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Development and characterization of Green synthesized Silver Nanoparticles of *Zingiber Officinale, Trachyspermum Ammi, Alium Sativum* for their Anti-Asthmatic effect, Anti-Cancer and Anti Inflammatory effect

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Abstract:- *Medicinal herbal drugs have significantly increasing importance in the pharmaceutical sciences. There are many herbal plants and their parts that are being used for different therapeutic purposes. Some of these ingredients include Ginger (Zingiber Officinale), Ajwain (Trachyspermum Ammi) & Garlic (Alium Sativum).These ingredients seem very simple but have wide pharmacological properties including anti oxidative, anti-inflammatory, hypo lipidemic, anti-viral, anti-cancer and more. The phytochemicals of these herbal ingredients have been studied in many scientific researches and are shown to be effective in treating various diseases. One of the several ways to deliver the phytochemicals in a novel way is by the formation of Nanoparticles. Nanoparticles are specialized novel delivery systems that to encapsulate the drug/active molecule within them and enhance absorption, metabolism, minimize or reduce the side/adverse effects and deliver drug to the site of action. This study further deals with the importance of Nanoparticles of the above stated herbal ingredients about how they could show significant use in treatment of conditions of inflammation, cancer and asthma.*

INTRODUCTION

Nanoparticles

Nanoparticles are the novel approach for the delivery of drug molecules. Nanoparticles are very small in sizes from 1-100nm. Nanoparticles are very useful in various fields of science involving pharmacy, medicines, biochemistry, pathology, nuclear science, physical science and more. Nanoparticles are made by the encapsulation of very small drug molecules in any type of polymers, metals or crystalline structures like diamond nanoparticles. Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties properties [Xi-Feng Zhang et al.]. These include optical, electrical, and thermal, high electrical conductivity, and biological properties [Xi-Feng Zhang et al.]. Silver nanoparticles are also effective against various fungi, virii, and algae [Jalpa Soni, 2007]. AgNPs can be obtained by physical, chemical, and biological methods, from which the chemical method is the most effective and popular. AgNPs are the most popular nano materials that are commonly used in modern pharma sciences, cosmetology, and medicinal sciences. Due to their antibacterial activity, they are widely being used in many cosmeceutical products and formulations, such as creams and ointments healing acne or dandruff, products intended for oral hygiene, and deodorants preventing excessive growth of flora [Alicja Kapuścińska, 2017].

Ginger

Ginger (Zingiber *officinales*) is a traditional medicinal herb that is widely used as a kitchen ingredient for preparation of many dishes in India. It is a widely used ingredient for traditional tratment of cough, cold, flu, body pain, and many conditions. Like in the treatment of asthma it was found in a study that Zingiber officinale reduces/prevents allergic asthma by the suppression of Thymine 2 mediated immune response [Khan AM et al. 2015 Mar]. Ginger contains many compounds around 60 out of which its chief constituents are *Aresesquiterpene hydrocarbons.* 6 shogaol, 6 gingerol are the major bioconstituents of ginger which have shown to suppress phthalate ester mediated (PEM) airway remodeling which shows its ability to prevent PEM asthma [Bhatt, Neeru et al. (2013)].

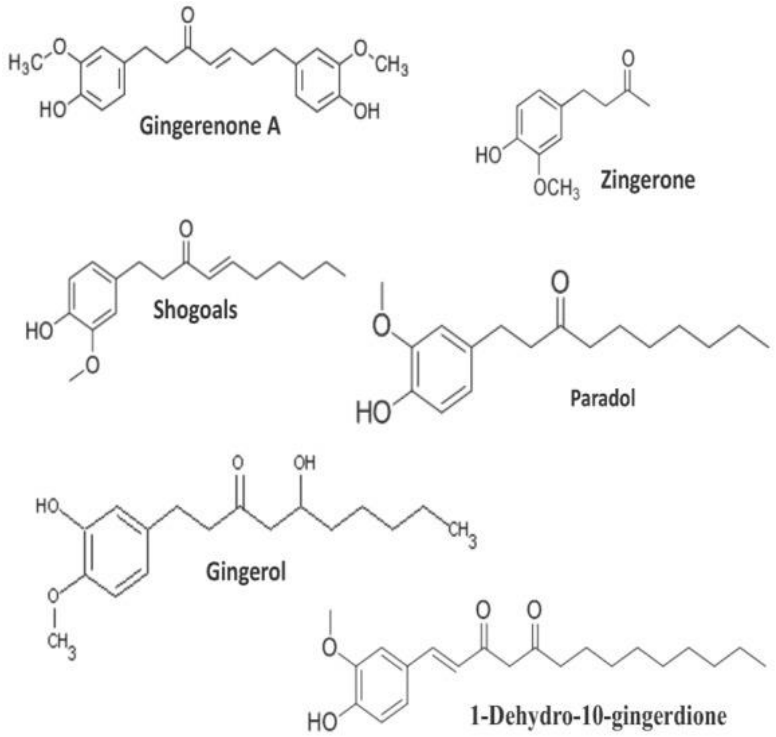


Fig 1. Phytoconstituents of Ginger [Arshad H Rahmani, 2014]

Medicinal properties of Ginger:-

- Ginger shows **anti oxidative** property [Masuda Y, et al. 2004]
- Ginger shows **anti-inflammatory** activity [Young et al. 2005]
- Ginger works as **anti emetic** agent [Shibata C, et al. 1999]
- Ginger has the capability to treat many heart related disease, and has activity of in vitro and animal data supporting the **anti-inflammatory, hypolipidemic, antiplatelet, hypotensive, and antioxidant** effects of this condiment have been reviewed [Nicoll and Henein 2009].
- **Antiasthmatic effect -** ginger and its active components induce bronchodilation by modulating intracellular calcium ($[Ca^{2+}]_i$) in airway smooth muscle (ASM)[Townsend EA et al. 2013 Feb]**.**

Garlic

Garlic also known as *Alium Sativum* is a rich medicinal plant that contains hundreds of phytoconstituents that are of various advantages. It is also a popular ingredient that is used in Indian kitchen for preparation of various dishes and in condiments. It has been used traditionally for medicinal purposes. Bulbs of *Alium Sativum* contain Quercetin which is the main flavonoid. Allicin is most active sulphur containing compound of garlic and Alliin is its precursor. [Batiha, Gaber et al. 2020].

Traditionally, garlic and its related constituents have showed to have many biological activities including antidiabetic activity, renoprotective action, anti-atherosclerotic activity, antibacterial action, antifungal action, hypertensive activities [Badal, D.S et al. 2019], anticarcinogenic activity, antioxidant activity [Rahman, K et al. 2006].

Pharmacological activities of Garlic:-

- **Antibacterial Activity** – Garlic has the potential to show antibacterial activity and has the capability to fight against many types of microbes and bacteria. [Ross, Z.M 2001] [Cutler, R.; 2004] [Wallock-R et al. 2014]
- **Anti-Protozoal Activity –** the studies of some scholars reported the anti-protozoal activity of garlic extracts and its phytoconstituents against several parasites of protozoa. [Batiha, Gaber et al. 2020]. Garlic shows activity against protozoa and stops their growth.
- **Antiviral Activity**- with activity to stop microbial infection the garlic phytoconstituents are also known to prevent viral infections [Batiha, Gaber et al. 2020].
- **Anti-Inflammatory Activity** - Hobauer et al. & Gu et al. observed that the constituents of garlic causes inhibition of the emigration of neutrophilic granulocytes into epithelia thus showing anti inflammatory activity [Batiha, Gaber et al. 2020].
- **Anticancer Activity**- garlic shows anti proliferative property and induce apoptosis. [Batiha, Gaber et al. 2020].
- **Antiasthmatic activity:** In a study of Lung function modification with increasing the Nitric Oxide production done by Md. Nazrul Islam and colleagues, it was found that garlic supplements had significantly increased NOs activity in smokers. By increasing the NOs activity there is pulmonary dilation which could lead to the antiasthmatic effect of garlic. [Islam MN et al. 2017 Jun; 11]

Ajwain

Ajwain (*Trachyspermum Ammi*) is a common ingredient of the Indian household that is used for variety of purposes including food preparation, condiments (masalas), traditional medicines for digestion or beneficial gastrointestinal properties. Ajwain has its natural odor and sharp tastes and that's why it is employed in India foods and dishes as a flavoring ingredient. The seeds of ajwain are used as flavoring agents in foods as preservatives, for the manufacture of vital oil in perfume industry [Hanif et al., 2021].

Pharmacological activities of ajwain:-

- **Antibacterial:-** the phytoconstituents of Ajwain showed antibacterial activities against many strains of *Klebsiella Pneumonia, Salmonella Typhi, Enterococcus Faecalis, Escherichia Coli, and Staphylococcus* bacteria .[Zaidi SF et al., 2009], [Hanif et al., 2021].

- **Antitussive action: -** Ajwain constituents have been reported to give antitussive action by the reduction of cough in old manuscripts, and even in a study it showed that conc of ajwain caused reduction in the formation of cough. [Boskabady MH et al., 2003] [Hanif et al., 2021].
- **Antioxidant: -** The Ajwain extract has been proven to show antioxidant property. It was evaluated by [Hanif et al., 2021] on hexachloro cyclohexane tempt oxidative stress. Which showed that the ajwain extract showed reduction in oxidative stress and thus reduce toxicity.
- **Bronchodilatory:**-The decoction of ajwain was evaluated in asthmatic patients for bronchodilatory effect and surprisingly it showed that ajwain constituents have bronchodilatory effect [Hanif et al., 2021].

METHODS AND PROCEDURES

Our study consists of the 4 steps in the preparation and evaluation of the Silver Nanoparticles of phytoconstituents of Ginger, Garlic and Ajwain

1. Extraction of Pharmaceutical Active constituents
   a. Extraction of Ginger
   b. Extraction of Garlic
   c. Extraction of Ajwain
2. Nano particle Encapsulation
   a. Silver Nitrate solution
   b. Microencapsulation technique
3. Centrifugation of the solution (Separation of Nano particles)
4. Observation of the Nanoparticles

1. Extraction of Pharmaceutical Active Constituents

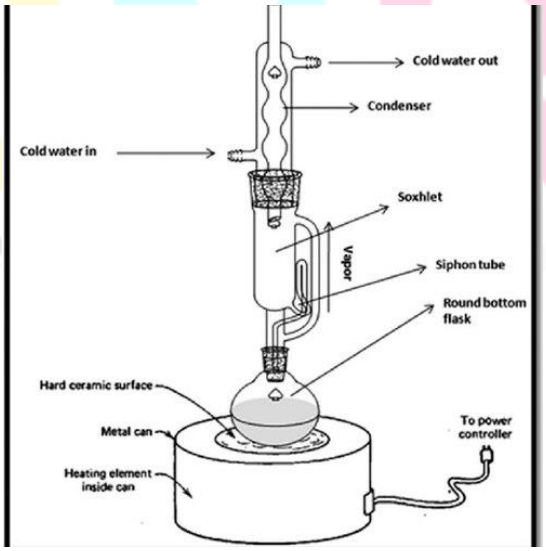


Fig 2. Soxhlet Apparatus Assembly[image- researchgate]

The extraction of the phytoconstituents of Zingiber Officinale, Trachyspermum Ammi and Alium Sativum has been performed by the use of Soxhlet apparatus.

- **Extraction of Ginger: -** Ginger extract was prepared by Soxhlet Apparatus after macerating ginger overnight.

  Procedure- Raw ginger rhizome 20g was crushed and soaked in 100ml ethanol for 24 hours. The next day Soxhlet extraction was carried out.

  - Place the ginger rhizome inside the vertical column of the Soxhlet apparatus with Steel wool and Filter paper.
  - Pour Ethanol 200ml over the rhizome carefully and it drips from the vertical column into the Round bottom flask.
  - Arrange the distillation tube and the heating panel as given in the standard.
  - Start the distillation water flow and then heating is started at 03:15 pm at 70°C.

Fig 3. Soxhlet Apparatus Assembly

  - The solvent (ethanol) evaporates and fills inside the vertical column by distillation and the rhizome is soaked again.
  - When the solvent reaches the level of the side tube, a flame is introduced below the small side tube.

- Then the solvent drips again inside the round bottom flask and 1st cycle completes.,
- This step is repeated 6 times again (total 7 times).
- And the timing for the completion of each cycle is given in the table below -

Day - 1	Started 3:15 pm
1st cycle	3:51 pm
2nd cycle	4:20 pm
Day - 2	Started 12:35 pm
3rd cycle	1:12 pm
4th cycle	1:44 pm
5th cycle	2:16 pm
6th cycle	2:51 pm
7th cycle	3:22 pm

Table no- 1: Extraction cycle of Ginger

- **Extraction of Garlic: -** Garlic extract was prepared by Soxhlet Apparatus after macerating garlic overnight.

  Procedure- Raw garlic bulb 20g was crushed and soaked in 50ml ethanol for 24 hours. The next day Soxhlet extraction was carried out.

  - Place the crushed garlic inside the vertical column of the Soxhlet apparatus with Steel wool and Filter paper.
  - Pour Ethanol 200ml over the garlic carefully and it drips from the vertical column into the Round bottom flask.
  - Arrange the distillation tube and the heating panel as given in the standard.
  - Start the distillation water flow and then heating is started at 02:10 pm at 70°C.
  - The solvent (ethanol) evaporates and fills inside the vertical column by distillation and the garlic is soaked again.
  - When the solvent reaches the level of the side tube, a flame is introduced below the small side tube.
  - Then the solvent drips again inside the round bottom flask and 1st cycle completes.
  - This step is repeated 6 times again (total 7 times).
  - And the timing for the completion of each cycle is given in the table below -

Day - 1	Started 2:10 pm
1st cycle	2:50 pm
2nd cycle	3:15 pm

3rd cycle	3:50 pm
4th cycle	4:22 pm
Day - 2	Started 12:58 pm
5th cycle	1:32 pm
6th cycle	1:58 pm
7th cycle	2:27 pm

Table no-2: Extraction cycle of Garlic

- **Extraction of Ajwain: -** Ajwain extract was prepared by Soxhlet Apparatus after macerating ajwain overnight.

  Procedure- Raw Ajwain seeds 20g was powered and soaked in 100ml methanol for 24 hours. The next day Soxhlet extraction was carried out.

  - Place the powered ajwain inside the vertical column of the Soxhlet apparatus with Steel wool and Filter paper.
  - Pour Methanol 200ml over the ajwain carefully and it drips from the vertical column into the Round bottom flask.
  - Arrange the distillation tube and the heating panel as given in the standard.
  - Start the distillation water flow and then heating is started at 1:53 pm at 70°C.
  - The solvent (methanol) evaporates and fills inside the vertical column by distillation and the ajwain is soaked again.
  - When the solvent reaches the level of the side tube, a flame is introduced below the small side tube.
  - Then the solvent drips again inside the round bottom flask and 1st cycle completes.
  - This step is repeated 6 times again (total 7 times).
  - And the timing for the completion of each cycle is given in the table below

Day – 1	Started 1:53 pm
1st cycle	2:25 pm
2nd cycle	3:00 pm
3rd cycle	3:30 pm
4th cycle	3:58 pm
Day – 2	Started 12:33 pm
5th cycle	1:00 pm

6th cycle	1:35 pm
7th cycle	2:09 pm

Table no-3: Extraction cycle of Ajwain

2. Nano Particle Encapsulation

- **Preparation of silver nitrate solution:-**

- Silver nitrate is taken in this research for encapsulating the phytoconstituents inside it.
- Silver nitrate is taken 75 mg and added water 80 ml and stir to dissolve.
- Make up the volume up to 100 ml by adding 20 ml water.
- Store the solution in a beaker and cover it with silver foil.

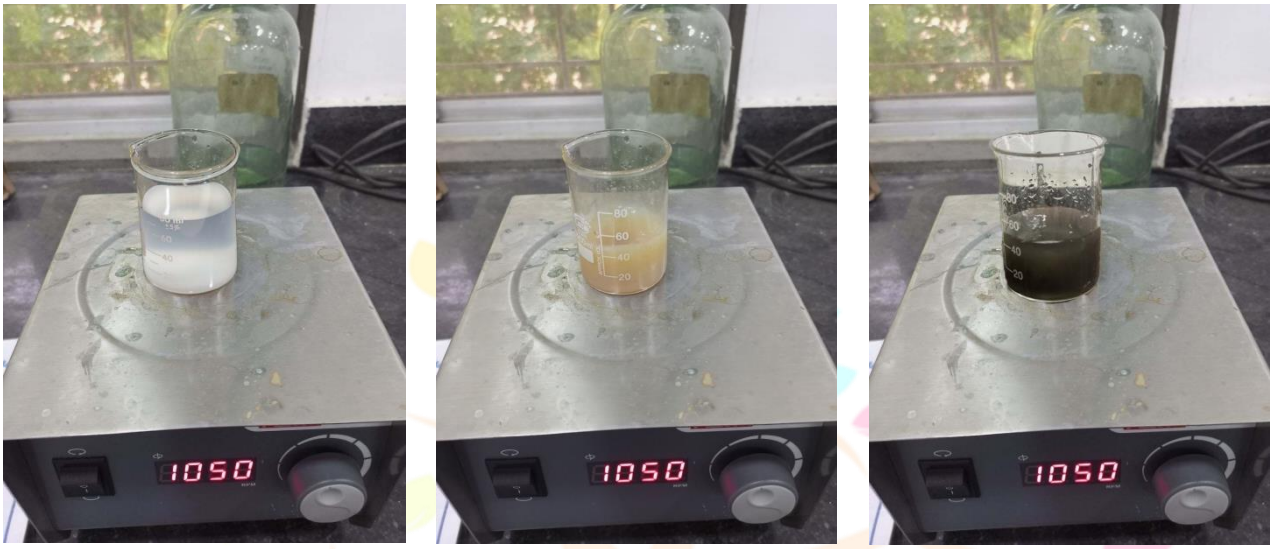
- **Microencapsulation technique:-**

Microencapsulation is a technique in which the phytoconstituents or the drug molecules are entrapped in a polymer or metal layer. This metal or polymer covers the drug and enhances its capability of absorption, penetration and effectiveness. The encapsulating technique also lets us to specifically target drug molecules to the site of desired action.

Fig 4. Magnetic Stirrer

- Take the Ginger extract 6 ml , Garlic extract 6 ml and Ajwain Extract 6 ml
- Mix the 3 extracts well in a Magnetic Stirrer
- Add Silver nitrate solution 25 ml in which the phytoconstituents will be encapsulated .

- Set the magnetic stirrer at 1000 rpm and start.
- After sometime the phytoconstituents start to get encapsulated and color of the solution starts to change.
- When the constituents start to form particles the magnetic stirrer is stopped and solution is well stored.



(a) **(b)** **(c)**

Fig 5- (a): Silver Nitrate solution without any added constituents

(b): solution on $AgNO_3$ and added Extracts of GGA phytoconstituents

(c): change in colour as a result of encapsulation (Indicates Formation of AgNPs)

Fig 6 :- Mixing of the GGA Extracts to silver nitrate solution

3. Centrifugation of the Solution

Fig 7:- Centrifuge (Centrifugation apparatus)

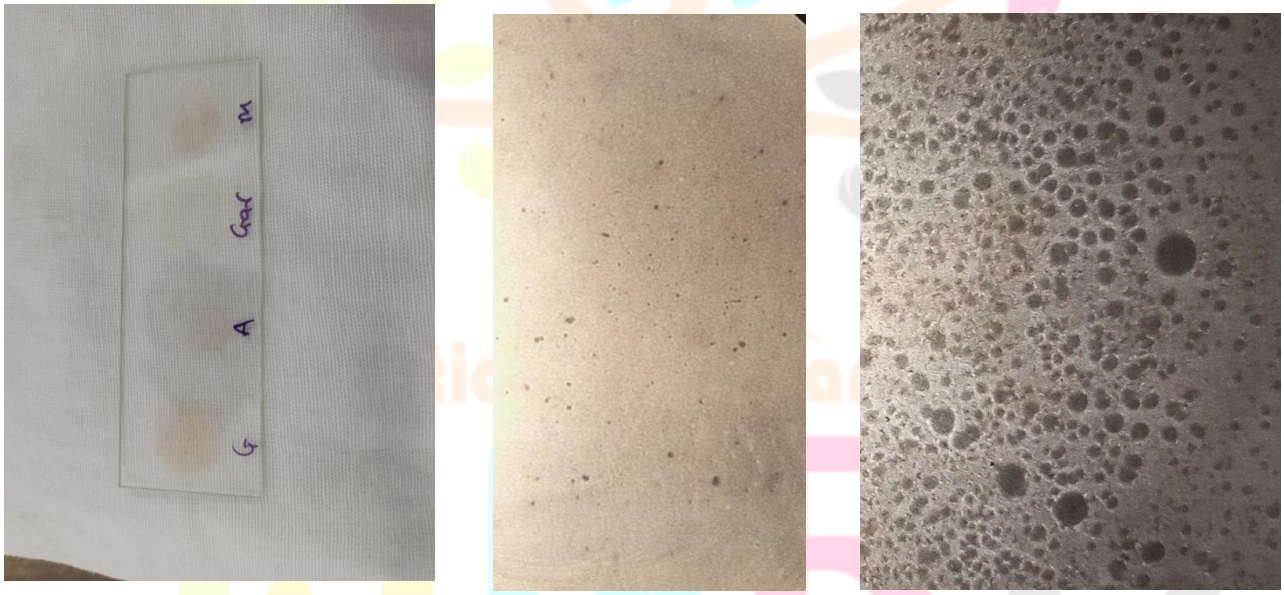
- The mixture (extracts +AgNO3 solution) is put into 4 tubes.
- All the tubes are placed inside the Centrifugation chamber.
- The Centrifugation apparatus is set at 2000 rpm for 5 minutes.
- After several minutes the tubes are removed
- We can observe particles settled at the bottom of the tubes.
- The tubes are well stored.

4. Observation Of the Nano Particles

To observe the particles formed in the solution:-

- Take a clean dry slide, free of dust particles or fingerprints.
- Place a drop of solution over the slide and spread carefully.
- Let the slide dry.
- Place the slide over the observing panel in the Projection Microscope.
- Set the slide above the light and switch ON the lights of the microscope.
- Carefully set the objective lens according to the required observation.
- Observe the image for the presence of nanoparticles.
- Look for the shape and structure of the particles.
- We saw that the nanoparticles formed were of spherical shaped encapsulating the drug inside them.

Fig 8:- Projection Microscope



(a) **(b)** **(c)**

Fig 9 :- (a)- slide prepared to observed the formed AgNPs under projection microscope

(b) & (c)- Observed AgNPs of the GGA phytoconstituents.

EVALUATION OF SILVER NANOPARTICLES FOR PHARMACOLOGICAL ACTIVITY

The evaluation of the nanoparticles was performed in the Pinnacle Biomedical Research Institute (PBRI) Bhopal M.P. , as suggested by our professor Dr. Ashish Garg Sir, where the drug samples (Ginger, Garlic, and Ajwain Nanoparticles) were evaluated for their *in vitro* pharmacological activity in 3 aspects:-

1. *Anti-inflammatory* activity
2. *Anti-asthmatic* activity
3. *Anticancer* activity

1. In vitro Anti inflammatory activity protocol

Method: - Denaturation of Protein

Denaturation is a process where the main structure of the protein is deformed/ degraded by the interaction with strong acid or strong base or when the atmospheric conditions are viable for the protein to be denatured. The protein denaturation with slight modifications was performed as described by Elisa et al., 1988. The procedure starts with the treatment of the sample (AgNPs) at different concentration (100-500 µg/ml) to the Egg Albumin solution which was then incubated at 27+/-1°C for 15 minutes. After this, denaturation of protein was induced by keeping the solution in a hot water bath at temperature 70°C for 10 minutes. The solution were taken out of the water bath and cooled, the turbidity of the solutions were measured in spectrophotometer at 600nm. This was compared with the normal untreated group in which also denaturation induced at same conditions. So this comparison tells us about the inhibition of denaturation of egg albumin by the AgNPs sample. This is a method for the measurement of anti-inflammatory of the AgNPs sample.

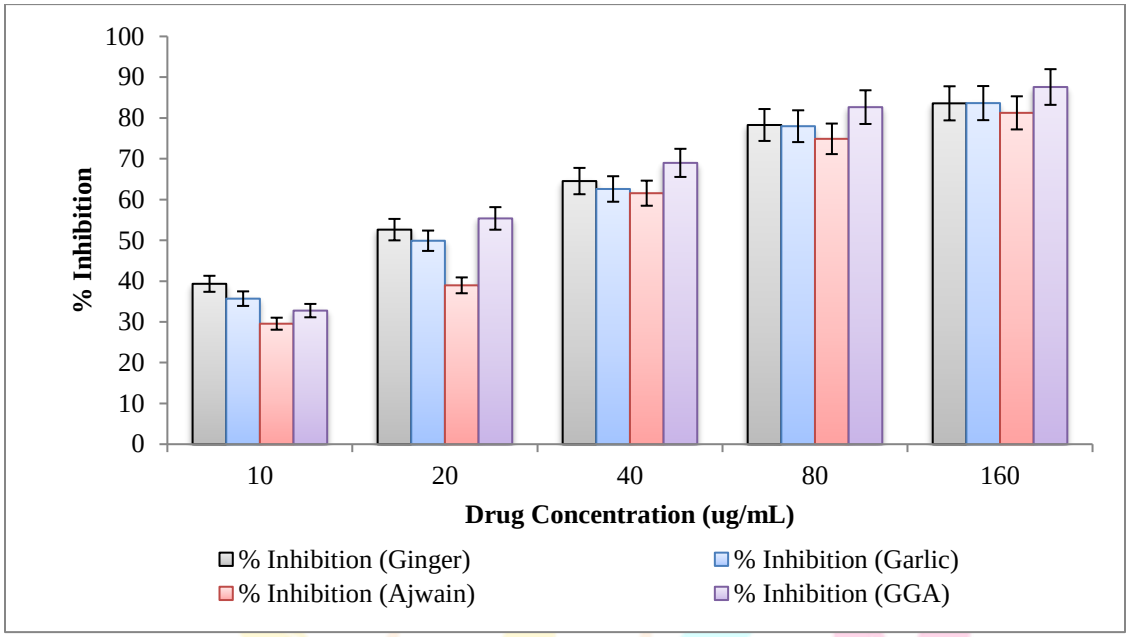
The membrane stabilization or inhibition of denaturation is calculated by-

%inhibition of denaturation= 100X(A1-A2/A1)

Protein Denaturation

Concentration (µg/mL)	%Inhibition (Ginger)	%Inhibition (Garlic)	%Inhibition (Ajwain)	%Inhibition (GGA)
10	39.331	35.689	29.547	32.758
20	52.614	49.889	38.958	55.365
40	64.527	62.584	61.549	68.985
80	78.279	77.982	74.884	82.654
160	83.588	83.647	81.251	87.589

Table no-4: *In vitro* anti-inflammatory activity



Graph no 1- *In vitro* anti-inflammatory activity

1. *In vitro* Anti-Asthmatic activity protocol

Preparation of Tracheal Chain from Goat Trachea

The in vitro activity against asthma of the Ag Nanoparticles was evaluated in the Tracheal Chain preparation of Goat which was formed by collecting the trachea of goat immediately after slaughtering. This trachea was immersed in cold Kreb's Solution. Which contains the following ingredients:- (Sodium chloride-114.0mM, Calcium chloride-2.5mM, Glucose- 11.7mM, Sodium Bicarbonate- 25mM, Magnesium Chloride- 1.2mM, Potassium dihydrogen Phosphate- 1.2mM).

Then cut into several strips (12 rings) of same width and they were joined with a silk thread.

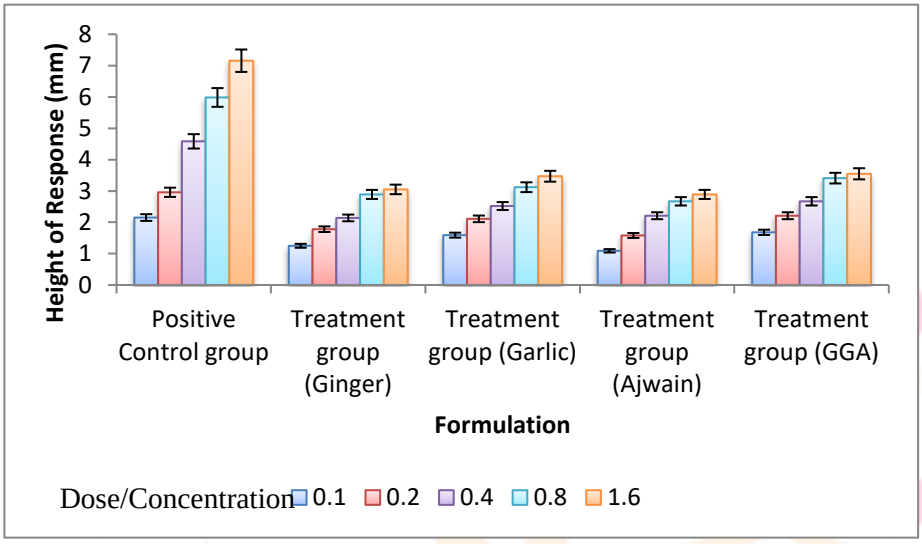
These strips were contained in organ tube which was filled with kreb's solution and temp 37+/- 0.5 °c was maintained along with bubble bath. These tissues were allowed to stay for 30 minutes. Then treated with Histamine (50 µg/ml) to check the height of response the tissues give with doses- 0.1ml, 0.2 ml, 0.4ml, 0.8 ml and 1.6ml & then the response before and after the treatment of histamine was checked.

These rings were treated with the Nanoparticles against positive control group, then after treating with histamine, the response was noted. The less the height of response the more is the antagonistic activity against histamine.

S.No.	Dose of Histamine (100µg/ml)	Height of response(mm)				
		Positive Control group	Treatment group (Ginger)	Treatment group (Garlic)	Treatment group (Ajwain)	Treatment group (GGA)
1	0.1	2.15	1.25	1.59	1.09	1.68
2	0.2	2.95	1.78	2.11	1.58	2.21

3	**0.4**	4.58	2.14	2.52	2.21	2.67
4	**0.8**	5.98	2.89	3.12	2.67	3.41
5	**1.6**	7.15	3.05	3.47	2.89	3.548

Table no-5: *In vitro* anti-asthmatic activity



Graph no 2- in vitro anti-asthmatic activity

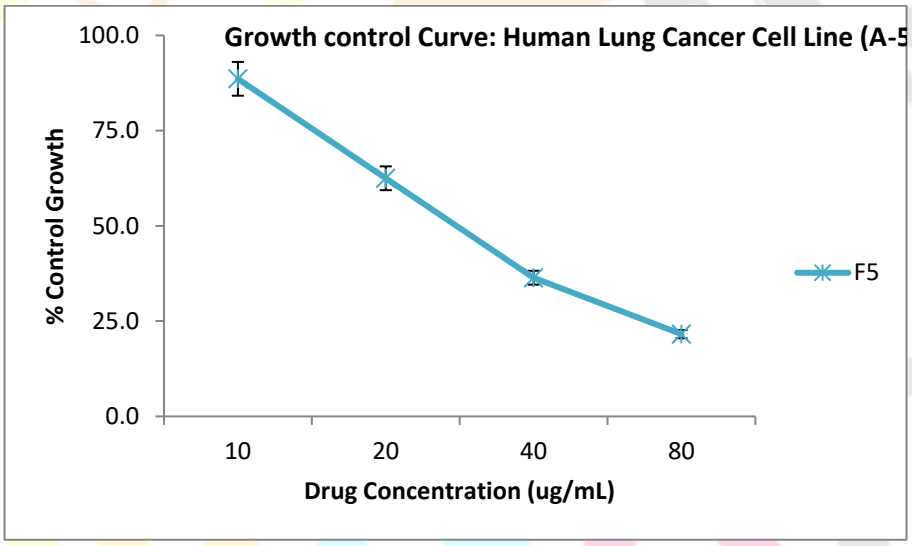
2. *In vitro* Anti-Cancer activity protocol

SRB(Sulforhodamine B) method

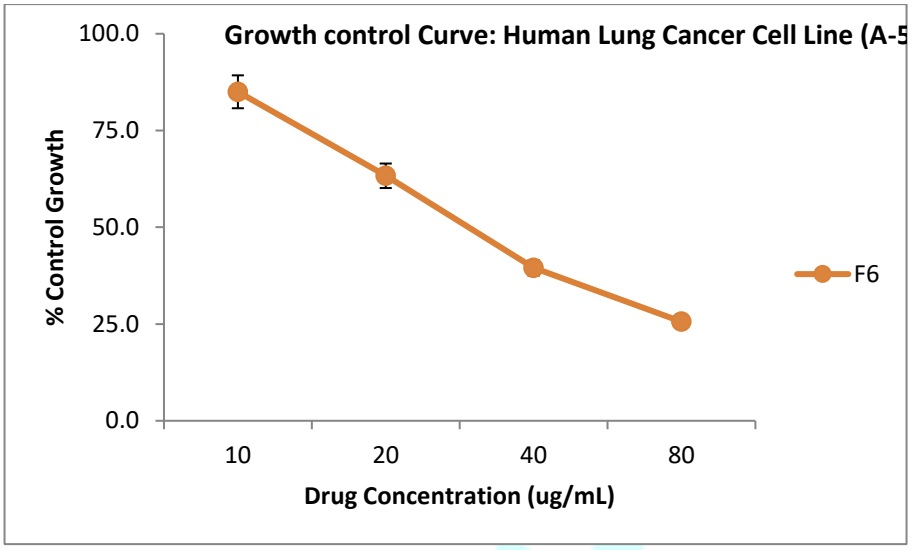
Sulforhodamine B assay is a type of in vitro evaluation in which the cytotoxic potential of chemical substances is evaluated. It is a reliable, fast and very sensitive process. This method is adequate for evaluation of anti-cancer activity of test substances in a laboratory scale as well as in large scale processes. It depends upon the evaluation of chemical substances in a 96 well plates containing suspension culture media. The desired human lung cancer cell was cultured in a flask containing a tissue culture media of a good atmospheric condition required for the cell growth (that is temperature 37°C, relative humidity 5%& CO_2 90%) . Then after this process the sample containing AgNPs solution was added to this cell culture at different concentration and incubated for 24 hours. The conc. used were- 10 µg/ml, 20µg/ml, 40µg/ml, and 80µg/ml. Then 24 hrs later the plates were removed from the incubator and treated with cold solution of TCA- tri chloro acetic acid at concentration of 50µl, 50% in the plates in order to stop the reaction. These treated plates were again incubated for 1hr at 4°C for fixing the cells at the bottom of the plates. These plates were washed many times and air dried. Then the plates were dyed with 100µl solution of dye in 0.4% acetic acid solution,

and a buffer solution100µl(10.5M) was added in each well and shaked in mechanical shaker 20 minutes. Enzyme linked Immuno Sorbent Essay (ELISA) was used for the determination of recording of the optical density of cell before and after the cell inhibition process at 540nm wavelength (Skehan et al., 1990;Masters,2000)

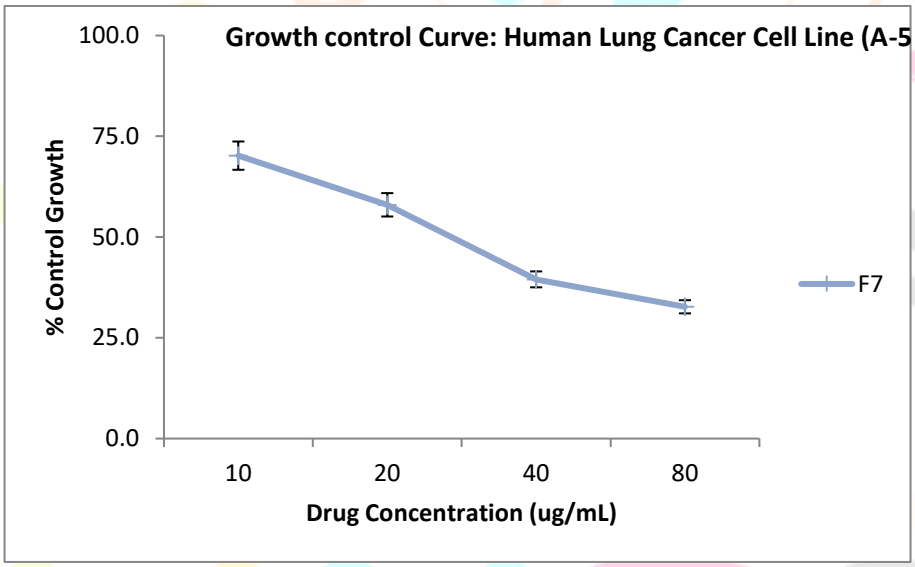
The in vitro cyto toxic characteristics of the silver Nanoparticles were tested in lung cancer cell line A549 by this assay. The assay performed showed the dose dependent cytotoxicity of the lung cancer cells, the decrease in the bioavailability of the cancer cells increases with the increase in the Ag Nanoparticles. The results of the SRB assay are given in the graphs (Graph no 3,4,5 & 6). The Nanoparticles concentration between 10- 80 micro gm/ml showed a greater amount of cytotoxicity than any other concentration. Thus it was found out that the Ag Nanoparticles showed high effect against the cancer cells of lung.



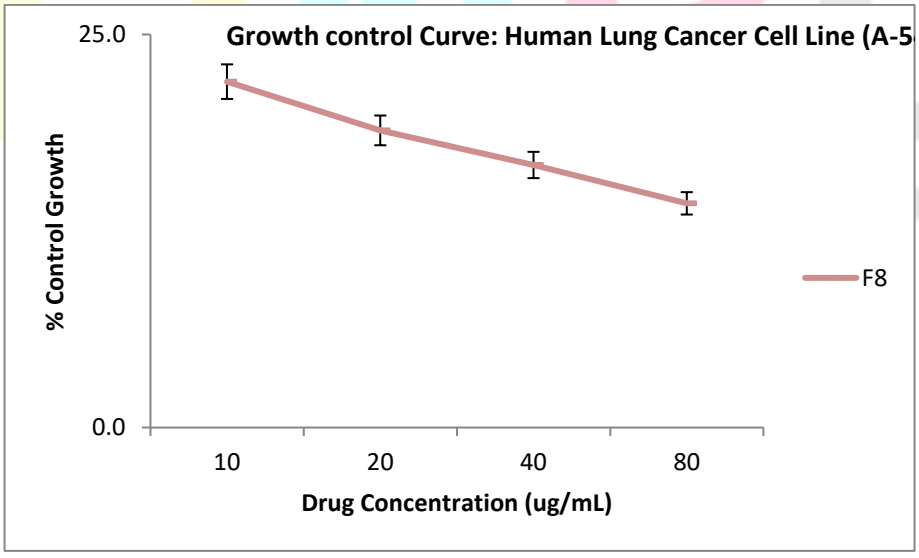
Graph no 3- *In vitro* anticancer activity of Ginger AgNPs



Graph no 4- ***In vitro*** **anticancer activity of Garlic AgNPs**



Graph no 5- ***In vitro*** **anticancer activity of Ajwain AgNPs**



Graph no 6- ***In vitro*** **anticancer activity of GGA mixed NPS**

Sulforhodamine B assay is a type of in vitro evaluation in which the cytotoxic potential of chemical substances is evaluated. It is an economic, fast and very sensitive process. This method is adequate for evaluation of anti-cancer activity of test substances in a laboratory scale as well as in large scale processes. It depends upon the evaluation of substances in a 96 well plates containing suspension culture media. The in vitro cyto toxic characteristics of the silver Nanoparticles were tested in lung cancer cell line A549 by this assay. The assay performed showed the dose dependent cytotoxicity of the lung cancer cells, the decrease in the bioavailability of the cancer cells increases with the increase in the Ag Nanoparticles. The results of the SRB assay are given in the graphs (Graph no 3,4,5 & 6). The Nanoparticles concentration between 10- 80 micro gm/ml showed a greater amount of cytotoxicity than any other concentration. Thus it was found out that the Ag Nanoparticles showed high effect against the cancer cells of lung.

DISCUSSION:-

The silver nanoparticles of our 3 active plant extracts were evaluated in 4 sets-

1. *Zingiber Officinale* Ag nanoparticles
2. *Alium Sativum* Ag nanoparticles
3. *Trachyspermum Ammi* Ag nanoparticles
4. *GGA (Ginger, garlic Ajwain)*mixed Ag Nanoparticles

These 4 Ag NPs were evaluated for 3 pharmacological activities namely:-

- *In vitro* Anti-inflammatory
- *In vitro* anti-Asthmatic Activity
- *In vitro* Anti-cancer Activity

- ***In vitro* Anti-inflammatory: -**

The *invitro A*nti-Inflammatory evaluation was carried out by the method of protein denaturation as explained by [Elias et al., 1988]. In this protocol the protein of egg albumin was used and the % of protein inhibition of 4 Ag nanoparticles set was measured.

Ginger Ag nanoparticles showed good results of inhibition for 10, 20, 40, 80, 160 µg/ml showing inhibition from 39.68%, 52.61%, 64.52%, 78.27%, up to 83.58%, respectively on increasing the concentration of NPs addition.[refer table no 4 & graph-1]

Garlic Ag nanoparticles showed good results of inhibition, for 10, 20, 40, 80, 160 µg/ml showing inhibition from 35.68%, 49.88%, 62.58%, 77.98%, up to 83.64% respectively on increasing the concentration of NPs addition.[refer table no 4 & graph-1]

Ajwain Ag nanoparticles showed good results of inhibition, for 10, 20, 40, 80, 160 µg/ml showing inhibition from 29.54%, 38.95%, 61.54%, 74.88%, and 81.25% respectively [table no 4 & graph-1]

And as for the activity of GGA mixed Ag nanoparticles showed inhibition, for 10, 20, 40, 80, 160 µg/ml showing inhibition from 32.75%, 55.36%, 68.98%, 82.65%, 87.58% respectively. [refer table no 4 & graph-1]

As we can observe from the evaluation results that all the AgNPs showed distinct protein denaturation activity. And the protein denaturation activities of the mixed GGA Ag NPs are more than the other nanoparticles set. Thus, showing high *in vitro* anti- inflammatory activity.

- ***In vitro* Anti-Asthmatic Activity:-**

The in vitro activity against asthma of the Ag Nanoparticles was evaluated in the Tracheal Chain preparation of Goat which was formed by collecting the trachea of goat immediately after slaughtering. This trachea was immersed in Kreb's Solution. Then cut into several strips (rings). These rings were treated with the Nanoparticles against positive control group, then after treating with histamine, the response was noted. The less the height of response the more is the antagonistic activity against histamine.

The response of positive control group for dose of histamine 10, 20, 40, 80, 160 µg/ml was 2.15 mm, 2.95mm, 4.58mm, 5.98mm, and 7.15mm respectively.[refer table no- 5, graph-2]

The Ginger Ag Nanoparticles showed the height of response for the same dose of histamine as 1.25mm, 1.78mm, 2.14mm, 2.89mm, and 3.05mm respectively.

The Garlic Ag NPs showed response for dose of histamine 10, 20, 40, 80, 160 µg/ml as 1.59mm, 2.11mm, 2.52mm, 3.12mm, and 3.47mm respectively.

The Ajwain Ag NPs showed response for dose of histamine 10, 20, 40, 80, 160 µg/ml as 1.09mm, 1.58mm, 2.21mm, 2.67mm, and 2.89mm respectively.

The GGA mixed Ag NPs showed the response for the same of histamine as 1.68mm, 2.21mm, 2.67mm, 3.41mm, and 3.54mm respectively.

As we can observe from the evaluated data that the height of response for the Ag NPs are comparatively less than the positive control group thereby showing comparative reduction in inflammation.

- ***In vitro* Anti-cancer Activity:-**

The in vitro anticancer study of the GGA AgNPs were performed in Lung Cancer cell line A549 through Sulforhodamine B Assay(SRB Assay). SRB Assay is a swift economic study for the evaluation of Cytotoxic Potential of various compounds. A549 was developed and cultured in a flask containing the tissue culture media at temp 37^0 c and relative humidity (CO_2 5%) was set. Then in a cell suspension containing 10,000 cells per 100µL was formed and poured in 96 well plates. To this he AgNPs were added at different concentration of 10, 20, 40, 80µg/mL.

The Ginger AgNPs showed cytotoxic effect and decline in the cancer cell growth from 85% to the level of 25% with increase in the concentration of added AgNPs- 10, 20, 40, 80 µg/mL. (refer graph no 3).

The Garlic AgNPs showed in vitro anticancer activity and decline in the cancer cell growth from 85% to the level of 25% with increase in the concentration of added AgNPs- 10, 20, 40, 80 µg/mL. (refer graph no 4).

The Ajwain AgNPs showed cytotoxic effect and decline in the cancer cell growth from 75% to the level of 30% with increase in the concentration of added AgNPs- 10, 20, 40, 80 µg/mL. (refer graph no 5).

The GGA AgNPs showed in vitro anticancer activity and decline in the cancer cell growth from 25% with increase in the concentration of added AgNPs- 10, 20, 40, 80 µg/mL. (refer graph no 6).

From the evaluated data it is clear that all the AgNPs showed cytotoxic effect/ anticancer effect by the cellular growth reduction. And the Ginger and Garlic AgNPs are found to have maximum anticancer activity than other AgNPs.

CONCLUSION:-

The nanoparticles are the novel drug delivery approach that are found to be beneficial in the delivery of drugs to the site of action, in terms of Targeting, Onset, Enhanced Absorption, and improved Bioavailability. We evaluated the phytoconstituents of 3 medicinal herbs- *Trachyspermum Ammi (ajwain), Zingiber Officinale (Ginger)s, and Alium Sativum(Garlic)* These herbs are effective for the treatment of many diseases and often found in the Indian kitchen for culinary purposes. The GGA nanoparticles are found to be useful for the treatment of cancer by the reduction of percent of cancerous cells. GGA NPs are found to be effective for the reduction of inflammation of the Goat tracheal chain as carried out in the above evaluation, and it was also seen that GGA NPs showed the reduction of asthmatic activity by the reduction of Histamine Activity. Although many further studies need to be done as the exact mechanism of action of the 3 constituents are still under discussion. In our study we conclude that the GGA nanoparticles are very effective for many pharmacological treatments and can be useful for cancer treatment, asthma treatment and anti-inflammatory action.

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