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Green synthesis of silver nanoparticles from seed extracts of *Cyperus esculentus* and *Butyrospermum paradoxum*

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***Objective*:** *To synthesize silver nanoparticles from two medicinally important plant seed extracts of Cyperus esculentus and Butyrospermum paradoxum for the first time and to check the potency of the crude methanolic extracts and their synthesized nanoparticles on ten different human pathogens. The percentage yields of two seed extracts were 15.10% and 18.08% respectively and subjected to phytochemical and antimicrobial screenings. For the synthesis of silver nanoparticles (AgNPs), different aqueous concentrations (1mM and 3mM) of silver nitrate solutions were prepared and added to the methanolic extracts of the two plants seeds under mild reaction conditions. Synthesis of AgNPs was confirmed from the change of colour of the reaction mixtures, change in pH, FTIR spectrum and UV-Vis study of the colloidal solutions and further subjected to antimicrobial screening.*

Methods: *Characterization of nanoparticles was done by using UV-visible spectroscopy, FTIR and pH meter to monitor and characteristics the nanoparticles.*

Result: *The phytochemical screening of the extracts revealed the presence of alkaloids, carbohydrate, cardiac glycosides, flavonoids, phenols, saponins, sterols, tannin, terpenoids and reducing sugar in the two samples. However, phlobatannins and quinone were absent in B. paradoxum seed extract. The presence of phenols, terpenoids and flavonoids showed that the seed extracts were viable for the synthesis of nanoparticles. Antimicrobial activities revealed t thepotency of B.paradoxum seed extract as compared to C. esculentus seed extract while the antimicrobial activities of the synthesized nanoparticles also revealed that the 3mM silver nanoparticles were more potent in inhibiting the growth of microorganisms than the seed extracts. This is an indication that silver nanoparticles could be used as antibacterial agents.*

Conclusions: *It can be concluded that the seeds of C. esculentus and B. paradoxum could be a good source for the synthesis of silver nanoparticles which shown high antimicrobial activity against ten different human pathogens. The important outcome of this study will be of high value products from medicinal plants C. s esculentus and B.paradoxum for biomedical and nanotechnology based industries.*

Keywords: *C. esculentus, B. paradoxum, phytochemical, antimicrobial, nanoparticles.*

I. Introduction

The development of green processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology [1, 2]. The research on synthesized nonmaterial and their characterization is an emerging field of nanotechnology from the past two decades, due to their huge applications in the fields of physics, chemistry, biology and medicine [3]. In recent years, noble metal nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic, and chemical properties that are significantly different from those of bulk materials [3]. Nanotechnology is a field that is mushrooming, making an impact in all spheres of human life. A number of approaches are available for the synthesis of silver nanoparticles viz, reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, microwave assisted process and recently via green chemistry route [4]

The biosynthetic method employing plant extracts [5] has received much attention recently owing to its simplicity, eco-friendliness and economically viable nature, compared to the other existing methods such as using bacteria and fungi [6] and the chemical [7, 8] and physical methods used for synthesis of metal nanoparticles. Understanding the biochemical processes that lead to the formation of nanoscale inorganic material is potentially appealing as an environment-friendly alternative to chemical methods [9].

Nanoscale materials have emerged as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties, which increases their contact with microbes and their ability to permeate cells [10]. Also, nanotechnology has amplified the effectiveness of silver particles as antimicrobial agents Many reports are available on the biogenesis of silver nanoparticles using several plant extracts, particularly neem leaf broth (*Azadirachta indica*), *Pelargonium graveolens*, geranium leaves, *Medicago sativa* (Alfalfa), *Aloe vera*, *Emblica officinalis* (Amla, Indian Gooseberry) and few microorganisms.

C. esculentus, also known as yellow nut sedge or earth almond, is a minor crop grown in temperate

and tropical zones of the world. In tile temperate zone, it is grown in Spain, Italy, and the United States. In the tropics, it is found largely in India and West Africa. It is an annual or perennial plant, growing to 90 cm tall, with solitary stems growing from a tuber. The plant is reproduced by seeds, creeping rhizomes and tubers *while B. paradoxum* (Shea butter) is a medium-sized deciduous tree belonging to the family Sapotaceae. It is believed that it is native to the West-African savannas and Central Africa. The shea tree has a rough and corky bark that is deeply cracked. It is usually characterized by milky latex in the stems and branches. The shea tree produces greenish yellow fruits, called shear fruit, which is of great economic importance. In Nigeria, it grows widely in the North, some parts of West and East of Nigeria. One of the most important characteristics of biological materials is their moisture content. The kernel is the source of the shea butter that is extracted through an arduous several hours of processing, over 22 steps, to produce 1 kg of the butter. The fruits are shaped like large plums and have smooth skin with an egg-shaped nut with the kernel that yields the fatty shea butter. The two plants B. paradoxum and C. esculentus parts have been extensively used in Nigeria traditional medicine as an antidiabetic, anti-inflammatory agent, and in the treatment of ulcer, diarrhea. Endometriosis or fibrosis activity of *C. esculentus* [11] and *B. paradoxum* is being use as a base for medicinal ointments, has also been claimed to have anti-inflammatory, emollient and humectants properties [12].

The presence of pharmacologically important compounds the two plants makes the plant more noteworthy in the field of nanochemistry. Hence the present study was aimed at the synthesis of silver nanoparticles using the methanolic extracts of the seeds of *B. paradoxum and C. esculentus.*

II. Materials and methods

Fresh seeds of *B. paradoxum* and *C. esculentus* seeds were bought from a Sango local market in Saki, Saki West Local Government Area of Oyo State, Nigeria in October, 2014 and identification was done at the Botany Department, University of Ibadan, Oyo State, Nigeria. The seeds were air-dried and screened to remove undesirable materials such as stones and other impurities, after which they were milled into powder using milling machine. The powder was air-dried further for 48 hours to prevent the formation of lump and kept at 4^oC for further use.

2.1 Preparation of the seed extracts

The methanolic extracts of the two seeds were prepared using cold extraction [13].1746.75g of C. esculentus and 1400g of B. Paradoxum powder was weighed and soaked with 3L of methanol in two different aspirator bottles with occasional shaking on daily basis for 7 days. The mixture of the two samples was subjected to filtration through whatman filter paper, followed by the evaporation of the solvent using rotary evaporator at 40^0C. The percentage yield of each seed was determined gravimetrely and the extracts were then stored in air-tight glass bottle in a refrigerator below 4^0C for future analysis.

2.2 Phytochemical screening

Phytochemical screening of the extracts of the C. esculentus and B. paradoxum seeds were carried out as described below [14]:

2.2.1 Test for alkaloids (Wagner's reagent)

A fraction of extract was treated with 3-5 drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100 ml of water) and observed for the formation of reddish brown precipitate (or colouration).

2.2.2 Test for carbohydrates (Molisch's test)

Few drops of Molisch's reagent were added to 2ml portion of the various extracts. This was followed by addition of 2ml of conc. H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for two-three minutes. Formation of a red or dull violet colour at the inter phase of the two layers was a positive test.

2.2.3 Test for cardiac glycosides (Keller Kelliani's test)

5ml of each extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides.

2.2.4 Test for flavonoids (alkaline reagent test)

2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

2.2.5 Test for phenols (ferric chloride test)

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

2.2.6 Test for phlobatannins (precipitate test)

Deposition of a red precipitate when 2ml of extract was boiled with 1ml of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

2.2.7 Test for saponins (foam test)

To 2ml of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

2.2.8 Test for sterols (Liebermann-Burchard test)

1ml of extract was treated with drops of chloroform, acetic anhydride and conc. H_2SO_4 and observed for the formation of dark pink or red colour.

2.2.9 Test for tannins (Braymer's test)

2mls of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

2.2.10 Test for terpenoids (Salkowki's test)

1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

2.2.11 Test for quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow precipitate (or colouration).

2.2.12.1 Test for oxalate

To 3ml portion of extracts were added a few drops of ethanolic glacial acetic acid. A greenish black colouration indicates presence of oxalates.

2.3 Preparation of silver nitrate solutions

1mM and 3mM of silver nitrate solutions were prepared by dissolving 0.017g and 0.051g of the salt respectively in a two 100ml standard flasks and made up to the mark with distilled water according to the formula given below.

$$\text{Molarity} = \frac{\text{Molecular weight} \times \text{required Morality} \times \text{Required Volume}}{1000}$$

2.4 Synthesis of silver nanoparticles

The silver nanoparticles were synthesized using a constant volume of the two extracts under various experimental conditions viz: room temperature (28 - 30°C), with different volumes of 1mM and 3mM silver nitrate solutions. The formation of reddish brown colour was observed after 24hrs which indicated formation of silver nanoparticles; the reaction was monitored by checking the P^H and absorbance of the reaction mixtures at regular time interval throughout the period of the reaction.

2.4.1 Separation of silver nanoparticles

The synthesized silver nanoparticles were separated by centrifuging at 13,000 rpm for 15mins. The pellets were collected by using acetone/alcohol followed by drying in a watch glass and the nanoparticles were then stored at -4^{o}C for further use.

2.4.2 UV-Visible spectra analysis of the nanoparticles

The reduction of pure silver ions was observed by measuring the UV-Visible spectrum of the spectrum of the reaction at different time intervals taking 1ml of distilled water used as blank and 1ml of the reaction mixture each for the analysis. This was done using an Elico spectrophotometer at a resolution of 1nm from 300 to 900nm.

2.4.3 FTIR analysis of the seed extract and nanoparticles

Perkin-Elmer spectrometer FTIR spectrum in the range of 4000-400cm^{-1} at a resolution of 4cm^{-1} was used for detection of various functional bonds in both the extracts and the nanoparticles being synthesized. The samples were mixed with KBr and thin sample disc was prepared by pressing with the disc preparing machine and placed in the Fourier Transform Infrared (FTIR) for the analysis of the nanoparticles.

2.4.4 Metal -plant interaction with colour formation

The seed extracts were mixed with the prepared silver nitrate solutions and incubated at room temperature. During the incubation, metal ions present in the solutions were interacted with the plant phytoconstituents and converted to pale yellow to dark brown colour. The intensity of the colour increased with time. The time duration for colour change is primarily due to the excitation of surface plasmon vibrations in silver nanoparticles, as shown in figures 1-4.

2.5 Antimicrobial activity

2.5.1 Antimicrobial study

Antimicrobial properties of the seed extracts and the synthesized nanoparticles were investigated against the following bacterial *spp*. *Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia*. The fungus *spp.* used for the test was

Candida albicans, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifer*. All the cultures were performed at the Department of pharmacceutical microbiology, University of Ibadan, Nigeria. The microorganisms were grown overnight at 37^{o}C in Mueller-Hinton Broth at pH 7.4 [15, 16].

2.5.2 Culture media and inoculums preparation

Nutrient agar /broth were used as the media for the culturing of bacterial strains. Loops full of all the bacterial cultures were inoculated in the nutrient broth and incubated at 37^{0}C for 72 hrs and Potato dextrose agar /and potato dextrose broth were used as the media for the culturing of fungal strains. Loops full of all the fungus cultures were inoculated in the potato dextrose broth (PDA) and incubated at room temperature for 72h.

2.5.3 Testing for antibacterial activity

The extracts and nanoparticles obtained above were screened for their antibacterial activity in comparison with standard antibiotic Gentamycin (30µg/mL) in-vitro by well diffusion method [17-18]. The cup-plate agar diffusion method was employed to assess the antibacterial activity of the prepared extracts and nanoparticles [19]. 20 ml of the inoculated nutrient agar were distributed into sterile petri dishes. The agar was left to set and in each of these plates, 5 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed [20]. Alternate cups were filled with 20µ L of each extracts and nanoparticles using microtiter-pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37^{0}C for 18 hours. The dimethylsulfoxide (DMSO) was used as a negative control. The diameters of the growth inhibition zones were measured at 24 hours of incubation averaged and the mean values were tabulated.

2.8. Antifungal activity

The extracts and nanoparticles were also screened for their antifungal activity in comparison with standard antibiotic Gentamycin (30µg/mL) in-vitro by well diffusion method [17-18]. Lawn culture was prepared using the test organism on potato dextrose broth (PDA). The inoculated plates were kept aside for a few minutes. Using well cutter, four wells were made in those plates at required distance. Using sterilized micropipettes 30µL of solvent with the extracts and nanoparticles were added in to the well. The plates with yeast like fungi were incubated at 37^{o}C for overnight. The plates with mold were incubated at room temperature for 48h. The activity of the extracts and nanoparticles were determined by measuring the diameters of zone of inhibition. For each fungal strain, controls were maintained where pure solvent (DMSO) was used instead of the extracts and the nanoparticles.

III. Results and discussions

3.1 Phytochemical screening

Phytochemicals in medicinal plants have been reported to be the active principles responsible for the pharmacological potentials of plants [21]. Phytochemical screening of C. esculentus and B. paradoxum seeds showed the presence of alkaloids, carbohydrate, cardiac glycosides, flavonoids, phenols, saponins, sterols, tannin, terpenoids and reducing sugar in the two plants (Table I). However, phlobatannins and quinones were absent in B. paradoxum seed extract. The therapeutic value of medicinal plants lies in the various chemical constituents in it. The bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane [22]. Flavonoids are a major group of phenolic compounds reported for their antiviral [23], antimicrobial and spasmolytic properties. Alkaloids isolated from plants are commonly found to have antimicrobial properties [24]. Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties [25]. They show marked physiological effects when administered to animals. The presence of alkaloids in the extracts showed that these plants can be effective anti-malaria, since alkaloids consist of quinine, which is anti-malaria [26]. The cardiac glycosides therapeutically have the ability to increase the force and power of the heart-beat without increasing the amount of oxygen needed by the heart muscle. They can thus increase the efficiency of the heart and at the same time steady excess heart beats without strain to the organ [27]. Saponin has relationship with sex hormones like oxytocin. Oxytocin is a sex hormone involved in controlling the onset of labour in women and the subsequent release of milk [28]. Another important action of saponins is their expectorant action through the stimulation of a reflex of the upper digestive tract [29]. Phenolic compounds possess hydroxyl and carboxyl groups, plants with high content of phenolic compounds are one of the best candidates for nanoparticles synthesis [30].

3.2 UV- visible spectroscopy

Reduction of silver ions into silver nanoparticles using methanolic extracts of seeds of C. esculentus and B. paradoxum was evidenced by the visual change of colour from yellow to reddish brown due to excitation of surface plasmon vibrations [31] in silver nanoparticles as shown in figures 1-4. The UV-visible

spectra show an absorption band at 421 nm which corresponds to the absorbance of silver nanoparticles (figure 5). After 24hrs, no significant colour change was observed and increased concentrations of silver nitrate resulted in a brown solution of nanosilver indicating the completion of reaction

3.3 Effects of pH on synthesized nanoparticles

pH of the solution is a critical factor in controlling the size and morphology of nanoparticles and the location of nanoparticles deposition [32]. For C. esculentus and B. paradoxum seeds extracts, the reduction of silver ions were observed at the pH of 5.46, 4.54 and 5.274, 4.53 from the lower concentration to higher concentration of silver nitrate in each of the extracts after 24 hrs of the reaction. Also similar conclusion was reached and reported [33] that pH is responsible for the formation of nanoparticles of various shapes and size for different plant extracts and that even the extracts coming from different parts of the same plant may have different pH values which further need optimization for the efficient synthesis of nanoparticles. It has been reported by several researchers that larger nanoparticles formed at lower pH as compared to higher pH [30]. However, higher pH facilitates the nucleation and subsequent formation of large number of nanoparticles with smaller diameter.

3.4 FTIR spectroscopy

The FTIR spectra of the crude extracts revealed the major peaks of some essential functional groups necessary for the formation of bonds between the phytoconstituents and the silver ions in solution (Figure 6a and 7a). In FTIR spectrum of C esculentus seed extracts the following strong bands of 3435.90cm^{-1} and 3400.07cm^{-1} (phenolic O-H stretching vibration), 2933.99cm^{-1} and 2925.37cm^{-1} (C-H stretching vibration of a methylene group), 1638.01cm^{-1} and 1622.98cm^{-1} (C=C stretching vibration of an aromatic or alkene), 1263.31cm^{-1} and 1237.74cm^{-1} (C-O stretching vibration) and 1053.88cm^{-1} and 1047.41cm^{-1} (O-H bending vibration) respectively while B. paradoxum seed extract displayed a strong band at 1709.20cm^{-1} (C=O carbonyl stretching vibration). This shows that a carbonyl compound is present in B. paradoxum seed extract. Also, C esculentus seed extract showed a C≡C or C≡N stretching vibration at 2078.51cm^{-1}. The probable functional group is the C≡N stretching vibration of the alkaloids present in the sample. Furthermore, the O-H absorbtion bands further confirms the presence of phenol in the two seed extracts. The synthesized silver nanoparticles were confirmed by the FTIR spectra (figure 6b, 6c for C. esculentus and figure 7b, 7c for B. paradoxum). Noticeable changes were observed in the absorption bands for all the spectra of nanoparticles and a prominent peak of 2091.78cm^{-1} which was all observed in all the four spectra suggesting the absorption band for AgNPs. The O-H absorbtion frequency band was also lowered in the four nanoparticles and this consequently led to line broadening. Studies have shown that the presence of hydrogen bonding leads to broad absorption bands. This can also be confirmed in the more concentrated 3mM nanoparticles where the broadening was more intense than the 1mM nanoparticles due to high concentration of silver ions as compared to 11mM nanoparticles.

3.5 Antimicrobial activity and MICs of the crude extracts and nanoparticles

The results of antimicrobial activities of the extracts are given in the Table 2, which clearly showed that all the extracts have shown significant antimicrobial activity equivalent to that of standard against the entire tested organisms. The measured diameter of the inhibition zones clearly indicated the antimicrobial effect of the extracts varied according to the type of bacteria and fungi used. The highest activity for C. esculentus seed extract was demonstrated against *S. aureus* with inhibition zone diameter of (24mm) while the lowest activity was demonstrated against *P. aeruginosa*, *K. pneumonia*, *S typhi*, *A. niger* and *R. stolonifer*. However, B paradoxum seed extract showed the highest activity activity against S. aureus with inhibition zone diameter of (26 mm) as compared to the standard (40 mm). The activity demonstrated against other organism are in decreasing order starting from *P. aeruginosa*, *K. pneumonia*, *A. niger*, *P. notatum* and *R. stolonifer*. The result on table 2 showed that the *B paradoxum* seed extract is more potent than C. esculentus seed extract.

MIC values are presented in table 4. The minimum inhibition concentration of *C. esculentus* seed extract was 12.5 mg/ml against *S. aureus* while that of *B. paradoxum* seed extract was also 12.5 mg/ml against *S. aureus*, *E. coli* and *B. subtilis*. Both samples had high MIC values against fungi at 50 mg/ml against C. *albicans* and *P. notatum* for *C. esculentus* seed extract and 25mg/ml against C. albicans for *B. paradoxum* seed extract. Experiment revealed remarkable antibacterial effect of *B. paradoxum* seed extract against the g+ strains of *S. aureus* and *B. subtilis* and g- strain of *E. coli*. *C. esculentus* seed extract also showed a remarkable inhibition against the g+ strain of S. aureus. It is expected that the g+ strains would have a

high concentration of the seed extract to inhibit their growth due to the lipopolysaccharide structure of these bacteria which notably restricts the penetration of external polar and hydrophilic materials through their cell wall [34]. This further confirms that B. *paradoxum* seed extract antibacterial activity and could therefore be employed as antibiotics. The antimicrobial activity of the nanoparticles and MICs were presented on table 4 and 5 respectively and it was revealed that the nanoparticles were more potent than the crude extracts against tested organisms. The 3mM silver nanoparticles in both extracts inhibited the growth of all the organisms significantly as compared to the standard antibiotics drugs. The 1mM silver nanoparticles were less potent than the crude seed extracts and this could be as a result of the low concentration of the silver nitrate in solution. This study has shown the phytochemicals, synthesized of nanoparticles and antimicrobial activity. It is concluded that the plant extract possess microbial activity against tested organisms. The zone of inhibition varied suggesting the varying degree of efficacy and different phytoconstituents of the extracts on the target organisms. The antimicrobial activity of the plants may be due to the presence of various active compounds in the seeds which are also responsible for the formation of nanoparticles.

References

[1]. Raveendran P, Fu J, Wallen SL. A simple and ''green'' method for the synthesis of Au, Ag, and Au-Ag alloy nanoparticles. Green Chem 2006; 8: 34-38.

[2]. Armendariz V, Gardea-Torresdey JL, Jose Yacaman M, Gonzalez J, Herrera I, Parsons JG. Gold nanoparticle formation by oat and wheat biomasses. Proceedings of Conference on Application of Waste Remediation Technologies to Agricultural Contamination of Water Resources; 2002.

[3]. Song JY, Kim BS. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng 2008; 32: 79-84.

[4]. A. J. Gavhane, P. Padmanabhan, S. P. Kamble and S. N. Jangle, Int J Pharm Bio Sci., **2012**, 3(3), 88 – 100.

[5]. V. Manonmani, Vimala Juliet, International Conference on Innovation, Management and Service, IPEDR, IACSIT Press. Singapore. 14, 2011**.**

[6]. T. Dhanalakshmi and S. Rajendran, Archives of Applied Science Research, **2012**, 4 (3), 1289-1293.

[7]. G. Oza, S. Pandey, R. Shah, M. Sharon, Advances in Applied Science Research, **2012**, 3 (3), 1776-1783. [5] A. A. El-Kheshen and S. F. Gad El-Rab, Der Pharma Chemica, **2012**, 4 (1), 53-65.

[8]. H. U. Igwe, E. I. Ugwu, Advances in Applied Science Research, **2010**, 1 (3), 240-246.

[9]. S. R. Bonde, D. P. Rathod, A. P. Ingle, R. B. Ade, A. K. Gade and M. K. Rai, Nanoscience Methods, **2012**, 1, 25–36.

[10]. K. Lamsal, S. W. Kim, J. H. Jung, Y. S. Kim, K. S. Kim And Y. S. Lee, Mycobiology, **2011**, 39 (3),194-199.

[11]. Chevallier A. The Encyclopedia of Medicinal Plants Dorling Kindersley. London. **1996,** 4:303148-9780751.

[12]. Akihisa T, Kojima N, Kikuchi T., Yasukawa K., Tokuda H., Manosroi A., Manosroi J. Journal of oleo Science. 2010; 59:273-80.

[13]. Tiwari et al. (2011).

[14]. Ugochukwu S., Arukwe U., Onuocha I. Preliminary phytochemical screening of different extracts of stem, bark and roots of Dennetia tripetala. G. Baker, Asian Journal Plant Science Research. 2013; 3:10-13.

[15]. Okunade MB., Adejumobi JA, Ogundiya MO, Kolapo AL. Journal of Phytopharmacotherapy and Natural products 2007;1(1): 49-52

[16]. Peter J.Petersen, C.Hal Jones, Patricia A.Bradford. In vitro by time-kill kinetic studies in fresh Mueller-Hinton broth. Diagnostic Microbiology and Infectious Disease. Volume 59, Issue 3, November 2007, Pages 347-349

[17]. E.A. du Toit, M. Rautenbach. A sensitive standardised micro-gel well diffusion assay for the determination of antimicrobial activity. J Microbiological Methods. Volume 42, Issue 2, October 2000, Pages 159-165.

[18]. Bagamboula, C.F.,Uyttendaela, M.,Devere, J., Inhibitory effect of Thyme and basil essential oil, carvacrol, thymol, estragol, inalool and p-cymene towards *Shigella sonnei* and *S. flexnerii*. Food Microbiol. 2004; 21, 33-42.

[19]. Bangar Raju, Mamatha Ballal, Indira Bairy. A novel treatment approach towards Emerging multidrug resistant enteroaggregative *Escherichia coli* (eaec) causing acute/persistent diarrhea using medicinal plant extracts. RJPBCS, Volume 2 Issue 1 January - March 2011, Page 15-23.

[20]. Popoola, T.O.S., Yangomodu O.D and Akintokun A.K. 2007. Research J Medicinal Plant, 1(2): 60-64.

[21]. Zare K., Nazemyeh H., Lotfipour F. Antibacterial activity and total phenolic content of the *Onopordon acanthium* L. Seeds. Pharmaceutical Sciences. 2014; 20:6-11.

[22]. R. G. Ayo. Phytochemical constituents and bioactivities of the extracts of Cassia nigricans Vahl: A review. J Medicinal Plants Research Vol. 4(14), pp. 1339-1348, 18 July, 2010.

[23]. EK Elumalai, M Ramachandran, T Thirumalai, P Vinothkumar. Antibacterial activity of various leaf extracts of *Merremia emarginata*. Asian Pacific J Tropical Biomedicine (2011)406-408.

[24]. Jose MA, Ibrahim, Janardhanan S. Modulatory effect of *Plectranthus amboinicus* Lour. on ethylene glycol induced nephrolithiasis in rats. Indian J Pharmacol, 2005; 37:43-4.

[25]. P.C. Njoku and M.I. Akumefula. Phytochemical and Nutrient Evaluation of Spondias Mombin Leaves. Pakistan J Nutrition 6 (6): 613-615, 2007.

[26]. Nisar Ahmad, Hina Fazal, Muhammad Ayaz, Bilal Haider Abbasi, Ijaz Mohammad, Lubna Fazal. Dengue fever treatment with Carica papaya leaves extracts. Asian Pacific J Tropical Biomedicine (2011)330-333.

[27]. Stray, F., The natural guide to medicinal herbs and plants. Tiger Books International, London 1998, pp: 12-16.

[28]. R. N. Okigbo, C. L. Anuagasi and J. E. Amadi. Advances in selected medicinal and aromatic plants indigenous to Africa. J Medicinal Plants Research Vol. 3(2), pp. 086-095, February, 2009.

[29]. P.B. Ayoola & A. Adeyeye. phytochemical and nutrient evaluation of *Carica papaya* (pawpaw) leaves. IJRRAS 5 (3) December 2010

[30]. Veerasamy R., Xin T., Gunasagaran S. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. Journal of Saudi Chemical Society. 2011;15:113-120.

[31]. A. Power, J. Cassidy, T. Betts, The Analyst, **2011**, 136, 2794-2801

[32]. Konishi Y., Ohno K., Saitoh N. Bioreductive deposition of platinum nanoparticles on the bacterium Shewanella algae. Journal of Biotechnology. 2007; 128:648-653.
[33]. Mock J., Barbic M., Smith D., Schultz D., Schultz S. Shape effects in plasmon resonance of individual colloidal silver nanoparticles. Journal of Chemical Physics. 2002; 116:6755-6759.
[34]. Russell A. Mechanisms of bacterial resistance to non-antibiotics - food-additives and food and pharmaceutical preservatives. Journal of Application Bacteriology. 1991;71:191- 201.

Table 1: Qualitative phytochemical screening of *C. esculentus* and *B. paradoxum* seed extracts

Phytochemicals	*C. esculentus*	*B. paradoxum*
Alkaloid	+	+
Carbohydrate	+	+
Cardiac glycosides	+	+
Flavonoids	+	+
Phenols	+	+
Phlobatannins	+	-
Saponins	+	+
Sterols	+	+
Quinone	+	-
Tannin	+	+
Terpenoids	+	+
Reducing Sugar	+	+

KEY: Present= (+), Absent = (-)

Table 2: Antimicrobial activity *C. esculentus* and *B. paradoxum* seed extracts against human pathogenic microorganisms

Test organisms	Zone of inhibition (mm)												
			Conc. of *C. esculentus*						Conc. of *B. paradoxum*				
	-ve	+ve	100	50	25	12.5	6.25	3.125	3.125	6.25	12.5	25	50	100
S. aureus	-	40	24	20	18	14	12	10	12	14	18	20	24	26
E. coli	-	40	22	18	16	14	12	10	10	12	14	18	20	24
B. subtilis	-	38	18	14	12	10	-	-	-	10	12	14	18	20
P.aeruginosa	-	40	14	12	10	-	-	-	-	-	10	12	14	18
S. typhi	-	38	14	12	10	-	-	-	-	10	12	14	16	18
K.pneumonia	-	40	14	12	10	-	-	-	-	-	10	12	14	16
C. albicans	-	28	18	16	14	12	10	-	10	12	14	16	18	20
A. niger	-	28	14	12	10	-	-	-	-	-	10	12	14	16
P. notatum	-	26	16	14	12	10	-	-	-	-	10	12	14	18
R.stolonifer	-	28	14	12	10	-	-	-	-	-	10	12	14	16

Key: -ve: - DMSO, +ve:- Gentamycin

Table 3: Minimum inhibitory concentration (MIC) *C. esculentus* and *B. paradoxum* seed extracts against pathogenic microorganisms

Test organisms	Minimum inhibitory concentration (MIC) mg/ml									
	Conc of *C. esculentus* seed extract					Conc. *B. paradoxum* seed extract				
	100	50	25	12.5	6.25	100	50	25	12.5	6.25
E. coli	-	-	-	+	+	-	-	-	-	+
B. subtilis	-	-	+	+	+	-	-	-	-	+
P.aeruginosa	-	+	+	+	+	-	-	+	+	+
S. typhi	-	+	+	+	+	-	-	-	+	+
K.pneumonia	-	+	+	+	+	-	-	+	+	+
C. albicans	-	-	+	+	+	-	-	-	+	+
A. niger	-	+	+	+	+	-	-	+	+	+
P. notatum	-	-	+	+	+	-	-	+	+	+
R. stolonifer	-	+	+	+	+	-	-	+	+	+

KEY: (+) no inhibition (growth of organism) and (-): inhibition (no growth of organism)

Table 4: Antimicrobial activity of 1mM AgNO3 nanoparticle *C. esculentus* and *B. paradoxum* seed extracts against pathogenic microorganisms

Test organisms	Zone of inhibition (mm)												
	1mM AgNO3 nanoparticles of *C. esculentus* seed extract							1mM AgNO3 nanoparticles of *B paradoxum* seed extract					
	-ve	+ve	100	50	25	12.5	6.25	3.125	3.125	6.25	12.5	25	50	100
S. aureus	-	38	18	16	14	10	-	-	-	10	12	14	18	20
E. coli	-	40	20	18	14	12	10	-	-	10	12	14	18	22
B. subtilis	-	38	18	16	14	10	-	-	-	10	12	14	18	20
P.aeruginosa	-	38	18	14	12	10	-	-	-	10	12	14	16	18
S. typhi	-	38	20	18	14	12	10	-	-	-	10	12	14	18
K.pneumonia	-	40	16	14	12	10	-	-	-	-	10	12	14	18
C. albicans	-	28	14	12	10	-	-	-	-	-	10	12	14	16
A. niger	-	26	14	14	12	10	-	-	-	-	-	10	12	14
P. notatum	-	28	16	14	12	10	-	-	-	-	10	12	14	18
R. stolonifer	-	28	16	14	12	10	-	-	-	-	10	12	14	16

KEY: -Ve= DMSO, +Ve =Gentamycin

Table 5: Antimicrobial activity of 3mM AgNO3 nanoparticles of *C. esculentus* and *B. paradoxum* seed extracts against pathogenic microorganisms

Test organisms	Zone of inhibition (mm)												
	3mM AgNO3 nanoparticle of *C. esculentus* seed extract							3mM AgNO3 nanoparticle of *B. paradoxum* seed extract					
	-ve	+ve	100	50	25	12.5	6.25	3.125	3.125	6.25	12.5	25	50	100
S. aureus	-	38	24	20	18	14	12	10	10	12	14	18	22	26
E. coli	-	40	26	22	18	14	12	10	10	12	16	18	20	24
B. subtilis	-	38	20	18	16	14	12	10	10	12	14	16	18	22
P.aeruginosa	-	38	20	16	14	12	10	-	10	12	14	16	18	20
S. typhi	-	38	24	20	18	14	12	10	10	12	14	16	18	20
K.pneumonia	-	40	18	16	14	12	10	-	-	10	12	16	16	18
C. albicans	-	28	16	14	12	10	-	-	-	-	10	14	14	18
A. niger	-	26	18	14	12	10	-	-	-	10	12	16	16	18
P. notatum	-	28	18	16	14	10	-	-	-	-	10	14	14	18
R. stolonifer	-	28	20	18	16	14	10	-	-	-	10	14	14	18

Table 6: pH effect on synthesized nanoparticles

Time (hours)	Samples	pH
0	1mM $AgNO_3$	8.683
	3mM $AgNO_3$	7.489
7	1mM $AgNO_3$ (A)	5.582
	1mM $AgNO_3$ (B)	4.601
	3mM $AgNO_3$ (A)	5.450
	3mM $AgNO_3$ (B)	4.642
24	1mM $AgNO_3$ (A)	5.466
	1mM $AgNO_3$ (B)	4.545
	3mM $AgNO_3$ (A)	5.274
	3mM $AgNO_3$ (B)	4.534

A = *C. esculentus*, B = *B. paradoxum*

Fig. 1: Initial colour of 1mM and 3mM silver nitrate solutions

Fig. 2a: Colour (pale yellow) of the reaction mixture of 1mM and 3mM silver nitrate and *C. esculentus* seed extract at 0 hour

Fig 2 b: Colour (brown) of the reaction mixture of 1mM and 3mM silver nitrate and *B. paradoxum* seed extracts after 0 hour

Fig 3a and 3b : Colour (brown) of the reaction mixture of 3mM silver nitrate, *C. esculentus* and *B. paradoxum* (B) seed extracts after 7 hours

Fig 4a: Colour (dark brown) of the reaction mixture of 3mM silver nitrate and *Cyperus esculentus* (A) nitrate and *Butyrospermum paradoxum* (B) seed extracts after 24 hours

Fig 4b: Colour (brown) of the reaction mixture of 1mM silver nitrate, *C esculentus (A)* and *B paradoxum* (B) seed extracts after 24 hours.

Fig 5: UV-visible spectra of synthesized nanoparticles after 24 hours

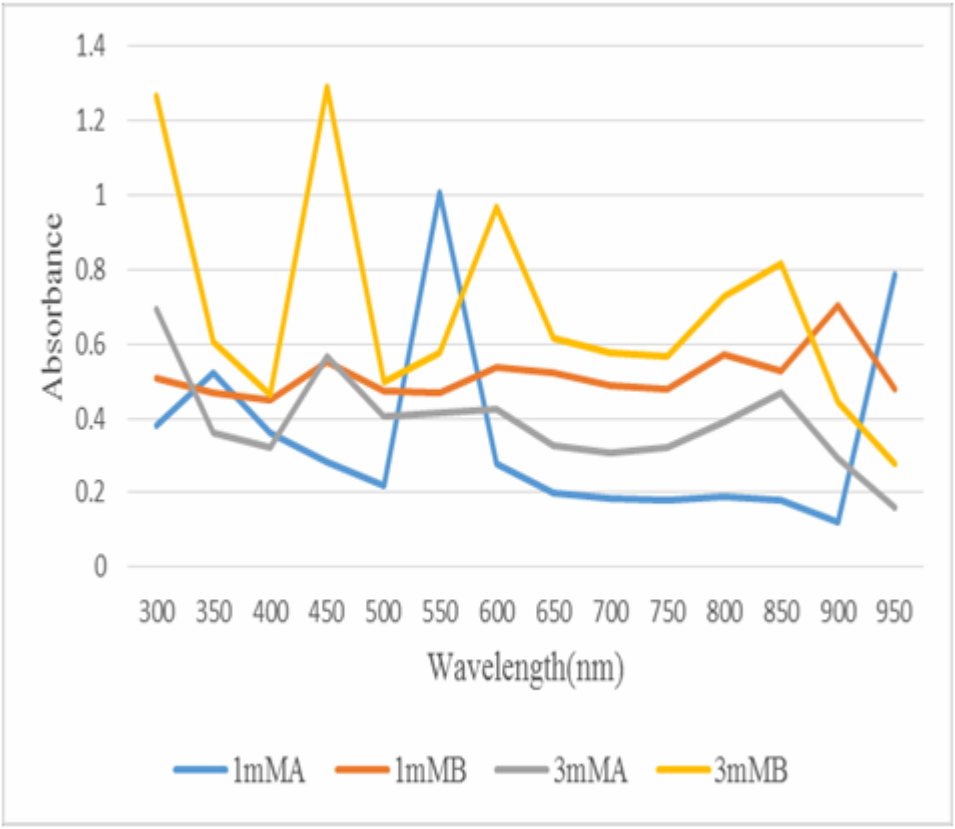

Figure 6a: FTIR spectrum of *C. esculentus* seed extract

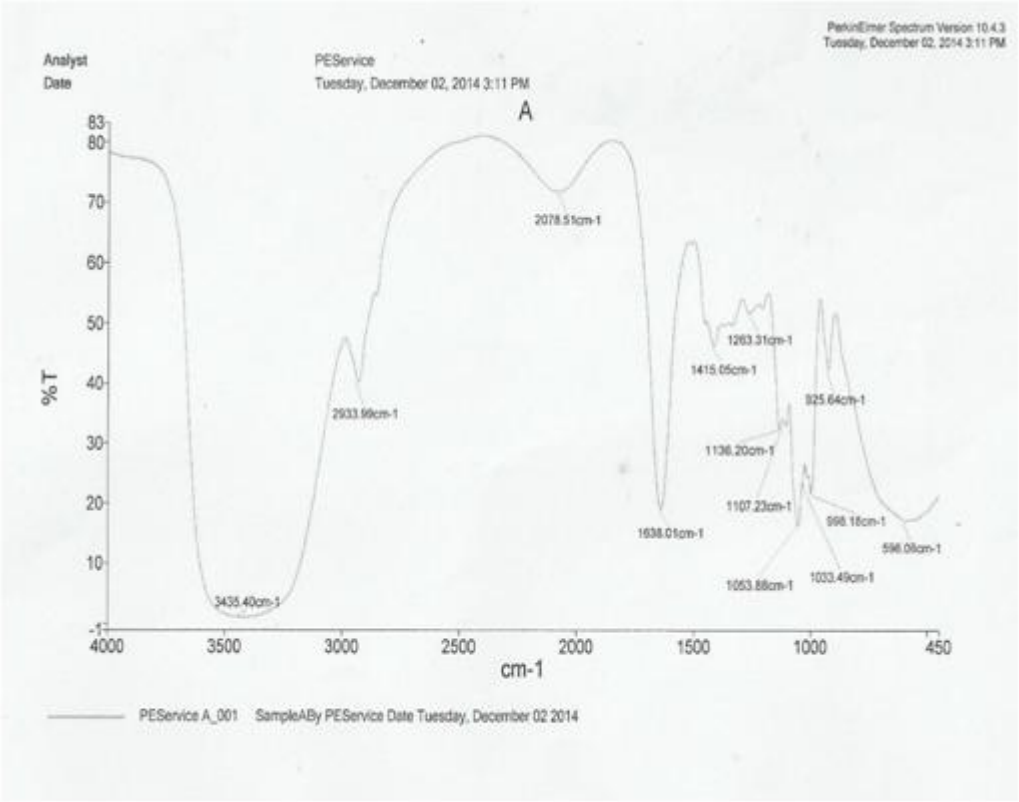

Fig.6b: FTIR spectrum of 1mM silver nanoparticles synthesized from *C. esculentus* seed extract

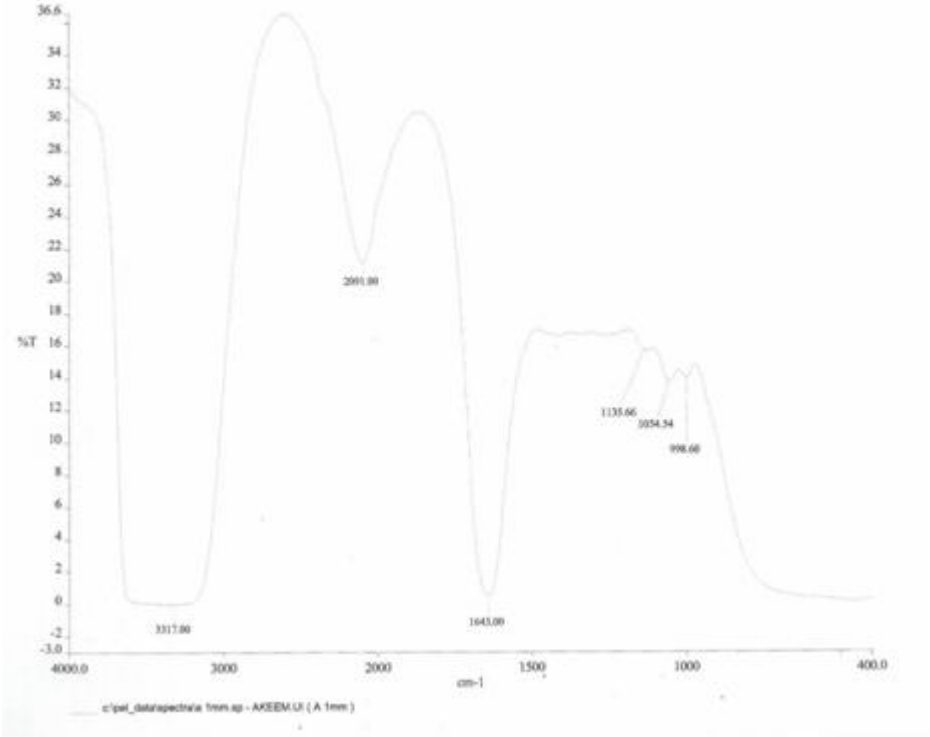

Fig. 6c: FTIR spectrum of 3mM silver nanoparticles from *C. esculentus* seed extract

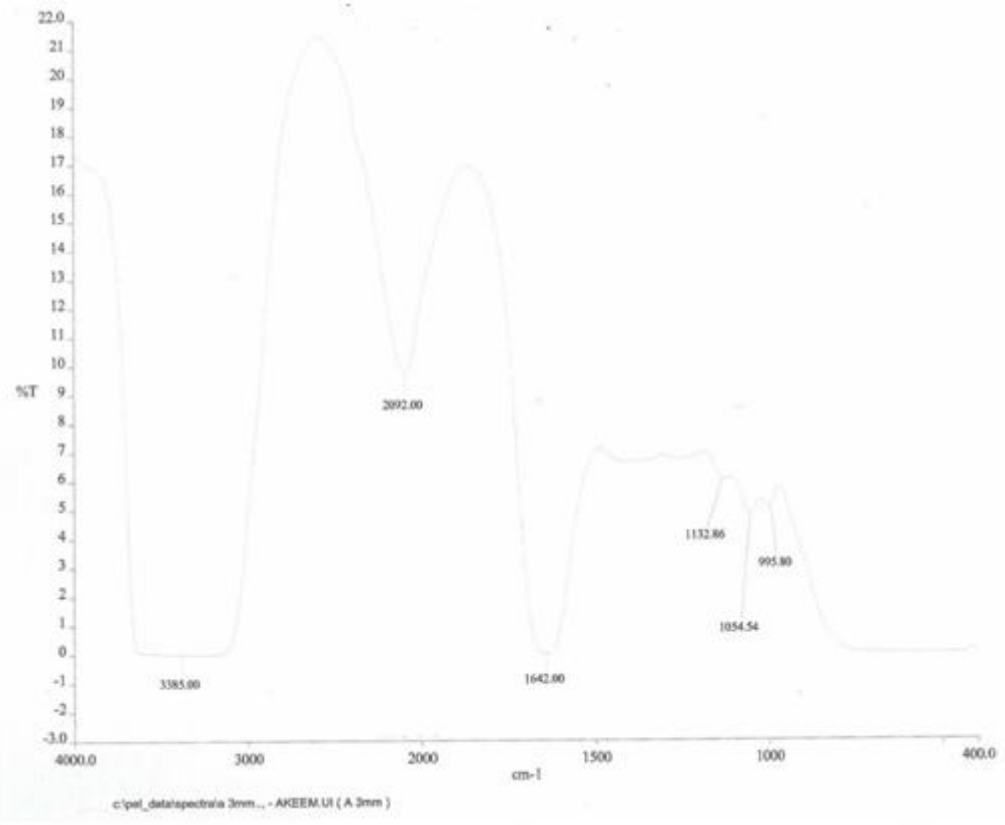

Fig 7a: FTIR spectrum of *B. Paradoxum* seed extract

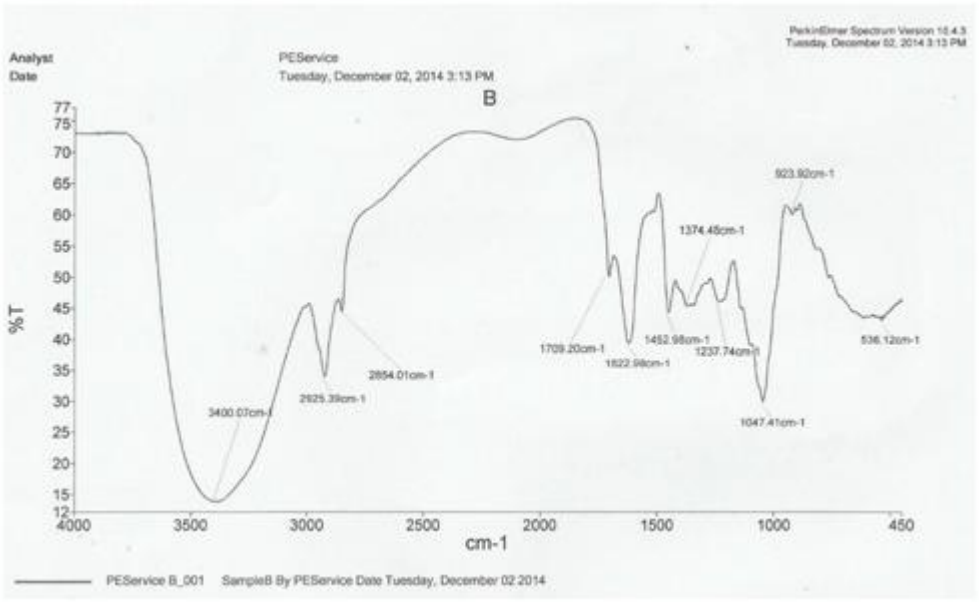

Fig. 7b: FTIR spectrum of 1mM silver nanoparticles synthesized *B. paradoxum* seed extract

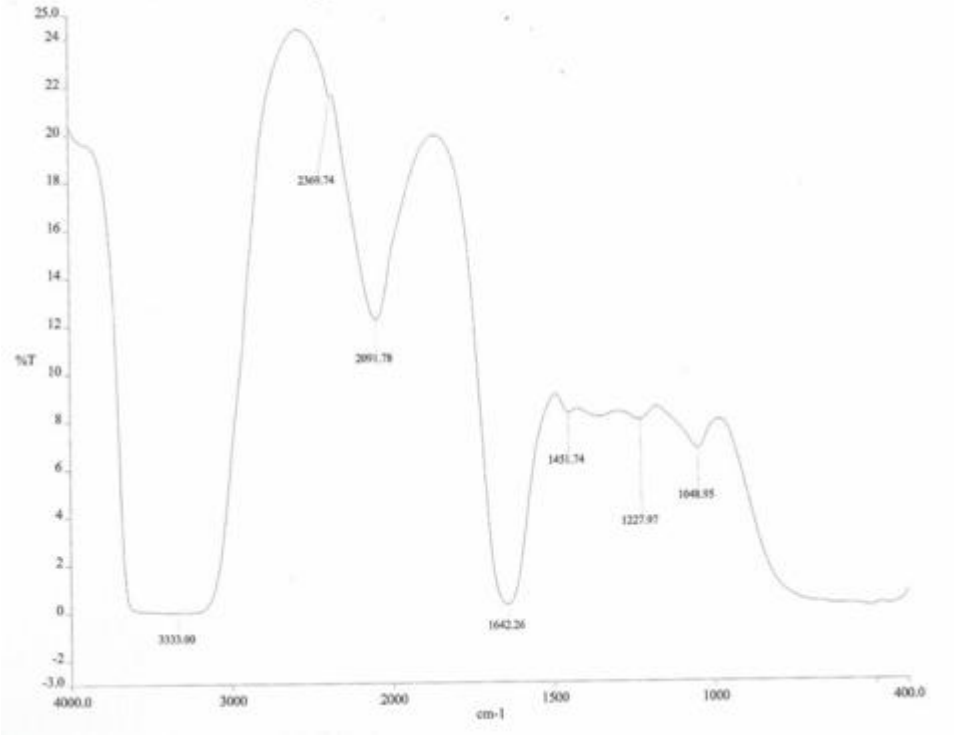

Fig.7b: FTIR spectra of 3mM silver nanoparticles synthesized from *B. paradoxum* seed extract