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Green Synthesis and Evaluation of Silver Nanoparticles using *Cyperus rotundus*

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Abstract

The study focuses on the green synthesis of silver nanoparticles using plant extract of *Cyperus rotundus* as reducing, capping and stabilizing agent. Aqueous silver ions when exposed to plant tubers extract [*Cyperus rotundus*] resulted in a color change indicating the formation of silver nanoparticles. The synthesized silver nanoparticles were characterized by UV-Vis spectrophotometer, Transmission Electron Microscopy (TEM) and Fourier Transform Infra-red Spectroscopy (FTIR) techniques. The UV-Vis spectra showed peak at 455 nm. TEM revealed that the synthesized silver nanoparticles are spherical and circular in morphology and ranges between 15nm to 100 nm. The particle size and zeta potential range of AgNPs monitored by particle size analyzer Mastsizer 2000 and result was found to be 15nm and -9.03mV. The plant extract of *Cyperus rotundus* possess wound healing property which may further be potentiated in the presence of silver nanoparticles. Synthesized silver nanoparticles were incorporated into gel base and different formulations were prepared from F1 to F4 having different carbopol concentration, out of which F2 was found to be optimal and further evaluated for its physical properties such as pH, viscosity & spreadability. The results obtained in the developed formulation showed no lumps, had uniform color dispersion and were free from any fiber and particle. It was also observed to have easy wash ability, good spreadability. Hence, silver nanoparticle of *Cyperus rotundus* in aqueous gel-base can be used as an appropriate formulation for Wound Healing.

Keywords: Green Synthesis, Silver Nanoparticles, *Cyperus rotundus*, NDDS

1. Introduction

Quest for new drug delivery system has got new impetus since early eighties to have improved therapeutic outcome from the same drug, because the NDDS has several advantages over conventional dosage forms [1]. Novel drug delivery systems have been developed in order to maintain greater control over a drug's pharmacokinetic and pharmacodynamics after administration, so that the various pharmaceutical and dermatological variables influence the choice of the system as per the demand of the drug and disease. The applications of such novel Nano-vehicle systems are able to deliver potent drugs to the preferred site in a very accurate manner. The design of Nano medicines based on Nano systems; probably control the release of a therapeutic moiety to the affected region at the skin site with localized effect by creating skin reservoirs [2]. It is a well-known fact that skin acts as a negatively charged membrane. The presence of charge on the surfaces of Nano carriers influences their drug diffusion through the skin. A positively charged delivery system would strongly interact with cells and has shown better permeability of the drug and prolonged pharmacological activity. Dosage form thus provided would be highly effective, safe and better than conventional products [3].

Extensive use of nanoparticles, especially silver nanoparticles (AgNPs) and Synthesis of nanoparticles using plant materials has been reported earlier in biology, pharmaceuticals and medicine [4]. Among the biosynthesis methods, which are used to prepare nanoparticles, the plant-mediated methods have gathered great attention due to several advantages over other method such as cost-effectiveness, availability, eco-friendliness and non-toxicity of plants [5]. Besides this, plant extracts are rich in different compounds which act as inhibitory and capping agents. The use of plant extracts for the synthesis of AgNPs is simple and cost-effective. Extracts of plant like *Syzygium cumin*, *basil*, *Saraca indica* and *Piper nigrum* had been used for the synthesis of metallic nanoparticles [7].

Cyperus rotundus commonly known as Nagarmotha is found throughout India. It belongs to the family Cyperaceae. Earlier the various parts of plant extract have been used as anti-noceceptive[9], as a tonic for the liver and heart, a digestion stimulant, and aid against hypertension[6].The constituents of C. rotundus were distinguished quantitatively with high amounts of sesquiterpenes[10] (70%), with a low proportion of oxygenated monoterpenes (10%) and monoterpene compounds (5%).The aerial parts of *Cyperus rotundus* Linn. contain sitosteryl (6'-hentriacontanoyl)-β-D-galactopyranoside and three furochromones [8]. It also found to contain proteins and traces of Mg, Vs, Cr, Mn, and Co. Major compounds isolated from the extracts of C. rotundus rhizome are α and β-cyperone, α and β-rotunol, β-pinene, β-selinene, camphene, limonene, linolenic-acid, myristic-acid, oleic-acid, pcymol, pectin, polyphenols[12], sugeonol, triterpenes including oleanolic acid and sitosterol, as well as flavonoids, sugars and minerals[6,7,8]. The rhizomes are used as a cooling, intellect promoting, nervine tonic, diuretic, antiperiodic, analgesic, anti-inflammatory, antipyretic and to treat diarrhea, dysentery, leprosy, bronchitis, amenorrhea, and blood disorders. The tuber part having anti-obesity properties [13], wound healing as an infusion or as soup in fever, diarrhea, dysentery, vomiting, and cholera [11,12]. In this article, we have reported the assisted rapid green synthesis of stable AgNPs using *Cyperus rotundus* tuber extract, antibacterial potential and effect on wound healing and reduction in silver ions have been reported. The potential benefits of AgNPs in all wounds can therefore be enormous.

In worldwide around one billion people are likely to suffer acute or chronic wounds. Wound may induce on multiple occasions in a person's lifetime. Current estimates indicate that approximately 6 million people suffer from chronic wounds worldwide. The ultimate goal for wound healing is a speedy recovery with minimal scarring. Wound healing proceeds through an overlapping pattern of events including coagulation, inflammation, proliferation and tissue remodeling [3].

2. Material & Methods

2.1 Chemicals

Silver nitrate was procured from Cosmas research limited, Ludhiana Punjab. Carbopol and sodium hydroxide was purchased from S D Fine Chem ltd, Mumbai. De-ionized water was purchased from Khullar Medicos, Pindi street, Ludhiana.

2.2 Plant Material

Fresh Tubers of *Cyperus rotundus* was collected from the herbal garden of PCTE group of institutes, Ludhiana, Punjab. It was identified and authenticated by performing different chemical test and macroscopic analysis.

2.3 Extract Preparation

For the preparation of aqueous extract dried tubers of *Cyperus rotundus* was used. 50gms of plant extract was weighed, thoroughly washed in DIW& converted into fine pieces. This was further subjected to boiling in 100 ml of water for 10 minutes. It was then filtered with Whatsmann No.1 filter paper (25 µm). Aqueous extract obtained was stored at 4°C for further use [14,15].

2.4 Phytochemical evaluation of tuber extract

Aqueous extract of the tubers of *Cyperus rotundus* was investigated for the presence of phytochemicals viz. polyphenols, alkaloids, triterpenoids, flavonoids, carbohydrates & steroids [15].

2.5 Synthesis of Silver Nanoparticles using Aqueous extracts of *Cyperus rotundus.*

Preparation of one milimolar silver nitrate solution was carried out by dissolving 0.017 gms of $AgNO_3$ into 100ml of deionized water and stored in amber colored bottle in cool and dry place. For the reduction of Ag^+ ions, different Concentrations of plant extract ranging from 1ml to 4ml was added drop wise into 50 ml aqueous solution of 1mM$AgNO_3$ at specific temperature with continuous stirring using magnetic stirrer [15,16].

2.6 Optimization of process variables for preparation of silver Nanoparticles.

For the optimization of silver nanoparticles plant extract, we choose two process variables i.e. effect of plant extract concentration and effect of reaction time [17].

2.6.1 Effect of Plant Extract Concentration-The reaction mixture was optimized at different concentration of tuber extract 1ml, 2ml, 3ml, 4ml of the total volume.

2.6.2 Effect of Reaction Time-The reaction mixture was optimized at different time intervals between 0 mins to 6 hr. The absorbance of the resulting reaction mixture at various optimized process parameters were measured spectrophotometrically.

Characterization

- **UV Spectroscopy**-The synthesized AgNPs was monitored using UV–VIS spectrophotometer and was also used to analyze Surface Plasmon Resonance (SPR) phenomenon. The small aliquot of samples was diluted first with DIW and absorbance was taken using UV-vis spectrophotometer at the wavelength ranging from 300-800 nm [16].
- **Transmission Electron Microscopy (TEM)** - HR-TEM (Tecnai, G2 20 with EDX, USA) was used to analyze the surface morphology and elemental silver in synthesized AgNPs. A thin film of each synthesized AgNPs sample was prepared by dropping a small amount on copper grid and extra sample was removed by using blotting paper and then kept for drying at room temp for 15-30 mins. Histogram and size distribution were calculated by measuring the diameter of nanoparticles using 'Image J 1.49' Analyzer Software, NIH, USA[15].
- **FTIR**-The infrared spectra for the green synthesized AgNPs were attained for the identification of functional groups in a (Perkin Elmer Spectrum 2, Germany) spectrophotometer IR affinity-1 by employing KBr pellet technique and registering amplitude waves ranging from 450 to 4000 cm [16].
- **Zeta potential**-The particle size range and the zeta potential of the synthesized AgNPs was determined by using particle size analyzer, Mastsizer 2000. The particle size was determined based on the Brownian motion of the nanoparticles.

Preparation of Nanogel

For the preparations of nanogel, four different formulations (F1, F2, F3, F4) was prepared using different amount of carbapol. Carbapol was mixed with 3 ml of optimal concentration of silver nanoparticle plant extract on the magnetic stirrer. After uniform dispersion of carbapol 1ml of NaOH solution was added for the gel formation [18,20].

Table 1: Preparation of Nanogel

Formulation code	Amount of carbapol added
F1	0.5gm
F2	1gm
F3	1.5gm
F4	2gm

Physiochemical evaluation of Topical Formulation

Physical parameters such as color, appearance and consistency were checked visually. The pH of various gel formulations was determined by using digital pH meter [19]. The measurement of pH of each formulation was done in triplicate and average values are calculated. The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer [21]. The gels were rotated at 0.3, 0.6 and 1.5 rotations per minute. At each speed, the corresponding

Viscosity values were noted. Other topical evaluation parameters such as spreadability & extrudability were also evaluated.

3. Results & discussion

3.1 Morphological characters of plant: Organoleptic characterization of tuber of *Cyperus rotundus* was carried out using color, odor, shape and surface as shown in Table no 2. The observe characters were found to be in accordance with the reported values in the literature.

Table 2: Morphological Characters of Tubers

Organoleptic Characters	Tubers of *Cyperus rotundus*
COLOR	Dark brown
ODOR	Pleasant
SHAPE	Elongated, broadly obovoid, trigonous
SURFACE	Slightly tuberous at the base

3.2. Phytochemical evaluation: Aqueous extracts of the tubers of *Cyperus rotundus* was evaluated for the presence of various phytoconstituents by performing different qualitative chemical tests. It showed the presence of various phytochemicals that is shown in Table 3. These constituents are thought to cause reduction of silver into silver ions.

Table 3: Phytochemical Parameters of Tubers

Phytochemical Parameters	Aqueous Extract
Alkaloids	--
Carbohydrates	++
Flavonoids	++
Tannins and phenols	--
Saponins	--
Triterpenoids	++

3.3 Synthesis of silver nanoparticles: Rapid synthesis of silver nanoparticles occurred in all the different concentrations of plant extract used for the formation of silver nanoparticles. The efficiency of aqueous extract of *Cyperus rotundus* tubers to synthesize silver nanoparticles was confirmed by the change in color from white [transparent] to reddish brown, which was recorded by visual observation.

Figure 1: Visual Observation of Synthesized of AgNPs

The results show that all the different concentrations were able to synthesize silver nanoparticles from the tuber extract of *Cyperus rotundus*. While comparing the different concentrations, an increase in the intensity of color was observed at 3ml concentration of the extract with reddish brown color. The notable change in color from white (Transparent) to reddish brown indicates the formation of silver nanoparticles. The intensity of reddish brown color was directly proportional to the increase in incubation period and temperature, which indicates the reduction of silver nitrate by the extract.

3.4 Optimization Concentration:

3.4.1. Effect of plant concentration: Various concentration of aqueous tuber extract was optimized during the preparation of synthesized AgNPs as shown in table.

Table 4: Effect of plant concentration on synthesized AgNPs:

Concentration of aqueous extract in ml	Intensity of color
1ml	Light brown
2ml	Light brown
3ml	Reddish brown
4ml	Reddish brown

3.4.2. Effect of Reaction Time: The peak of the resulting reaction mixture at various optimized process parameters were measured spectrophotometrically as shown in table.

Table 5: Effect of reaction time on synthesized AgNPs

Reaction time	Synthesized AgNPs	Peak
10 min	No color change	No peak
30 min	Reddish brown	Intense peak
60 min	Reddish brown	Intense peak
1hr	Reddish brown	No peak
2hr	Dark brown	No peak
4hr	Dark brown	Tilted peak
6hr	Dark brown	Tilted peak

3.5 Characterization of AgNPs: Among the different methods used for the synthesis of silver nanoparticles from the literature, the maximum intensity of color was observed using green synthesis. Green nanotechnology is also known as photobiological approach which utilizes plants or their extracts as reducing and capping agents in the synthesis of AgNPs. Since the reaction takes place in one step. Therefore, this method was used for the synthesis of silver nanoparticles for further study and characterization.

- **UV- Visible Absorption Spectroscopy:** The optical properties of the synthesized silver nanoparticles were analyzed using this technique. It was reviewed that the reduction of silver ions occurs rapidly and more than 90 % of reduction of silver ions is complete within 30 mins respectively, after adding the aqueous plant extract to the metal ion solutions. So in this study an immediate reduction of silver ions within 30 min was observed which is attributed because of the presence of water soluble phytochemicals like carbohydrates, flavonoids and triterpenes in the *Cyperus rotundus* plant extract. The characteristic absorption peak was found at 455 nm in UV-visible spectrum as shown in fig 2 and fig 3.

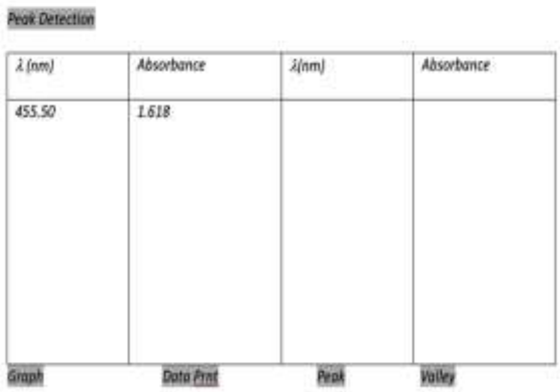

Figure No- 2

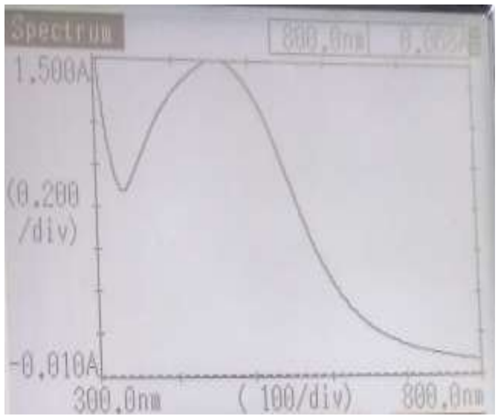

Figure No - 3

FTIR Analysis: The Fourier transform infrared spectrum (FTIR) measurement was done to identify the reducing, capping and stabilizing capacity of biomolecules in synthesized silver nanoparticles using *Cyperus rotundus* plant extract. After reaction with silver nitrate the peaks are shifted to higher wave number side. The silver nanoparticles of O-H stretching in carboxylic acids and flavonoids in the plant extract is shifted from 3417cm-1 to 3337.1cm-1.the strong peak at 1683cm-1 to 1493cm-1. The peak at 575 and 667 cm-1 are of N-H stretching vibrations shows the presence of aromatic amines in flavonoids and cluster of silver nanoparticles with sharp peak at 565cm-1. The immediate reduction and capping of silver ion into silver nanoparticles in the present analysis is might be due to carbohydrates and flavonoids. The presence of polyphenolic biomolecules in *Cyperus rotundus* plant extract and their interaction with the surface of the silver nanoparticles was confirmed by FTIR spectra as shown in table no 6.

Table 6: FTIR Analysis of Synthesized AgNPs

Functional group	Types of vibration	Peaks
OH	Stretching	3490-3500
CH	Stretching	1500-1550
NH	Stretching	1450-1500
For Synthesized AgNPs- OH	Stretching	3331.71
CH	Stretching	1493.03
AgNPs	Stretching	565.14

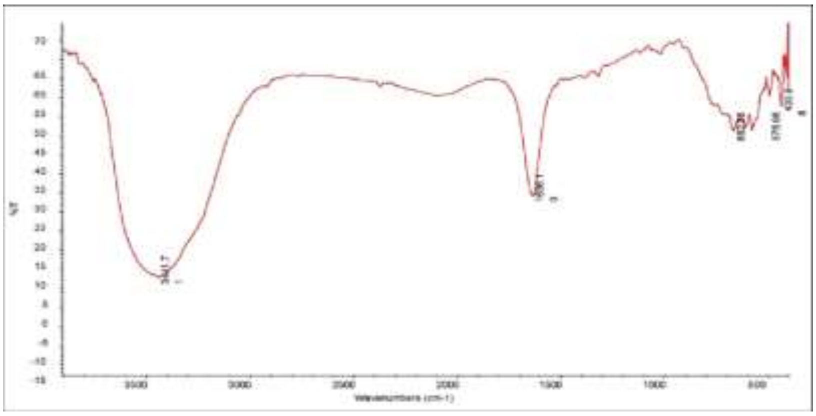

Figure 4: FTIR analysis of synthesized AgNPs

- **TEM [Transmission electron microscope]:** TEM has been employed to characterize the size, shape and morphology of synthesized AgNPs. The TEM micrograph of the synthesized AgNPs at 100 nm and 500nm scales as shown in fig. It was found that the AgNPs were spherical in shape with maximum particles in the size range of 15nm-100nm nm having average diameter 15.75 nm and also shows the SAED pattern of synthesized AgNPs exhibits the dotted concentric rings, it also suggests the polycrystalline nature of AgNPs. Histogram and size distribution were calculated by measuring the diameter of nanoparticles using 'Image J 1.49' Analyzer Software, NIH, USA.

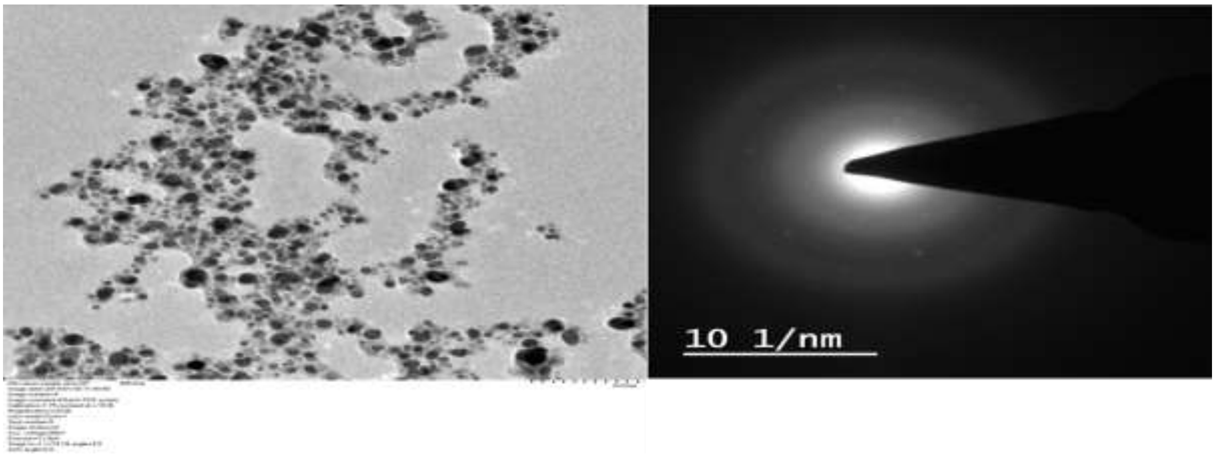

Figure 5: TEM of Synthesized AgNPs and SAED pattern

- **Zeta potential:** The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. The size and zeta potential measurement of synthesized silver nanoparticles was 15 nm and average zeta potential is -9.03mV.

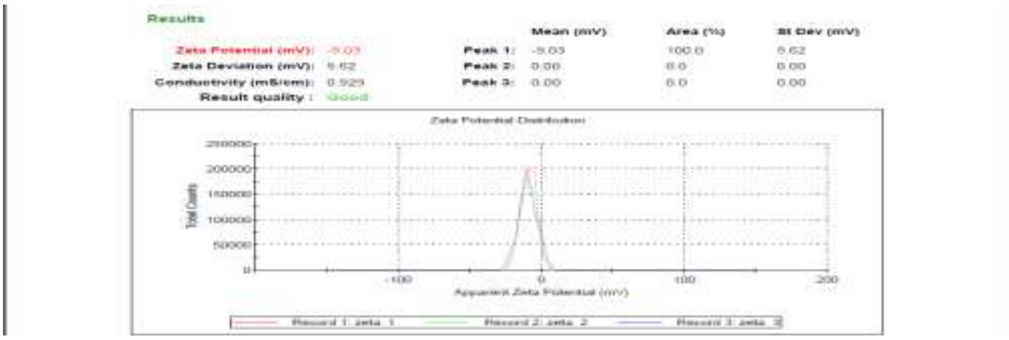

Figure 6: Zeta Potential of Synthesized AgNPs

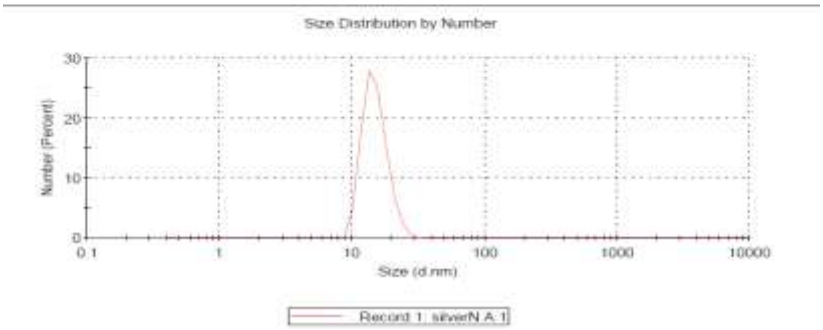

Figure 7: Particle size of synthesized AgNPs

3.6 Optimization parameters for Nanogel:

❖ **Macroscopic analysis**: Physicochemical parameters such as homogeneity of color, presence of any foreign particle and fibers, washing ability, pH and viscosity are evaluated. Visual inspection results indicate that prepared topical gel formulation has uniform color distribution and free from any lumps, fibers and foreign particles. Formulation was easily washable, as the four of the formulations were prepared by carbapol as shown in table.

Table 7: Macroscopic Analysis of Nanogel

Formulation code	Color	odor	Grittiness	stickiness	washability
F1	Light brown	odorless	smooth	Non greasy	Easily Washable
F2	Greenish brown	odorless	Smooth	Greasy	Easily Washable
F3	Greenish brown	odorless	Smooth	Greasy	Easily Washable
F4	Dark Brown	Bitter smell	Lumps were found	More greasiness	No Easily Washable

3.7 Physiochemical Evaluation of Nanogel

- **pH-** pH of the synthesized AgNPs nanogel was found to be 6.2 and 6.8 for formulation F1and F2 which is near to the pH of the skin and hence is found to be compatible as it contains less amount of carbapol in it.as we increase the amount of carbapol in formulation F3and F4, pH increases i.e. incompatible with the skin as shown in table.

Table 8: pH of Nanogel

Formulation code	Ph
F1	6.2
F2	6.8
F3	7.0
F4	7.4

- **Viscosity:** Viscosity was found to be 64066 for nanogel prepared by Carbapol. The observed results are comparable with the earlier literature and results are shown in table 6.8.

Table 9: Viscosity of Various Nanogel Formulations

Formulation code	0.3	0.6	1.5
F1	59987	39991	29994
F2	64066	54322	38772
F3	60983	43563	23976
F4	39886	23832	19999

- **Spreadability:** Bioavailability and therapeutic property of the topical formulation depends upon the spreadability. The spreadability is expressed of time in seconds based on the slip off from the gel by upper slide under certain load. Time taken for the separation of the two slides is less which indicates the topical formulation has better spreadability as shown in table.

Table 10: Spreadability of Nanogel

Formulation code	Time taken to spread
F1	1 sec
F2	2sec
F3	2sec
F4	2.5sec

- **Extrudability:** The method adopted for evaluating nanogel formulation for extrudability is based upon the quantity in percentage of nanogel and nanogel extruded from lacquered aluminum collapsible tube on application of weight in grams required at least 0.5cm ribbon of nanogel in 10 sec. The amount of gel extruded were collected and weighed. The % of gel extruded was calculated; and grades were allotted (+ + + + excellent, + + + Good, + + fair, + Poor) as shown in table.

Table 11: Extrudability of Nanogel.

Extrudability	Results
F1	++
F2	+++
F3	+++
F4	++

4. Conclusion

The article entitled "Green synthesis and Evaluation of Silver Nanoparticles using *Cyperus Rotundus*" was carried out to study the synthesis, characterization of silver NPs and synthesized nanoparticles was then incorporated into gel and further investigated for its evaluation parameters. As the *Cyperus Rotundus* tubers extract contain water soluble phytochemicals like carbohydrates, flavonoids and triterpenes. The salient findings of this investigation are summarized below:

- The AgNPs was synthesized successfully by green synthesis method, using *Cyperus Rotundus* tubers extract. The aqueous plant extract showed change in color from white [transparent] to reddish brown when exposed to 1 mM silver nitrate solution. UV-visible spectroscopy was used to monitor the formation of AgNPs and the absorption spectrum was found to be 455nm.
- TEM analysis determines the NPs are spherical in shape and size range was 15nm to 100nm. The size and zeta potential measurement of synthesized silver nanoparticles was 15 nm and average zeta potential is -9.03mV.
- Nanogel was prepared by adding carbapol 934 and the F2 Formulation was found to be optimal as compared to others. Visual inspection results indicate that prepared topical gel formulation has uniform color distribution and free from any lumps, fibers and foreign particles. Formulation F2 was easily washable and the pH was found to be 6.80 for gel which is near to the pH of the skin and hence is found to be compatible with skin.

References

1. Davis ME, Chen Z. Shin DM Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat Rev Drug Discov. 2008; 7(9):771-82. https://doi.org/10.1038/nrd2614

2. Schmid MH, Korting HC. Therapeutic progress with topical liposome drugs for skin disease. Adv Drug Deliv Rev 1996; 18:335-42. https://doi.org/10.1016/0169-409X(95)00019-4

3. Nacht S, Katz M. The microsponge: A novel topical programmable delivery system. In: Osborne DW, Amman AH, editors. Topical Drug Delivery Formulations. Marcel Dekker Basel, New York; 1990. p. 299-325. https://doi.org/10.1201/b14194-19

4. A. Freitas, What is nanomedicine? Nanomed. Nanotechnol. Biol. Med. 1(2005) 2-9. https://doi.org/10.1016/j.nano.2004.11.003

5. Aruna MS, Sravani A, Resshma V. Formulation and evaluation of herbal acne gel. World J Pharm Res. 2015; 4(5): 2324-30.

6. David WH, Vernon VV, Jason AF. Purple nutsedge, Cyperus rotundus L.Florida (U.S.A): Institute of Food and Agricultural Sciences, University of Florida; 2012. p. 02-15.

7. Parotta JA. Healing Plants of Peninsular India. New York: CABI Publishing; 2001. p. 02-66. https://doi.org/10.1079/9780851995014.0000

8. Honey J, Neha B. A review on pharmacognosy of Cyperus species. Available from: http://www.pharmatutor.org/articles/pharmacognosy-of-cyperus-species. [Last cited on 2013 Apr 10].

9. Imam MZ, Sumi CD. Evaluation of antinociceptive activity of hydromethanol extract of Cyperus rotundus in mice. BMC Complement Altern Med 2014; 14:83. https://doi.org/10.1186/1472-6882-14-83

10. The btaranonth C, Thebtaranonth Y, Wanauppathamkul S,Yuthavong Y. Antimalarial sesquiterpenes from tubers of Cyperus rotundus: Structure of 10,12-peroxycalamenene,a sesquiterpene endoperoxide. Phytochemistry 1995; 40:125-8. https://doi.org/10.1016/0031-9422(95)00260-E

11. Pal D, Dutta S, Sarkar A. Evaluation of CNS activities of ethanol extract of roots and rhizomes of Cyperus rotundus in mice. Acta Pol Pharm 2009; 66:535-41.

12. Nagulendran KR, Velavan S, Mahesh R,Das, Hazeena BV. In vitro antioxidant activity and total polyphenolic content of Cyperus rotundus rhizomes. EJ Chem 2007; 4:440-9. https://doi.org/10.1155/2007/903496

13. Athes K, Divakar M, Brindha P. Anti-obesity potential of Cyperus rotundus L. aqueous tuber extract in rats fed on high fat cafeteria diet. Asian J Pharm Clin Res 2014; 7:88-92.

14. Raghuwanshi, N.; Pathak, A.; Patel, A.; Vashisth, P.; Singh, H.; Srivastava, A.K.; Pruthi, V. Novel biogenic synthesis of silver nanoparticles and their therapeutic potential. Front Biosci Elite, 2017; 79:33-43. https://doi.org/10.2741/e783

15. Raghuwanshi N, Patel A, Arora N, Varshney R, Srivastava AK, Pruthi V. Antineoplastic and Antimicrobial Potential of Novel Phytofabricated Silver Nanoparticles from Pterospermum acerifolium Leaf Extract. Nanoscience & Nanotechnology-Asia. 2018 Aug 1; 8(2):297-308. https://doi.org/10.2174/2210681207666170607154529

16. Chandran SP, Minakshi Chaudhary, Renu Pasricha, Absar Ahmad, Murali Sastry. Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotech Prog. 2006; 22:577-583. https://doi.org/10.1021/bp0501423

17. Christopher, J.S.G., Banerjee, S. and PanneerSelvam, E. Optimization of parameters for biosynthesis of silver nanoparticles using leaf extract of Aegle marmelos. Braz. Arch. Biol. Technol., 2015; 58:702-710. https://doi.org/10.1590/S1516-89132015050106

18. Prabu SL, Umamaheswari A, Rajakumar S, Bhuvaneswari PL, Muthupetchi S. Development and Evaluation of Gel Incorporated with Synthesized Silver Nanoparticle from Ocimum gratissimum for the Treatment of Acne Vulgaris. American Journal of Advanced Drug Delivery. 2017; 5:107-7. https://doi.org/10.21767/2321-547X.1000018

19. Chandira RM, Pradeep PA, Bhowmik D, Chiranjib JB, Tripathi KK, Sampath Kumar KP. Design, development and formulation of antiacne dermatological gel. J Chem Pharm Res. 2010; 2(1):401-14.

20. Prusty A, Parida P. Development and Evaluation of gel incorporated with biogenically synthesized silver nanoparticles. J. Appl. Bio. Pharm. 2014; 3:1-6. https://doi.org/10.14205/2309-4435.2015.03.01.1

21. Niyaz BB, Kalyani P and Divakar G. Formulation and evaluation of Gel containing Fluconazole-Antifungal Agent.Int J Drug Dev and Res 2011; 3:109-128.