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SYNTHESIS AND CHARACTERISATION OF SILVER NANOPARTICLES FROM RICE FLATSEDGE [*CYPERUS IRIA* (L)] STEM EXTRACT

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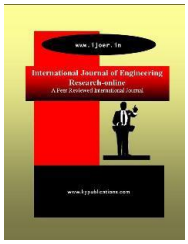

ABSTRACT

The biosynthesis of nanoparticles has been proposed as a cost effective and environmental friendly alternative to chemical and physical methods. The present research explores a plant mediated synthesis of silver nanoparticles (SNPs) as a green chemistry approach that interconnects nanotechnology and plant biotechnology. It has been demonstrated using *Cyperus Iria* (L) stem n-hexane extract. The AgNPs were characterized by Ultraviolet-Visible spectrometer, Scanning electron microscopy (SEM), Energy Dispersive X-ray Analysis (EDAX), Selected Area Diffraction Pattern (SAED), Fourier Transform infrared spectroscopy (FT IR) analysis was used to characterize the silver nanoparticles formed. TEM micrographs showed spherical particles with an average size of 32 nm. The XRD pattern showed the characteristic Bragg peaks suggested that the face center cubic (fcc) silver nanoparticles and confirmed that these nanoparticles are crystalline in nature.

Key words: *C.iria* stem extract, Nanoparticle synthesis, Characterisation, antimicrobial study

1. Introduction

Green synthesis of silver nanoparticles is evolving into an important branch of nanotechnology. Plant based synthesis of silver nanoparticles is gaining more importance owing to its simplicity, rapid rate of synthesis of nanoparticles (NPs) of attractive and diverse morphologies and elimination of elaborate maintenance of cell cultures and Eco friendliness. It has many advantages such as, ease with which the process can be measured up, economic viability and etc. Presently, the researchers are looking into the development of cost-effective procedures for producing reproducible, stable and biocompatible metal NPs. Aluminum, Gold, Zinc, Carbon, Titanium, Palladium, Iron and Copper have been routinely used for the synthesis of NPs. Metal nanoparticles are of use in various catalytic applications, electronics, biology and biomedical applications, material science, physics, environmental remediation fields[1].

Presently available literature revealed that the metal NPs synthesis using plants, microorganisms and algae as source has been unexplored and underexploited. Resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years and is a major health problem. The development of green processes for

the synthesis of NP is evolving into an important branch of green nanotechnology. Plants have evolved in the presence of natural nanomaterials. A variety of preparation routes have been reported for the preparation of silver nanoparticles. Silver salt reduction was the most used[3,5].

Cyperus Iria L. [(Family: Cyperaceae) commonly known as Rice flatsedge] grows well in moist to wet soil in annual and plantation crops. It is one of the most common weed in rice fields and other flooded crops. It is found nearly everywhere in irrigated rice fields. The stem is full, trigonal, thin, smooth, non-winged angles, 1 to 2 mm in diameter and 10 to 50 cm high (figure 1). *C. iria* contains a high concentration of juvenile hormone (JH) III, which plays important biological role(s) in the plant mechanism perhaps through plant-insect, plant-plant or other interactions. In our current study, the silver nanoparticles have been synthesized using screened extract of the rice flatsedge plant stem and report that silver nanoparticles can be applied effectively in the control of microorganisms and the prevention of deleterious infections.

2. Materials and Method

All chemicals and reagents had analytical grade. Silver nitrate, n-hexane with high purity purchased from Sd Fine/Merck India Chemicals, India.

2.1 Apparatus and Instruments: The conventional Soxhlet extraction apparatus was used, which consists of a condenser, a Soxhlet chamber, and an extraction flask. The extractor thimble was permeable one with 44 mm internal diameter and 200 mm external length. The rotary evaporator was used for evaporation of solvent of extracted material.

2.2 Sampling and extraction: The fresh sample of *Cyperus Iria* (L) stem powder was collected at the end of September 2013 in local agricultural fields (Giddaluru revenue sub-division. The plant material was completely dried in sublight and cut into small pieces, ground in grinding mill with particle size of less than 2 mm (Figure 1). The raw grinded sample was sealed and stored in desiccators for further usage. 30 gm homogenized *Cyperus Iria* (L) stem sample was extracted with 500 ml n-hexane for 2hour. The extraction was repeated for 3 times and then the extracts were filtered through whatman filter paper no 42. Then the filtered extract was stored in refrigerator at 4°C for further use in synthesis of silver nanoparticles.

2.3 Synthesis of AgNPs (SNPs): The synthesis of silver nanoparticles was done by mixing *C.iria* (L) stem extract and 1 mM of aqueous silver nitrate solution ($AgNO_3$) in the ratio 1:20 added to plant extract ethanolic solution and heated at 80 ± 2°C until the colour of the solution was changed from colour less to thick brown (Figure 2). Resulted solutions were settled for 24 hours in dark to avoid any further photochemical reactions, after that the solution was centrifuged at 5000 rpm for 10 minutes with magnetic shaker. The supernatant was discarded and the pellet was air dried in the incubator.

The bio reduction of Ag^+ ions was monitored by periodic sampling by the UV spectrophotometer. The AgNPs in the freeze-drying bottle were suspended in ultrahigh purity water for all characterization methods and antibacterial assays. During biosynthesis of silver nanoparticles when stem extract was added to 100 ml of 1 mM $AgNO_3$ salt, the ionization took place as follows:

$$AgNO_3\,(aq) \leftrightarrow Ag^+\,(aq) + NO_3^-(aq)$$

$$e^- + Ag^+ \rightarrow Ag^o$$

It is assumed that the silver ions enter inside the plant cell via the H^+ATPase protein embedded in the thylakoid membrane by an electro genic pump[4]. Synthesis of silver nanoparticles is a photochemical reduction reaction.

2.4 Characterization techniques

- UV–visible spectroscopy: The formation of dark brown color during the synthesis was confirmed as the formation of AgNPs. The reduction of the pure AgNPs was recorded under UV–visible spectroscopy using ELico model UV–visible spectrophotometer between 300 nm and 700 nm. The UV–visible spectra of the plant leaf extract and silver nitrate solution were also recorded.
- FTIR analysis was done using Perkin Elemer Spectrum-1, and was used to identify the chemical constituents in the region of 400-4000 cm^{-1} of the Ag-NPs

- XRD measurement: XRD measurements of Ag-NPs were cast into glass slides were done by Phillips PW 1830 instrument. The operating voltage of 40 kV and current of 30 mA with Cu kα radiation of 0.1541 nm wavelength, in the 2θ range 10- 80º, step size 0.02/θ.
- The morphology of the Ag-NPs was analyzed using an SEM. The powdered Ag-NPs were uniformly spread and sputter coated with platinum in an ion coater for 120 seconds, then observed by SEM JEOL-JSM 6360 MODEL, JAPAN). The size distribution of the nanoparticle was obtained by counting 150 particles from an enlarged SEM image.32 Elemental analysis of the powdered Ag-NPs was conducted using an EDX detector (EDS, EDAX Inc., Mahwah, NJ, USA) attached to the SEM machine.
- TEM analysis of Ag-NPs: Sample for TEM analysis was prepared, as mentioned in IR sample preparations. The sample was first sonicated (Vibronics VS 80) for 5 minutes. Ag-NPs were loaded on carbon coated copper grids, and solvent was allowed to evaporate under Infra light for 30 minutes. TEM measurements were performed on Phillips modle CM 20 instrument, operated at an accelerating voltage at 200 kV.

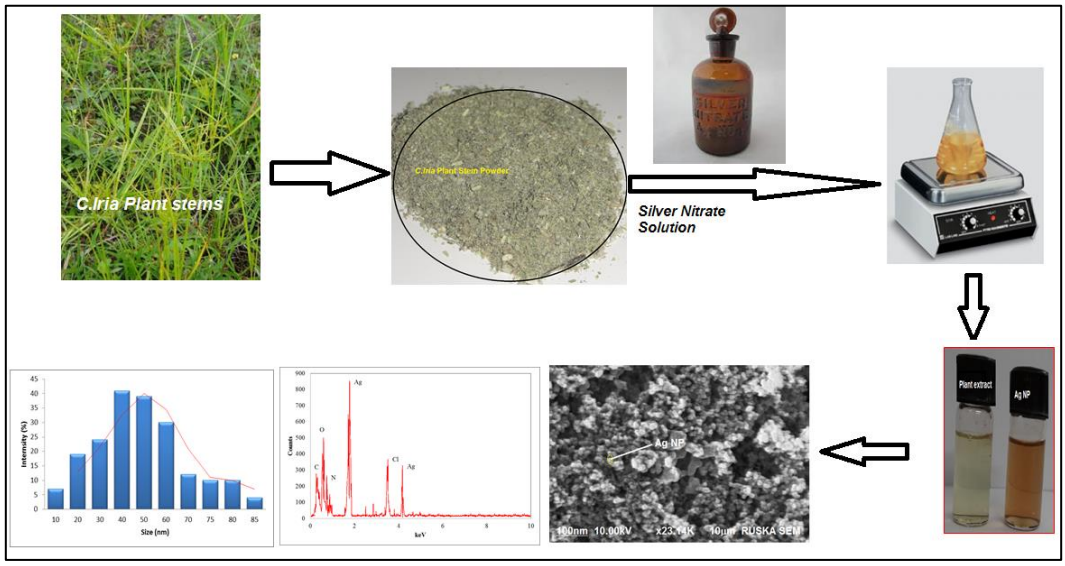

Figure 1. Graphical representation of green and chemical syntheses of silver nanoparticles (SNPs) using *C.ira* L stem and evaluation of their surface characteristics

3. RESULTS AND DISCUSSION

In the present study, n-hexane stem extract of *C.iria* was effectively used for the synthesis of SNPs. After treatment of aqueous plant leaf extract with 1mM $AgNO_3$ colour change was observed in the reaction mixture from light -green to dark- brown (Figure 1). It takes about 25-30 min to complete the reaction with the 5 min exposure to sunlight. The change in colour indicated the formation of SNPs which occurs due to excitation of surface plasma resonance in metal nanoparticles[8].

3.1. UV-Vis Spectrophotometric studies

It is well known that silver nanoparticles exhibit reddish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. Ag^{2+} ions of silver nitrate are found to be reduced to Ag atoms. It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions. The formation of SNPs was investigated by UV-Vis spectroscopy technique. Absorption measurement was carried out using UV-Visible spectrophotometer at a resolution of 1 nm. The UV- Visible spectra of synthesized AgNPs showed absorption peak at 449.3 nm which is specific for SNPs. Obtained results showed good resemblance with many previous studies6,7. The UV- Visible spectra of AgNPs was shown in Figure 2.

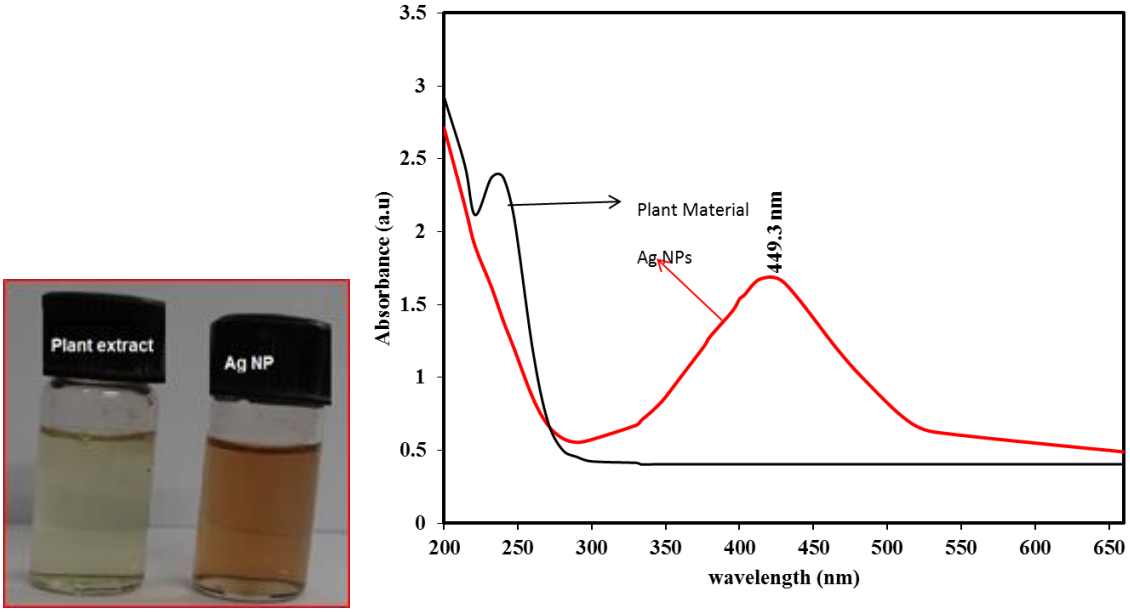

Figure 2: Silver nanoparticles before and after bio reduction (Ag^+ to Ag^o) (left); (b) UV absorption spectra of SNPs after bio reduction (right)

3.2 FT-IR Analysis

FT-IR study of SNPs was carried out to identify the possible biomolecules responsible for synthesis and stabilization of AgNPs. Spectra of pure *C.iria* L stem extract and chemically synthesized SNPs as shown in Figure 3.

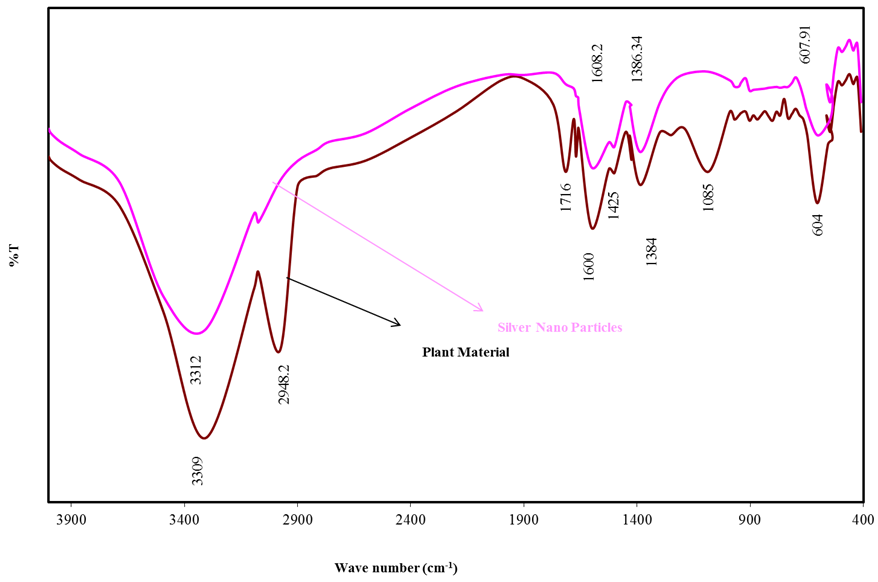

Figure 3: Comparison of the IR spectra of pure *C.iria* L stem extract and chemically synthesized SNPs

As shown in Figure 3, the FT-IR spectrum of pure *C.iria* L stem extract is remarkably similar to the FT-IR spectrum of SNPs, except slight minimal shifts in a few peaks. This striking resemblance between these two spectra clearly suggests that some of the residual phytomolecules of the S *C.iria* L stem remained attached on the surface of the synthesized SNPs. Therefore, the FT-IR spectrum of *C.iria* L stem exhibit several absorption peaks at different locations including at, 3309 cm^{-1} (due to alcoholic or phenolic-OH), ~2948 cm^{-1} (-C–H, asymmetrical stretch), ~1716 cm^{-1} (due to C=O stretch), The narrow peaks at 1600 and 1445 regions clearly evince the presence of stretches of C=C-C and C-H bonds in aromatic rings that are found in Coumaric acid,

Ferulic acid etc[9] 1384 cm^{-1} (C–O stretch) ~1085 cm^{-1} (C–O) which correspond to various oxygen containing functional groups. The peak at 604 cm^{-1} is corresponds the alcohol and OH out-of-plane bending. Majority of these peaks are also present in the IR spectrum of SNPs spectra with some minimal shifts. For instance, all the aforementioned peaks are slightly shifted in the IR spectrum of SNPs and appeared at ~3312 cm^{-1}, ~2946 cm^{-1}, ~1608 cm^{-1}, ~1386 cm^{-1}. Therefore, the presence of these peaks in the IR spectrum of SNPs clearly points towards the successful dual role of the *C.iria* L stem extract, both as a reducing and capping agent.

3.3 X-ray Diffraction al analysis

The diffractogram of SNPs exhibited five intense diffractions (Figure 4), which not only confirms the crystallinity of the sample but also established the identity of the NPs. In Figure 4 of XRD pattern is shown typically peaks at 19.7°, 33.8°, 38.2°, 63.6° and 78.1° corresponding to the (111), (200), (220), (311) and (222) diffractions for face centered cubic (fcc) silver phase (JCPS 04-0786), in a similar way as recently published by *laccase* Ag/AgCl nanoparticles synthesis or from *F. oxysporum*.

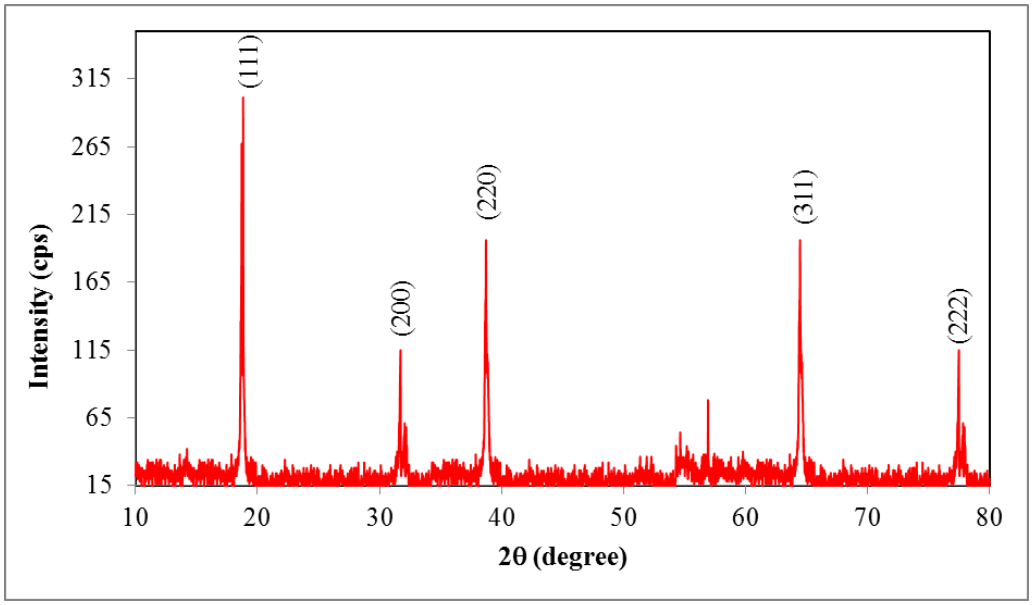

Figure 4: XRD patterns of SNPs synthesized by treating *C.Iria* L. extract with 1 mM silver nitrate

The unassigned peaks could be due to the crystallization of bioorganic phase that occurs on the surface of the nanoparticle. Two small insignificant impurity peaks observed at 50˚ and 60˚ are attributed to the presence of other organic substances in culture supernatant. The X-ray diffraction peaks were found to be broad around their bases indicating that the silver particles are in nanosizes. The peak broadening at half maximum intensity of the X-ray diffraction lines is due to a reduction in crystallite size, flattening and micro-strains within the diffracting domains[13].

3.4 SEM-EDX studies

SEM technique was employed to visualize the size and shape of silver nanoparticles. The SEM images of the AgNPs are shown in fig. 5. The formation of silver nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the average size was from 32-35 nm with inter-particle distance. It is seen that AgNPs of different shapes were obtained in stem extract being used as reducing and capping agents *C.iria* (L) stem extract formed approximately tubular and cuboidal AgNPs, respectively. This may be due to availability of different quantity and nature of capping agents present in the stem extract. This is also supported by the shifts of the peaks obtained in the FTIR analysis. EDX spectra recorded from the silver nanoparticles were shown in Figure 5 (right). From EDX spectra, it is clear that silver nanoparticles reduced by *C.iria* (L) stem extract have the weight percentage of silver as 78.12%.

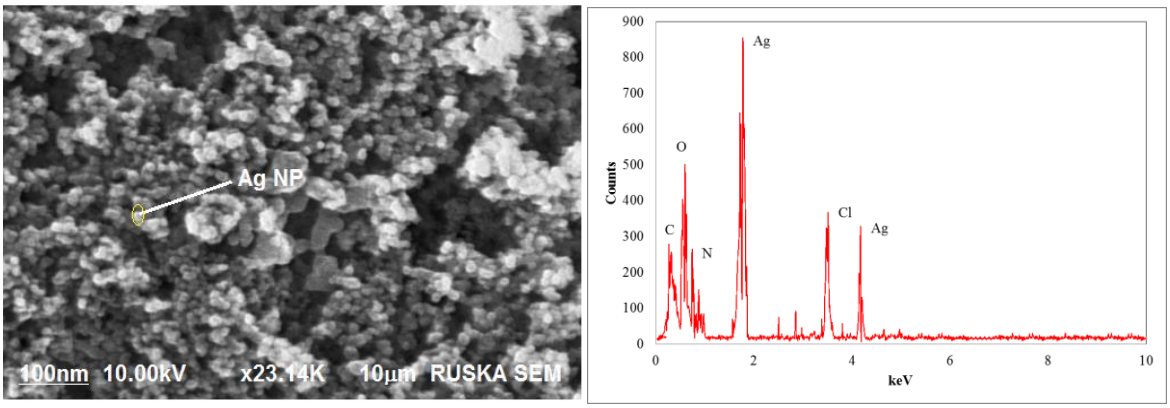

Figure 5: SEM micrograph of agents *C.iria* (L) stem extract-silver nanoparticles (left) and corresponding EDX spectra (right)

3.5 TEM Studies

The size distribution of nanoparticles in general is an important issue as nanoparticles exhibit different physical and chemical properties depending on their shape and size. Transmission electron microscopy (TEM) is therefore one of the most adapted techniques to study the size and shape of the nanoparticles and provide their distribution. It is important to note that the majority of the TEM studies were performed on plant extracted green synthesis of silver nanoparticles. The use of medicinal plants in the synthesis of Ag NPs is not only used for size and shape control but also used to provide properties to the Ag NPs along with the antimicrobial properties of the plant. The TEM images provide monodispersed nanoparticles in each case, indicating that the polyphenols act not only as a reducing agent but also as a capping agent and therefore restrict their growth to 60 nm (Figure 6).

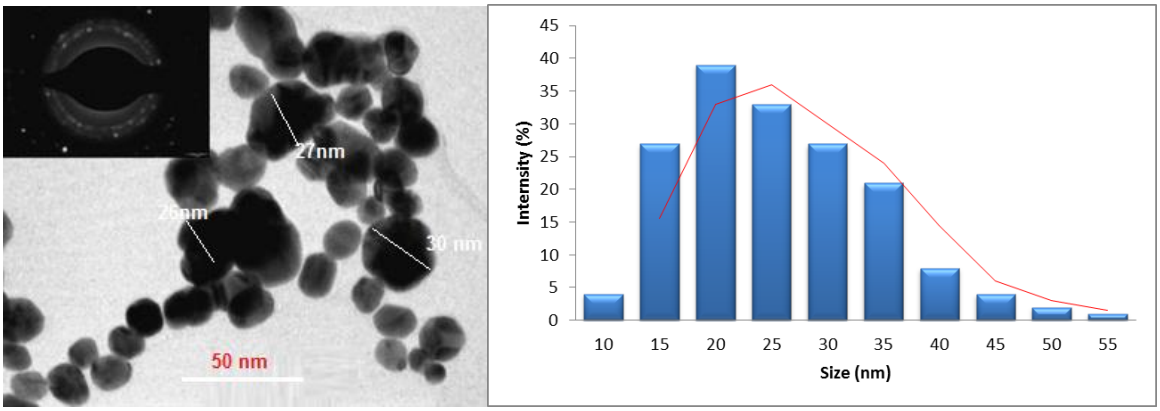

Figure 6: TEM image of silver nanoparticles synthesized using *C.iria* L stem and particles size distribution (right) inset SAED pattern

The TEM images of the particles thus obtained show spherical particles with a wide size distribution with 75% of the nanoparticles presenting particle size between 10 and 35 nm (Figure 6). Less than 10% of the nanoparticles were under 10 nm in size and between 40 to 55 nm. Most of the particles in the TEM image are polydispersed, nevertheless spherically shaped. Crystalline structure was confirmed by SAED pattern (inset picture). SAED pattern shows that green synthesized silver nanoparticles are of crystalline nature and few of them were recorded in the form of aggregates. The four diffraction ring (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) lattice planes could be indexed on the basis of the face-centered cubic (fcc) structure for silver. SAED shows five diffraction rings of fcc for silver. SAED report clearly indicates that synthesized silver nanoparticles are of crystalline structure.

4 Conclusion

During the last decades, many efforts were put into the development of new green synthesis methods. This present study stated the green-mediated synthesis of silver nanoparticles using the extract of easily available *C.iria* weed. The broad peak was observed at 447 nm for silver nanoparticles. Thus, the synthesized nanoparticles showed cubical and spherical-structured nanoparticles with agglomeration which was characterized by SEM and TEM. Purity and component of silver nanoparticles were confirmed by EDX.

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Estimation of Capciacin content in different Red Chili varieties by UV – Spectrophotometer

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Key words: *C chinense*, capsaicin, UV spectrophotometer.

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Abstract:

The major focus of the present study was to estimation and comparison of Capsaicinoids in different verities of Chillies samples. For this, popular agricultural verities of Chili (Ellachipur sannam, Kashmir chili, Byadagi, Gutnur sannam, Hindupur variety, Birds eye chilli, Jwala) samples have been collected. The grounded chilli samples were subjected to soxhlet extraction with Tetrahydrofuran as a solvent. Measurements of the concentration of capsaicin in the extracts were evaluated through their absorbencies measured on λ=280nm by UV spectrophotometer. The amount of capsaicin in sample extracts was calculated using the following equation (y = 0.755x + 0.047). Among the seven verities of chilli samples Birds Eye Chili (0.71%), Jwala (0.61%) verities were found high yield of Capsaicinoid and Byadagi (0.22%), Hindpur (0.21%) verities were found less in capsaicinoid quantity.

Capsaicin Introduction:

Capsaicin is an active component of chili peppers, which plants are belonging to the genus *Capsicum.* Pure capsaicin is a non-volatile, hydrophobic, colorless, and highly pungent [1], crystalline to waxy compound and chemical name of the Capsaicin is 8-methyl-*N*-vanillyl-6-nonenamide. Capsaicin is present in large quantities in the placental tissue (which holds the seeds), the internal membranes and, to a lesser extent, the other fleshy parts of the fruits of plants in the genus *Capsicum* [2]. Capsaicin and several related compounds are called Capsaicinoids and are produced as secondary metabolites by chili peppers, probably as deterrents against certain mammals and fungi [3]. The most commonly occurring Capsaicinoids are capsaicin (69%), Dihydrocapsaicin (22%), Nordihydrocapsaicin (7%), Homocapsaicin (1%), and Homodihydrocapsaicin (1%) [4].

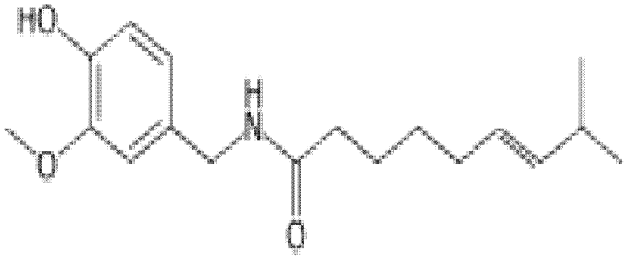

Figure 1: Chemical structure of Capsaicin

It is an irritant for mammals, including humans, and produces a sensation of burning in any tissue with which it comes into contact. Because of the burning sensation caused by capsaicin when it comes in contact with mucous membranes, it is commonly used in food products to provide added spice or "heat" (piquancy), usually in the form of spices such as chili powder and paprika [5]. Capsaicin is also an active ingredient in riot control and personal defense pepper spray agents [6, 7]. Capsaicin is used as an analgesic in topical ointments, nasal sprays (Sinol-M), and dermal patches to relieve pain, typically in concentrations between 0.025% and 0.1% [8]. It may be applied in cream form for the temporary relief of minor aches and pains of muscles and joints associated with arthritis, backache, strains and sprains, often in compounds with other rubefacients. it's been used for many years to treat pain related to **osteoarthritis, rheumatoid arthritis** and **fibromyalgia**, as well as certain kinds of joint pain. Capsaicin is also used by people with the skin disease psoriasis to decrease itching and inflammation. Some research has also suggested that capsaicin can also help with appetite suppression. As Capsaicin has been have many food and medicinal application the present study is aimed to study the Capsaicin content in different varieties of chilies [9]. Different analytical instruments were previously reported [10] for the estimation of Capsacin content in chili samples.

Instrumentation:

Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10mm path length are used for analysis. Soxhlet apparatus is used to extraction of capsaicin from chili samples. Standard chromium was weighed by using Denver electronic analytical balance (SI-234).

Chemicals used:

Standard capsaicin, purchased from Sigma Chemical, Bengaluru. Tetrahydrofuran of make Merck purchased from Mumbai, Different varieties of chilli samples (Ellachipur sannam, Kashmir chilli, Byadagi, Gutnur sannam, Hindupur variety, Birds eye chili, Jwala) (figure 1) were collected from Agricultural market yard, Guntur, AP.

Preparation of standard Capsaicin:

The standard Capciacin (10mg) was weighed accurately and transferred to volumetric flask (10ml). It was dissolved properly and diluted up to the mark with diluents prepared by mixing methanol to obtain final concentration of 1000 µg /ml.

Extraction:

Preparation of Chilli Powder extracts:

The extract was prepared employing Soxhlet extraction method. About 5gm of dried chilli powder material was uniformly packed into a thimble and extracted with THF (figure 2). The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4^0C for their future use in Capciacin analysis.

Results and discussion:

Measurements of the concentration of capsaicin in the extracts were evaluated through their absorbencies measured on λ=280nm. A simple linear regression curve was plotted using standard capsaicin, purchased from Sigma Chemical. A stock solution of 1mg/ml capsaicin in Tetrahydrofuran prepared and different concentrations from 0.2-1.2µg/ml were prepared from the stock solution (table 1). The absorbencies for standard dilutions were also measured and used to prepare the linearity curve presented on figure 3. The Capsaicinoid extracted from solvents was estimated by UV visible spectrophotometer. The crude extract was diluted by using the respective solvent. The optical density was recorded at 280nm. The amount of capsaicin in sample extracts was calculated using the following equation ($y = 0.755x + 0.047$) for UV spectrophotometer estimation (Figure 3) for the total capsaicin estimation.

Various Agricultural verities of Chili samples (Ellachipur sannam, Kashmir chili, Byadagi, Gutnur sannam, Hindupur variety, Birds eye chilli, Jwala) have been selected for estimation of capsaicinoid concentrations. All the samples were grounded and subjected to Soxhlet extraction by using the tetrahydrofuran. Capsaicinoid concentrations in the samples were estimated calculated using capsaicin linear regression equation. The capsaicinoid content was found to be Birds Eye Chili (0.71%), Byadagi (0.21%), Guntur Sannam (0.28%). Hindpur (0.21%), Jwala (0.61%), Kashmir variety (0.37%) and Ellachipur Sannam(0.25%) respectively. Among the seven verities of chili samples Birds Eye Chilli (0.71%), Jwala (0.61%) verities were found high yield of Capsaicinoid and Byadagi (0.22%), Hindpur (0.21%) verities were found less in Capsaicinoid quantity.

Conclusion:

Capsicum spp. are well known for their ability to cause an intense organoleptic sensation of heat when consumed. Chillies are the berries of the genus Capsicum (family: Solanaceae) and they are used variously as a pungent flavor in food, natural plant color, pharmaceutical ingredient and as sprays for riot control and self-defense. Pungent flavor of chilies is due to a group of closely related alkaloid called Capsaicinoids found only in the genus Capsicum. The Capsicum content in different red chilly varieties was studied using UV spectrophotometer. Standard calibration curve was obtained within the concentration range of 0.2-1.2µg/ml. regression equation of standard calibration curve ($y = 0.755x + 0.047$; $R^2 = 0.999$) was used for the determination of Capsacin content in Chili powder samples. Among the seven verities of chilli samples studied, Birds Eye Chilli (0.71%), Jwala (0.61%) verities were found high yield of Capsaicinoid and Byadagi (0.22%), Hindpur (0.21%) verities were found less in Capsaicinoid quantity.

Figure 1: Different verities of chili samples selected for analysis

Figure 2: Soxhlet extraction of Capsaicinoid from chili samples

S No	Parameter	Results
1	Wavelength maxima	280nm
2	Beers Law Range	0.2-1.2µg/ml
3	Slope	0.755
4	Intercept	0.047
5	r^2	0.999

Table 1: Regression equation of Capsaicin standard

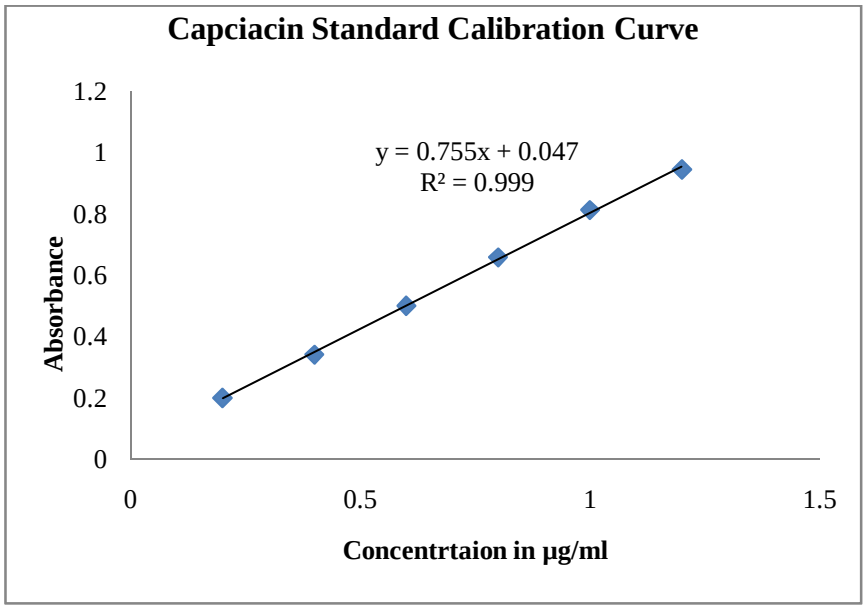

Figure 3: standard calibration curve of Capsaicin

S No	Name of the variety	% Capsacin Content
1	Birds Eye Chilli	0.71
2	Byadagi	0.22
3	Guntur Sannam	0.28
4	Hindpur	0.21
5	jwala	0.61
6	Kashmir variety	0.37
7	Ellachipur Sannam	0.25

Table 3: Amount of Capsacin found in the selected verities of chili samples

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RESEARCH ARTICLE

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B. Krupa Karuna Vani, Int.J.A.PS.BMS,2017,Vol.6(1) 022-029

UV and Derivative Spectrophotometry Method for the estimation of Canagliflozin in pharmaceutical formulations

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Abstract:
Simple, fast and reliable UV and derivative Spectrophotometric methods were developed for determination of Canagliflozin in pharmaceutical formulation. Canagliflozin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor used for treatment of type 2 diabetes mellitus. Absorption for NH was measured at maximum wavelength 227nm. Analytical Calibration curves were linear within a concentration range from 2-12µg/ml. The developed method was applied to directly and easily to the analysis of the pharmaceutical tablet preparations. The percentage recovery of Canagliflozin in pharmaceutical dosage form was found to be 98.57-100.75% for UV, 98.50-99.880% for first derivative and 98.87-99.90% for second derivative methods respectively. The method was completely validated and proven to be rugged. The results obtained were statistically evaluated and were found to be accurate and reproducible. The excipients did not interfere in the analysis. The results showed that this method can be used for rapid determination of Canagliflozin in pharmaceutical tablet.

Key words: Canagliflozin, Ultraviolet and derivative spectropscopy, ICH guidelines, method development, validation.

Drug Introduction:
Canagliflozin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor [1,2], which belongs to a new class of anti-diabetic drugs approved in 2013 for treating type 2 diabetes in certain patients.
Canagliflozin is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus [3]. SGLT2 is a transport protein found in the kidney and is responsible for reabsorbing glucose that has been filtered. Canagliflozin works by decreasing the amount of sugar the body absorbs, and increasing the amount of sugar that leaves the body in the urine [4-6]. Canagliflozin is sold as a tablet dosage form and it is also available along with other drugs like metformin as combined dosage forms. The drug is associated with side effects like increased incidence of urinary tract infections, fungal infections of the genital area, thirst, elevations in LDL cholesterol, increased urination and episodes of low blood pressure. There are concerns that it may also increase the risk of diabetic ketoacidosis [7-9]. There are very few analytical methods [10-15] have reported for analysis of Canagliflozin. Among them only there spectropotometry methods have been reported [10-12]. Hence the present work is aimed to development and validation of colorimetric method for estimation drug in pharmaceutical dosage forms.

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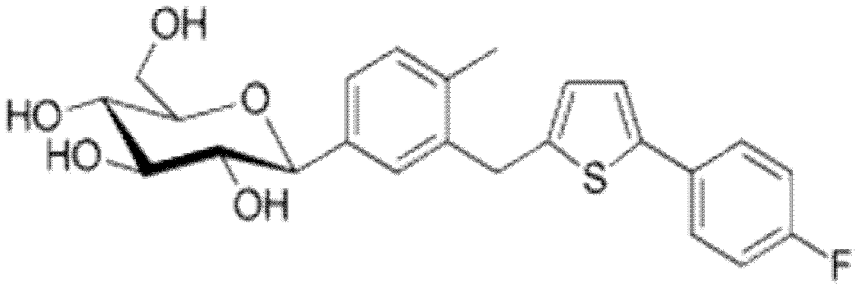


Figure.1: Chemical structure of Canagliflozin

Material and Methods:

Instrumentation:

Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10mm path length are used for analysis. Standard chromium was weighed by using Denver electronic analytical balance (SI-234).

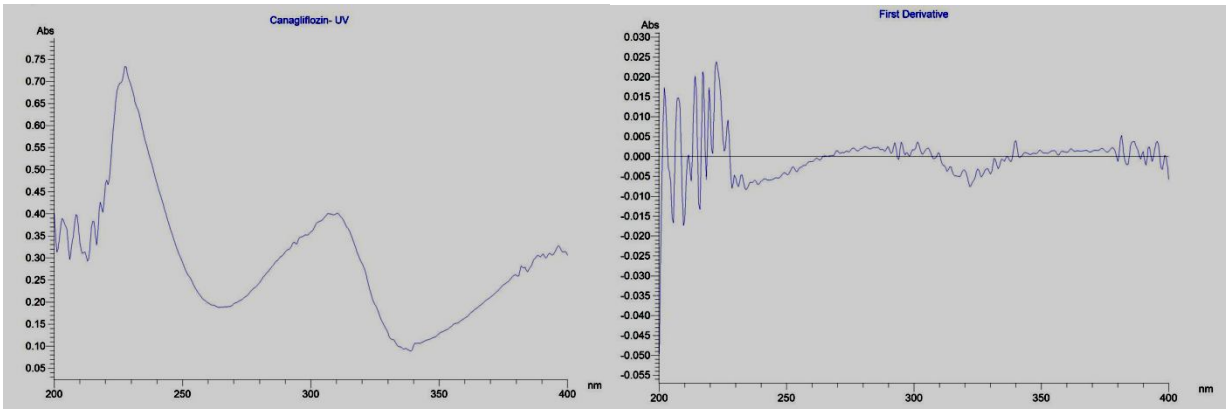
Chemicals and reagents:

All the chemicals used were of laboratory reagent grade and were purchased from Merck chemicals private limited, Mumbai, India. The marketed formulation of Canagliflozin was purchased in local pharmacy.

Preparation of standard stock solution:

Standard drug solution of Canagliflozin was prepared by dissolving 10mg of Canagliflozin in 50ml methanol and was transferred to 100ml volumetric flask, it was sonicated for 5min to dissolve the drug completely in the solvent and the volume was made up to mark with methanol to obtain stock solution of 100µg/ ml concentration. The solution was scanned (figure 1) in the range of 200-400 nm against blank.

The developed methods have been validation for linearity, precision, ruggedness, sensitivity etc parameters under ICH guidelines [16, 17].



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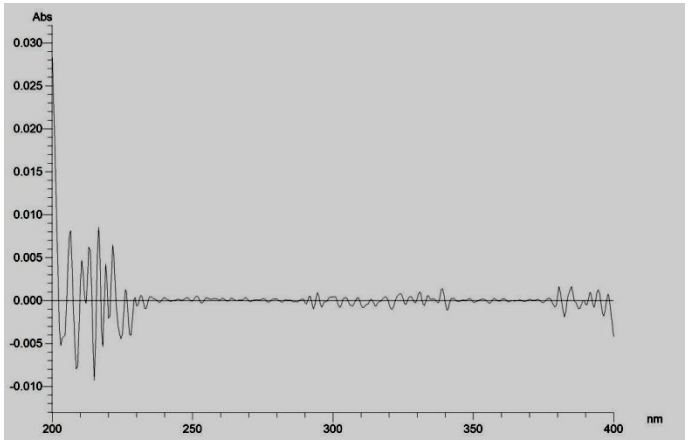

Figure 2: Scanning spectra of Canagliflozin for UV [M1], First derivative [M2] and Second derivative method [M3]

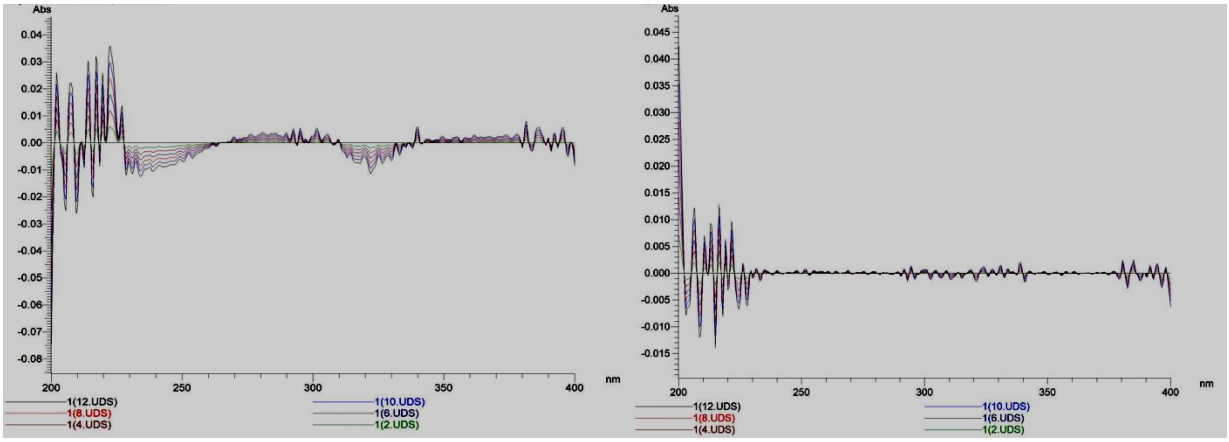

Figure 3: Overlay of scanning spectra of Canagliflozin for First derivative [M2] and Second derivative method [M3]

Method Validation:

Linearity:

The linearity of the response of the drug was verified at 2 to 12µg/ml concentration. The absorbance for 2, 4, 6, 8, 10 and 12ml concentrations (Table 1) were measured at 227nm by using UV spectrophotometer. The calibration curve was obtained by plotting the absorbance versus concentration data and was treated by linear regression analysis (figure 3).

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Table 1: Results of Linearity test for Canagliflozin:

S NO	Concentration in µg/ml	Absorbance obtained		
		UV	First Derivative	Second derivative
1	2	0.261	0.00813	0.00023
2	4	0.373	0.01405	0.00215
3	6	0.470	0.01899	0.00408
4	8	0.567	0.02515	0.00619
5	10	0.664	0.03106	0.00836
6	12	0.761	0.03679	0.01030

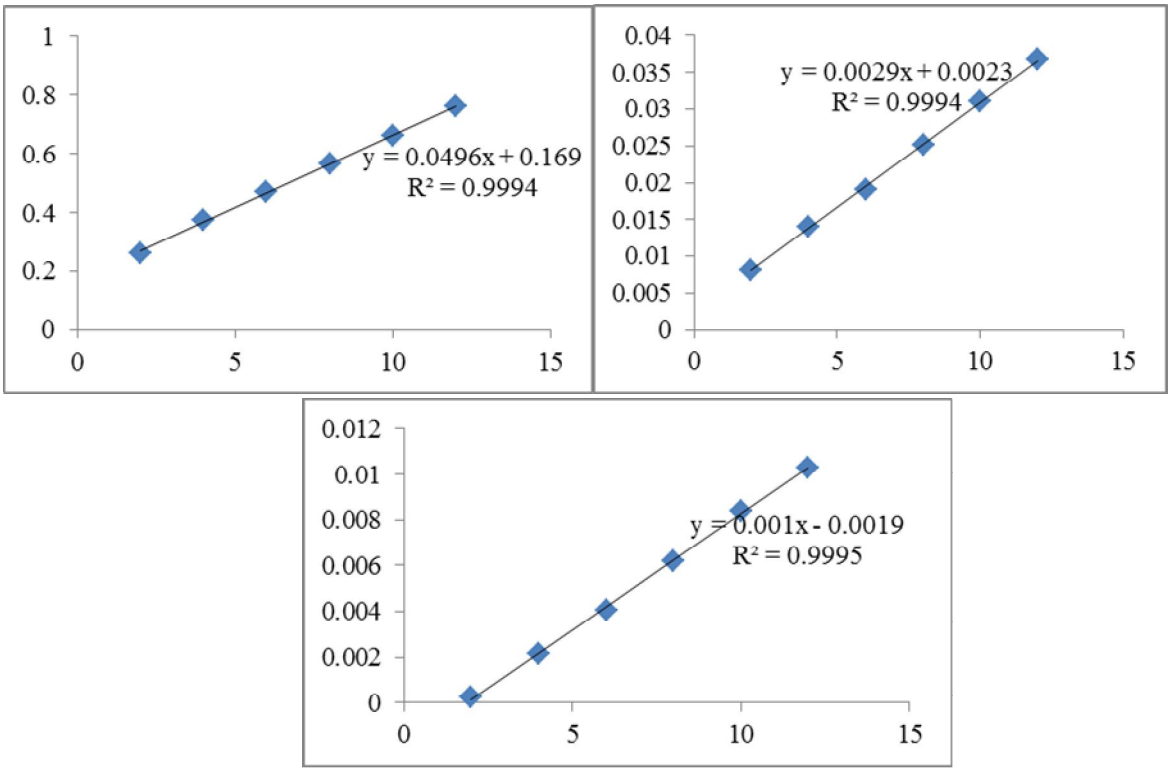

Figure 3: Linearity graph of Canagliflozin for UV, First Derivative and second derivative method

Accuracy (Recovery Test):

Recovery experiment was conducted to confirm the accuracy of the method. The recovery experiments were performed by adding known amounts to tablet. The recovery was performed at three levels 50, 100 and 150% of

Canagliflozin standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Canagliflozin are listed in the Table 2.

Table 2: Results of recovery test for Canagliflozin:

Level	Concentration in µg/ml			UV		First Derivative		Second derivative	
	T	S	F	Amount found	% Recovery	Amount found	% Recovery	Amount found	% Recovery
50%	4	2	6	5.98	99.78	5.93	98.83	5.96	99.33
	4	2	6	5.97	99.57	5.91	98.50	5.94	99.00
	4	2	6	5.93	98.93	5.92	98.67	5.98	99.67
100%	4	4	8	7.88	98.58	7.91	98.87	7.95	99.37
	4	4	8	8.01	100.17	7.94	99.25	7.98	99.75
	4	4	8	7.94	99.29	7.95	99.37	7.91	98.87
150%	4	6	10	9.98	99.84	9.98	99.80	9.99	99.90
	4	6	10	10.06	100.60	9.91	99.10	9.96	99.60
	4	6	10	10.07	100.75	9.93	99.30	9.94	99.40

T=Target Concentration
S= Spiked
T=Total concentration Prepared

Precision:

Assay of method precision (intra-day precision) was evaluated for three independent assays of test samples of NH. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, systems and different days in the same laboratory. The precision data is reported in the table 3.

Formulation Assay:

Initially ten tablets (sulisent-100mg) were weighed and powdered. The amount of tablet powder equivalent to 10 mg of Canagliflozin was weighed accurately and transferred to 10ml volumetric flask. To this 5ml diluents was added and were keep it for solubility for 24H. The solution was then filtered through whatmann filter paper # 41. This filtrate was diluted suitably with the media to get the solution of 6µg/ml concentration .The absorbance was measured against blank. The drug content of the preparation was calculated using standard calibration curve. Amount of drug estimated by this method is given in Table 4.

Discussion of Results:

All the validation studies have been conducted at the 227nm maximum wavelength for Canagliflozin was considered for the analytical method development (figure 2). A linear correlation was found which obeys Beer Lambert's Law in the concentration range of 2-12 µg/ml (Figure 2). Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and the correlation coefficient (r^2). Where the slope was found to be y= 0.049x with intercept 0.169 and the correlation coefficient was found to be 0.99 for UV method. For first derivative method the slope was found to be y= 0.002x with intercept 0.002 and the correlation coefficient was found to be 0.99. For second derivative method the slope was found to be y= 0.0021 with intercept -0.001 and the correlation coefficient was found to be 0.99. Overlay of first and second derivative spectra were presented in figure 3. Precision results with all the methods were found within the limit of RSD below 2. Recovery studies were found in the range of 98.57-100.75% for UV method, 98.50-99.880% for first derivative and 98.87-99.90% Second derivative methods respectively. The value of LOD (0.05) and LOQ (0.10) are determined for all the methods. Percentage of assay for formulation assay with proposed method was found to good (98.67%-UV, 98.83%- first derivative and 99.33% for Second derivative methods respectively.

Table 3: Summary of validation results for Canagliflozin:

S. No	Parameter	Ultraviolet	First Derivative	Second derivative
1	λ max	227nm	227nm	227nm
2	Beers Law Range	2-12 µg/ml	2-12 µg/ml	2-12 µg/ml
3	Slope	0.049	0.002	0.001
4	Intercept	0.169	0.002	-0.001
5	r^2	0.999	0.999	0.999
6	%RSD of Precision Intraday Interday Ruggedness	 0.45 0.53 1.19	 0.20 0.23 0.37	 0.36 0.62 0.60
7	Recovery range (50-150%)	98.57-100.75%	98.50-99.880%	98.87-99.90%
8	LOD LOQ	0.05µg/ml 0.10µg/ml	0.05µg/ml 0.10µg/ml	0.05µg/ml 0.10µg/ml
10	Formulation assay	98.67%	98.83%	99.33%

Conclusion:

From the results obtained with proposed methods, it can be concluded that spectrophotometric method of using the Methanol as the media was found to be new, accurate, precise and economic for the determination of Canagliflozin. The proposed method shows better sensitivity. In addition, the proposed method employs an inexpensive instrument. Overall the proposed new and eco-friendly spectrophotometric method is economical and suitable for quality control of Canagliflozin in fixed-dose combination tablets.

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RESEARCH ARTICLE

B. Krupa Karuna Vani , The Experiment,2017,Vol 40 (3),2376-2381

Extractive Visible Spectrophotometre Method development and validation for the estimation of Canagliflozin in pharmaceutical formulations

Abstract:

This work describes the development, validation and stable studies of a new, simple and reliable visible spectroscopy procedure for the analysis of Canagliflozin in pharmaceutical formulations. Studies were carried out to investigate the reaction between Canagliflozin with 5 different chromogenic dyes. All these dyes shown specific colors on reaction with Canagliflozin and prominent wavelength maxima was observed. All the developed methods were validated as per the ICH guidelines and results shows that the methods were valid. Formulation analysis shows good argument with the true values. Hence the proposed methods for the estimation of Canagliflozin are simple, rapid, accurate, economical and are useful for the estimation of Canagliflozin in pharmaceutical formulation.

Key words: Canagliflozin, colorimetry, ICH guidelines, method development, validation.

Drug Introduction:

Canagliflozin belongs to a new class of anti-diabetic drugs approved in 2013 for treating type 2 diabetes in certain patients. Canagliflozin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor [1,2], which is a transport protein found in the kidney and is responsible for reabsorbing glucose that has been filtered. Canagliflozin works by decreasing the amount of sugar the body absorbs, and increasing the amount of sugar that leaves the body in the urine [3,5]. Canagliflozin is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus [6]. The drug is available as a tablet dosage form and it is also available along with other drugs like metformin as combined dosage forms. The drug is associated with side effects like increased incidence of urinary tract infections, fungal infections of the genital area, thirst, elevations in LDL cholesterol, increased urination and episodes of low blood pressure. There are concerns that it may also increase the risk of diabetic ketoacidosis [7-9]. There are very few analytical methods [10-15] have reported for analysis of Canagliflozin. Among them only there spectropotometry methods have been reported [10-12]. Hence the present work is aimed to development and validation of colorimetric method for estimation drug in pharmaceutical dosage forms.

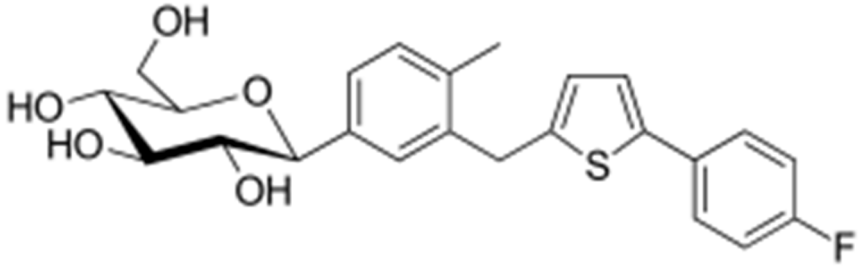

Figure.1: Chemical structure of Canagliflozin

Material and Methods:

Instrumentation:

Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10mm path length are used for analysis. Standard chromium was weighed by using Denver electronic analytical balance (SI-234).

Chemicals and reagents:

All the chemicals used were of laboratory reagent grade and were purchased from Merck chemicals private limited, Mumbai, India. The marketed formulation of Canagliflozin was purchased in local pharmacy.

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Preparation of regents:

ARS solution: weigh 200 mg of Alizarin Red S Reagent and is dissolved in 100ml of distill water.
Woll Faster Blue Black (WFBBL) solution: weigh 200 mg of WFBBL and is dissolved in 100ml of distill water.
Eryochrome Black T (EBT) solution: weigh 300 mg of EBT and is dissolved in 100ml of distill water.
HCL Solution: dissolve 8.6 ml of concentrated hydrochloric acid in 1000ml of distill water

Preparation of standard stock solution:

Standard drug solution of Canagliflozin was prepared by dissolving 10mg of Canagliflozin in 5ml methanol and was transferred to 10ml volumetric flask, it was sonicated for 5min to dissolve the drug completely in the solvent and the volume was made up to mark with methanol to obtain stock solution of 1000µg/ ml concentration. From 1000µg/ml, 250, 150 and 100µg/ml solution was prepared by selective dilution.

ARS Method [M1]:

In a series of 125 ml separating funnels containing aliquots of standard drug [0.5-3.0ml; 150µg/ml] solution was taken. To this 6ml of HCl solution and 2ml of dye solutions were added successively. The total volume of the aqueous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel 10ml of Chloroform was added and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 443nm against a similar reagent blank.

WFBBL Method [M2]:

In a series of 125 ml separating funnels containing aliquots of standard drug solution (0.5-3ml; 100µg/ml) was taken. To this 6ml of HCl solution and 2ml of WFBBL solutions were added successively. The total volume of the aqous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel 10ml of Chloroform was added and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 584nm against a similar reagent blank.

EBT Method [M3]:

In a series of 125 ml separating funnels containing aliquots of standard drug solution (5-30ml; 250µg/ml) was taken. To this 6ml of HCl solution and 2ml of EBT solutions were added successively. The total volume of the aqueous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel 10ml of Chloroform was added and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 410nm against a similar reagent blank.

All the developed methods have been validation for linearity, precision, ruggedness, sensitivity etc parameters under ICH guidelines [16, 17].

Result and Discussion:

Method Development:

The drug Canagliflozin is in basic nature and forms an ion association complex with acid dyes ARS, WFBBL and EBT. The formed complex is extractable in to Chloroform from the aqueous phase. The protonated nitrogen positive charge of the drug molecule in acid medium is expected to attack the positive charge of the dye. Hence form a colored complex which is extracted with Chloroform. The obtained color chromogen shows absorbance at 443nm for ARS Method, 584nm for WFBBL method and 410nm in EBT method.

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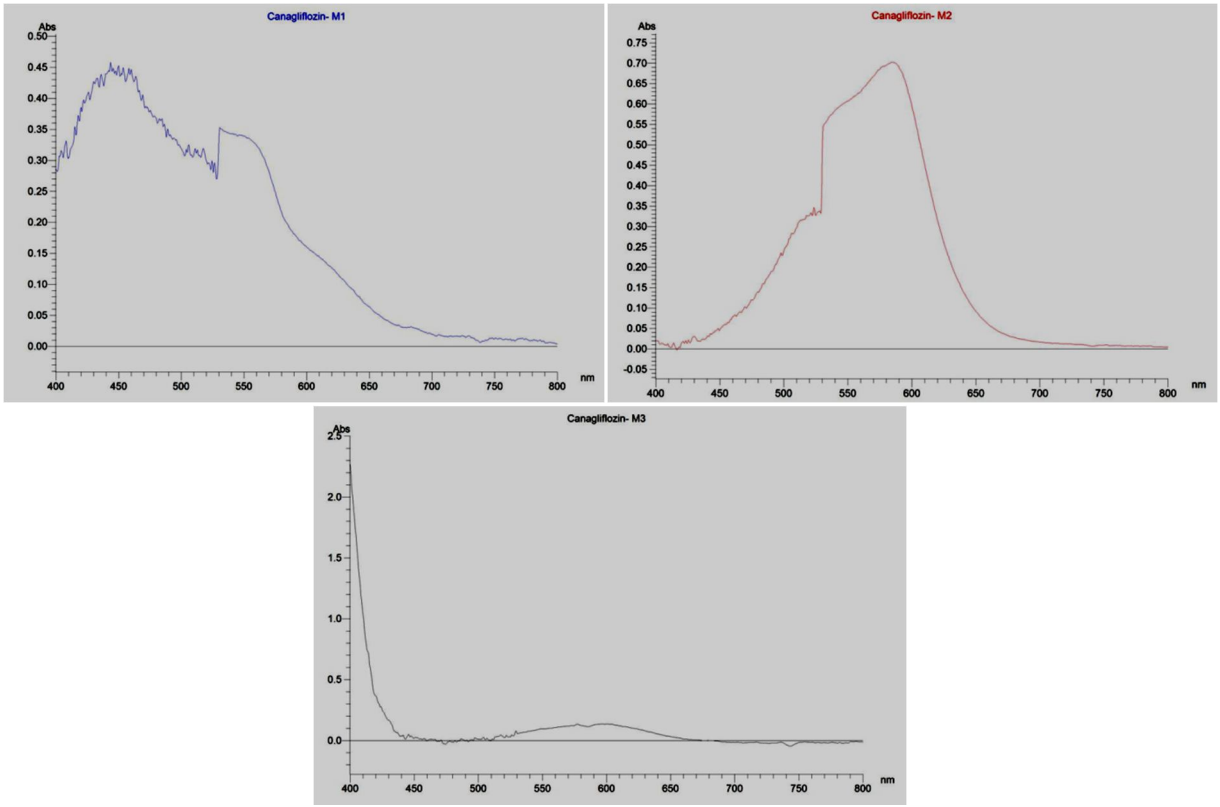

Figure 2: Colorometric spectra of Canagliflozin for ARS Method [M1], WFBBL Method [M2] and EBT Method [M3]

Method Validation:

Linearity test was evaluated by measuring the absorbance values of standard solutions. For Canagliflozin linearity was found to be in the range of 3-18 µg/ml for ARS Method[M1], 2-12 µg/ml for WFBBL Method [M2] and 5-30 µg/ml for EBT Method [M3] respectively measured at specific wavelength of the method. Linearity graph was constructed by taking concentration versus absorbance in each method. It was found that all the developed methods show good linear relation with in the concentrations under the study. Summary results of the Linearity were shown in table 1. Wavelength scanning spectra was shown in figure 2. Accuracy of the developed methods was confirmed by performing recovery studies 50%, 100% and 150% to the standard level. Each level was performed in triplet. Average recovery in each level by the method was compared with the standard values. Average recovery was found to be 99.73- 100.61 % for ARS Method [M1], 99.79-100.82% for WFBBL Method [M2] and 100.18-100.72% for EBT Method [M3]. All the results were within the acceptance limit this indicates that the proposed methods are accurate. Summary results of accuracy were shown in table 2.

In order to assess the intra and inter-day precision of the assay, standard concentration of Canagliflozin in each method was prepared as described above. Absorbance of the each solution at 9µg/ml for M1 and 6µg/ml for M2 and M3 were measured at corresponding wavelength; % RSD of the six repeated values in each method was calculated and was found to be within the acceptance limit. This indicates that the proposed methods were precise. Results of the precision were shown in table2. The limit of detection was calculated by LOD = 3.3σ/S, and found to 0.07µg/ml for M1, 0.05µg/ml for M2 and 0.10µg/ml for M3 respectively, which shows the sensitivity of the methods. The limit of quantification was calculated by LOQ = 10σ/S and found to 0.25µg/ml for M1, 0.20µg/ml for M2 and 0.40µg/ml for M3 respectively. Percentage of recovery was found to be more than 98% in all the methods when proposed methods were applied to its marketed formulation (sulisent – 100mg). This indicates

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that the proposed method can successfully applied for thee routine estimation of Canagliflozin in pharmaceutical dosage forms. Results of the formulation analysis were shown in table 2.

Conclusion:

These Visible spectrophotometric methods proposed for estimation of Canagliflozin on their pharmaceutical dosage forms were accurate, precise, reproducible and sensitive. All the methods have good linearity range to analysis and high degree of accuracy. All methods were found sensitive and successfully used for quantification of Canagliflozin in its pharmaceutical dosage forms. The validation procedure confirms that this is a workable methods for their quantification in the formulations.

Table 1: Results of Linearity test for Canagliflozin:

S No	M1		M2		M3	
	Concentration in µg/ml	Absorbance	Concentration in µg/ml	Absorbance	Concentration in µg/ml	Absorbance
1	3	0.156	2	0.142	5	0.174
2	6	0.264	4	0.256	10	0.286
3	9	0.379	6	0.364	15	0.412
4	12	0.485	8	0.478	20	0.546
5	15	0.596	10	0.586	25	0.665
6	18	0.702	12	0.698	30	0.795

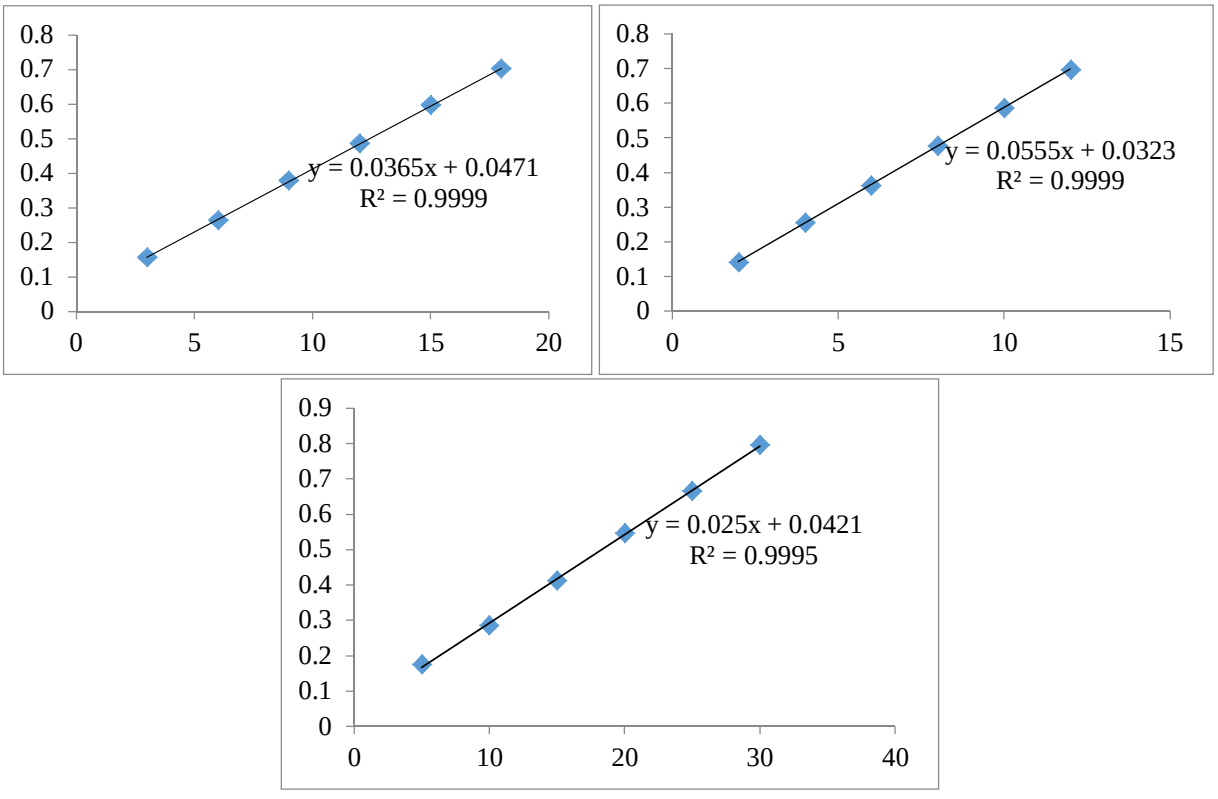

Figure 3: Linearity graph of Canagliflozin for ARS Method [M1], WFBBL Method [M2] and Canagliflozin for EBT Method [M3]

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Table 2: Summary of validation results for Canagliflozin:

S. No	Parameter	ARS Method	WFBBL Method	EBT Method
1	λ max	443nm	584nm	410nm
2	Linearity Range	3-18 µg/ml	2-12 µg/ml	5-30 µg/ml
3	r^2	0.999	0.999	0.999
4	Slope	0.036	0.055	0.025
5	Intercept	0.047	0.032	0.042
6	%RSD of Precision Intraday Interday Ruggedness	 0.496 0.694 0.786	 0.510 0.708 0.54	 0.45 0.773 0.785
7	Recovery range (50-150%)	99.73- 100.61 %	99.79-100.82%	100.18-100.72%
8	LOD	0.07µg/ml	0.05µg/ml	0.10µg/ml
	LOQ	0.25µg/ml	0.20µg/ml	0.40µg/ml
9	Stability Period	90min	80min	35min
10	Formulation assay	99.89%	99.67%	99.93%

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