Research Article

Antipyretic Activity of Acanthophora spicifera and Padina tetrastromatica in Crude and Silver Nanoparticles Containing Extracts

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ABSTRACT

Green synthesis of silver nanoparticles was carried out using seaweed extracts. The presence of silver nanoparticles was confirmed by FTIR, UV—Vis, SEM and XRD. The antipyretic effect of these silver nanoparticles was compared to the crude extracts when the experiment was carried out in wistar rats. The extracts containing the silver nanoparticles were more effective in bringing down the temperature than the crude extracts. Further, these were very much active in smaller doses and also took much less time to bring their antipyretic effect.

Keywords: Silver nanoparticle, seaweeds, antipyretic activity, SEM, XRD.

INTRODUCTION

The problem faced today in the pharmaceutical industry is that more and more pathogens are becoming resistant to drugs day by day. So there is a need to find out new possibilities and new sources from which these drugs can be isolated. Seaweeds are marine algae which are the potent source of secondary metabolites as they live in harsh climatic conditions. Not only drugs from natural sources have new structural features, with novel biological activity but phytochemicals derived from them are also extremely useful as lead structures for synthetic modification and optimization of bioactivity. This fact implies that seaweed cells have some protective mechanisms and compounds[1]. They have a rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential[2, 3].

Similarly, biosynthesis of nanoparticle is also gaining more importance. The advantages being cost wise cheaper, ecofriendly and also without any sideeffects. Recently there are a few reports that algae are being used as a biofactory for synthesis of metallic nanoparticles. Recent researchers[4-6] reported the synthesis of silver bionanoparticles using *Sargassum wightii, Kappaphycus alvarezii* and *Gelidiella acerosa* crude extracts, respectively. The present study was designed to find out the antipyretic properties of biosynthesised silver nanoparticles using seaweeds.

MATERIALS AND METHODS

Seaweed (*Acanthophora spicifera and Padina tetrastromatica)* samples which were healthy and fully grown and submerged underwater were collected from the tidepools from Tuticorin coast in Hare Island (9.20°N; 78.08°E). The samples were washed with seawater and freshwater to remove salt, epiphytic microorganisms and other suspended materials. The clean algae were frozen and lyophilized. The dry material was stored at -20°C until further use.

Preparation of Extract

Extracts of the freeze dried and powdered biomass were prepared using ethanol as solvent using a soxhlet apparatus. The resultant crude extracts were filtered and then concentrated in a rotary evaporator at a temperature less than 40°C. The crude extracts were weighed and deep frozen (-20°C) until further use.

Biosynthesis of silver nanoparticles

5 gm of seaweed powder was mixed in 100 ml distilled water and the mixer was allowed to stand at 100°C for 10 min and filter out the seaweed extract using Whatman no.1 filter paper. To a clean and surface sterilized burette, 50 ml of seaweed extract was taken and allowed to fall in drops to the beaker containing 100 ml of 1 mM of silver nitrate. The beaker was kept on hot magnetic stirrer at 70°C. The change in colour from colourless to brown colour was taken for visible confirmation of formation of silver nanoparticles. Then the sample was subjected to further characterization.

Characterization of bionanomaterial

Fourier transform infra red spectroscope (FTIR)

To identify silver nanoparticles associated biomolecules, the Fourier transform infra red spectra of washed and green synthesised silver nanoparticles powder were recorded on the Nicolet Avatar 660 FT-IR Spectroscopy (Nicolet, USA) using KBr pellets. To obtain good signal to noise ratio, 256 scans of silver nanoparticles were taken in the range of 400-4000/cm and the resolution was kept as 4/cm.[7] (fig 1 and 2).

UV-visible spectroscopy analysis

After complete reduction, the reaction mixture was treated with NaCl to precipitate unreacted silver ions and the precipitate was removed by filtration through Whatman filter paper No.1. Silver nanoparticles were concentrated by repeated (4–5 times) centrifugation of the reaction mixture at 10,000 rpm for 10 min. The supernatant was replaced by distilled water each time and subjected to UV–Vis analysis. Then the sample was analyzed in UV-visible spectrophotometer Perkin Elmer (Lamda-25) from 200nm - 600nm (fig 3 and 4).

Scanning Electron Microscopy (SEM)

This study was undertaken to know the size and shape of the silver nanoparticles biosynthesized using the seaweeds. SEM analysis was done using FEI Quanta 200 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper. Then the film on the SEM grid was allowed to dry and the images of nanoparticles were taken (fig 5 and 6).

X-Ray Diffraction (XRD)

The silver nanoparticles solution obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of silver nanoparticles in 10 ml of deionized water. After freeze drying of the purified silver nanoparticles, the structure and composition were analyzed by XRD (fig 7 and 8).

Antipyretic activity (Yeast induced pyrexia)

All animal experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee, strictly adhering to the guidelines of committee for the purpose of control and supervision of experiments on animals constituted by the Animal Welfare Division of Government of India.

A brewer's yeast-induced pyrexia model in female mice was used to test the antipyretic activity of seaweed extracts. After the induction of pyrexia in all the groups by injection of brewer's yeast suspension (10 mg/kg) subcutaneously in the back below the nape of the neck, the rats were treated with standard drug and seaweed extracts. When the rectal temperature peaked after 24 h, 150 mg and 200 mg of extracts per kg body weight were administered intra-peritonealy, and the rectal temperature (^{o}C) was recorded after an additional 18 hours with an interval of one hour, four times, using an electric thermometer connected to a probe, inserted 2 cm into the rectum.

Relative antipyrexia (%) was expressed as (value of the control−value of the extract / value of the control × 100. Paracetamol (10 mg/kg, p.o.) was used as a standard.

Treatment protocol

- **Group-I:** (Normal control) consist of normal rats treated with 10 ml/kg of normal saline, orally.
- **Group-II:** (Standard) rats received 10mg/kg of paracetamol intra peritoneally.
- **Group-III:** (*A. spicifera* Dose I) rats received SNP containing extract of *A. spicifera* at a dose of 150 mg/kg b.w. intra peritoneally.
- **Group-IV:** (*A. spicifera* Dose II) rats received crude extract of *A. spicifera* at a dose of 200 mg/kg intra peritoneally.
- **Group-V:** (*P. tetrastromatica* Dose I) rats received SNP containing extract of *P.tetrastromatica* at a dose of 150mg/kg intra peritoneally.

- **Group-VI:** (*P. tetrastomatica* Dose II) rats received crude extract of *P.tetrastomatica* at a dose of 200mg/kg intra peritoneally.

RESULT

Both the seaweeds were given as crude and SNP containing extracts in two different concentrations of 150 mg/kg p.o. and 200 mg/kg p.o. (Dose I and Dose II). The standard drug paracetamol was given in the dosage of 10 mg/kg p.o. Normal body temperature of the experimental animals was 36-37^{o}C and their mean body weight was 120 g. With the help of yeast, the temperature increased to 40^{o}C at the end of 18 hours and then the animals were injected intraperitoneally with the seaweed extracts. The animals were divided into six groups namely control, standard and the two experimental groups containing two sets of animals for each seaweed extract in two different concentrations of 150 mg/kg p.o. and 200 mg/kg p.o.

At the end of each hour the rectal temperature of the animals were recorded. Even though the dosage was less, the sample containing SNP showed great antipyretic activity. The *A.spicifera*-SNP was more effective in controlling the temperature at a lower dose (150 mg/kg) when compared to crude seaweed samples with 200 mg/kg concentration (Dose II). The rectal temperature which was elevated to 40.2^{o}C after 18 hours of administration of yeast was effectively reduced to 38.4±0.2^{o}C in the SNP treated groups after one hour while the temperature remained at 39.7±0.1^{o}C in the normal seaweed samples for the same time. At the end of second hour, the temperature reduced to 37.2±0.2^{o}C in the SNP treated group while it lowered only to 39.5±0.1^{o}C in the crude extract. At the end of fourth hour, the SNP treated group recorded a temperature of 37.2±0.2^{o}C while the crude extract group temperature was 38.3^{o}C. (fig 9 and 10).

Similarly, *P. tetrastromatica*-SNP was highly effective in bringing down the temperature in the pyretic rats. 38.2±0.3^{o}C was obtained for the first hour and a significant drop in temperature to 36.9±0.3^{o}C was recorded at the end of second hour. In the Dose II group which contained only crude seaweed extract showed a temperature of 38.5±0.1^{o}C was recorded at the end of one hour, which remained at 37.1±0.1^{o}C at the end of two hours.

A remarkable result was obtained in this experiment where the seaweed extracts containing the SNP reduced the temperature in roughly half the time which was taken by the crude extract. So, the SNP containing extracts were effective both in terms of low dose and less time taken for treatment. This was further elicited by the statistical data obtained when comparing the treated samples using crude and SNP. They both varied significantly ($p<0.05$) in reducing the temperature in the different hours of recorded values.

DISCUSSION

Yeast induced pyrexia in rats is a suitable and sensitive model for evaluating antipyretic effects of compounds. Yeast induces both TNF-α and prostaglandin synthesis. Among the seaweeds analyzed *A. spicifera* and *P. tetrastromatica* showed good antipyretic effects. When the mice were injected with brewer's yeast, the rectal temperature peaked at 39.19±0.07^{o}C, which was above the normal (38.45±0.06^{o}C), at 24 h. and oral administration of the ethanol extracts of *E. cava* marginally lowered rectal temperatures in hyperthermic mice in the work done by researchers[8]. The antipyretic activity of *Hypnea musciformis* was determined in Brewer's yeast induced pyrexia on albino mice[9]. 400mg/kg methanol extract of *Hypnea musciformis* showed significant decrease in body temperature while 200 mg/kg methanol extract showed less effect. 400 mg/kg methanol extract exhibited closely significant ($p<0.05$) decrease in elevated body temperature as compared to standard drug.

Kang *et al*. studied the effect of *S. fulvellum* and *S. thunbergii* extracts on antipyretic activities against pyrexia induced in mice[10]. The dichloromethane extract of these seaweeds (4 g/kgbw) lowered rectal temperature in hyperthermic mice to 36.38^{o}C and 36.82^{o}C from 40^{o}C. The effect of methanol extract on yeast-induced pyrexia showed that the rectal temperature was markedly elevated to 41.7^{o}C, after 18 h the subcutaneous injection of yeast suspension decreased to 40.7^{o}C within 1 h of 200 mg/kg methanol extract of *Gracilaria corticata* treatment followed by 39.6^{o}C at 2 h and further reduced to 38.2^{o}C at 4 h showing a considerable decrease in compared to paracetamol in the study conducted by Paul[11]. This may be attributed to the presence of various secondary metabolites present in the ethanol extract which may be involved in inhibition of prostaglandin synthesis.

Furthermore, when the antipyretic activity of the crude and biosynthesized SNP containing extracts was compared, the SNP containing

sample was able to reduce the pyrexia even in small dose and in half the time taken. This is a remarkable innovation in the field of pharmacognosy which can be further explored in the future.

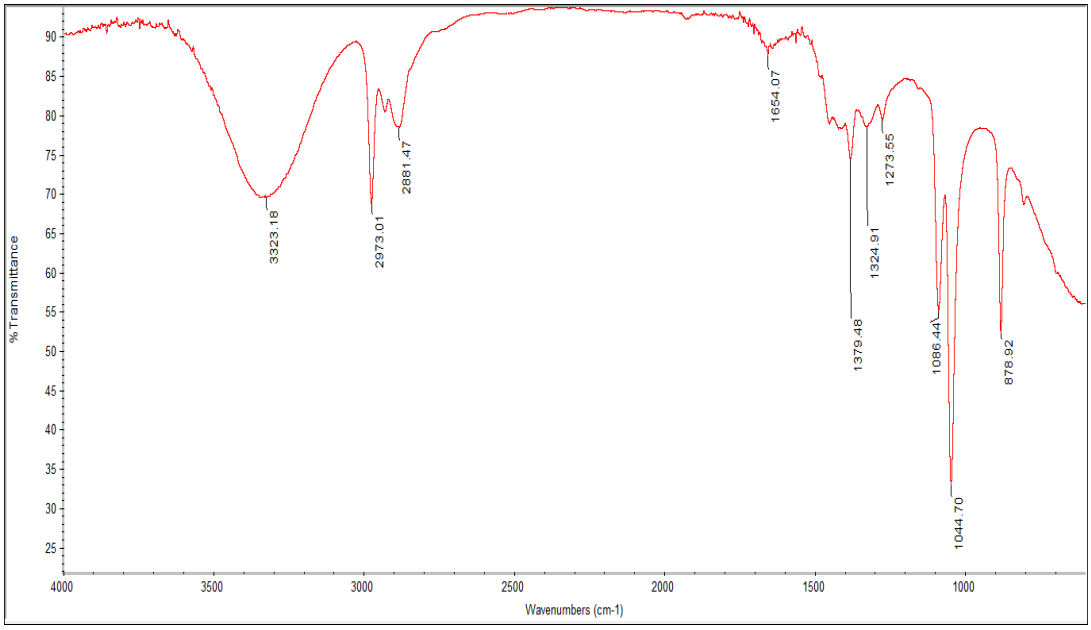


Fig. 1: FTIR spectrum of silver nanoparticles synthesized by the reduction with *A. spicifera* extract

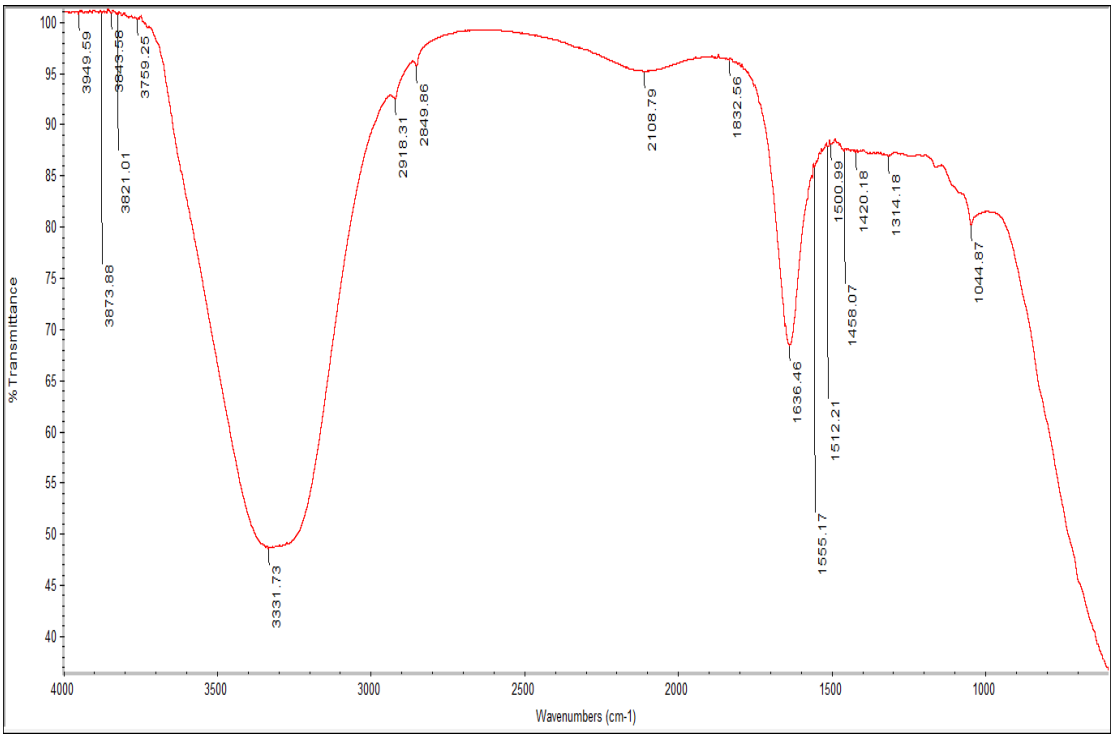


Fig. 2: FTIR spectrum of silver nanoparticles synthesized by the reduction with *P. tetrastromatica* extract

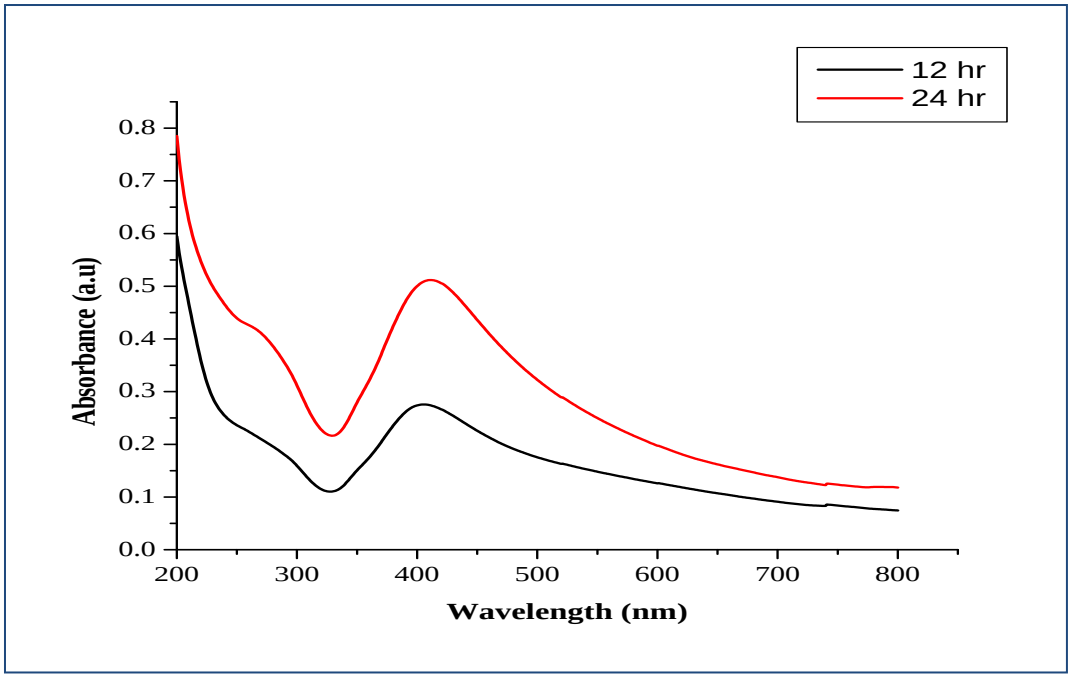


Fig. 3: UV-Vis absorption spectrum of silver nanoparticles synthesized by treating with *A. spicifera* extract

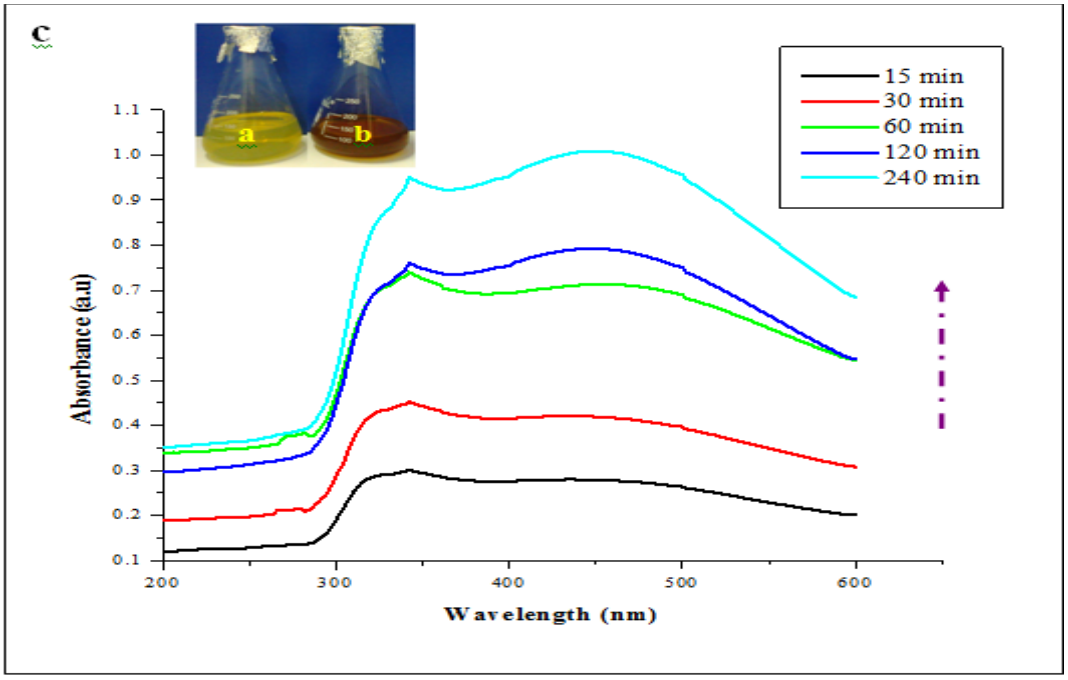


Fig. 4: UV-Vis absorption spectrum of silver nanoparticles synthesized by treating with *P. tetrastromatica* extract

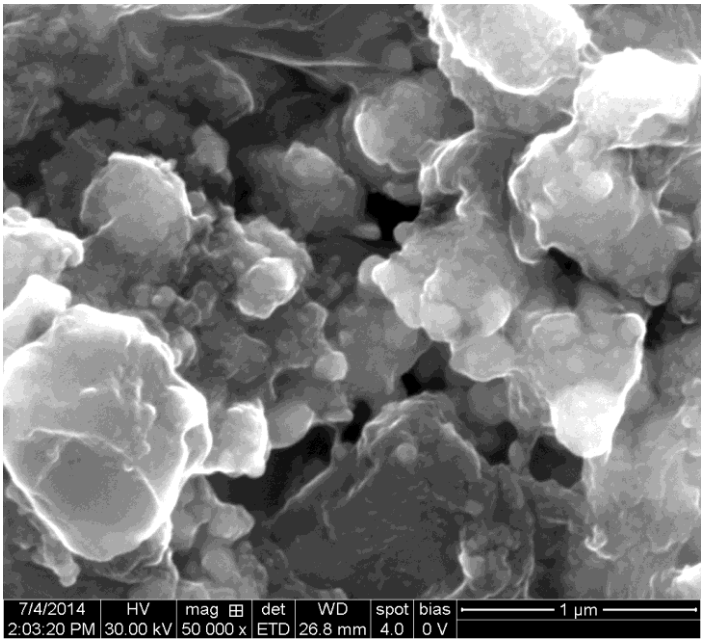


Fig. 5: Scanning Electron Microscope (SEM) image of silver nanoparticles obtained by green synthesis using *A. spicifera* extract

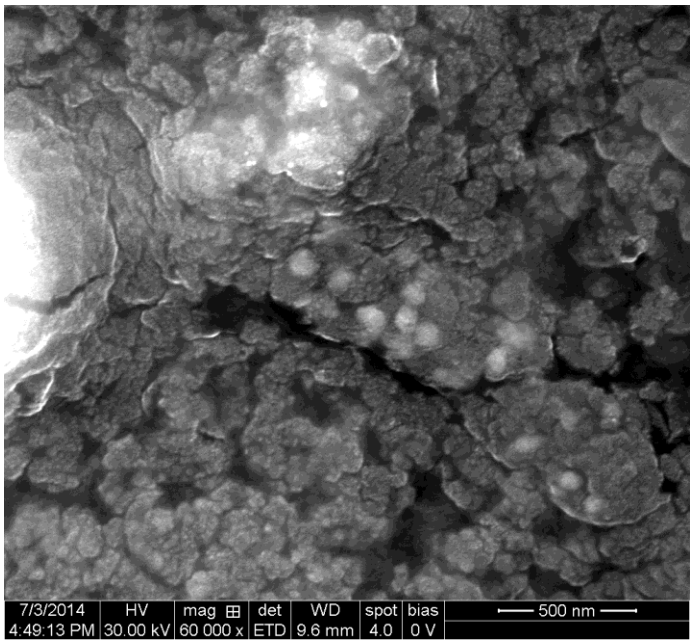
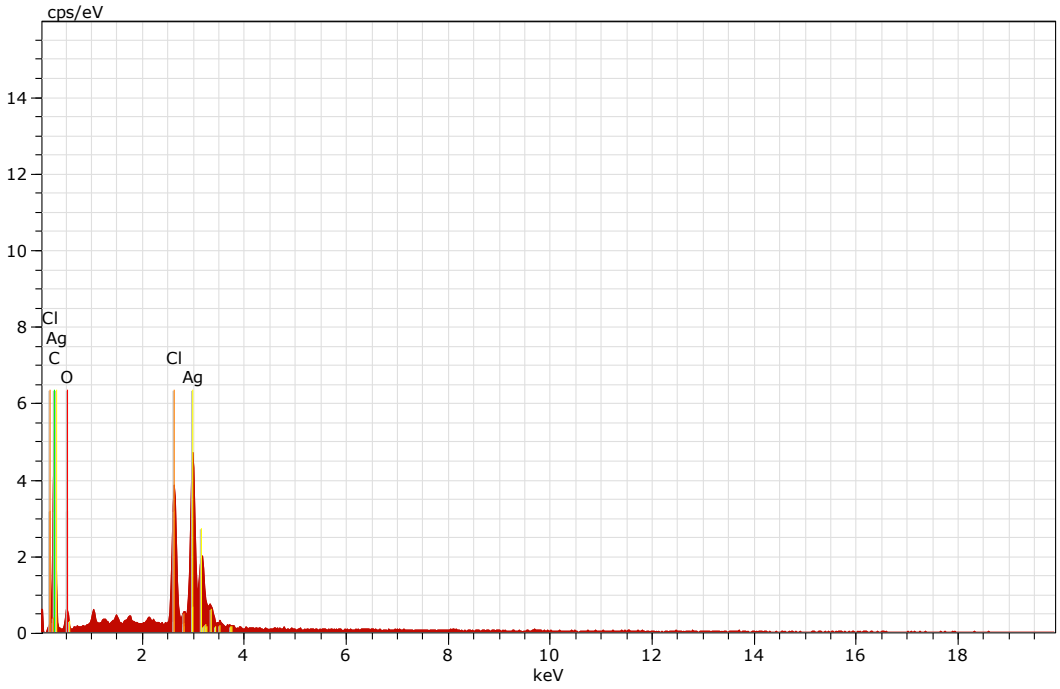


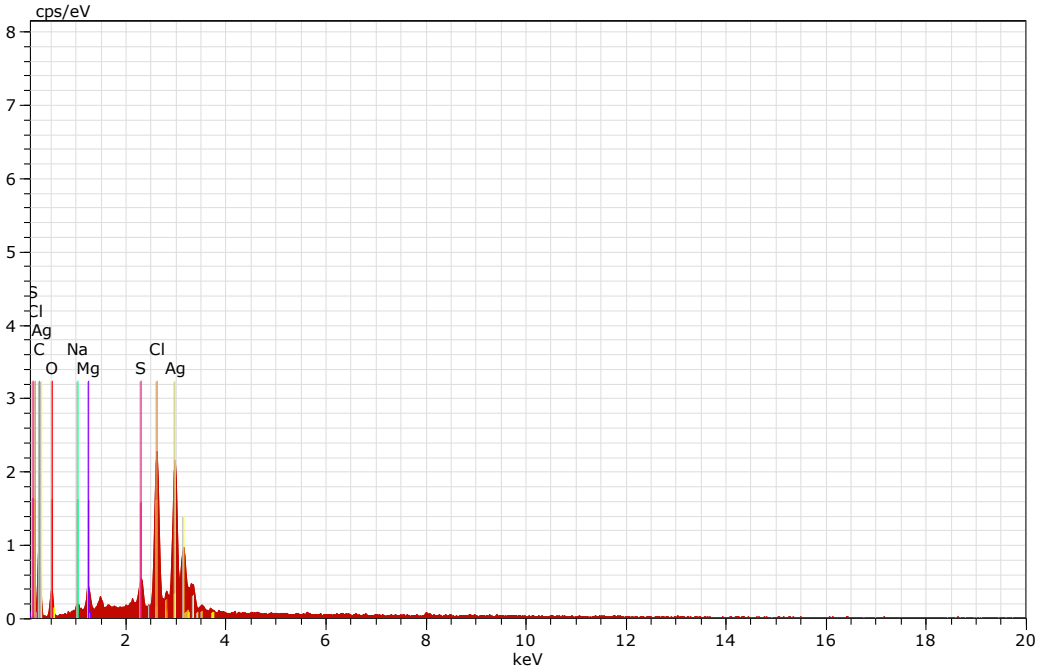
Fig. 6: Scanning Electron Microscope (SEM) image of silver nanoparticles obtained by green synthesis using *P. tetrastromatica* extract



Spectrum: 3 2128

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
C	6	K-series	60.94	57.45	79.35	10.17
Ag	47	L-series	24.56	23.15	3.56	0.86
O	8	K-series	14.95	14.10	14.61	4.16
Cl	17	K-series	5.62	5.30	2.48	0.24
		Total:	106.07	100.00	100.00	

Fig. 7: X-Ray Diffraction pattern of silver nanoparticles obtained by green synthesis using *A. spicifera* extract



Spectrum: 3 2124

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
C	6	K-series	24.58	41.03	64.50	4.71
Ag	47	L-series	15.27	25.49	4.46	0.54
O	8	K-series	11.41	19.05	22.48	2.78
Cl	17	K-series	5.88	9.82	5.23	0.25
Mg	12	K-series	1.19	1.98	1.54	0.12
S	16	K-series	0.97	1.62	0.95	0.07
Na	11	K-series	0.61	1.02	0.84	0.09
		Total:	59.90	100.00	100.00	

Fig. 8: X-Ray Diffraction pattern of silver nanoparticles obtained by green synthesis using *P. tetrastromatica* extract

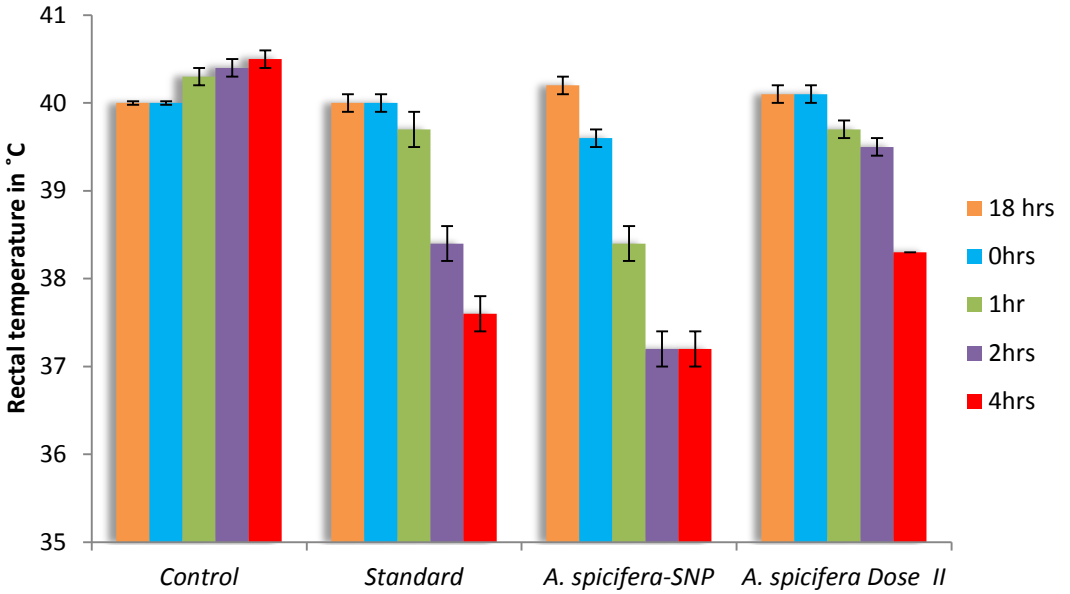


Fig. 9: Showing the antipyretic activity of green synthesized silver nanoparticles using *A. spicifera* extract

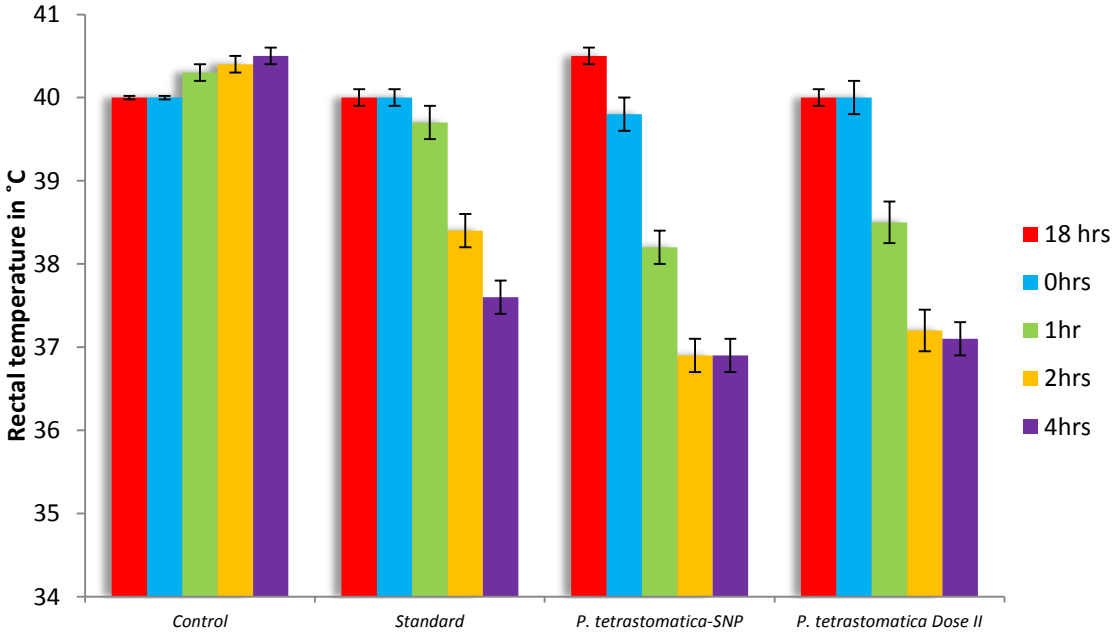


Fig. 10: Showing the antipyretic activity of green synthesized silver nanoparticles using *P. tetrastromatica* extract

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