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SYNTHESIS, CHARACTERIZATION AND STUDY OF APPLICATION OF RAJATHA BHASMAM (SILVER SULPHIDE ASH) AN AYURVEDIC SUPPLEMENT

Dr.PadamataSai Sudhakar[1], Dr. K. BalaMurali Krishna[2], Prof. B. SyamaSundar*
1 Professor, K C Department, Dr N R S Ayurvedic Medical College, Bandar Road, Vijayawada 2 Department of nanotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur-522510, Andhra Pradesh, INDIA.
*Former Vice Chancellor, Yogi Vemana University, Kadapa, A.P

Abstract:
RajathaBhasma is an Ayurvedic supplement prepared from Silver useful in cardio-vascular diseases, improves skin complexion, digestion power, body strength and immunity. The present study deals with the synthesis, characterization and study of application of RajataBhasma. The synthesized bhasma was subjected to physical and structural characterization studies. Application of nanosilver has been studies with RajathaBhasma in order to evaluate use a medicinal supplement. The characterization of prepared bhasma was also done by using modern analytical techniques such as EDAX, SEM, XRD, FTIR, which shows that Rajathabhasma mainly consists of silver in nano size. Application studies like antimicrobial, antioxidant studies reveal that high potency of RajathaBhasma use as medicinal supplement.

Key words: RajathaBhasma, Ayurvededa, anti-oxidant activity, antibactrial activity, Silver.

1. Introduction of RajathaBhasma:

RajathaBhasma is an Ayurvedic supplement prepared from Silver. It is widely used in the natural treatment of memory loss, dizziness, excessive thirst and diabetes. It has balancing effect on Tridosha, as it balances Vata, Pitta and Kapha naturally (1, 2). RajathaBhasma boosts the immune system, body strength, brain activity, digestion and skin color. It is an astringent and bitter-sweet in taste. It is a natural supplement that produces a cooling effect. According to Ayurveda, it is a very useful natural supplement and without any known side effects. RajathaBhasma is the incinerated powder of Silver (3). As per Ayurveda, this natural Bhasma is beneficial for strength promoting and rejuvenation. In Ayurvedic treatment dosage of 65 mg to 125 mg twice a day with recommended adjuvant for specific diseases (4). There are no side effects observed since medieval times. It is well tolerable almost in all cases and patients.

Very few studies have been reported with the RajataBhasma. *RekhaChaturvedi et al* (5) reported standard manufacturing procedures for the manufacture of RajataBhasma and also attempts to study the effect of Shodhana process on Rajata. *Sunil Kumar Dalal* (6) reported that RajataBhasma it contains Total. Ag- 80.12%, S- 9.426%, Al-0.363%,As- 1.892%, Hg-0.000076%, Cu- 0.866%, Fe- 3.082%, Pb- 0.202% and Cd- 0.0016% . In RajataBhasma heavy metals like Lead, Mercury, Arsenic and Cadmium are in permissible limits. Particle size of RajataBhasma found in nanometre range. *Mamta Parikh et al* (7) prepared and analyzedRajata (Silver) Bhasma and found 73.594% of silver, while sulphur was present at 17.246% which may be in sulphide form of silver. Quantity of iron, copper and platinum was 0.43585%, 0.29944% and 0.0014752% respectively. To evaluate the use of RajataBhasma the resent work is aimed to synthesis, characterization and anti-oxidant activity, antibactrial activity application have been studied.

2. Materials and Methods

2.1 Chemicals and solvents:

Chemical used for the preparation of Bhasma (As_2S_3 and S) were of analytical grade was purchased from Indian Research Products, Chennai. Standard silver nano particles of <100nm particle size was purchased from sigma Aldrich (576832-5G). Composition for the preparation of nutrient agar medium Peptone, beef extract, agar and NaCl were purchased

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form Fisher Scientific Company, Mumbai. The standard antibiotic ciprofloxacin for antibiotic standard was obtained from Dr. Reddy's laboratories, Hyderabad.

2.2 Instrumentation:

Teccomp UV-2301 double beam UV-Visible spectrophotometer with HITACH software manufacture by Teccompindiapvt ltd. Denver electronic analytical balance (SI-234) manufacture by Denver instruments. BRUKER VERTEX 80/80v FT-IR spectrometer manufacture by Bruker Corporation, Bruker- D4 ENDEAVOR manufacture by Bruker Corporation, LEO 1420 VP Compact variable pressure Digital SEM manufacture by Leo Electron Microscopy Ltd, BRUKER EDX Two-dimensional VANTEC-500 detector manufacture by Bruker Corporation, Hitachi H7500 TEM manufacture by Hitachi High-Technologies corporation are used.

2.3 Preparation of solutions:

Iodine [0.1 N] solution: Weigh 40 g of potassium iodide (KI) in a 500 mL glass-stoppered flask and dissolve in 100 mL of purified water. Cool the solution up to room temperature, add 12.7 g of resublimed iodine (I_2), restopper the flask, and swirl the flask until the iodine is completely dissolved.

Standard anti bacterial drug solution:

Ciprofloxacin was used as standard drug for anti bacterial activity. 10mg of standard ciprofloxacin drug was weighed accurately and was dissolved in 10ml distilled water to get a standard drug concentration of 1000µg/ml. from this required dilutions was prepared by serial dilution method for the evaluation of anti bacterial activity.

Standard anti fungal drug solution:

Fluconazole was used as standard drug for anti fungal activity. 10mg of standard Fluconazole drug was weighed accurately and was dissolved in 10ml distilled water to get a standard drug concentration of 1000µg/ml. from this required dilutions was prepared by serial dilution method for the evaluation of anti fungal activity.

Dimethyl sulfoxide [1%] solution:

1ml of 100% Dimethyl sulfoxide (DMSO) was measured accurately and was made up to 100ml in a calibrated volumetric flask using distilled water.

DPPH [0.1 mM] solution:

Weigh accurately 0.394mg of DPPH accurately and was dissolved in 100ml of 95% methanol to get DPPH stock solution concentration of 10mM. 10ml from this stock solution was further diluted to 100ml to get DPPH solution having 1mM concentration. This solution was used for the free radical scavenging activity.

2.4 Preparation of RajathaBhasmam

Silver leaves were cut into small pieces and mix equal quantity of SuddhaHarithala (As_2S_3) and Sulphur. The content was put in earthern pot with silver. Seal the earthern pot with another pot and do Kukutaputam (furnace) for three times. Dark coloredRajathaBhasmam was obtained.

2.5 Physical characterization of RajathaBhasmam:

Bulk Density:

The Bulk density of the synthesised Rajatha Bhasmam was measured in a graduated cylinder.

Bulk density = Mass of sample/ volume as a whole

$$Pb = \frac{Ms}{Vt}$$

Moisture Content:

Moisture content was determined by drying replicate samples for twelve hours in an oven set to 150°C.

Loss on Ignition:

1gram of Rajatha Bhasmam was ignited at 750±50°C. loss in mass is expessed as % of the total initial mass.

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$$\% \text{ Loss on Ignition} = \frac{\text{Weight before ignition}}{\text{weight after ignition}} \times 100$$

Ash Content:

1gram of Nanoparticle was burnt on a ashless filter paper. % Ash content was calculated by using formula

$$\% \text{ Ash Content} = \frac{\text{Weight before burnt}}{\text{weight after burnt}} \times 100$$

***p*H :**

4grams of syntheisised silver nanopartilces was taken in a 250ml beaker, and 100ml of distilled CO_2 free (boiled) water, cover with a watch glass and boil on a hot plate for 5min. insert the thermometer and set aside for a few minutes to allow the bulk of the nanoparticle to settle. Pour off the supernatant liquid as soon as possible and measure the pH.

Point of zero charge (PZC):

RajathaBhasmam (1g/L) was added to water within sealed polystyrene bottles and dispersed by shaking on a shaker table. After two days the water was decanted by pipet to a plastic test tube and the pH measured.

Iodine Number :

5Gm of RajathaBhasmam sample was weighed accurately and was grind enough quantity to pass through 75 micron US sieves (about 1gm), weigh very accurately 0.2gm of sample and introduce it in the iodine flask. Introduce 40ml of 0.1N iodine solution. Shake the contents for exactly four minutes. Filter through Whatman filter paper No. 1. 10 ml of filtrate was titrated against standard sodium thiosuphate solution.

Decolorizing Power :

Weigh accurately about 0.1gram of the nanoparticle sample, with accuracy of 0.1gram and transfer to 50ml glass stoppard flask. Add from a burette 10 ml of methylene blue solution and shake for 5 minutes. After the first 10 ml are decolorized continue to add methylene blue solution (1 ml at a time) till the blue color disappears for 5 minutes. Decolorizing power of nanoparticle sample expressed in terms of milligrams of methylene blue adsorbed by 1gram of synthesized Ag RajathaBhasmam.

$$\text{Decolorizing Power} = \frac{15 \times V}{10 \times M}$$

Where V = volume in ml of methylene blue solution consumed
M = mass in gram of the material taken for the test.

Surface Functional groups:

The acid/basic properties of the nanoparticle sample were studied by measuring the surface functional groups of treated RajathaBhasmam samples using the multi-basic titration method of Boehm and Diehl (H.P. Boehm, E. Diehl, Angew. Chem. Int. Ed. 3 (1964) 669–677). According to this method, about 0.1g of nano-particles sample was placed into a 200 mL conical flask containing 100 mL of each of the following 0.05N aqueous solution: sodium hydroxide, sodium carbonate, sodium bicarbonate, sodium ethoxide and hydrochloric acid. The flask was then sealed and the mixture was shaken for 24 h at 25°C. The carbon sample was removed by filtration, and about 15 mL of accurately withdrawn aliquot of filtrate was titrated by 0.01N hydrochloric acid in order to estimate the excess base or 0.01N sodium hydroxide for the excess acid. The amount of acidic/basic functional groups was then calculated using the titration data.

2.6. **Structural Characterization:**

The synthesized silver RajathaBhasmam were characterized by using different techniques including UV–Visible Spectroscopy, scanning electron microscopy and energy dispersive X-ray spectroscopy.

Ultraviolet-Visible Spectrophotometer: The reduction of silver ions in the colloidal solution was confirmed by UV–Visible spectroscopy. A small aliquot of sample was taken in a quartz cuvette and observed for wavelength scanning between 300-800 nm with distilled water as a reference.

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Fourier Transform Infrared Spectroscopy (FTIR) Measurements: After the synthesis of RajathaBhasmam was freeze-dried and pelleted with potassium bromide in the ratio of 1:10 and subjected for FTIR spectroscopic measurement. The wave number ranged from 450 to 4000cm^{-1} with the resolution of 4cm^{-1} and was analyzed by subtracting the spectrum of pure KBr.

X-ray diffraction (XRD) analysis: The analyzed material is finely ground, and average bulk composition is determined. The particle or grain size of the silver RajathaBhasmam was determined using Debye Sherrer's equation.

$$D = \frac{0.94\lambda}{B\cos\theta}$$

Where D is the crystal size, λ is the wavelength of X-ray, Θ is the Braggs angle in radians and B is the full width at half maximum of the peak in radians.

Scanning electron microscopy: Surface morphology of silver RajathaBhasmam was demonstrated by scanning electron microscopy. The sample was prepared by centrifuging colloidal solution after 6 h of reaction at14000 rpm for 4 min. The pellet was re-dispersed in deionized water and again centrifuged. The process was repeated three times and finally washed with acetone. The purified silver RajathaBhasmam were sonicated for 10 min for making the suspension and then a drop from the suspension was placed on the carbon coated copper grid. The sample was kept under lamp until completely dry. 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 and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5min. The prepared sample was subjected to SEM analysis.

Energy-Dispersive X-Ray (EDX) Analysis: Energy-Dispersive X-Ray analysis conform the presence of elemental silver in the synthesized silver RajathaBhasmam. The sample was kept on copper grid stained with uranyl acetate and lead citrate.

Transmission Electron Microscopy (TEM): The size and shape of the synthesized silver RajathaBhasmam were determined by transmission electron microscopy. Prior to analysis, RajathaBhasmam were sonicated for 5 minutes, and a drop of appropriately diluted sample was placed onto a carbon-coated copper grid. The liquid fraction was allowed to evaporate at room temperature. Then the size and shapes of the RajathaBhasmam was observed.

2.7 Studies pertaining to biological activity and anti-oxidant activity of the synthesized RajathaBhasmam in case of RajathaBhasmam:

Assessment of Antibacterial Activity: Antibacterial activity of the synthesized RajathaBhasmam was determined by using the Kirby-Bauer disc diffusion method [8, 9] against different pathogenic bacteria and fungi. The anti fungal zone inhibition activity was studied against *Aspergillusniger* (MTCC 282) and *Rhizopusoryzae*(MTCC 262).The anti bacterial zone inhibition activity was studied against five human pathogenic bacteria namely, *Bacillus Subtilis* (MTCC 10619), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 3160), and *Bacillus cereus* (MTCC 1305). The zone of inhibition was observed and measured in presence ofRajathaBhasmam.

Antioxidant activity (DPPH method):

The free radical scavenging activity of the synthesized AgNPs was determined by using DPPH method described by Marsden S. Blois et al 1958 [10]. Briefly, DPPH solution of 0.1 mM was prepared in 95% methanol and 1 ml of this solution was added to 3.0 ml of synthesized AgNPs solution of 5–250µg/ml. The solution was incubated for 30min at dark conditions at room temperature and absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Ascorbic acid was used as a standard. The experiment was repeated triplicate and the DPPH scavenging activity was calculated by using the formula

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$$\% \text{ Inhibition} = \frac{A0 - A1}{A0} \times 100$$

Where A_o is the absorbance of the control and A1 the absorbance of the AgNPs solution

3. Results and Discussions:

BhasmaKalpana is a unique part of AyurvedicRasashastra. It is a method of converting metals into metallic Bhasma. Metallic Bhasmas are well known for its quick effectiveness, smaller dose and a long shelf life. However if these bhasmas are not well prepared and analyzed they can be toxic to human body. Therefore BhasmaPariksha is given in Ayurveda to confirm the well prepared metallic Bhasma, but in this era we need to analyze the Bhasmas on modern parameters too to make it acceptable globally. So in this study prepared RajataBhasma was analyzed on various physical and chemical parameters.

The Physical characterization results of the synthesized nano-particles were given in table 1. Physical characterization reveals the nano silver properties of the RajataBhasma. The RajataBhasma was characterized using different techniques likes UV absorption, TEM, SEM, EDX and XRD and further anti microbial and anti oxidant activities were studied. The surface plasmon resonance (SPR) band (λ max) UV-visible spectroscopy scan around 447nm broadened and slightly moved to the long wavelength region, indicating the presence of nano size silver in rajathabhasma (figure A). The surface plasmon absorption of silver RajathaBhasmam have the short wavelength band in the visible region around 447nm is due to the transverse electronic oscillation (figure B). TEM analysis reveals that morphology of silver particles is almost spherical, and exhibited high surface areas, an average size of 146nm in the range of 95–210nm. The TEM image confirms the particles are spherical in shape. The SEM studies confirmed the formation of silver particles in the size range of 90- 200nm, a clear indication that particles of RajathaBhasma (figure C) are in nano size. The RajathaBhasma particles are irregular and slightly spherical in shape. Higher magnification showed the average diameter of these RajathaBhasmam to be about 145nm. Silver ions were not well separated from each other in the nanotriangles and dimensions are capped with smaller particles due to the presence of small crystal and hexagonal particles of approximately. EDX analysis confirmed the presence of elemental silver as the major constituent. The insignificant amounts of observed S, C and O are attributed to the plant biomass attached to the NPs. The EDX profile of RajathaBhama was shown in Figure D. XRD patterns of RajathaBhasma reveals that diffraction peaks appeared at 24.15, 30.91 and 41.50 corresponding to 111, 120 and 130 Plane for silver. Prominent Peaks at 32.52° and 48.52° were observed corresponding to -121 and -212 Plain representing the Polycrystalline Structure of RajathaBhasma (figure E).

Antimicrobial activity study by disc diffusion test results indicate that the maximum zone of inhibition against *Escherichia coli* is 8.6mm, while *Pseudomonas aeruginosa* requires 9.6mm at a concentration of 100µg/ml. These results support the traditional use of RajathaBhama as antimicrobial agent exhibits significant antimicrobial activity against E. coli and multidrug-resistant bacteria. Table 2 shows the MIC values of RajathaBhama for different bacteria. This work had been found that the RajathaBhama effectively inhibited the growth and multiplication of human pathogenic bacteria and fungi. Results of antibacterial activity, antifungal activity were presented in figure F, G, H, I and table 2, 3. The % DPPH inhibition was found to be very high for crude RajathaBhama.The IC_{50} value for DPPH scavenging activity of RajathaBhama was found to be less than 50µg/ml, while the IC_{50} value for ascorbic acid was found to be 42.68µg/ml. Hence RajathaBhama shows very high DPPH inhibition activity. Results were given in table 4 and comparative figure was given in figure J.

4. Conclusion:

The prepared RajathaBhasma was found nano in size (90- 200nm) and particles are irregular and slightly spherical in shape. Structural and physical characterization of RajathaBhasma found that Ayurvedic method of silver has meet all the characteristic properties of nanosilver particles synthesized by various physical, chemical and biological methods. High DPPH inhibition activity and Antimicrobial activity (antibacterial and antifungal) proves its medicinal use of RajathaBhasma is an Ayurvedic supplement.

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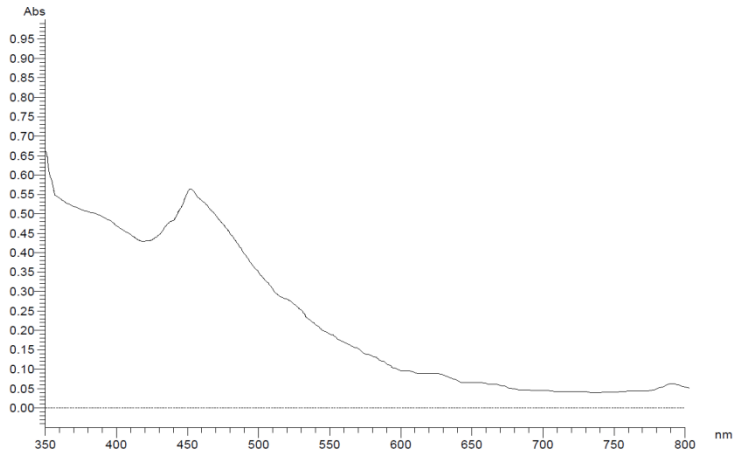

Figure A: UV scanning spectra of RajataBhasma

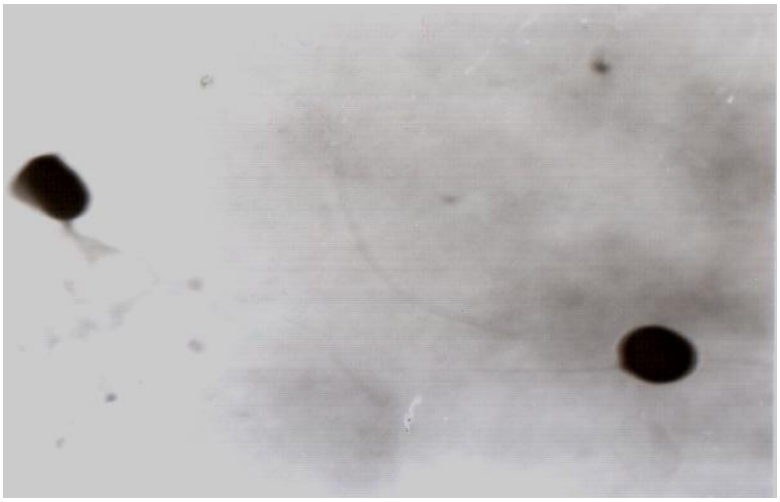

Figure B: TEM images for RajataBhasma

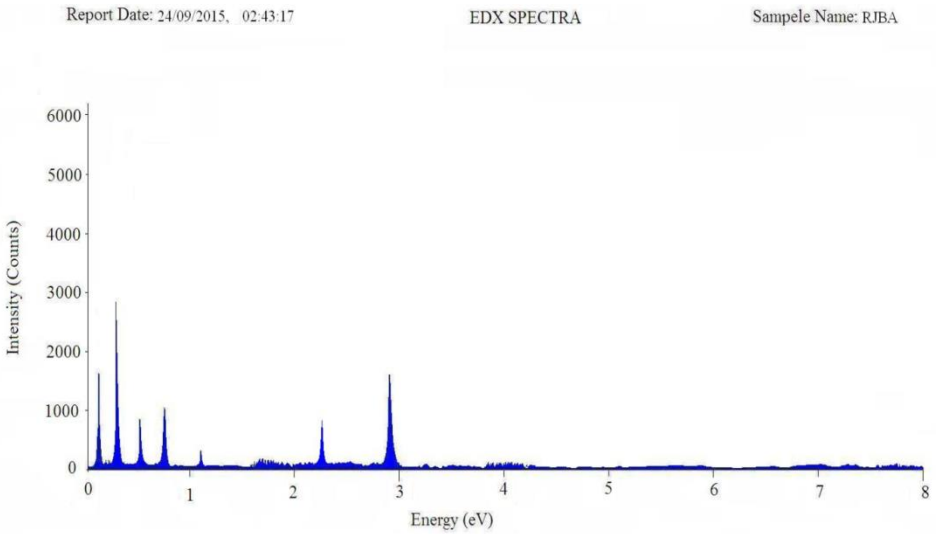

Figure D: EDX spectra for RajataBhasma

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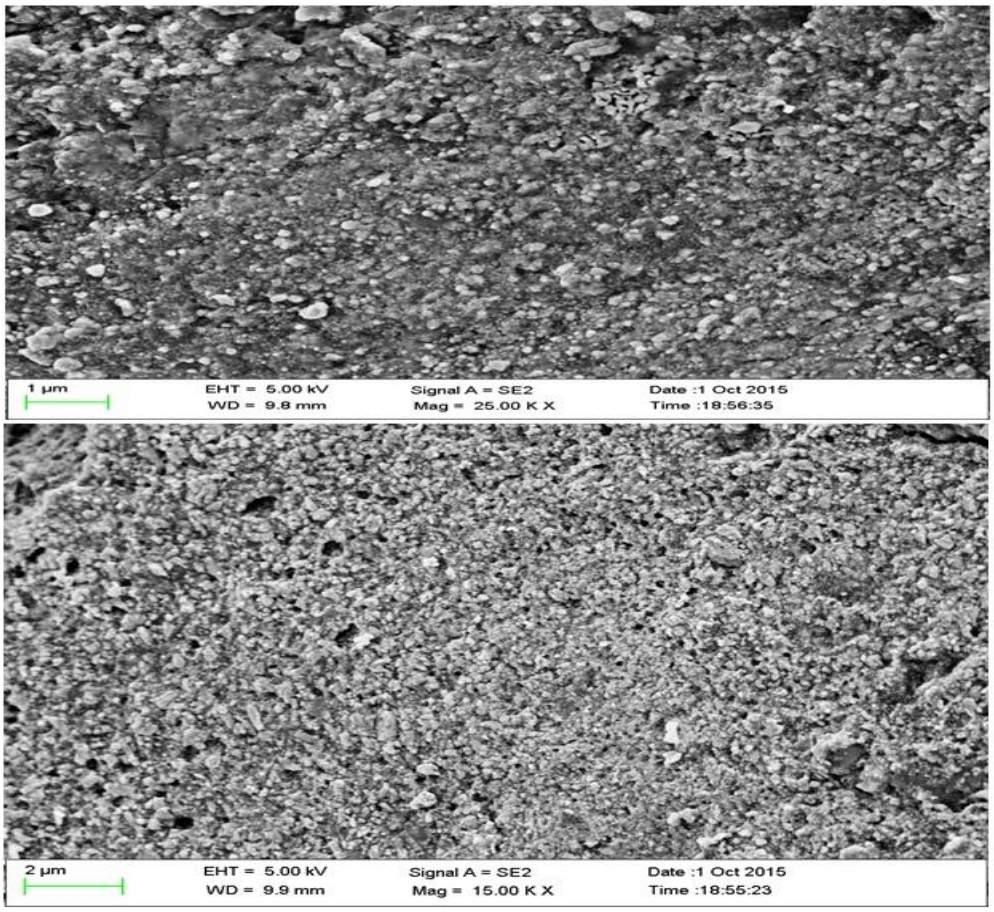

Figure C: SEM image for RajataBhasma

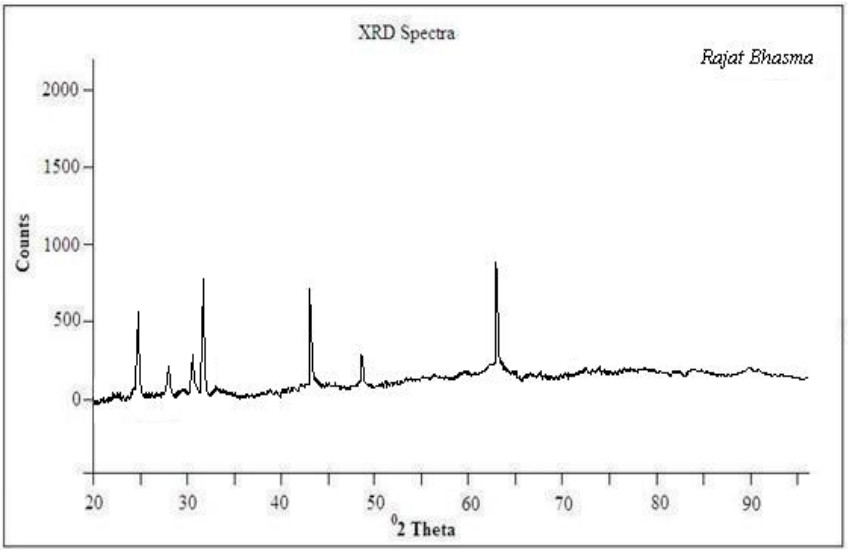

Figure E: XRD Spectra for RajathaBjhasma

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Table 2: Results of studies of anti bacterial activity of RajathaBhasmam followed by graphical representation and pictorial representation

S No	Name of the Organism	Zone inhibition observed for concentration (µg/ml) of RajathaBhasmam					
		1000	500	250	100	10	1
1	*Bacillus Subtilis*	17.2	15.6	11.8	7.4	3.6	
2	*Escherichia coli*	18.9	16.5	12.6	8.6	5.8	2.3
3	*Pseudomonas aeruginosa*	15.8	12.5	11.2	9.6	4.9	
4	*Staphylococcus aureus*	16.2	14.3	11.5	8.7	5.3	
5	*Bacillus cereus*	13.6	10.1	7.5	5.1	3.2	

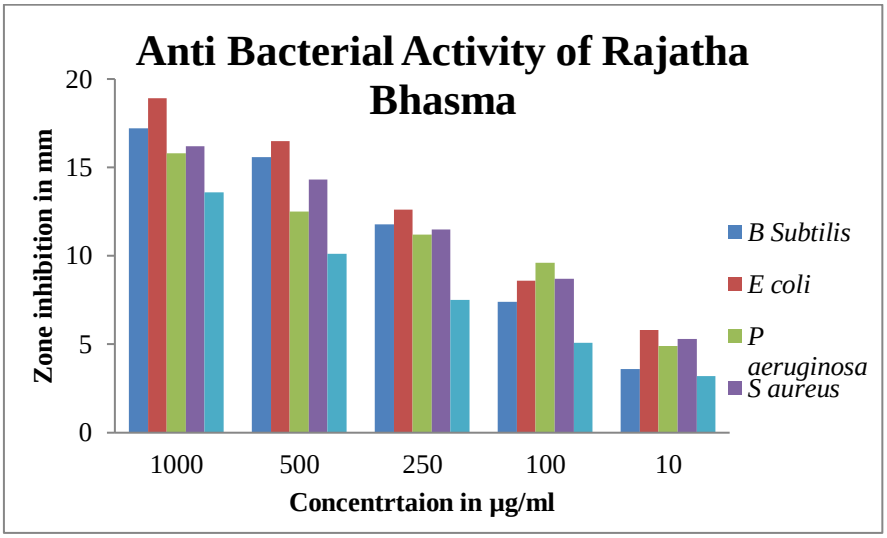

Figure F: Comparative anti bacterial activity of RajathaBhasma

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Figure G: Pictorial representation with regard to studies of anti bacterial activity in case of RajathaBhama

Table 3: Results of studies of anti fungal activity of RajathaBhama followed by pictorial representation

S No	Name of the Organism	Zone inhibition observed for concentration (µg/ml) of AgNPs					
		1000	500	250	100	10	1
1	*Aspergillusniger*	15.1	12.7	9.6	6.4	3.2	---
2	*Rhizopusoryzae*	12.5	9.4	6.7	4.1	---	---

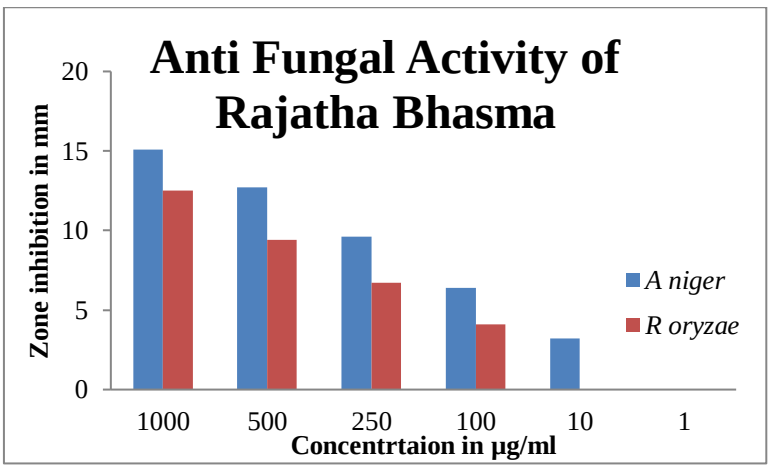

Figure H: Comparative anti-fungal activity of RajathaBhasma

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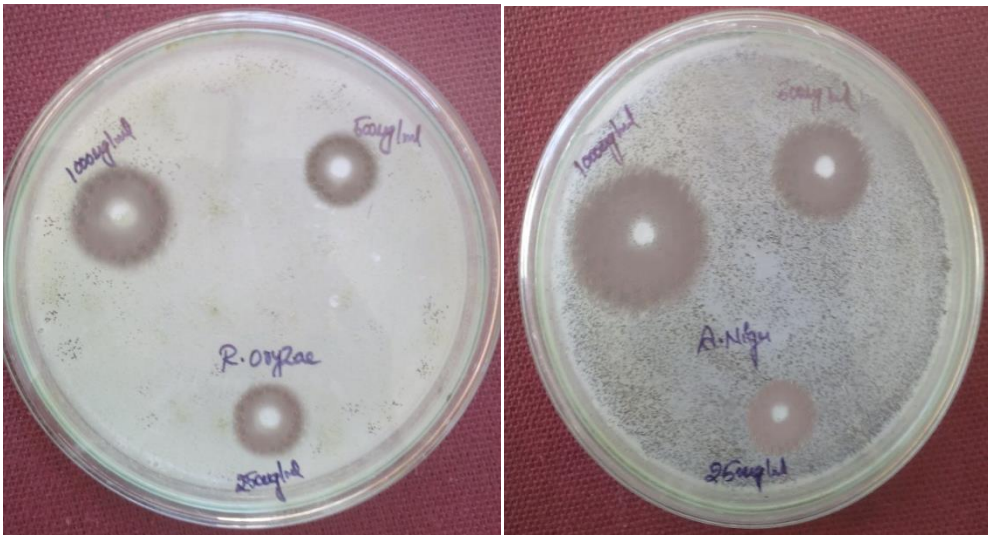

Figure I: Pictorial representation with regard to studies of anti fungal activity in case of RajathaBhama

Table 4: Results of studies of anti oxidant activity (DPPH method) of synthesized silver nano particles in case of *RajathaBhasma* followed by graphical representation

S NO	Concentration in µg/ml	%DPPH Inhibition Observed	
		Ascorbic acid	RajathaBhama
1	5	13.26203	--
2	10	21.39037	5.240642
3	15	36.25668	19.67914
4	20	45.34759	34.4385
5	25	52.72727	39.35829
6	30	60.53476	45.24064
7	50	69.94652	65.24064
8	100	86.63102	76.36364
9	150	91.3369	85.13369
10	200	93.79679	89.09091
11	250	97.54011	92.62032

Table 7.4: DPPH Activity results

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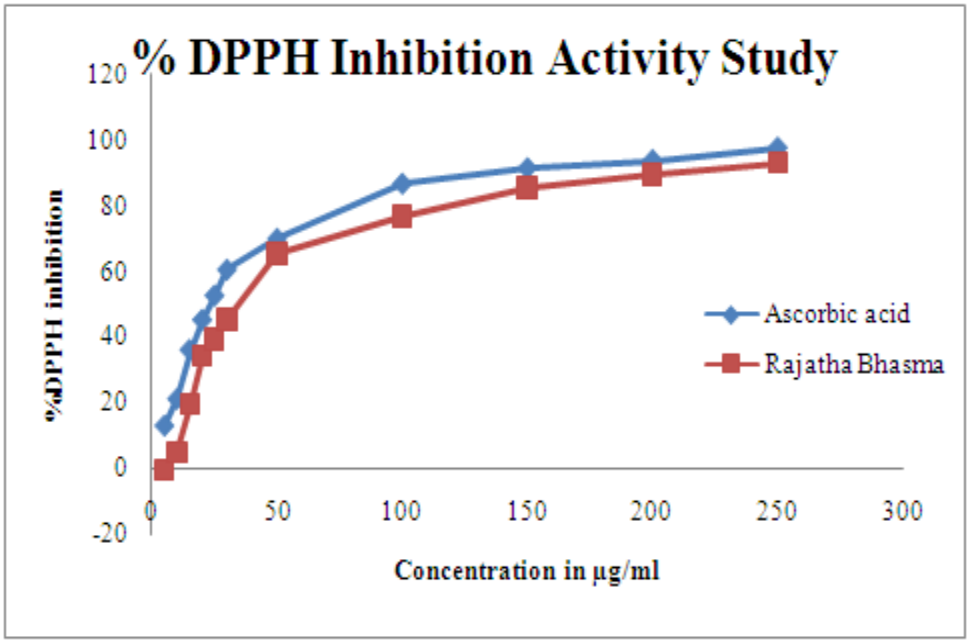

Figure J: Graphical representation with regard to anti oxidant activity (DPPH method) of synthesized silver nano particles in case of RajathaBhasma

5. References:

1. Barnes PM, Bloom B, Nahin R., Complementary and alternative medicine use among adults and children: United States, 2007, 361KB PDF CDC National Health Statistics Report #12. 2008
2. Mishra, L.; Singh, B. B.; Dagenais, S., Healthcare and disease management in Ayurveda, Alternative therapies in health and medicine., 2001, 7(2): 44–50
3. Chopra, Ananda S., Ayurveda, In Selin, Helaine. Medicine across cultures: history and practice of medicine in non-western cultures. Kluwer Academic., 2003, pp. 75–83.
4. Underwood, E. Ashworth, Rhodes, P., History of Medicine, Encyclopædia Britannica (2008 ed.).
5. RekhaChaturvedi and C. B. Jha, Standard manufacturing procedure of RajataBhasma , AYU, 2011, 32(4); 566-571
6. Sunil Kumar Dalal, pharmaceutico- analytical study of rajatabhasma, ayush doctors, July 23, 2015
7. Mamta Parikh, AK Choudhury, B J Patgiri and P K Prajapati, Analytical Assessment of RajataBhasma, International Journal of Pharmaceutical & Biological Archives 2012; 3(6):1512-1517
8. Bauer, A. W., D. M. Perry, and W. M. M. Kirby (1959), Single disc antibiotic sensitivity testing of Staphylococci. A.M.A. *Archives of Internal Medicine*, 104:208–216.
9. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck (1966), Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology,* 36:493-496.
10. Marsden S. Blois (1958), Antioxidant Determinations by the Use of a Stable Free Radical, *Nature*, 181, 1199 – 1200.