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Biosynthesis of Nanoparticles from *Ficusbenjamina* (Fig Tree) and comparing AgNP's Synthesized by Cocktails of plant extracts

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Abstract

The main purpose of this paper is to synthesize and determine the characteristics of NP's synthesised from Ficus benjamina (fig tree). And also to compare as well as characterise the NP synthesized by different cocktails of plant extracts, hence discerning the best phenolic for NP synthesis. The method used in this paper is a basic extraction protocol of leaves followed by addition of different concentrations of silver nitrate solution to determine the concentration at which maximum amount of Silver (Ag) NP's are produced. The second part of the experiment is to analyze whether addition of polyphenols from other plant sources increased the efficiency of Ag NP production. The results show that Ficus benjamina is able to synthesise NP's and addition of polyphenols increases the efficiency of production; although characterisation through FTIR and SEM is yet to be done. In conclusion, as the absorbance values of the three different cocktails of polyphenols is similar, these different cocktails are equally good at improving the efficiency of production.

Keywords: Fig, neem, onion, green tea, phenolic, nanoparticles (NP).

Introduction

Nanotechnology is an emerging field with wide applications due to the unique properties of nanoparticles not found in the bulk. The nanomaterial properties depend on their size, surface and morphology[1]. Silver nanoparticles have applications in electronics, chemistry, energy, medicine, physics, and material science[2-5]. Originally, synthesis of the nanoparticles was a chemical based approach. These included thermal decomposition in organic solvents, chemical and photo-reduction using reverse micelles. These processes had many disadvantages such as, expenditure, toxicity, usage of hazardous chemicals with high risk, release of dangerous byproducts[6-8]. Hence an alternative synthesis approach which is comparatively safer had to be used. Microorganisms have been used for the synthesis of metal nanoparticles[9- 12].

Now another method of green synthesis is being developed. Here plant extracts are used for the nanoparticle synthesis, by reducing silver ions to produce silver nanoparticles[13,14]. This approach unlike the microbial synthesis does not involve long procedures for cell culturing[9]. Green synthesis is safer, ecofriendly and economic. The nanoparticles synthesized by the green synthesis approach are non-toxic on human cell lines[15]. As silver nanoparticles show antimicrobial activity, fewer amounts are required compared to the bulk[16].

The biosynthesized silver nanoparticles are used in nonlinear optics, bio-labeling, optical receptors, coating for solar energy, electrical batteries[2]. Some of the plant extracts reported to produce the silver nanoparticles is Neem, *Hibiscu*s, *Rosa sinensis*, *Murraya koenigii*[17,18]. In this paper, the ability of *Ficus benjamina* to synthesize silver nanoparticles is discussed. Other known ficus species reported to produce silver nanoparticles are *Ficus benghalensis*, *Ficus religiosa. Ficus benjamina* is a Fig plant belonging to the family of *Moraceae*. The plant is native to South Asia and Northern Australia. It is commonly known as weeping fig. It is used as an ornamental plant[9].

The synthesis of the silver nanoparticles from plant extracts can be indicated by conversion of the aqueous silver ion solution to yellow brown. The color change is due to surface plasmon resonance of the silver nanoparticles[9,20]. Localized SPR is the oscillations of conduction electrons confined to metallic nanoparticles[6,20,21]. Proteins, enzymes and phenolics present in the plant extract are known to cause the reduction of silver ions and hence the formation of silver nanoparticles[9]. The nanoparticles produced can hence be characterized for determining their specific properties. Since polyphenols are one of the compounds involved in synthesizing the nanoparticles, addition of polyphenols to the plant extracts can be studied for changes in absorption peak or reaction rate.

Polyphenols are a structural class of natural synthetic chemicals with multiple phenolic units exhibiting antioxidant properties[22]. Polyphenols occur as anthocyanins, flavanoids, flavonols and isoflavones. The phenolic compounds have hydroxyl and ketonic groups that bind to metals. This chelating ability is due to the high nucleophilic character of the compounds. Flavanoids scavenge molecular species of active oxygen, donate electrons or hydrogen atoms [9] and hence are involved in the reduction of silver ions. Polyphenols are found in food sources. Some of them are carrot, green tea, onion, grape, garlic. The polyphenols are first extracted from the sources for adding on to the plant extract. In this paper, the extraction of polyphenols from neem, onion, green tea is investigated, the three being rich sources of polyphenols[23]. Polyphenols in green tea are known as GTPs. They include epigallocatechingallate (EGCG), epigallocatechin (EGC), epicatechingallate(ECG), epicatechin(EC), flavanoids[22]. In onion the polyphenols present are flavonols, anthocyanins, as glycosyl derivatives[24]. Neem leaves contain quercetin, a flavanoid and limonoids(nimbin) and nimbosterol (β-sitosterol)[25].

This paper focuses initially on the synthesis of nanoparticles by *Ficus benjamina*. The NP's synthesized by them were measured by using UV-spectrometer. In the second part of our experiment, we extracted polyphenols from onion, green tea and neem. We added these extracts to a solution of $AgNO_3$ and *Ficus benjamina* extract. We measured the NP's synthesized using UV-Spectrometer.

Material and Methods

Synthesis of NP's by *Ficus benjamina*: $AgNO_3$ crystals were taken. Three different concentrations of $AgNO_3$ were prepared using distilled water. These were – 100 mL each of 0.8 mM (0.014g of $AgNO_3$), 1 mM (0.017g of $AgNO_3$), and 10 mM (0.17g of $AgNO_3$). *F. benjamina* leaves were rinsed thoroughly by deionized water. Leaf extract was prepared by taking 5 g of leaf thoroughly washed, dried, cut into fine pieces, mixed with 20 mL of deionized water in a beaker and mixture was boiled for 5 min before decanting. For reduction of silver ions, 1 ml of *Ficus benjamina* leaf extract was added drop wise into 9 ml of 0.8 mM, 1 mM, and 10 mM aqueous solution of $AgNO_3$ with constant stirring at 50–60ºC. As soon as, *F. benjamina* extract was mixed in aqueous solution of silver ion complex, it starts to change color from light yellow to yellowish brown due to excitation of surface plasmon resonance which indicates the formation of silver nanoparticles. The synthesized nanoparticles by *F. benjamina* leaf extract were centrifuged at 5500 rpm for 15 min and subsequently redispersed in deionized water to get rid of any uncoordinated biological molecules. All was done in triplicate. Now the analysis was performed.

Plant Extracts: Onion: Samples: The material used in this study was obtained from the greenhouse in VIT (12.97° N, 79.16° E) after processing of brown skin onion bulbs. The material was transferred to the laboratory immediately after processing and stored at −20°C until analyzed.

Green Tea: Samples: The samples of green tea leaves were obtained from the VIT greenhouse (12.97° N, 79.16° E). They were washed with distilled water, dried and were cut. Extracts then made from these leaves.

Neem: Samples: The samples of Neem leaves were obtained from the VIT greenhouse (12.97° N, 79.16° E). They were washed with distilled water, dried and cut. Extracts were then made from these leaves.

Extraction procedure: Onion: Onion was thawed and ground in a domestic blender. An amount of approximately 3 g of the ground material was placed in a 100-mL conical flask with 30 mL of solvent stirred using vortex for 20 min and then filtered through Whatman filter paper. This procedure was repeated twice more. The filtrates were then combined in a 100 mL volumetric flask and made to the volume. All samples were filtered through Whatman filters before analyses. 1 mL of the filtrate was taken for addition.

Neem and Tea leaves: The method described by the International Organization for Standardization (ISO) 14502-1 was used. Briefly, 0.200 mM (0.001 g of each sample was weighed in an extraction tube, and 5 mL of 70% methanol) at 70°C was added. The extract was mixed and heated at 70°C on a vortex for 10 min. After cooling at room temperature, the extract was centrifuged at 2000 rpm for 10 min. The supernatant was decanted. The extraction step was repeated twice. Both extracts were pooled and the volume adjusted to 10 mL with cold 70% methanol. One milliliter of the extract was diluted with water to 100 mL. 1mL of this solution was taken for addition.

Addition to *Ficus benjamina*: We added 1 mL of each extract to 1.5 mL of 1 mM $AgNO_3$and 1.5 mL of 1 mM *Ficus* extract. The reaction started and we waited for half an hour for it to progress. Then we took a quartz cuvette and performed the analysis. All was done in triplicate.

Analysis: The NP's synthesized by all the samples were measured in the UV-Spectrometer at wavelengths ranging from 200 nm to 600 nm. The absorbance values were recorded for every 50 nm increase and in triplicate.

Results and Discussion

Synthesis of Ag NP's by *F. benjamina*: The figure 1 shows the absorbance values for the 3 different concentrations of $AgNO_3$ (0.8 mM, 1 mM, 10 mM) at wavelengths ranging from 200 to 600 nm. The $AgNO_3$ concentration of 10 mM shows peaks at 250 and 450 nm, which indicate 2 different SPR wavelengths for the NP's. The peaks showed an O.D. of 3 which indicates that it is out of the range of the UV-Spectrometer. Further study along these lines can be seen only after FTIR, TEM analysis.

The shape of the curve is a double bell shaped curve. The peak for 1 mM is at 1.2 O.D., and we observe a single bell shaped curve. The peak for 0.8 mM is at 0.6 O.D., and again we observe a single bell shaped curve. We can also see that even the lowest value of O.D. for 10 mM concentration is higher than the peaks for both 0.8 and 1 mM. Thus, greater amount of AgNP's are synthesized when the concentration of $AgNO_3$ is more.

Table-1
Absorbance readings of Ag NP synthesis from *Ficus benjamina* using 3 different concentrations of $AgNO_3$

Conc (OD)	200 nm	250 nm	300 nm	350 nm	380 nm	400 nm	420 nm	440 nm	460 nm	480 nm	500 nm	550 nm	600 nm
0.17 (1)	3.00	3.00	1.743	1.295	1.785	3.00	3.00	3.00	2.801	2.221	1.714	0.984	0.573
0.17 (2)	3.00	3.00	1.745	1.298	1.784	3.00	3.00	3.00	2.809	2.221	1.711	0.984	0.576
0.17 (3)	3.00	3.00	1.747	1.301	1.786	3.00	3.00	3.00	2.805	2.218	1.717	0.978	0.573
Mean	**3.00**	**3.00**	**1.745**	**1.298**	**1.785**	**3.00**	**3.00**	**3.00**	**2.805**	**2.220**	**1.714**	**0.982**	**0.574**
0.017 (1)	1.139	0.538	0.451	0.407	0.437	0.454	0.445	0.437	0.435	0.430	0.404	0.659	0.269
0.017 (2)	1.142	0.530	0.453	0.415	0.436	0.445	0.461	0.446	0.446	0.429	0.411	0.364	0.267
0.017 (3)	1.124	0.537	0.467	0.411	0.426	0.451	0.459	0.443	0.441	0.437	0.407	0.367	0.256
Mean	**1.135**	**0.535**	**0.457**	**0.411**	**0.433**	**0.450**	**0.455**	**0.442**	**0.440**	**0.432**	**0.407**	**0.363**	**0.264**
0.014 (1)	0.641	0.401	0.435	0.317	0.269	0.247	0.227	0.203	0.198	0.176	0.165	0.146	0.092
0.014 (2)	0.641	0.405	0.432	0.318	0.262	0.244	0.229	0.203	0.198	0.179	0.167	0.145	0.093
0.014 (3)	0.639	0.401	0.429	0.313	0.264	0.250	0.223	0.212	0.186	0.179	0.161	0.132	0.085
Mean	**0.640**	**0.403**	**0.432**	**0.316**	**0.265**	**0.247**	**0.226**	**0.206**	**0.194**	**0.178**	**0.164**	**0.141**	**0.090**

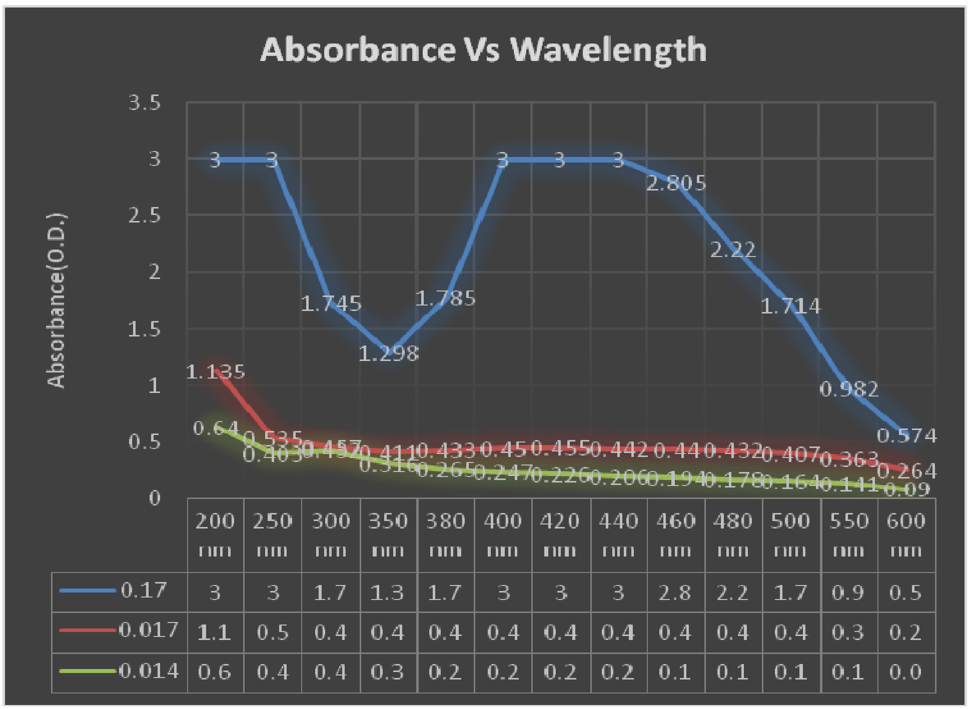


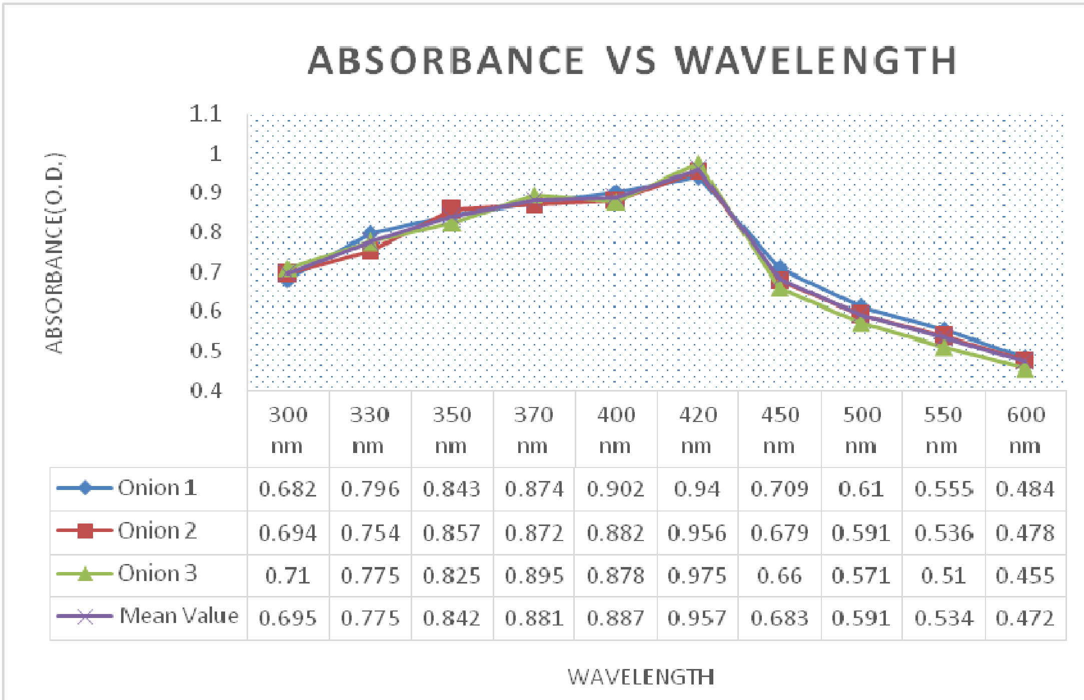
Figure-1
Graph of OD vs. Wavelength for Nanoparticle synthesis by *Ficus benjamina*

Synthesis of Ag NP's by a mixture of *F. benjamina* and polyphenol extract from Onion, Neemand Green Tea

Table-2
Synthesis of Ag NP by addition of Onion, Neem and Green Tea extracts to *F. benjamina* and $AgNO_3$ solution

Samples	300 nm	330 nm	350 nm	370 nm	400 nm	420 nm	450 nm	500 nm	550 nm	600 nm
Onion 1	0.682	0.796	0.843	0.874	0.902	0.94	0.709	0.61	0.555	0.484
Onion 2	0.694	0.754	0.857	0.872	0.882	0.956	0.679	0.591	0.536	0.478
Onion3	0.71	0.775	0.825	0.895	0.878	0.975	0.66	0.571	0.51	0.455
Mean	**0.695**	**0.775**	**0.842**	**0.881**	**0.887**	**0.957**	**0.683**	**0.591**	**0.534**	**0.472**
Neem 1	0.752	0.813	0.882	0.924	0.986	1.051	0.765	0.627	0.527	0.445
Neem 2	0.768	0.804	0.894	0.931	0.973	1.185	0.709	0.618	0.518	0.445
Neem 3	0.75	0.795	0.875	0.91	0.953	1.025	0.8	0.605	0.545	0.445
Mean	**0.757**	**0.804**	**0.884**	**0.922**	**0.971**	**1.087**	**0.758**	**0.617**	**0.53**	**0.445**
Tea 1	0.767	0.799	0.842	0.897	0.948	1.022	0.759	0.622	0.511	0.436
Tea2	0.712	0.843	0.832	0.891	0.937	1.036	0.764	0.631	0.52	0.431
Tea3	0.732	0.821	0.823	0.898	0.94	1.029	0.756	0.645	0.498	0.447
Mean	**0.743**	**0.816**	**0.833**	**0.895**	**942**	**1.056**	**0.76**	**0.633**	**0.51**	**0.437**

The Ag NP's were synthesized and the figure 2, 3, 4 of the O.D. values of each extract versus the wavelengths were drawn. We see that the peak for onion is 0.95 O.D., neem is 1.2 and tea is 1. For all of them, peaks are at 425 nm. In onion, the peaks for all the triplicate curves are uniform, while in neem one of them is at 1.2 while the other is 1.05, and tea is the most uniform. The mean graph can be easily seen from the combined graph for the triplicate, hence was not drawn separately. The O.D. values at the different wavelengths are very similar for all the extracts (if we ignore the high peak for one of the neem extracts), indicating that the phenolic cocktail of all of them are equally good at synthesising NP's.



	300 nm	330 nm	350 nm	370 nm	400 nm	420 nm	450 nm	500 nm	550 nm	600 nm
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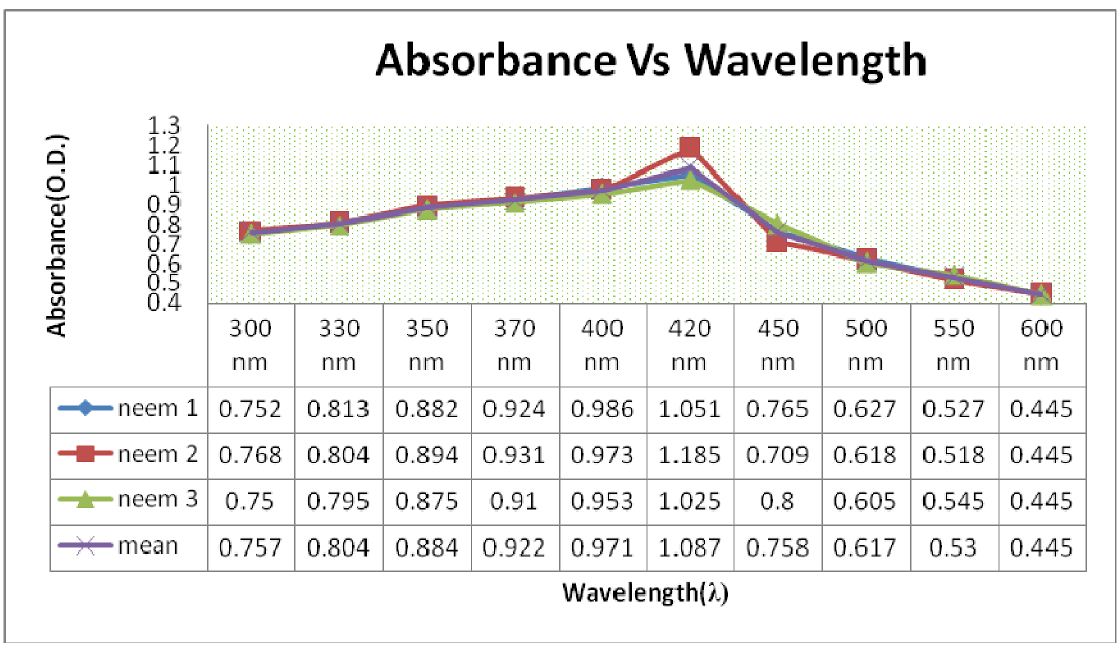
Onion 3	0.71	0.775	0.825	0.895	0.878	0.975	0.66	0.571	0.51	0.455
Mean Value	0.695	0.775	0.842	0.881	0.887	0.957	0.683	0.591	0.534	0.472

Figure-2
Graph of OD vs. Wavelength for a mixture of polyphenol extract of Onion and *F. benjamina*



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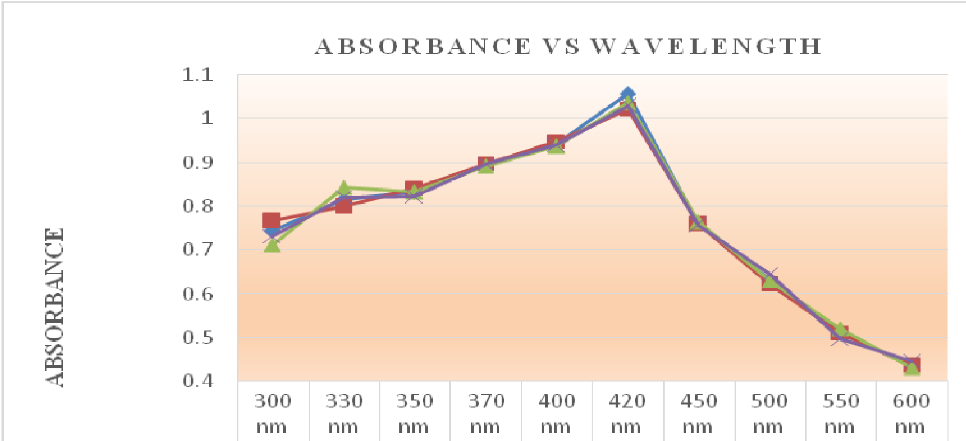
neem 2	0.768	0.804	0.894	0.931	0.973	1.185	0.709	0.618	0.518	0.445
neem 3	0.75	0.795	0.875	0.91	0.953	1.025	0.8	0.605	0.545	0.445
mean	0.757	0.804	0.884	0.922	0.971	1.087	0.758	0.617	0.53	0.445

Figure-3
Graph of OD vs Wavelength for a mixture of polyphenol extract of Neem and *F. benjamina*



	300 nm	330 nm	350 nm	370 nm	400 nm	420 nm	450 nm	500 nm	550 nm	600 nm
Mean Value	0.743	0.816	0.833	0.895	0.942	1.056	0.76	0.633	0.51	0.437
Tea 1	0.767	0.799	0.842	0.897	0.948	1.022	0.759	0.622	0.511	0.436
Tea 2	0.712	0.843	0.832	0.891	0.937	1.036	0.764	0.631	0.52	0.431
Tea 3	0.732	0.821	0.823	0.898	0.94	1.029	0.756	0.645	0.498	0.447

Figure-4
Graph of O.D. vs Wavelength for a mixture of polyphenol extract of Green Tea and *F. benjamina*

Comparative study of *F. benjamina* and Polyphenol extracts: We used a solution of 1 mM of $AgNO_3$, *Ficus benjamina* extract, and one polyphenol extract. The figure 2, 3, 4) of the O.D. versus the wavelength for each extract was drawn. We found that Neem peaked at around 1.05, tea and onion both peaked at 1 O.D. All of them peaked at 425 nm compared to the *Ficus benjamina* 1 mM concentration of $AgNO_3$, which peaked at 200 nm. Since the $AgNO_3$ concentration taken is the same, we see that on addition of the polyphenol extracts, there is a shift in the peaks from 200 nm to 425 nm. This shift is due to a change in the Surface Plasmon Resonance wavelength. This shift is toward a higher wavelength, or toward a lower energy state. This indicates greater stability of the AgNP's formed.

Conclusion

The main objective of this paper is biosynthesis and characterisation of NP's from *Ficus benjamina* (fig tree).The method used in this study is a standard extraction protocol followed by addition of different concentrations of silver nitrate solution to determine the concentration at which maximum Silver (Ag) NP's are produced. Since the absorbance values of the three different cocktails of polyphenols is similar, these different cocktails are equally good at improving the efficiency of production.

Work yet to be done: The samples of *Ficus benjamina* NP's and the NP's synthesized by the polyphenol extracts have yet to be subjected to XRD, TEM, SEM and FTIR analysis.

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