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Evaluation of Antibacterial Potential & Phytochemical Screening by the Medicinal Plant of *Acorus Calamus & Agaricus Bisporus* & Their Synthesis of Herbal Silver Nanoparticles with Different Solvents

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Abstract:- *Agaricus bisporus* and *Acorus calamus* are used medicinally and ayurvedic medicines. The present study was aimed to carry out for preliminary phytochemical screening and evaluate in vitro antibacterial potential of various solvent extracts. The synthesis, characterization and application of biologically synthesized nanoparticles have become an important branch of nanotechnology. Various plant materials have the potential in synthesizing silver nanoparticles thus providing an alternative to the conventional chemicals methods. Antibacterial activity of *Acorus calamus* rhizomes & *Agaricus bisporus* extracts obtained with methanol and aqueous solvent was evaluated in which found to be much greater in methanol extracts as compared to aqueous and the activity of the synthesized silvernanoparticles was more efficient and more zone of inhibition as compared to medicinal plants. The phytochemical analysis of *Acorus calamus plant & Agaricus bisporus* disclosed the presence of major phytoconstituents viz., alkaloids, steroids, phenols, flavonoids. As similar way the major presence of phytoconstituents in silver nano extract in steroids, alkaloids, inulin, glyocsides & saponin. Silver nano extracts of plants showed a maximum phytoconstituents in phytochemical screening test.

Key Words: Phytochemical Screening, Antibacterial Activity, Acorus calamus, Agaricus bisporus and Silver nanoparticles.

1. INTRODUCTION

Herbal plants are used in a world wide. Medicinal plant are easily found and has a huge variety of natural chemistry for their therapeutic properties & uses. Mostly medicines i.,e alleopathic, ayurvedic & homoepathic of the past were extracted from plants. Medicinal plants possess secondary metabolites which are the main sources of medicinal drugs having curative nature. 7500 species are being used as medicinal plants in India [1]. *Acorus calamus* family Araceae is a well known plant in Indian traditional medicines [2] for centuries. *Acorus calamus*, is a semi aquatic perennial, aromatic herb with creeping rhizomes [3] which has been used traditionally as a medicine and also the powder of rhizome has spicy flavor in it. Edible mushrooms are important source of biologically active compounds [4]. Edible mushrooms are used medicinally for diseases involving depressed immune function, cancer, allergies, fungal infection, frequent flu and colds, bronchial inflammation, heart disease, hypertension, infectious disease, diabetes and hepatitis [5]. Most attention has been paid to the investigation of natural drugs from various edible mushrooms. It is quite interesting that silver nanoparticles can also be synthesized from plants [6,7,8]. The development of reliable green process for the synthesis of silver nanoparticles is an important aspect of current nano biotechnology research. Biosynthesis of nanoparticles from green synthesis is advantageous over chemical and physical methods as it is a cost effective and environmental friendly method and it is not necessary to use high pressure, energy, temperature and toxic chemicals. Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganism isolation and culture media enhancing the cost competitive feasibility over nanoparticle synthesis by microorganisms. In this work, we report the biosynthesis of silver nanoparticles using a plant and mushroom extract of *Acorus calamus* (Vach) & *Agaricus bisporus* (Button Mushroom). Their antimicrobial activity has also been reported against gram positive and gram negative bacteria.

2. MATERIAL & METHODS

2.1. Collection of Plant Materials

Materials used in the present study were fruiting bodies of *Agaricus bisporus* procured from the local market and *Acorus calamus* procured from TERI, Gurugram, Haryana, India.

Collected samples were identified and authenticated by the taxonomist of the same department.

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Fig. 1 Fresh *Acorus calamus & Agaricus bisporus*

2.2. Bacterial Strains

The bacterial cells of *Escherichia coli* (DH5α)(ATCC No. 68368), *Bacillus amyloliquefaciens*, (ATCC No. 23842), *Staphylococcus aureus* (ATCC No. 25923), and *Pseudomonas aeruginosa* (ATCC No. 27853), were obtained from Helix BioGenesis Pvt. Ltd., Noida, U.P., India. They were sub cultured freshly in Nutrient Broth and used further for research work.

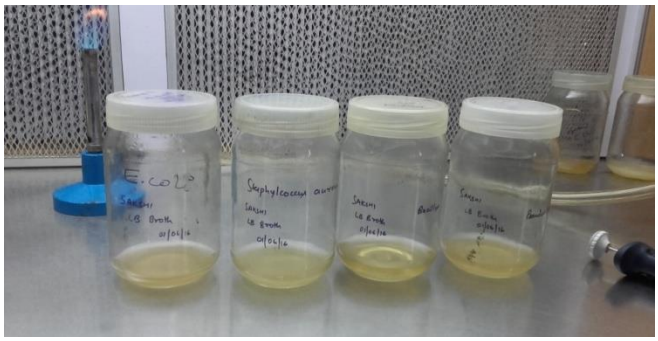

Fig. 2 Bacterial strains i.e., *Escherichia coli*, *Bacillus amyloliquefaciens*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

2.3. Sample preparation

Plant samples were washed, dried and crushed by an ordinary grinder (Philips HL 7720) to make fine powder. The 50g of dried rhizome of *A.calamus* and *A.bisporus* was weighed in weighing balance (High Precision Balance) and placed in a Soxhlet extractor [9] containing 800ml of methanol and pouch of extracts for 24h to 48h (22 cycles). Then obtained methanol extracts was evaporated to dryness and extract amount was measured. Extract was stored for the further use.

The 50g of rhizome crushed powder was dissolved in 500ml of distilled water to form crude extract by maceration method by boiling continuously for 30minutes in water bath (Thermo Scientific). The conical flasks of the extracts were covered by cotton plugs to avoid the evaporation. The extracts were placed in shaking incubator at 250rpm for 48h. After shaking they were filtered with muslin cloth and with filter paper twice. Prepared crude extracts were evaporated to dryness amount was measured [10] and was stored at 4°C [11].

Fig. 3 Dried form of *Acorus calamus & Agaricus bisporus* for the preparation of extracts

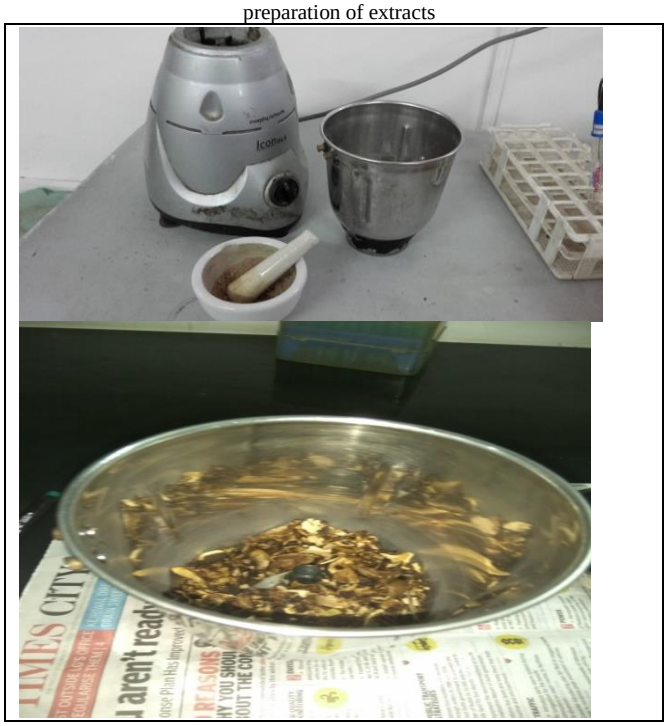

Fig. 4 Crushed form of *Acorus calamus & Agaricus bisporus* in grinder

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Fig. 5 Soxhlet extractor for methanol extraction

Fig. 6 Maceration method for aqueous extraction

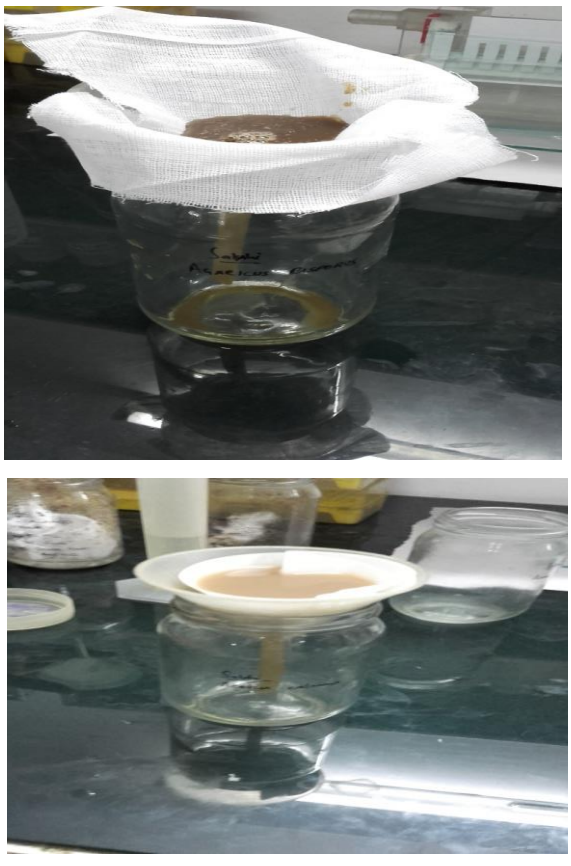

Fig. 7 The filtration of extract prepared from aqueous *Acorus calamus* & *Agaricus bisporus*

Fig. 8 The Preparation form of aqueous extract for dryness

3. ANTIBACTERIAL TEST

3.1. Agar well diffusion method

Muller Hinton Agar media were prepared and autoclaved at 121°C for 15 minutes at 15 lbs and poured in sterile petri plates up to a uniform thickness of approximately 10-15 minutes and the agar was allowed to set at ambient temperature. This method is suitable for the organism to grow rapidly overnight at 35-37°C. The wells were made in medium after inoculation with the microorganism. 200µl of inoculums were spread over Muller Hinton Agar plates using sterile spreader, after few minutes four wells were made in each petri plated and loaded with different concentration of plant extracts with control. Plates were incubated at 37°C for 24 h. Antibacterial activity was observed by measuring its inhibition length. Inhibition length against bacteria was calculated [12].

The experiments were done in triplicate.

Inhibition length = Zone of Inhibition (mm) – Well diameter (mm)

3.2. Phytochemical analysis of different crude extract

Extract were tested for the presence of active principle such as steroid, tannins, phenols, flavonoid, alkaloids, glycoside, triterpinoids, carbohydrates and proteins. Following standard procedures were used [13].

3.3. Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles (AgNPs), 50ml stock solution of 1mm $AgNO_3$ was prepared in distilled water. 1ml of aqueous extract of *A.calamus* and *A.bisporus* each was added to 9ml of stock solution of $AgNO_3$ in conical flask. The mixture was stirred continuously for 5-10 minutes and then incubated in dark room, at 37ºC under static condition. Suitable controls were maintained throughout the experiment. Reduction of silver nitrate to silver ions was confirmed by the color changes to light yellow color to brown color. Simultaneously, the positive control was maintained with the extract and de-ionized water used as negative control containing only silver nitrate solution [14].

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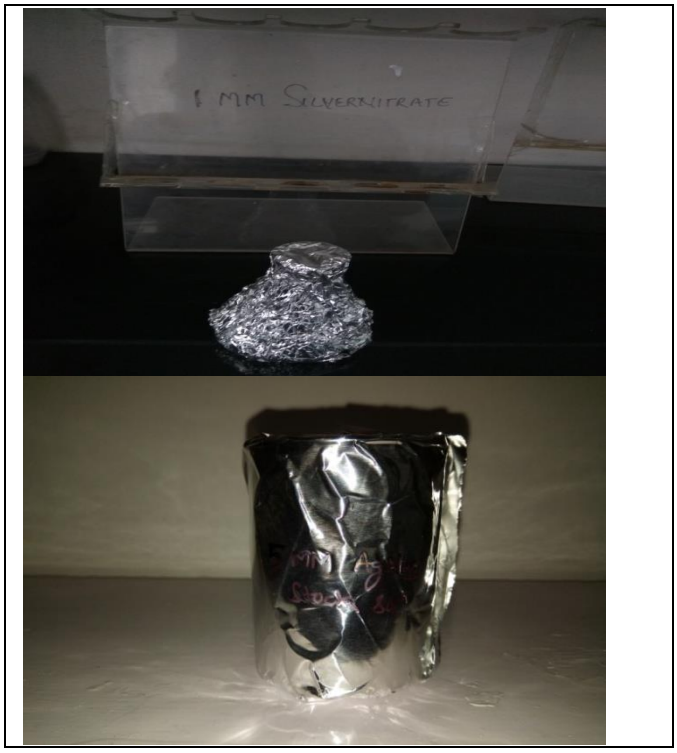

Fig. 9 The synthesis of AgNP's of *Acorus calamus* & *Agaricus bisporus*

3.4. Characterization:

UV-Visible spectra analysis: The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles [15]. UV-Visible absorbtion spectrophotometer with a resolution of 1nm between 300 to 700nm was used [16]. The reduction of pure Ag+ions was monitored by measuring the UV-Vis spectrum of the reaction medium after diluting small aliquot of the sample into deionized water. 1ml of the sample was pipetted into test tube and diluted with 4ml of deionized water and subsequently analyzed at room temperature, UV-Vis spectral analysis was done by using UV-Vis spectrophotometer 117 by recording the absorbance from 200-700nm and the strong Plasmon absorbance band was observed at 420-430nm in positive mushroom samples and sweet flag rhizomes indicating the production of silver nanoparticles from the extracts [17].

4. RESULTS & DISCUSSION

4.1. Antibacterial Screening test by Agar Well Diffusion Method

Morchella esculenta and *Ganoderma lucidum* [25], have been reported against *S.aureus* and *E.coli* [26] have reported that extract of *Clavaria vermicularis* and *Marasmium oreades* offered more inhibition to gram-negative bacteria (*E.coli* and *Pseudomonas aeruginosa*) as compared to gram-positive bacteria (*Bacillus sutilis* and *Staphylococcus aureus*). [27] also reported the antibacterial

Plants are a source of large amount of drugs which are claimed to possess the antibiotic properties in the traditional system. These plants have some kind of secondary metabolites that are responsible for their antibacterial, antifungal, antiulcer, antifeedant, repellent, and pesticidal properties and thus treat large number of diseases.

The antibacterial activity of different extracts of rhizomes of the *Acorus calamus plant & Agaricus bisporus* by agar well diffusion method with different concentration. Inhibition length was calculated in order to reveal its inhibitory effect against two gram positive (*Bacillus amyloiquefaciens, Staphylococcus aureus*) and two gram negative bacteria *(Escherichia coli, Pseudomonas aeruginosa*) and its antibacterial activity (Table 1 & 2).

Extracts prepared from methanol has shows maximum antimicrobial sensitivity against *S.aureus* i.e. 16mm (200mg/ml) zone of inhibition & 24mm (400mg/ml) (Table 2A) as compared to the aqueous extract which gave minimum activity against *E.coli* i.e. 15mm (200mg/ml) zone of inhibition & 16mm (400mg/ml) in *Acorus calamus* (Table 1A) whereas no zone of inhibition against for *Pseudomonas aeruginosa* & *Bacillus amyloiquefaciens*. Our results supports the finding of [18]. The zone of inhibition find out by them against E.coli. previously [19] on antimicrobial activities of *A.calamus* had reported lack of antibacterial activity while [20] have observe very less antibacterial activity in his study on antimicrobial properties of *A.calamus* rhizome.

Extract prepared from aqueous gave minimum activity 15mm (200mg/ml) & 16mm (400 mg/ml) zone of inhibition against *S.aureus* in *Agaricus bisporus* (Table 1B), whereas *B.amyloliquifaceins* has shown the maximum percentage of inhibition i.e., 16mm (200mg/ml) & 20mm (400mg/ml) (Table 2B) resepectively. *Agaricus bisporus* extract prepared from methanol has shown the maximum percentage of inhibition against *Pseudomonas aeruginosa* i.e., 30mm (200mg/ml) & 35mm (400mg/ml).whereas minimum activity shown for *E.coli & S.aureus*. The results of the present study are in agreement with the work of the earlier workers [21, 22], who have also reported strong antibacterial activity of methanol extract of *G.lucidum* against gram negative bacteria (*E.coli*) and comparatively less activity against gram-positive (*S.aureus*) bacteria. Similar trend in antibacterial activity of methanol extract of *Lactarius delicious* [23], *Sparassis crispa* [24],

potential of ethanol extract of *Pleurotus florida* and *Pleurotus ostreatus.*

Overall studies of antibacterial activity that methanol is quite effective against *S.aureus & P.aeruginosa* maximum zone of inhibition has shown the antibacterial activity is present in both plant of extracts. It will help to evaluate the medicinal & antibiotic properties with the help of plant extracts.

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Table 1. Antibacterial activity of aqueous extracts of *Acorus calamus* & *Agaricus bisporus*

Table 1A.

Bacterial Strain	Aqueous Extract of *Acorus calamus*					
	200mg/ml			400mg/ml		
	Well Diameter (mm)	Zone of Inhibition (mm)	Inhibition Length (mm)	Well Diameter (mm)	Zone of Inhibition (mm)	Inhibition Length (mm)
E.coli	9mm	15mm	6mm	9mm	16mm	7mm
S.aureus	9mm	17mm	8mm	9mm	19mm	10mm
Pseudomonas aeroginosa	9mm	NI	NI	9mm	NI	NI
Bacillus amyloliquefacien	9mm	NI	NI	9mm	NI	NI

Table : 1B

Bacterial Strain	Aqueous Extract of *Agaricus bisporus*					
	200mg/ml			400mg/ml		
	Well Diameter(mm)	Zone of Inhibition (mm)	Inhibition Length (mm)	Well Diameter (mm)	Zone of Inhibition (mm)	Inhibition Length (mm)
E.coli	9mm	NI	NI	9mm	NI	NI
S.aureus	9mm	15mm	6mm	9mm	16mm	7mm
Pseudomonas aeroginosa	9mm	NI	NI	9mm	NI	NI
Bacillus amyloliquefacien	9mm	16mm	7mm	9mm	20mm	11mm

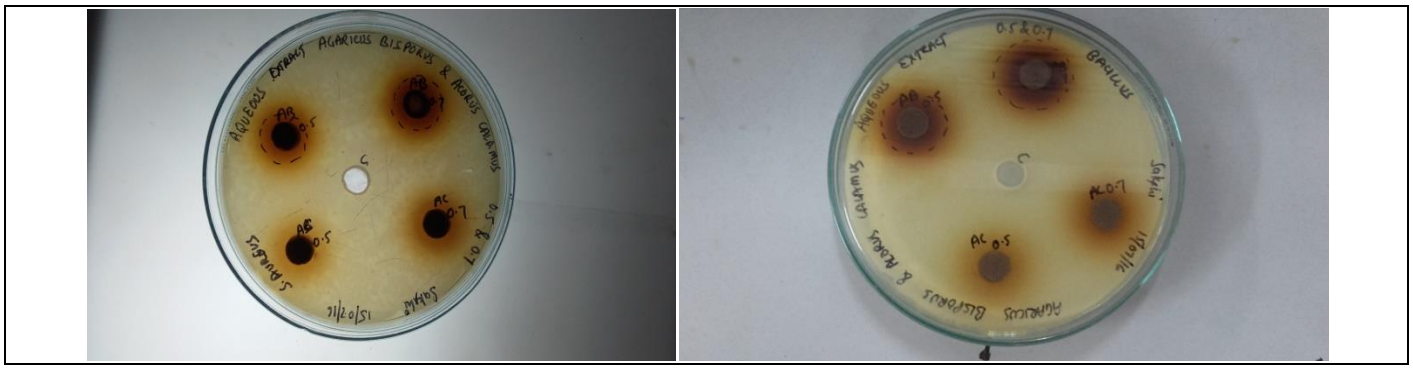

Fig. 10 Antibacterial activity against of *S.aureus, Bacillus amyloliquefacien, E.coli* & *Pseudomonas aeroginosa* of aqueous & methanol extract with the different concentrations 0.2gm/ml (A) & 0.4gm/ml (B), Sterile H_2O (C) is showed in as negative control.

Table 2. Antibacterial activity of methanol extracts of *Acorus calamus* & *Agaricus bisporus*

Table 2A.

Bacterial Strain	Methanol Extract of *Acorus calamus*					
	200mg/ml			400mg/ml		
	Well Diameter (mm)	Zone of Inhibition (mm)	Inhibition Length (mm)	Well Diameter (mm)	Zone of Inhibition (mm)	Inhibition Length (mm)
E.coli	9mm	14mm	5mm	9mm	22mm	13mm
S.aureus	9mm	16mm	7mm	9mm	24mm	15mm
Pseudomonas aeroginosa	9mm	14mm	5mm	9mm	16mm	7mm
Bacillus amyloliquefacien	9mm	14mm	5mm	9mm	18mm	9mm

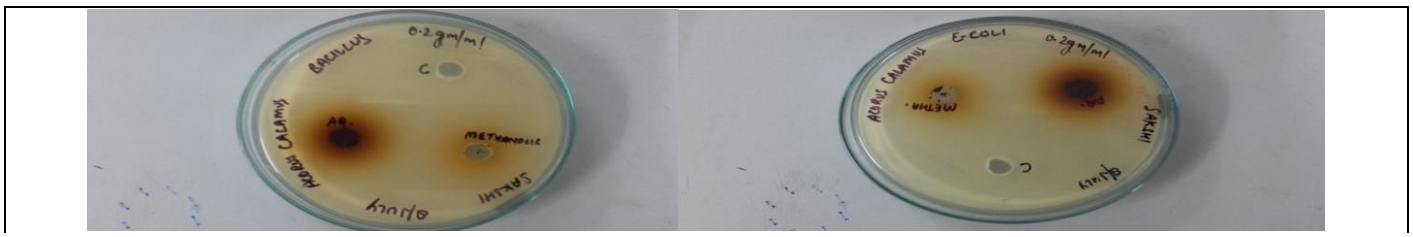

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Fig. 11 Antibacterial activity against of *S.aureus, Bacillus amyloliquefacien, E.coli & Pseudomonas aeroginosa* of aqueous & methanol extract with the different concentrations 0.2gm/ml (A) & 0.4gm/ml (B), Sterile H_2O disc (C) is showed in as negative control.

Table 2B.

Bacterial Strain	Methanol Extract of *Agaricus bisporus*					
	200mg/ml			400mg/ml		
	Well Diameter (mm)	Zone of Inhibition (mm)	Inhibition Length (mm)	Well Diameter (mm)	Zone of Inhibition (mm)	Inhibition Length (mm)
E.coli	**9mm**	**13mm**	**4mm**	**9mm**	**15mm**	**6mm**
S.aureus	**9mm**	**13mm**	**4mm**	**9mm**	**13mm**	**4mm**
Pseudomonas aeroginosa	**9mm**	**30mm**	**21mm**	**9mm**	**35mm**	**26mm**
Bacillus amyloliquefacien	**9mm**	**12mm**	**3mm**	**9mm**	**15mm**	**6mm**

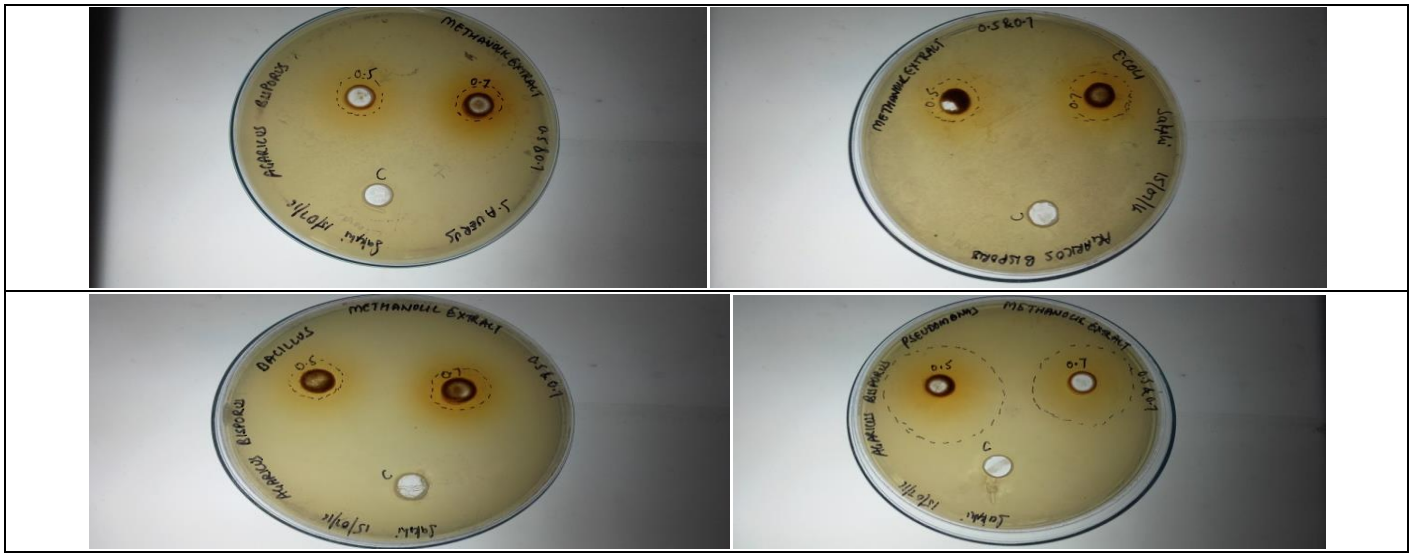

Fig. 12 Antibacterial activity against of *S.aureus, Bacillus amyloliquefacien, E.coli & Pseudomonas aeroginosa* of aqueous & methanol extract of *Agaricus bisporus,* with the different concentrations 0.2gm/ml (A) & 0.4gm/ml (B), Sterile H_2O disc (C) is showed in as negative control.

4.2. Antibacterial activity of biologically synthesized AgNP's extracts by Well Diffusion Method

Nanotechnology provides a platform to develop nanomaterials with promising application in different fields. In the present study the history of conventional use of mushrooms in ancient times was revealed from tribals and local hakims and it was found that mushrooms were used for their nutritional and medicinal values. The present study reveals the production of silver nanoparticles from edible mushrooms and their potent inhibitory activity against *Staphylococcus aureus, E.coli, Pseudomonas aeroginosa & B.amyloiquefaciens* strains. The AgNPs extracts from these medicinally and nutritionally important mushrooms will act as an alternative to antibiotics which could be effective, safe and cost effective for the treatment of infections.

The antibacterial effects of biologically synthesized silver nanoparticles have been investigated against *Bacillus amyloiquefaciens, Staphylococcus aureus, E.Coli* and *Pesudomonas aeruginosa.* The test was performed with loading the biologically synthesized nanoparticles into the well 'A', followed by the plant extract in 'B' and the sterile distilled water (blank) in the well C'. In this susceptibility test, *E.Coli* showed (Table 3) more sensitive in biologically synthesized nanoparticles 23mm zone of inhibition of *Acorus calamus* and followed by *Bacillus amyloiquefaciens* showed (Table 4) more sensitive in biologically synthesized nanoparticles 22mm zone of inhibition of *Agaricus bisporus* all the experimental bacteria showed resistant biologically synthesized silver nanoparticle for the throughout experiment. It shows the highest efficacy of silvernanoparticles individually or with plant extracts.

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Table: 3 Antibacterial activity of biologically synthesized AgNP's of *Acorus calamus* extract

Bacterial Strain	Zone of Inhibition (mm in diameter)		Control
	Synthesized AgNPs extract	Plant Extract	Sterile Distilled Water
E.coli	**23**	**6**	**NI**
S.aureus	**20**	**4**	**NI**
Pseudomonas aeroginosa	**19**	**NI**	**NI**
Bacillus amyloliquefacien	**16**	**NI**	**NI**

Fig. 13 Antibacterial activity against of *S.aureus* & *E.coli* of synthesized silver nano extract of *Acorus calamus*

Table: 4 Antibacterial activity of biologically synthesized AgNP's of *Agaricus bisporus* extract.

Bacterial Strain	Zone of Inhibition (mm in diameter)		Control
	Synthesized AgNPs extract	Plant Extract	Sterile Distilled Water
E.coli	**15**	**NI**	**NI**
S.aureus	**18**	**6**	**NI**
Pseudomonas aeroginosa	**13**	**NI**	**NI**
Bacillus amyloliquefacien	**22**	**11**	**NI**

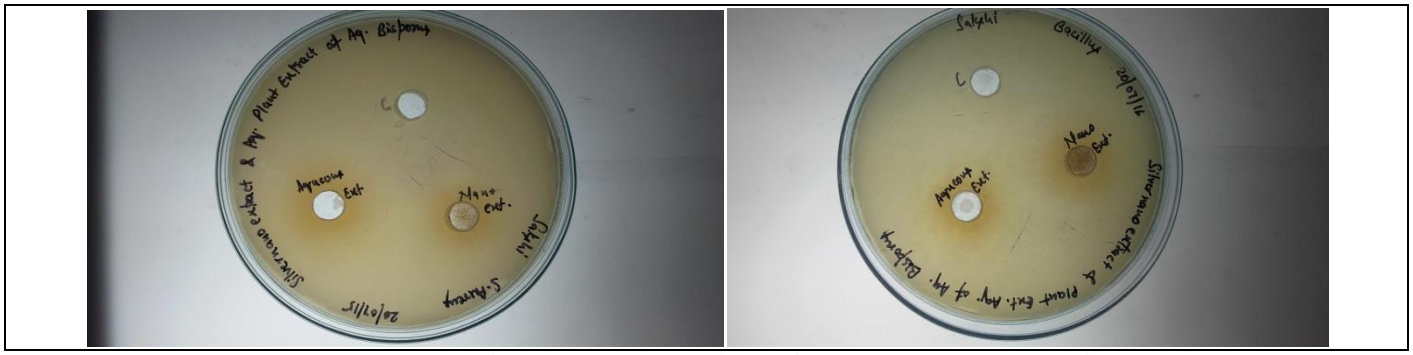

Fig. 14 Antibacterial activity against of *S.aureus* & *B.amyloliquefaciens* of synthesized silver nano extract of *Agaricus Bisporus*

4.3. Phytochemical Screening Test for AgNP's extracts of Acorus calamus & Agaricus bisporus & their plants

The phytochemical analysis of silver nano extract of *Acorus calamus plant* & *Agaricus bisporus* disclosed the presence of secondary metabolites. Among the three solvents for extraction methanol and ethanol showed more number of phytoconstituents as compared to aqueous in case of silver nano extracts of *Acorus calamus* i.e., steroids, alkaloids, carbohydrates & terpenoids. On the other hand, among the three solvents for extraction aqueous and ethanol showed more number of phytoconstituents as compared to methanol in case of silver nano extracts of *Agaricus bisporus* i.e., steroids, alkaloids, inulin, glyocsides, phlobtannins & terpenoids.

Flavonoids screened positive in extracts made from methanol & aqueous being absent in the extracts made in ethanol & silver nano *A.calamus* of flavonoids are absent in the same extracts. Tanins, napthoquinone & amino acids were absent whereas total phenolic count & alkaloids showed positive results in all three extracts & silver nano *A.calamus* were also absent on the same of *A.calamus.* Cabohydrates were screened positive in all extracts of *A.calamus* & silver nano *A.calamus.* Saponins and steroids were present only in ethanol of *A.calamus* whereas both were present in all extract made from aqueous, ethanol and methanol extracts of silver nano A.calamus. Inulin were screened positive in extract made from ethanol in *A.calamus* & silver nano *A.calamus* whereas reducing sugar were only absent only in aqueous extract of *A.calamus.*

These phytoconstituents play a vital role in medicinal properties of plants. The methanol extract of *A.calamus* rhizome display anticellular and immunomodulatory properties [28]. The anti-inflammatory properties of the extract have been using RT-PCR, ELISA, Immunoblotting, and immunofluorescence staining techniques which revealed that *A.Calamus* leaf extract inhibits the production of pro-inflammatory cytokines through multiple

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mechanisms [29]. The saponins found in ethanol extract of *A.calamus* demonstrate hypolipidemic properties. The water extract of *A.calamus* at high concentration have also demonstrated hypolipidemic activity [30]. *A.calamus* improves post prandial hyperglycemia and cardiovascular complications [31]. The aqueous extract of *A.calamus* has also demonstrated allelopathic effects on the growth of two water bloom-forming algal species *Microcystisaeruginosa* and *Chlorella pyrenoidosa* [32].

The phytochemical screening done is to evaluate the secondary metabolites. Tanins, napthoquinone & amino acids were absent whereas total phenolic count & alkaloids showed positive results in all three extracts & silver nano *A.bisporus* were also absent on the same of *A.bisporus*. Flavonoids screened positive in extracts made from ethanol & methanol being absent in the extracts made in aqueous & silver nano *A.bisporus* of flavonoids are absent in all extracts. Glycosides were absent in all extracts of *A.bisporus* & but screened positive for ethanol & aqueous extract of silver nano *A.bisporus.* Alkaloids and steroids were screened positive both in methanol & aqueous of *A.bisporus* & silver nano *A.bisporus* only absent in ethanol extract. Inulin were screened positive in extract made from ethanol & methanol in *A.bisporus* & silver nano *A. bisporus* whereas reducing sugar, carbohydatre and starch were absent but saponin were screened positive only in aqueous extract of silver nano *A.bisporus.*

Bioactive compounds found in edible mushroom *A.bisporus* are known to play a vital role in promoting health. The presence of essential nutrients and minerals in the edible mushroom (*A.bisporus*) imply they could be utilized to improve health [33]. These phytochemicals play a vital role in medicinal properties of plants. Saponins for instant comprise a large family of structurally related compounds containing a steroids or triterpenoid. They are reported to have a wide range of pharmaceutical properties, such as anti-inflammatory and anti-diabetic effects. Thus *A.bisporus* can be used in the management of diabetes and inflammation related diseases. Terpenoids have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti-inflammatory and anti-cancer effects among others. Phenolic compound are antioxidant and exhibit a wide range of spectrum medicinal properties such as anti cancer and anti-inflammatory. *A.bisporus* can be therefore being harnessed in the management of oxidative stress induced disease since phenol and flavonoids have been shown to posses various anti-oxidant functions [34]. The carbohydrate content of edible and non-edible mushrooms represents the range of 4-13% on dry weight basis [35]. However the soluble glucose content was very less than the protein content.

Table 5. Comparison study of phytochemical screening for *Acorus calamus* & silver nano *Acorus calamus*

S.No.	Phytochemical (Phyto-constituents)	Biochemical Test	*Acorus calamus* Extracts			Silver nano Extracts of *Acorus calamus*		
			Methanol Extract	Ethanol Extract	Aqueous Extract	Methanol Extract	Ethanol Extract	Aqueous Extract
1	Saponins	Forth Test	-	+	-	+	+	+
2	Tannins	Ferric Chloride Test	-	-	-	-	-	-
3	Steroids	Salkowski Test	-	+	-	+	+	+
4	Flavonoids	Alkaline Reagent	+	+	+	-	-	-
5	Terpenoids	Salkowski Test	+	+	-	+	+	-
6	Napthoquione	Dam-Karrer Test	-	-	-	-	-	-
7	Inulin	Napthol Test	-	+	-	-	+	-
8	Glycosides	Sodium Nitroprusside Test	-	-	+	-	+	+
		Kellar-Killiani Test	-	-	+	-	-	-
9	Reducing Sugar	Fehling's Test	+	+	-	+	+	+
10	Alkaloids	Wagner's Reagent	-	-	+	+	+	-
11	Phenols	Ferric Chloride Test	+	+	+	+	+	+
12	Amino Acids	Ninhydrin Test	-	-	-	-	-	-
		Biuret Test	-	-	-	-	-	-
13	Carbohydrates	Molisch's Test	+	+	+	+	+	+
		Fehling's Test	+	+	-	+	+	+
14	Phlobtannins	Hydro chloride Test	-	-	-	-	-	-
15	Starch	Iodine Test	-	-	-	-	-	-

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Table 6. Comparison study of phytochemical screening for *Agaricus bisporus* & silver nano extract of *Agaricus bisporus*

S.No.	Phytochemical (Phyto-constituents)	Biochemical Test	*Agaricus bisporus* Extract			Silver nano extract of *Agaricus bisporus*		
			Methanol Extract	Ethanol Extract	Aqueous Extract	Methanol Extract	Ethanol Extract	Aqueous Extract
1	Saponins	Forth Test	-	-	-	-	-	+
2	Tannins	Ferric Chloride Test	-	-	+	-	-	-
3	Steroids	Salkowski Test	+	-	+	+	+	+
4	Flavonoids	Alkaline Reagent	+	+	-	-	-	-
5	Terpenoids	Salkowski Test	+	-	-	+	+	+
6	Napthoquione	Dam-Karrer Test	-	-	-	-	-	-
7	Insulin	Napthol Test	+	+	-	-	+	+
8	Glycosides	Sodium Nitroprusside Test	-	-	-	-	+	+
		Kellar-Killiani Test	-	+	-	-	-	-
9	Reducing Sugar	Fehling's Test	-	-	-	-	-	-
10	Alkaloids	Wagner's Reagent	+	-	+	+	+	+
11	Phenols	Ferric Chloride Test	+	+	+	+	+	+
12	Amino Acids	Ninhydrin Test	-	-	-	-	-	-
		Biuret Test	-	-	-	-	-	-
13	Carbohydrates	Molisch's Test	-	-	+	-	-	-
		Fehling's Test	-	-	-	-	-	-
14	Phlobtannins	Hydro chloride Test	-	-	+	-	-	-
15	Starch	Iodine Test	-	-	-	-	-	-

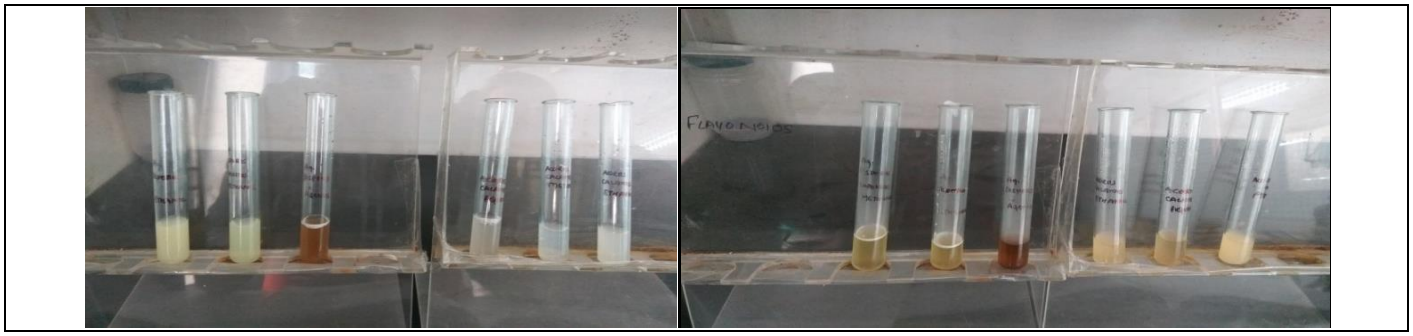

Fig. 15, 16, 17 & 18 The phytoconstituents of flavonoids & saponin in aqueous, methanol & ethanol extract of *Acorus calamus* & *Agaricus bisporus*.

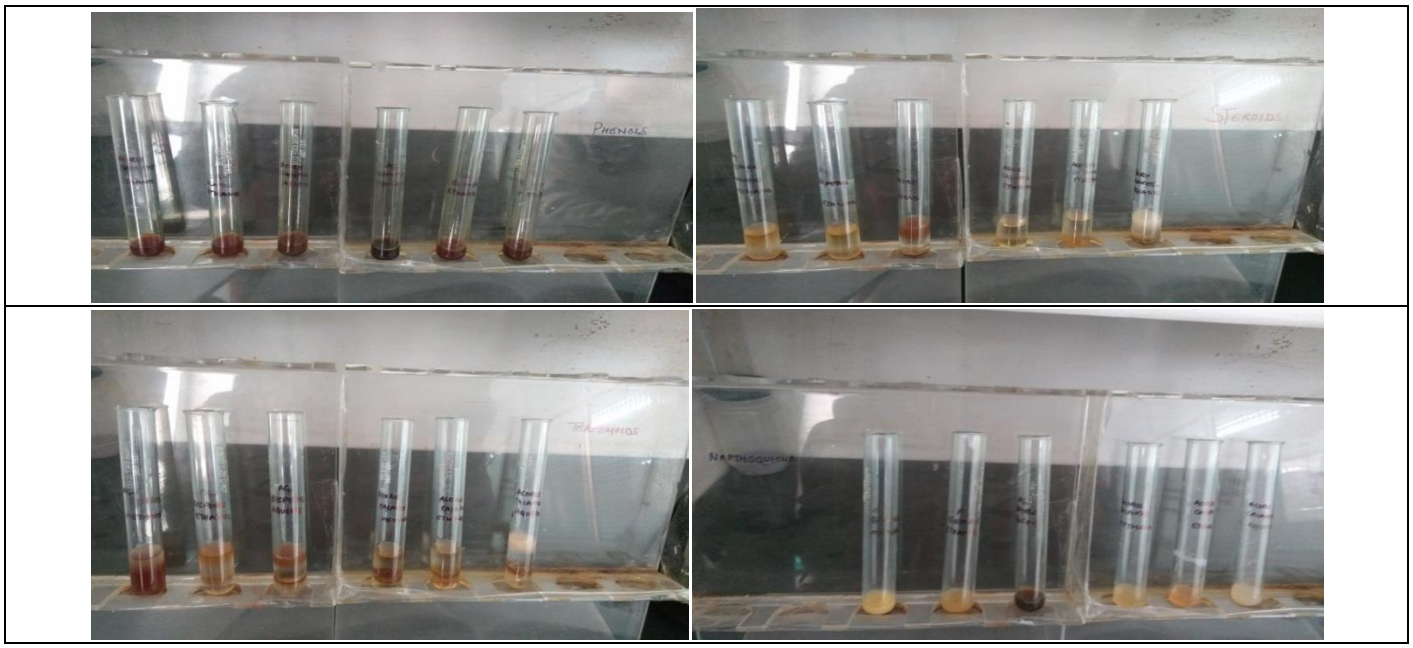

Fig. 19, 20, 21 & 22 The phytoconstituents of terpenoids, steroids, napthoquionone & phenols in aqueous, methanol & ethanol extract of *Acorus calamus* & *Agaricus bisporus.*

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Fig. 23, 24 & 25 The phytoconstituents of carbohydrate, starch & tanins in aqueous, methanol & ethanol extract of *Acorus calamus & Agaricus bisporus.*

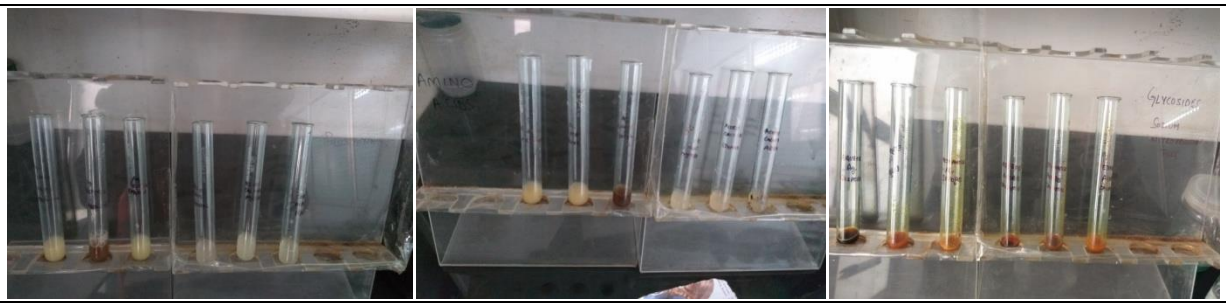

Fig. 26, 27 & 28 The phytoconstituents of glycosides, amino acids & phlobtanins in aqueous, methanol & ethanol extract of *Acorus calamus & Agaricus bisporus.*

Fig. 29 & 30 The phytoconstituents of tannins & steroids in aqueous, methanol & ethanol extract of silver nano *Acorus calamus & Agaricus bisporus.*

Fig. 31, 32 & 33 The phytoconstituents of terpenoids, flavonids & napthoquinone in aqueous, methanol & ethanol extract of silver nano *Acorus calamus & Agaricus bisporus.*

Fig. 34, 34 & 35 The phytoconstituents of inulin, phenol & alkaloids in aqueous, methanol & ethanol extract of silver nano *Acorus calamus & Agaricus bisporus.*

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Fig. 36, 37 & 38 The phytoconstituents of starch, amino acids & phlobtanin in aqueous, methanol & ethanol extract of silver nano *Acorus calamus & Agaricus bisporus.*

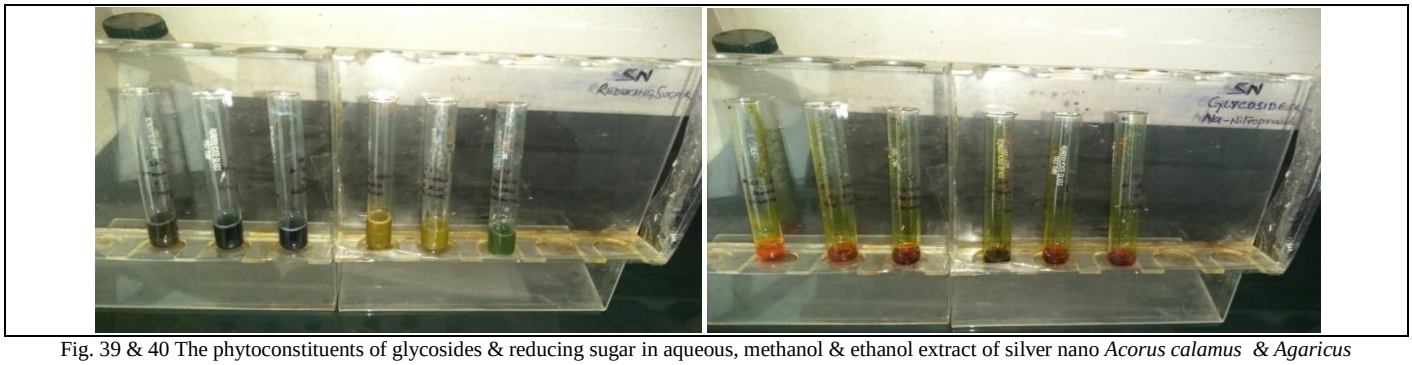

Fig. 39 & 40 The phytoconstituents of glycosides & reducing sugar in aqueous, methanol & ethanol extract of silver nano *Acorus calamus & Agaricus bisporus.*

5. CONCLUSION

Based on the results obtained from the present study, it can be concluded that the methanol extract of *Agaricus bisporus* (mushroom) & aqueous extract of *Acorus calamus* (Vach) can be successfully applied in the development of more potent and efficient antimicrobial agents. The results of preliminary phytochemical analysis of the plants revealed some differences in the

constituents of the two plants tested. *Acorus calamus* tested positive for most of the phytochemicals tested. *Agaricus bisporus* showed the absence of amino acids. The presence of alkaloids in extract may be participating in plant metabolism sequences and the presence of terpenoids may be show cytotoxic activity against wide range of organisms, ranging from bacteria and fungi. This data can also help us to choose the superior race of this valuable plant with greater quantity of medically and therapeutically important phytochemicals. Biologically synthesized silver nanoparticles could be immense use in medical for their efficient antimicrobial properties. The nanosilver was found to have wider antimicrobial activity in against *S.aureus* in *Acorus calamus* & *Bacillus*

amyloliquefacien in *Agaricus bisporus.* We believe that the silver nanoparticle has great potential for applications in catalysis, biomedical and pharmaceutical industries. Further work is therefore under progress to identify their mechanism of action to scavenge the free radicals.

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