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Letter

Green Synthesis of Silver Nanoparticle Using *Tephrosia tinctoria* And Its Antidiabetic Activity

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Abstract

The present study focuses on the green synthesis of silver nanoparticles using aqueous extract of *Tephrosia tinctoria* (TT). This medicinal plant was rich in phenol and flavonoids groups of compounds. They reduced the silver nitrate into silver nanoparticle (AgNPs) and which were characterized using UV-Vis spectrophotometry, X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive X-Ray (EDAX) patterns and FT-IR. The XRD analysis illustrated that the silver nanoparticles were crystalline in nature. The SEM and TEM analysis revealed that the synthesized silver nanoparticles were spherical in shape and the particle size was found to be less than 100nm. The antidiabetic ability of the AgNPs was tested and the results showed significant free radical scavenging ability, Inhibition of carbohydrate digestive enzymes (α-Glucosidase and α-Amylase) and enhancement of Glucose uptake rate. The FT-IR result revealed that the presence of various functional groups around AgNPs.

Key Words: Nanoparticles, Biomaterials, Particles, nanosize, Green synthesis**,** Glucose uptake, *Tephrosia tinctoria*

1 Introduction

Green synthesis of nanoparticles was more preferred than chemical synthesis since it is involved in the reduction of metals using various hazardous chemicals. Naturally, plants possess both the primary and secondary metabolites to carry out the green synthesis. The green synthesized silver nanoparticles were widely used for many applications such as antimicrobial [1], antioxidant [2,3], anticancer [4] and also used in industries. This medicinal plant was reported for antioxidant and antidiabetic activity [5]. In the present study, the silver nanoparticles were synthesized using aqueous stem extract of *Tephrosia tinctoria* for antidiabetic activity. The antidiabetic targets like, α-glucosidase, α-amylase, free radical inhibition and glucose uptake were used as a model for the present study.

2 Materials and Method

2.1 Plant material

T. tinctoria was collected from the Kolli hills, Tamil Nadu, India during November and authenticated by the Botanical Survey of India, Coimbatore, India (No BSI/SRC/5/23/09-10/TECH - I569).

2.2 Green Synthesis and Characterization of Silver nanoparticles (AgNPs)

The shade dried stem portion of TT was ground to powder. Approximately 20gm of the powder was mixed with 300ml of distilled water for 2 hrs and centrifuged at 5000 rpm for 10 mins. The supernatant was collected and filtered using Wattman filter paper No.1. 100ml of permeate was mixed with 100ml of 1mM $AgNO_3$ and incubated at 37°C for 24 hr in Rotary shaker. Later the incubated mixture was centrifuged at 9000 rpm for 10 min. The pellet was collected and washed with distilled water for five times by centrifuging at 9000

rpm for 10 min for each cycle. Then the purified AgNPs was analyzed in UV-Vis spectrophotometer and the functional groups were studied using the Bruker – Alpha-T FTIR spectrum. The X-ray Diffraction (XRD) patterns of the AgNPs were measured using an analytical 'X' Pert PRO diffractometer with Cu Kα radiation (λ = 0.15418 NM). The size of the AgNPs was measured using SEM and TEM and the spectrum of Energy-Dispersive X-ray spectroscopy was recorded using Oxford instrument, UK. The total phenol [6] and flavonoid [7] contents were estimated before and after the synthesis of AgNPs process.

2.3 Antidiabetic activity of AgNPs

2.3.1 α-Glucosidase and α-Amylase inhibitory activity

The α-Glucosidase inhibitory [8] and α-Amylase inhibitory [9] assays were carried out by standard procedures.

2.3.2 Glucose uptake assay

This protocol is the modified method of Klepper et al., [10]. Human blood (4ml) was collected and mixed with an anticoagulant (Heparin). The blood cells were washed with phosphate buffer saline (PBS) and suspended with PBS. The blood suspension was mixed with plant extracts/AgNPs and incubated for 20 mins at 37^0C. The glucose (0.5% in PBS) added to the mixture and again incubated for 20 mins at 37^0C. The blood cells washed with PBS and lysed using 50µl of 0.1N NaOH for 3 mins and 50µl of 0.1N HCl for 3 mins. The lysed blood was centrifuged and the glucose content of supernatant was analyzed using GOD-POD method.

3 Results and Discussion

3.1 Characterization of AgNPs

The AgNPs formation started after adding $AgNO_3$ to aqueous extract and incubated for 24hrs. The raddish brown colour was observed after the incubation and it might be due to the formation of AgNPs [11]. The AgNPs showed strong absorption peak at 480 nm [12].

During the synthesis of nanoparticles, the absorbance of the spectra increases with an increment of higher concentrations of $AgNO_3$. Further SEM and TEM have provided insight into the morphology and the details of size of the synthesized nanoparticles. The synthesized silver nanoparticles were well dispersed without any aggregation and possess a spherical shape. The approximate size of the nanoparticle was found to be around 73 nm (Fig. 1C, 1D and 1E).

The crystalline nature of the AgNPs was elucidated using XRD analysis. The peaks at 38.32, 44.49, 64.65 and 77.51 were corresponded to 111, 200, 220 and 311 respectively according to JCPDS no. 04-0784 (Fig. 2A). Apart from these, there were also few other sharp peaks seen, which might be due to the existence of the organic phytochemicals in the mix [13].

The EDAX profile evidently depicts the optical absorption of Ag. At 3Kev, 49.86% of Ag is present in AgNPs and besides Cl, S and O were also found in AgNPs (Fig. 1B). The functional groups attached to the AgNPs were identified by FTIR analysis. The phenolic group present in the crude aqueous extract has shown an affinity in binding with the AgNPs. Apart from this, the AgNPs has also attached with other functional groups such as Phenol (-OH) at 3442 cm^{-1}, carboxylic acids at 2923 cm^{-1}, alkynes at 2141 cm^{-1} and ethers at 1019 cm^{-1} respectively (Fig. 3).

The phenol and flavonoid contents in the supernatant after the green synthesis of AgNPs were found to be less than the crude aqueous extract (Fig 2B). Hence, it was substantiated that the phytochemical contents may be attached with the AgNPs during green synthesis process.

3.2 Bioactivity of TT and AgNPs

The free radical scavenging effect was observed in an increasing order, AgNPs<TT<GA (Table 1). The lower antioxidant potential of AgNPs may be due to the

attachment of fewer antioxidant molecules (i.e. Phenol & Flavonoids) when compared to the crude aqueous extract of TT. During the green synthesis of AgNPs, the free antioxidant compounds in the crude aqueous extracts were removed by washing. This results in lower levels of antioxidant activity in AgNPs. The AgNPs prepared using TT showed higher percentage of DPPH free radical scavenging activity than the green synthesized AgNPs of *Iresine herbstii* [3].

The inhibition of carbohydrate digesting enzymes (α - Glucosidase & α – Amylase) is an important target to prevent a sudden increase of glucose level in blood. In the present study the enzyme inhibition was observed in an increasing order, TT<AgNPs<Acarbose (Table 1). The AgNPs significantly inhibits carbohydrate digesting enzymes than the crude aqueous extract of TT.

The glucose uptake in RBC is a very basic step to identify the hypoglycemic effect of the plant extracts [14]. At 75µg/ml, glucose uptake assay in AgNPs showed the maximum 3.80±0.028 fold increase compared with crude aqueous extract of TT (2.61±0.07) (Table 1). There was no haemolysis observed in all the concentrations tested during the glucose uptake assay. Further studies may focus on identifying mechanism of action of AgNPs in glucose uptake.

4 Conclusion

The green synthesis of AgNPs is cost effective and eco-friendly. Hence, the AgNPs was synthesised using aqueous extract of TT and characterized using UV, FTIR, SEM, TEM, EDAX and XRD. The AgNPs showed significant carbohydrate digestive enzyme inhibition and increase the glucose uptake in cells than the crude extract and also it has the antioxidant activity. The enhanced bioactivity of AgNPs due to the phenol and flavonoid compounds attachment and it was confirmed by FTIR and phytochemical analysis.

References

1. Bal KE, Bal Y, Cote G, Chagnes A. Morphology and antimicrobial properties of *Luffa cylindrical* fibers/chitosan biomaterial as micro-reservoirs for silver delivery. Mater Lett 2012;79:238-241.
2. Dipankar C, Murugan S. The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from *Iresine herbstii* leaf aqueous extracts. Colloid Surface B 2012;98:112-119.
3. Velavan S, Arivoli P, Mahadevan K. Biological Reduction of Silver Nanoparticles Using Cassia Auriculata Flower Extract and Evaluation and Evaluation of Their in vitro Antioxidant Activities. Nanoscience and Nanotechnology: An International Journal 2012;2(4):30-35.
4. Babu G, Arulvasu C, Prabhu D, Jegadeesh R, Manikandan R. Biosynthesis and characterization of silver nanoparticles from *Datura inoxia* and its apoptotic effect on human breast cancer cell line MCF7. Mater Lett 2014;122:98-102.
5. Rajaram K, Suresh KP. *In-vitro* antioxidant and antidiabetic activity of *Tephrosia tinctoria* PERS. an endemic medicinal plant of South India. J Pharm Res 2011;4:891-893.
6. Amin I, Norazaidah Y, Emmy HKI. Antioxidant activity and phenolic content of raw and blanched Amaranthus species. Food Chem 2006;94:47–52.
7. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 2005;91:571-577.
8. Gao H, Kawabata J. Alpha-Glucosidase inhibition of 6-hydroxyflavones. Part 3: Syntheis and evaluation of 2,3,4-trihydroxybenzoyl containing flavonoid analogs and

6-aminoflavones as alpha-glucosidase inhibitors. Bioorg Med Chem 2005;13(5):1661-1671.

9. Conforti F, Scatti G, Loizzo MR, Sacchetti GA, Poli F, Menichini F. *In vitro* antioxidant effect and inhibition of α-amylases of two varieties of *Amaranthus caudatus* seeds. Biol Pharm Bull 2005;28(6):1098-1102.
10. Klepper J, Garcia-Alvarez M, O'Driscoll KR, Parides MK, Wang D, Ho YY, De VDC. Erythrocyte 3-o-methyl-D-glucose uptake assay for diagnosis of glucose transporter-protein syndrome. J Clin Lab Anal 1999;13(3):116-121.
11. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloid Surface B 2003;28:313-318.
12. Sastry M, Mayyaa KS, Bandyopadhyay K. pH Dependent changes in the optical properties of carboxylic acid derivatized silver colloidal particles. Colloid Surface A 1997;127:221-228.
13. Shankar SS, Ahmad A, Parsricha R, Sastry M. Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. J Mater Chem 2003;13:1822-1826.
14. R. Yeong and B. Ardith, Flaxseed lignin increased glucose uptake by human red blood cells, Open Nutraceuticals J. 2 (2009), pp. 81-85.

Figure Captions

Fig. 1 A) UV-Vis spectrum of AgNPs; B) EDAX spectrum of AgNPs; C) SEM image of AgNPs; D) TEM image of AgNPs; E) TEM image of AgNPs at 100nm magnification.

Fig. 2 A) XRD pattern of AgNPs; B) Quantification of flavonoid and phenol contents before and after AgNPs synthesis; E – Aqueous extract; S – Supernatant of green synthesized AgNPs

Fig. 3 FT-IR analysis of AgNPs

Table

Table 1. Free radical scavenging and antidiabetic activity of TT and AgNPs

Conc. (µg/ml)	Percentage of DPPH free radical scavenging activity (%)			Antidiabetic activity							
				Percentage of α-glucosidase inhibitory activity (%)			Percentage of α-amylase inhibitory activity (%)			Fold of glucose uptake (Fold)	
	TT	AgNPs	GA	TT	AgNPs	Acarbose	TT	AgNPs	Acarbose	TT	AgNPs
25	49.24 ±0.70	30.66 ±0.96	77.32 ±1.41	16.53 ±1.27	67.81 ±0.27	73.41 ±1.41	31.15 ±0.99	69.70 ±0.82	75.68 ±0.78	0.90±0 .040	3.08±0. 014
50	63.62 ±1.15	42.03 ±1.76	88.08 ±0.70	36.3± 0.83	74.34 ±0.74	85.35 ±1.69	63.78 ±0.89	87.02 ±1.17	89.89 ±0.48	1.83±0 .044	3.41±0. 012
75	85.35 ±0.65	60.16 ±1.22	95.09 ±0.35	50.74 ±1.08	83.52 ±0.71	96.25 ±1.40	74.07 ±0.81	94.76 ±0.86	97.79 ±0.63	2.61±0 .070	3.80±0. 028

Data is mentioned as Mean±SD; GA-Galic acid

Highlights

- ✓ *Tephrosia tinctoria* has been reported of its significant antioxidant and antidiabetic potential. In connection with the previous study, we have tested the green synthesised AgNPs for antioxidant and antidiabetic activity.
- ✓ There was an increase in the free radical scavenging, carbohydrate digestive enzyme inhibition and glucose uptake rate in the AgNPs addition than crude aqueous extracts of TT.
- ✓ During the green synthesis, isoflavonoids and phenolic groups were attached with the Ag to form AgNPs, it was confirmed by Phytochemical quantification and FTIR analysis.

Graphical Abstract (for review)

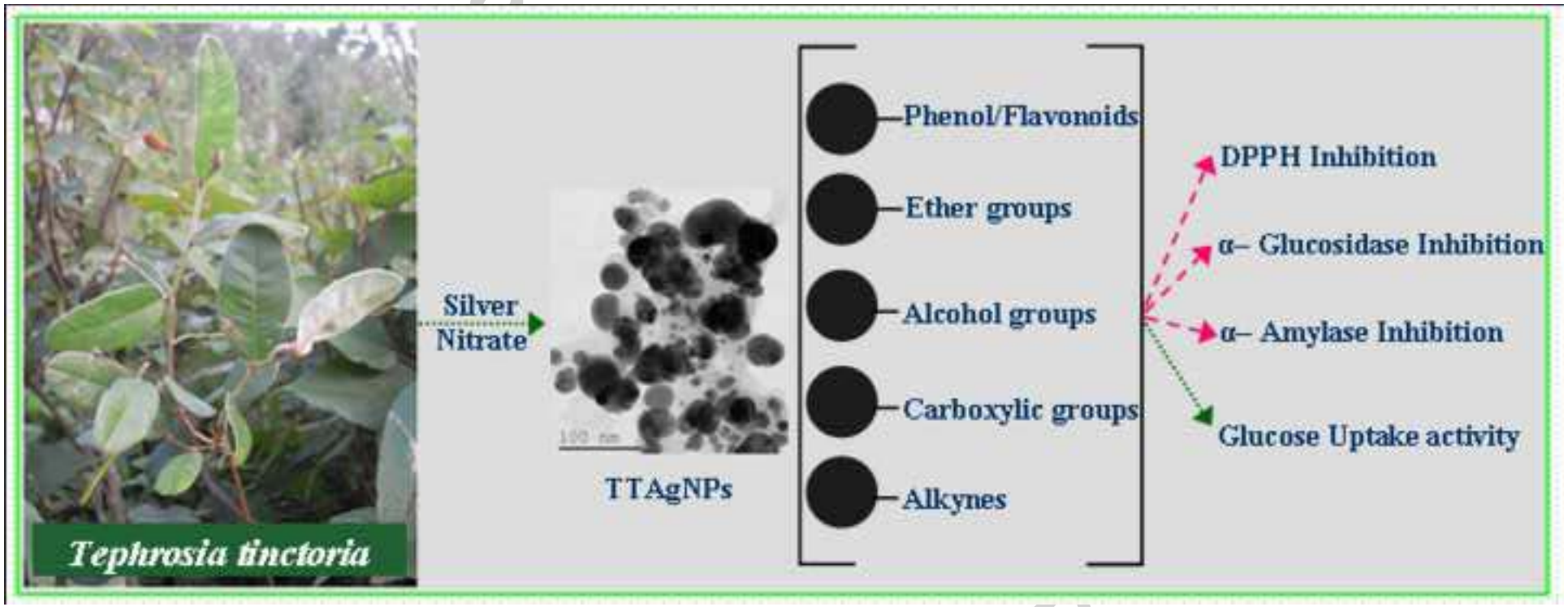
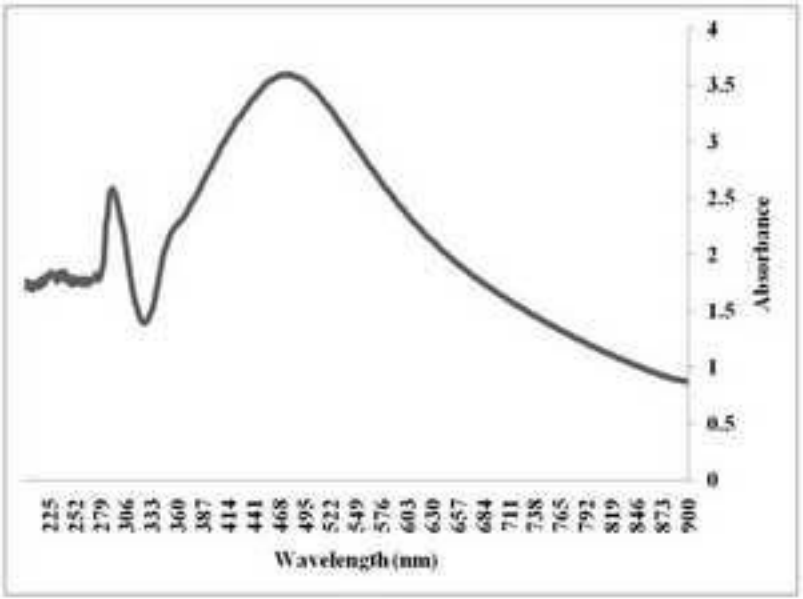


Figure 1



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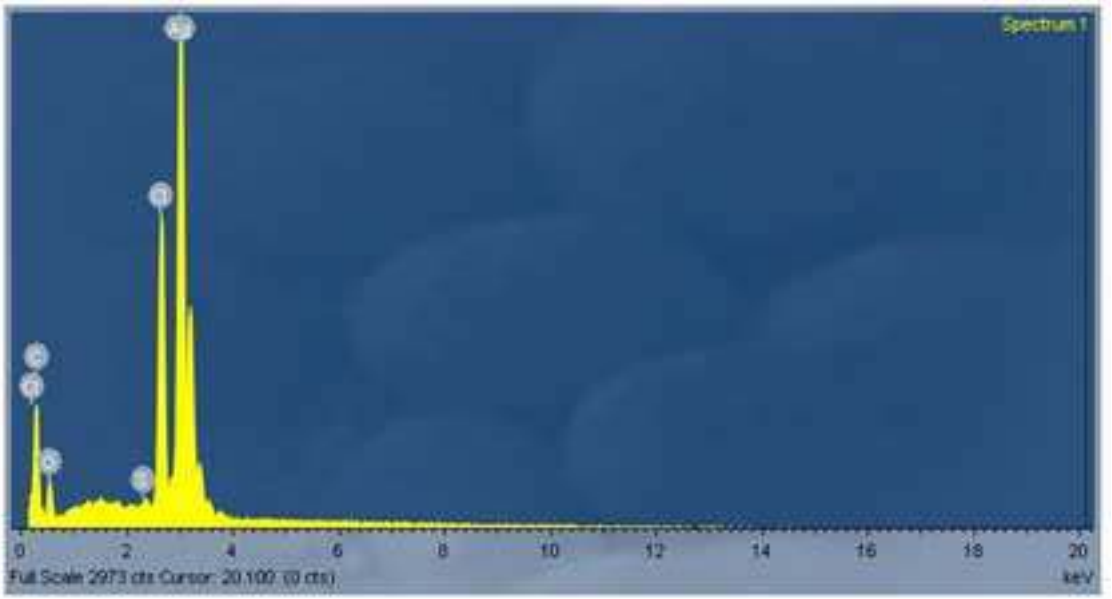

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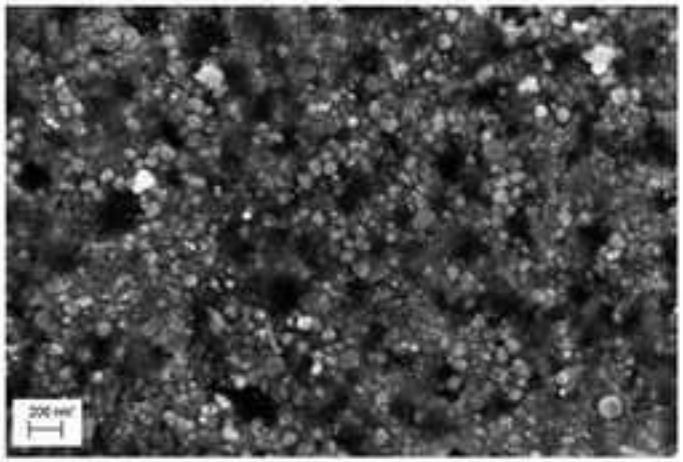

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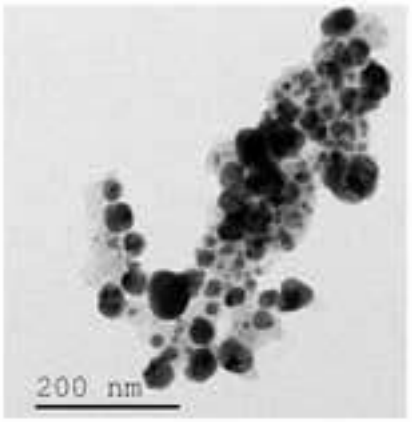

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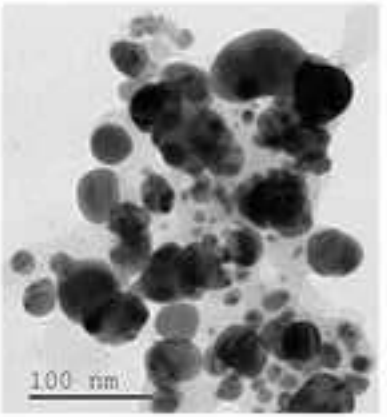


E

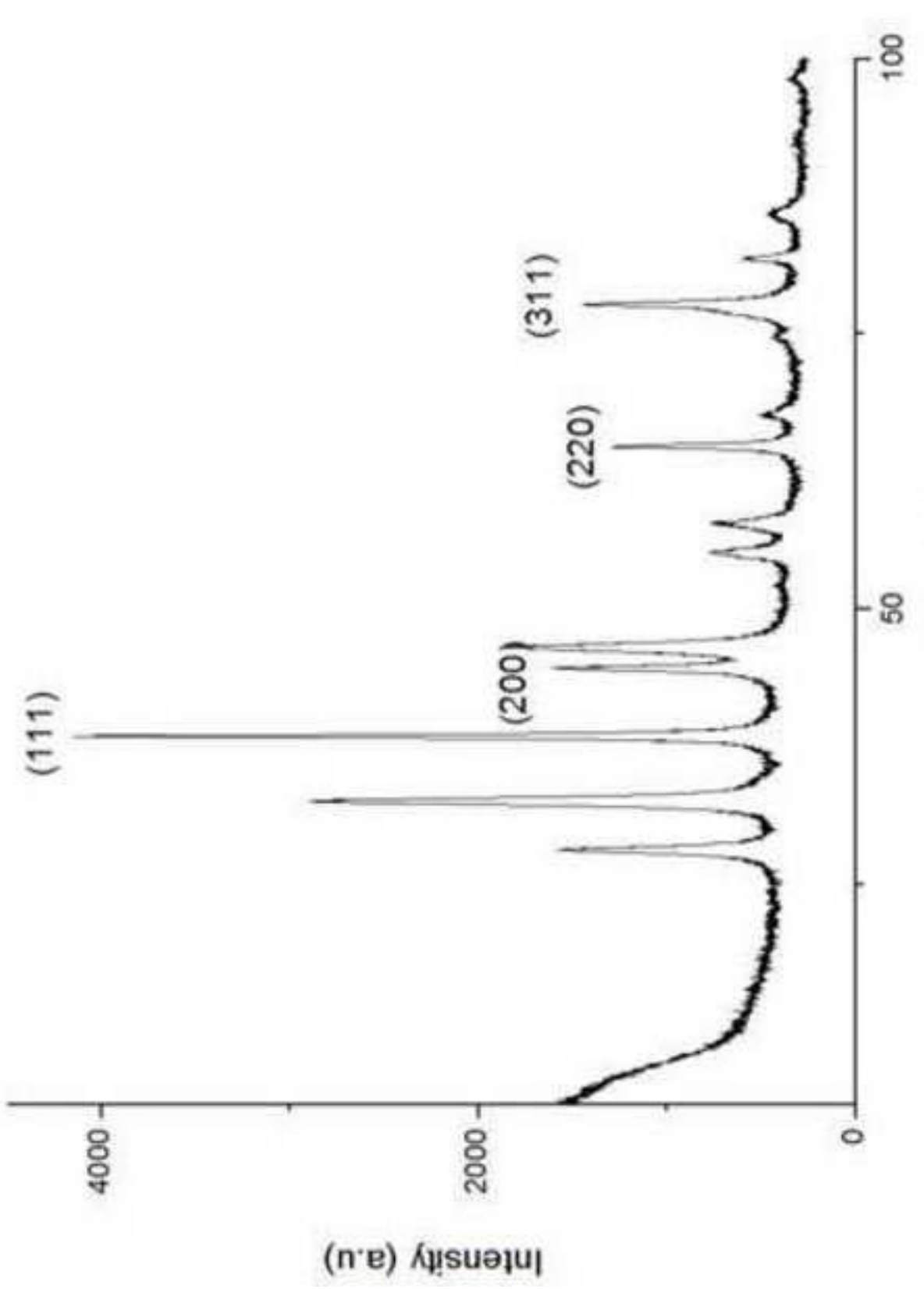


Figure 2 (A)

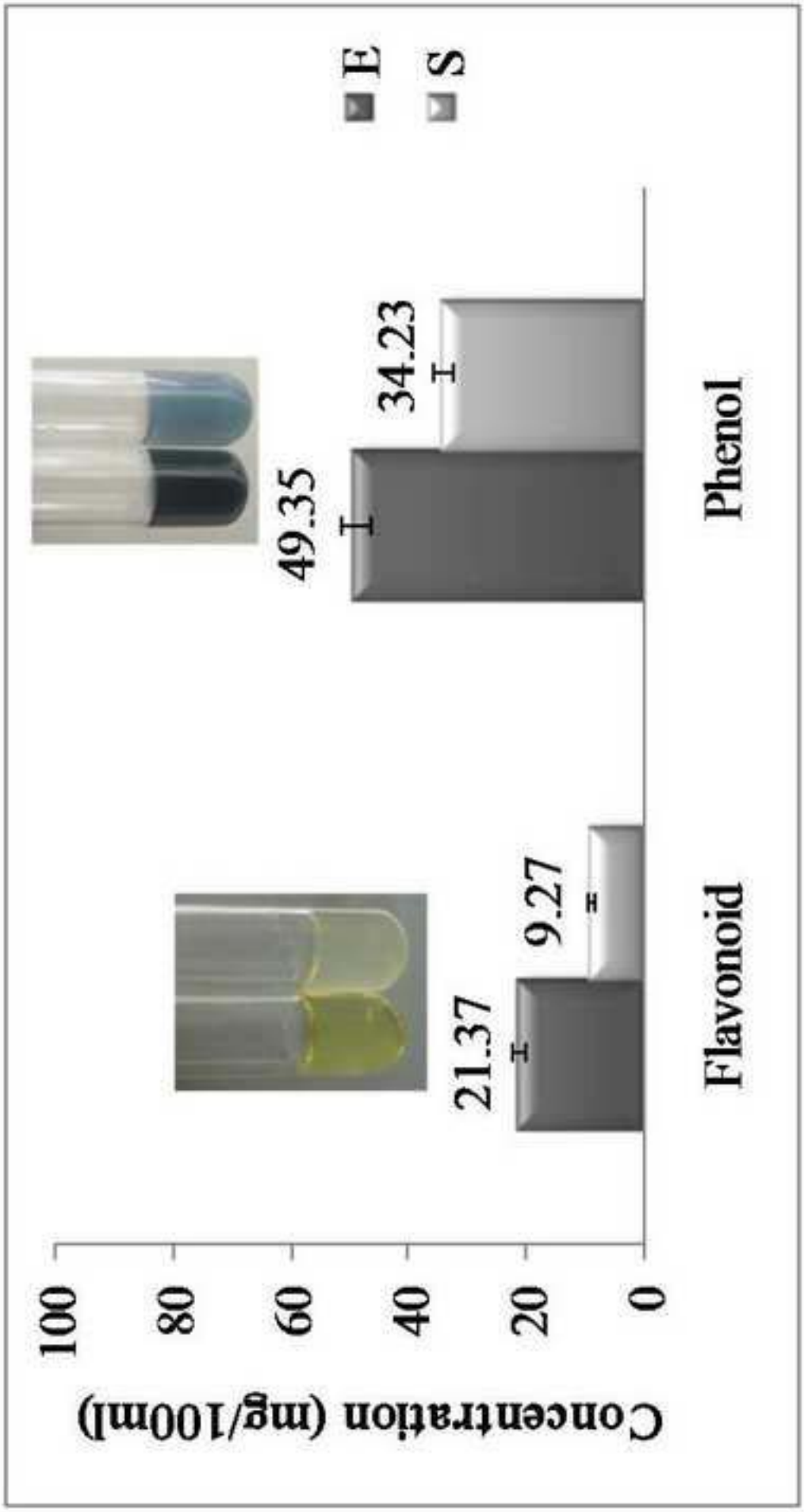
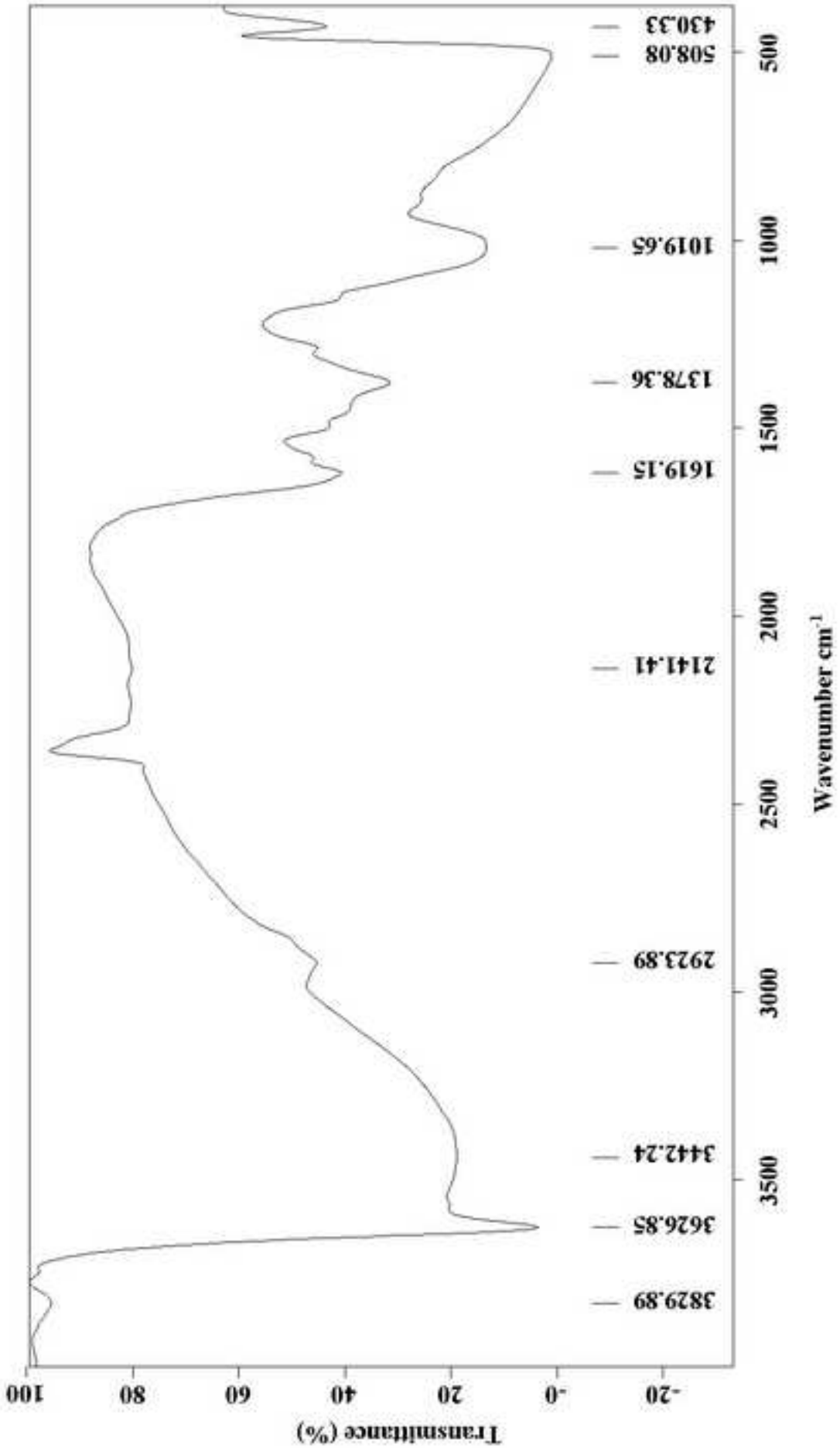

Concentration (mg/100ml)
100
80
60
40
20
0
E
S
49.35
34.23
21.37
9.27
Phenol
Flavonoid
B

Figure 2 (B)


Transmittance (%)
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80
60
40
20
0
-20
Wavenumber cm-1
500
1000
1500
2000
2500
3000
3500
430.33
508.08
1019.65
1378.36
1619.15
2141.41
2923.89
3442.24
3626.85
3829.89

Figure 3