.e. CETW – U1, CETE – U5 &STE – U6) gave positive results. This shows that these samples – FeNPs has the ability to breakdown the clot. Earlier worker performed using streptokinase as reference standard for aqueous and methanolic crude extracts of *Camellia sinensis* which exhibited the thrombolytic activity[18].

Antioxidant Activity

Table 6 % of inhibition for DPPH- scavenging activity

Samples	% of Inhibition
U1 1:1 (CETW)	67.5%
U1 1:3 (CETW)	64%
U5 1:2 (CETE)	52%
U6 1:3 (STE)	52%

Table 6 shows that the CETW (at 1:1 and 1:3) showed higher anti oxidant activity of 67.5% & 64%. The earlier worker performed using green and black tea (methanol, ethyl acetate and hot aqueous). Higher level of activity for both green and black tea extracts (0.08mg/ml)using methanol and ethyl acetate solvents[3].

Anti Diabetic Assay

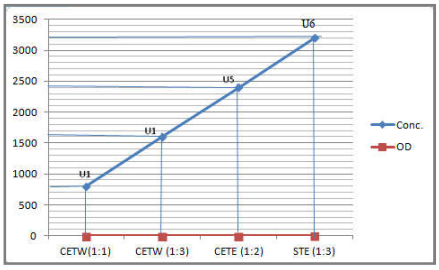


Figure 24 Anti diabetic activity of the FeNPs extract

Table 7 Anti diabetic activity of the FeNPs extract

Sl.No.	Extracts – FeNPs (sample)	Total activity of the sample (in IU)
1	U1 1:1 (CETW)	175,000
2	U1 1:3 (CETW)	50,000
3	U5 1:2 (CETE)	216,666.66
4	U6 1:3 (STE)	565,972.22

Figure 24 and table 7 shows that the highest activity was observed in STE with 565,972.22 IU. The earlier worker performed using *Adenanthera pavonina* leaf extracts. This study showed the in vitro assay of alpha amylase inhibitory activity[11].

Anti Inflammatory test

Table 8 Anti inflammatory activity

Samples	% of inhibition
U1 1:1 (CETW)	17.69%
U1 1:3 (CETW)	47.03%
U5 1:2 (CETE)	36.64%
U6 1:3 (STE)	22.41%

This depicts the highest % of inhibition at CETW (47.03%) at 1:3.The earlier worker performed using methanolic extract of *Enicostemma Axillare*. The results showed that the sample exhibited this activity at varying concentrations[5].

Water Hardness Removal

Table 9 % of water hardness removal

Samples	% of removal
U1 1:1 (CETW)	77.7%
U1 1:3 (CETW)	68.8%
U5 1:2 (CETE)	66%
U6 1:3 (STE)	55.5%

Table 9 shows that water hardness was more efficiently removed by CETW (77.77%) at 1:1.The earlier work performed by using *M.oleifera* seeds for treating surface water, synthetic water and ground water which gave better results at higher dosage [19].

Larvicidal Activity

Table 10 Larvicidal activity of the *C.sinensis* extracts - FeNPs

Sl. No.	Extracts – FeNPs	Time duration (in minutes)
1	CETW (1:1)	-
2	CETW (1:3)	-
3	CETE (1:2)	-
4	STE (1:3)	Within 10 minutes

Table 10 shows that only STE had most effective larvicidal activity (against malarial larvae). Earlier worker performed against *Anopheles subpictus* & *Culex tritaeniorhynchus* using methanol, ethyl acetate and acetone extracts of *A.squamosa* L., *C.indicum* L., and *Tridax procumbens*[1].

Removal of Heavy Metals

Table 11 % of Removal of heavy metals

Samples	% of removal
U1 1:1 (CETW)	66.86%
U1 1:3 (CETW)	70.41%
U5 1:2 (CETE)	45.56%
U6 1:3 (STE)	76.33%

Table 11 shows that STE & CETW removed the heavy metals efficiently with 76.33% and 70.41% compared to the other extracts. The similar work was performed using green tea leaves for the removal of chromium[7].

Anticancer Activity

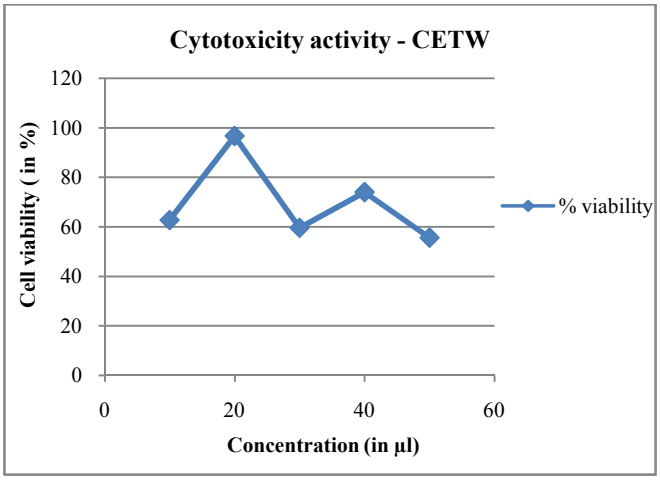


Figure 25 Cytotoxic activity of *C.sinensis*– FeNPs

Table 12 % of cell viability

Samples	% of cell viability				
	10µl	20 µl	30 µl	40 µl	50 µl
CETW(1:1)	62.71%	96.61%	59.60%	74.01%	55.64%

Figure 25& Table 12 shows that the FeNPs extracts were non-toxic to the VERO III cells, and didn't have any anti- cancer property. The cell viability was higher in CETW having 96.61% of viable cells at 20 µl compared to the other

concentrations. Similar work was performed using marine yeast by MTT assay in Vero, HepG2 and MCF -7 cell lines[14].

CONCLUSION

The extracts were prepared from used *C.sinensis* leaves by cold and soxhlet method of extraction. This process was carried out using ethanol and aqueous solvents. The phytochemical analysis was performed, showing the presence of alkaloids, tannins, flavonoids, glycosides, steroids, terpenoids, proteins, carbohydrates, saponins and phenols. The iron nanoparticles were synthesized in varied concentrations and this was characterized using SEM + EDAX (Energy Dispersive X-Ray Analysis), UV- Visible spectroscopy, FTIR (Fourier Transform Infrared Spectroscopy) & XRD (X-Ray Diffraction). The presence of nanoparticles was confirmed using the morphological shape, size, its chemical composition etc. These synthesized nanoparticles were used for several applications such as removal of water hardness and heavy metals, antibacterial, antifungal, anti coagulant, anti oxidant, thrombolytic, anti diabetic, larvicidal, anti inflammatory and anti cancer activities showing positive results.

References

1. C. Kamaraj, A. Bagavan, G. Elango, A. Abduz Zahir, G. Rajakumar, S. Marimuthu, T.Santhoshkumar, and A. Abdul Rahuman, Larvicidal activity of medicinal plant extracts against *Anopheles subpictus*&*Culex tritaeniorhynchus*, *Indian J Med Res. 2011 Jul; 134(1): 101–106*.
2. Dharmalingam Subha, Nallasamy Chandralega and Natesan Geetha, Phytochemical Screening of *Tanacetum parthenium* L. (Feverfew) Leaves: An Important Medicinal Plant, *Ijppr.Human, 2015; Vol. 2 (2): 98-106.*
3. D.Shalini and G.Sudha, Antioxidative activity of various Tea extracts (*Camellia sinensis*), *J. Bio sci. Res., 2010. Vol. 1(4):271-278*.
4. G. Krisgnaveni, O. Sailaja, K. Mounika, Identification And Estimation Of Phytochemicals From The Plant *Pedicularis Bicornuta* Leaf Extract By Uv-Spectrophotometry, *Research Desk, 2014, 3(1). 410-418*.
5. G. Leelaprakash, S. Mohan Dass, Invitro Anti-Inflammatory activity of Methanol extract of *Enicostemma Axillare*, Int. J. Drug Dev. & Res., 2011, 3(3): 189-196.
6. Gottimukkala KSV, Harika Reddy P, Deeveka Zamare, Green Synthesis of Iron Nanoparticles Using Green Tea leaves Extract, *J. Nanomedine Biotherapeutic Discov. 2017, 7: 151.*
7. Jeyaseelan and Gupta. Green Tea Leaves as a Natural Adsorbent for the Removal of Cr(VI) From Aqueous Solutions. *Air, Soil and Water Research 2016:9 13–19*.
8. Kartheek Chegu, K. Mounika, M. Rajeswari, N. Vanibala, P. Sujatha, P. Sridurga, D. R. Brahma Reddy, IN VITRO STUDY OF THE ANTICOAGULANT ACTIVITY OF SOME PLANT EXTRACTS, *World Journal of Pharmacy and Pharmaceutical Sciences, 2018, Volume 7, Issue 5, 904-913*.
9. Lebogang Katata-Seru, Tshepiso Moremedi, Oluwole Samuel Aremu, Indra Bahadur, Green synthesis of iron nanoparticles using *Moringa oleifera* extracts and their applications: Removal of nitrate from water and antibacterial activity against *Escherichia coli*, *Journal of Molecular Liquids, 2017.*
10. Marion M. Bradford, A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding, *Analytical Biochemistry, 1976, 72, 248-254*.
11. M. Nirmali Wickramaratne, J. C. Punchihewa and D. B. M. Wickramaratne, In-vitro alpha amylase inhibitory activity of the leaf extracts of *Adenanthera pavonina*, 2016, *BMC Complementary and Alternative Medicine, 16:466.*
12. Neeru Agrawal, Divya K. Minj, Khushboo Rani, Estimation of Total Carbohydrate Present In Dry Fruits, *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, *2015, Volume. 1 Issue 6, PP 24-27*.
13. Nur Faezah Omar, Siti Aishah Hassan, Umi Kalsom Yusoff , Nur Ashikin Psyquay Abdullah, Puteri Edaroyati Megat Wahab and Uma Rani Sinniah; Phenolics, Flavonoids, Antioxidant Activity and Cyanogenic Glycosides of Organic and Mineral-base Fertilized Cassava Tubers, *Molecules* 2012, *17*, 2378-2387.
14. P. Senthilraja and K. Kathiresan, In vitrocytotoxicity MTT assay in Vero, HepG2 and MCF-7 cell lines study of Marine Yeast, Journal of Applied Pharmaceutical Science, 2015, Vol. 5 (03), pp. 080-084.
15. Raj kumar Tiwari, Udayabanu M and Silpi chanda,Quantitative analysis of secondary metabolites in aqueous extracts of *Cledodendrum serratum*, Int. Res. J.Pharm.2016; 7(12):61-65.
16. S.A Emmanuel,O.O Olajide, S. Abubakar, I.D Idowu, A.T Orishadipe and S.A Thomas**,** Phytochemical and Antimicrobial Studies of Methanol, Ethyl acetate, and Aqueous Extracts of *Moringa oleifera* Seeds, *American Journal of Ethnomedicine, 2014, Vol. 1, No. 5, 346-354.*
17. S.E. Abah and L.O. Egwari, Methods of Extraction and Antimicrobial Susceptibility Testing of Plant Extracts, *African J. Basic &Appl.* Sci., *2011,3 (5): 205-209.*
18. Sikandar Khan Sherwani, Asma Bashir, Syeda Sadaf Haider, Muhammad Ajmal Shah, Shahana Urooj Kazmi, Thrombolytic Potential of Aqueous and Methanolic Crude Extracts of *Camellia sinensis* (Green Tea): In vitrostudy, *Journal of Pharmacognosy and Phytochemistry, 2013, Vol. 2 No. 1, 125-129*.
19. Suleyman A. Muyibi and Lilian M. Evison, Moringa oleifera seeds for softening hardwater. *Wat. Res. Vol. 29, No. 4, pp. 1099-1105*.
20. Tariq. A. L and Reyaz. A. L "Phytochemical analysis of Camellia sinensis Leaves" *Int. J. Drug Dev. & Res., 2012, 4(4):311-316.*
21. Vijay D Tambeand Rajendra S. Bhambar**,** Estimation of Total Phenol, Tannin, Alkaloid and Flavonoid in *Hibiscus tiliaceus* Linn. Wood extracts, *Journal of Pharmacognosy and Phytochemistry (RRJPP), 2014, Volume 2, Issue 4, 41-47*.