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Drug Discovery

Green synthesis of sliver nanoparticles using *piper betle* aqueous leaf extract

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ABSTRACT

In the present work was undertaken with a preliminary phytochemical screening of betel leaves showed the presence of active compound in the aqueous extract. The aqueous extract of betel leaves showed the presence of preliminary phytochemical such as

alkaloids flavonoids, Saponins, tannins terpenoids and glycoside substance. In the present work demonstrated an eco-friendly and convenient green method for the synthesis of silver nanoparticles using betel leaf aqueous extracts and found suitable reducing agent for the green synthesis at room temperature itself. Colour change occur due to surface plasmon resonance during the reaction with the ingredients present in the aqueous extract solutions resulting in the formation of silver nanoparticles, which is confirmed by UV-vis spectroscopy. FT-IR spectra of betel leaf mediated biosynthesized silver nanoparticles indicate various functional biomolecules groups present at different position.

Keywords: Silver NanoParticles, Antioxident, Antibacterial activity, *Piper Betle*.

1. INTRODUCTION

Nanotechnology has dramatically developed as an important field of modern research with potential effects in electronic and medicine. Nanomaterials are the particles with a characteristic size range from 1-100 nanometers and they are at the leading edge of nanoscience and nanotechnology. Recently, metal nanoparticles have received particular interest in various fields ranging from material science to biotechnology (Guo *et al*., 2005; Daniel and Astruc, 2004; Huang *et al*., 2007). The properties of many conventional materials change when they formed from nanoparticles. This is because nanoparticles have a greater surface area per weight than larger particles this causes them to be more reactive to certain other molecules. Nanoparticles are effectively a bridge between bulk materials and atomic or molecular structures.

Plant materials are used throughout the world as home remedies, over the counter drug products and raw substances for the pharmaceutical industry, cosmetics industry and represent a substantial proportion of the world drug market. It is therefore significant to establish their quality. In this study we selected a widely available plant material such *as betel nut* and *betel leaf*. India is one of the largest populated countries in the world and it has eight different geographical zones. Over 100 varieties of *Piper betel* has been distributed in both of the hemispheres of world of these 40 species have been recorded in India (Rai *et al*., 2011).

A preliminary study has reported *Piper betel* leaves extracts contains large numbers of bioactive molecules like polyphenols, alkaloids, steroids, saponins and tannins (Koff *et al*., 1971). It exhibit biological capabilities of detoxication, antioxidantion and antimutation that suggested the chemopreventive potential of extracts against various ailments including liver fibrosis and carcinoma.

Nanotechnology is one of the most important and active area of research and development of new biomedical products. Several new products are using silver nanoparticles to generate antimicrobial action due to a large surface area. Silver nanoparticles and silver based compound have been reported to have good antimicrobial activity against an extensive range of microorganisms. In current research, the progress in the field of nanotechnology and nanoscience has brought to fore the nanosized organic and inorganic nanoparticles which are finding increasing applications in medicine, therapeutics, and food packaging and synthetic textiles (Prabhu and Poulose, 2012). Nanocoating the surface of clothing, textiles and textiles for footwear is one approach to the production of high active surface to have UV-blocking and antimicrobial properties (Kathirvelu *et al*., 2009; Gupta *et al*., 2008). Duran *et al*. (2007) study reported that the cotton fabric incorporated with biological synthesized silver nanoparticles exhibited antibacterial activity against human pathogens. A new generation of dressing incorporating agents such as silver to prevent or reduced infection from human pathogens (Yin *et al.,* 1999).

2. MATERIALS AND METHODS

Collection of Plant material and Identification

The Betel leaf of (*Piper betel*) was collected from Malaiyandigondanur, Udmalipat district, Tamilnadu, India. The plant was identified *Piper betel* (Fig. 1) Dr. Arumugamsamy at the Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

Preparations of the Plant extract

Apparently healthy *betel* leaves were collected from local market and washed thoroughly in tap water to remove dirt and other attached particles. The *betel* leaves extracts was prepared by taking 20 g of thoroughly washed and finely cut betel leaf in a 250 mL Erlenmeyer flask with 100 mL of sterile distilled water and then boiled the mixture for 10 min. The solution was then removed from the head source and left at room temperature. Following this step the extract was then filtered through a Whatman filter paper No.1. The extract was kept in refrigerator at 4ºC for further experiments.

Figure 1 Betel leaf

Qualitative Phytochemical Analysis

Phytochemical components of the aqueous extracts of *betel* leaf were screened by using standard methods. The components analyzed were Alkaloids, Flavonoids, Saponins, Tannins, Triterpinoids and Glycosides.

Alkaloids

Solvent free extract, 50 mg of the plant sample was stirred with one mL of dilute hydrochloric acid and filtered. The filtrate was tested for alkaloids.

Mayer's Test: To the filtrate, a drop of Mayer's reagent was added along the sides of the test tube. A white precipitate indicates the test as positive

Flavonoids

Alkaline reagent test: Two mL of aqueous solution of the extract was treated with 1 mL of 10 % ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Saponins

Fifty mg of the plant sample was ground with 3 mL of distilled water and diluted with the same, made-up to 20 mL. The suspension was shaken in a graduated cylinder. After 15 min, a two cm layer of foam indicates the presence of saponins.

Glycosides (Keller-kilani test)

Crude extract was mixed with 2mL of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was then poured into another test tube containing 2mL of concentrated H2SO4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Tannins

One mL of water and 1-2 drops of ferric chloride solution was separated and 1 mL of aqueous extract of the plant sample. Blue color was observed for gallic tannins and green black for catecholic tannins.

Terpinoids (Keller-kilani test)

To 4 mg of the sample was treated with 0.5 mL of acetic anhydride and 0.5mL of chloroform. Concentrated sulphuric acid was added slowly along the sides of the test tube. The presence of red violet colour was observed for terpinoids.

Biosynthesis of AgNPs from *betel* leaf

The aqueous solution of 1mM concentration silver nitrate (AgNO3) was prepared to synthesize silver nanoparticles from betel leaves. For the experiment briefly, 5mL of betel leaves aqueous extract was slowly added to 100mL of aqueous solution of 1mM concentration AgNO3 while stirring, for reduction into Ag ions. The formation of dark brown colour was observed after 8 h incubation at room temperature and λ max was taken using UV-Visible spectroscopy (UV-2600 series shimadzu UV-vis spectrophotometer from 200-800 nm at a resolution of 1nm). Then the silver nanoparticles solution was purified by repeated

centrifugation at 10,000 rpm for 20 min to isolate Ag nanoparticles free from other bioorganic compounds present in the solution. After centrifugation the obtained particles were washed with distilled water for 2 to 3 min and kept it in Hot air oven for drying at 60ºC for 2 hours. The effectiveness and accuracy in results without any contamination, each and every steps of the experiment were maintained under sterility conditions.

Characterization techniques

UV-Visible spectroscopy

Formation of silver particles (After 24h incubation at room temperature) was confirmed by the colour change of the solution and the surface plasmon resonance band was obtained by UV-Visible spectral analysis which was done by using UV-Visible spectrophotometer (JASCO, V-670) from 300-700 nm at a resolution of 1 nm.

FTIR- spectrum

Fourier transform infrared spectroscopy (FTIR) analysis of aqueous extract of *Betel leaf* using FTIR Shimadzu-8400S was carried out at PSG College of Arts and Science, Coimbatore, Tamilnadu, India. The FTIR was recorded in the range of 400 to 4,000 cm-1. The various modes of vibrations were identified and assigned to know the different functional groups present in the extract**.**

X-ray Diffraction spectrum

X-ray diffraction (XRD) measurement of the green synthesized using *betel leaf* extract reduced silver particles was carried out using X'Pert Pro X-ray diffractometer (PAN analytical BV, The Netherlands) equipped with Cu/Kα radiation source using Ni as filter at a setting of 30kV/30mA. All X-ray diffraction data were collected under the experimental conditions in the regular angular range.

The crystalline silver nanoparticle was calculated from the width of the XRD peaks, using a Debye-Scherer formula,

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

Where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X ray wave length, β is the full width at half maximum and θ is the diffraction angle.

Scanning Electron Microscopy

Each of the colloidal solution containing silver nanoparticles synthesis using *Betel leaf* extract was centrifuged at 5,000 rpm for 20 min. The supernatants were discarded and the final pellets were dissolved in 1000µL of deionized water. The pellet was mixed properly and carefully placed on a glass cover slip followed by air-drying. The cover slip itself was used during scanning electron microscopy (SEM) analysis. The images of silver nanoparticles were obtained in a scanning electron microscope (Fb-Quanta 200 SEM machine). The details regarding applied voltage, magnification used and size of the contents of the images were implanted on the images itself.

Energy-dispersive X-ray (EDX) analysis

Energy-dispersive X-ray (EDX) analysis referred to as EDS, is an x-ray technique used to identify the elemental composition of materials. The bio reduction synthesized silver nanoparticles using *Betel leaf* extract subject to the EDX spectrum using Fb-Quanta 200 resolution attached scanning electron microscope to confirm the presence of silver in the particles as well as to detect other elementary compositions of the particle.

3. RESULTS AND DISCUSSION

Phytochemical screening

The preliminary photochemical screening of the aqueous extraction of *betel* leaf was reported (Table I). The positive result for the presence of Alkaloids, Saponins and Glycosides substance are observed in aqueous extract of *betel* of leaf. However, the negative results for the absence of flavonoids, tannins, triterpinoids, phlobatannins and acids substance in aqueous extract of *betel* leaf. Periyanayagam *et al*. (2012) study revealed that the preliminary phytochemical screening of *Piper betle* L. showed the presence of

Alkaloids Flavonoids, Saponins, Tannins Terpenoids and Glycosides, investigation on *betel* leaves revealed the presence of Alkaloids, Carbohydrate, Amino acids, Tannins and Steroidal components. Previously many researches works indicated the betel leaves contains starch, diastases, sugars and an essential oil composing of safrole, allyal pyrocatechol monoacetate, eugenol, terpinen-4-ol, eugenyl acetate, etc. as the major components and the middle part of the vine contains major superiority of tannin (Chopra and Chopra, 1958; Kanjwani *et al.*, 2008). Hence, our present study revealed that the aqueous extract of *betel* leave showed presence of Alkaloids, Saponins, Coumarin, and Glycosides substance (Fig. 1a).

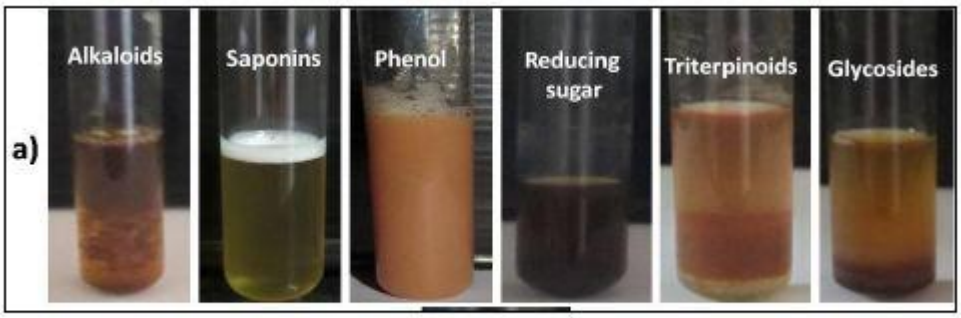

Fig 2: Phytochemicals of aqueous extract of (a) *betel* leaf

Table 1. Preliminary Phytochemical analysis *betel* leaf

S.No	Phytochemicals compound	Aqueous Extraction of *Betel* Leaf
1	Alkaloids	+
2	Flavonoids	-
3	Saponins	+
4	Tannins	+
5	Triterpinoids	+
6	Glycosides	+

Biosynthesis of AgNPs from betel leaf

The aqueous extract of *betel* leaves was used as reducing agent for the synthesis of AgNPs using 1mM concentration of AgNO3. The crude aqueous extract was light brown colour however after addition of AgNO3 the colour of the reaction mixture turned dark brown colour which indicated the formation of AgNPs after 24h incubation period (Fig. 3). The synthesized AgNPs by reduction of silver nitrate during exposure to *betel* leaves aqueous extract was confirmed by UV-Vis spectral analysis, the surface plasmon resonance peak observed at 430 nm (Fig. 4). *Piper betel* leaf petiole extract and ionic surfactants such as cetyl trimethyl ammonium bromide and sodium dodecyl sulphate were used to prepare the stable AgNPs and the obtained AgNPs are in the size of 80nm. Green synthesis silver nanoparticles using aqueous seed extract of *J. curcas* and no toxic chemicals are used as reducing and stabilizing agent during the synthesis (Bar *et al.*, 2009).

The main mechanism considered for the process is plant-assisted reduction due to phytochemicals. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids and quinones are water-soluble phytochemicals that are responsible for the immediate reduction of the ions. Studies have revealed that xerophytes contain emodin, an anthraquinone that undergoes tautomerization, leading to the formation of the silver nanoparticles. In the case of

mesophytes, it was found that they contain three types of benzoquinones and cyperoquinone. It was suggested that the phytochemicals are involved directly in the reduction of the ions and formation of silver nanoparticles. Biodiversity of plants and their potential secondary constituents, plants and plant parts have gained attention in recent years as medium for nanoparticles synthesis (Madhumitha and Selvaraj, 2013).

Fig 3: Colour change of *betel* leaf extracts containing AgNPs before and after synthesis. a) *Betel* leaf extracts b) 1mM $AgNO_3$ c) after 24h incubation

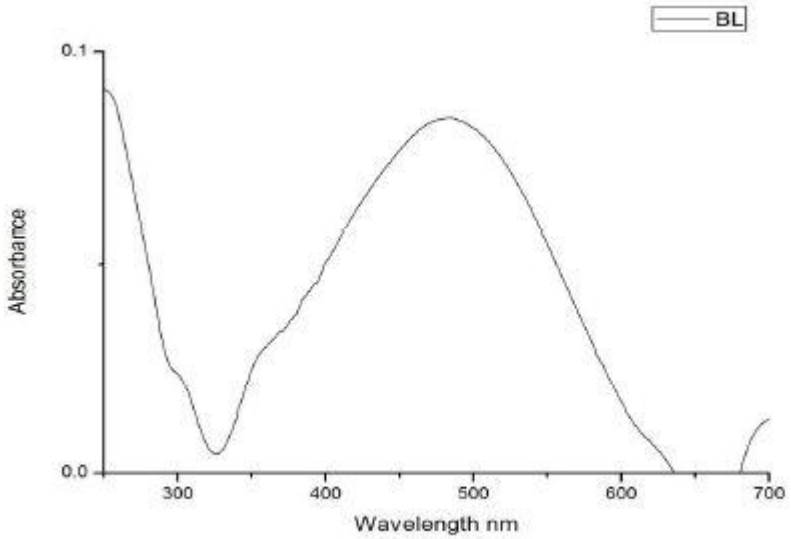

Fig 4: UV absorption of biogenic AgNPs showing surface plasmon peak at 457 nm

Characterization

Fourier Transmission Infra-Red Spectroscopy (FTIR)

FTIR spectrum analysis of the reaction mixture has helped to understand the nature of biomolecules involved in the formation of silver nanoparticles. The FTIR Spectrogram of the betel nuts extract mediated bio inspired synthesized AgNPs has showed prominent sharp absorption peaks located 3450.80, 1634.74, 1519.97 and 682.83 (Fig. 5). The absorption peak at 3450.80 cm-1 1 may be assigned to the O-H stretch, H-bonding function group of alcohols, phenols and the peak at 2924.09 cm-1 is assigned to C-H stretch function group alkanes compound. The absorption peak at 3450.80, cm-1 at close to O-H group and peak at 1519.97 cm-1 and peak at 1634.74 cm-1 indicated N-H bend function group 1° amines and 163473.32 indicated the presence of NO2 stretching.

The light absorption peaks 68283cm-1 may be assigned H-C=O:C-H stretch function group aldehydes and -C≡C-stretch function group alkynes. Moreover, the FT-IR spectra indicate various functional groups present at different position. Vanaja *et al.* (2014) reported that the functional biomolecules are hydroxyl, carboxylic, phenol, and amine group in *M. tinctoria* leaf extract involved in

the reduction of silver ions which was confirmed by FTIR spectrum. FTIR graph of silver nanoparticles obtained from areca nut extract under microwave assistance showed ketone and ester groups which suggest the presence of flavanone layer on the bare nanoparticle (Bhat *et al*., 2013).

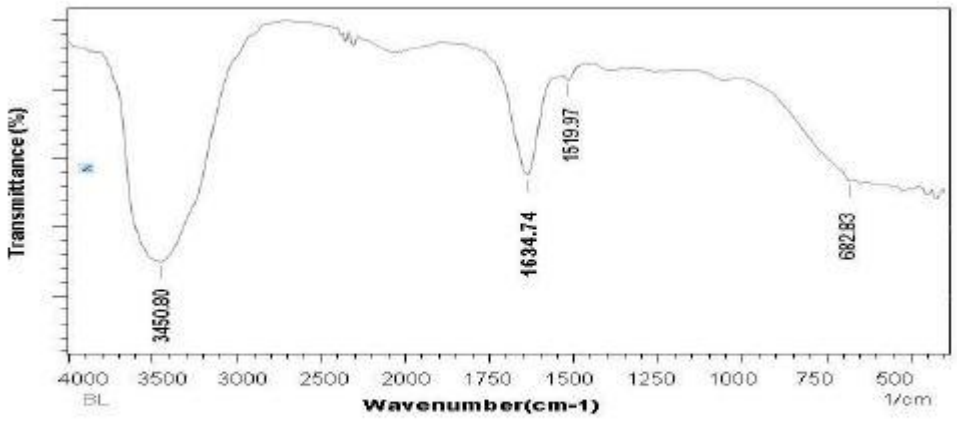

Fig 5: FTIR spectrum of betel leaf mediated bioinspired AgNPs

XRD (X-Ray Diffraction Measurement)

Analysis through X-ray diffraction was carried out to confirm the crystalline nature of the silver nanoparticles. The XRD pattern of *betel* leaf mediated bioinspired synthesized silver nanoparticles showed numbers of Bragg reflections that may be indexed on the basis of the face-centered cubic structure of silver (Fig. 6). A comparison of obtained XRD spectrum with the standard confirmed that the silver particles formed in present experiments were in the form of nanocrystals, as evidenced by the peaks at 2θ values 111, 200, 220 and 311) Bragg reflections, respectively, which may be indexed based on the face-centered cubic structure of silver (JCPDS file nos. 04-0783). X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag+ ions by the betel leaf extract are crystalline in nature. It was found that the average size from XRD data and using the Debye-Scherrer equation was approximately 15 nm. The presence of structural peaks in XRD patterns and the average crystalline size around 15nm clearly illustrate that the AgNPs synthesized by bioinspired method were nanocrystalline in nature. The average particle size of silver nanoparticles synthesized by the present green method can be calculated using the Fe Debye-Scherrer equation (Ahmad *et al*., 2010; Nabikhan *et al*., 2010).

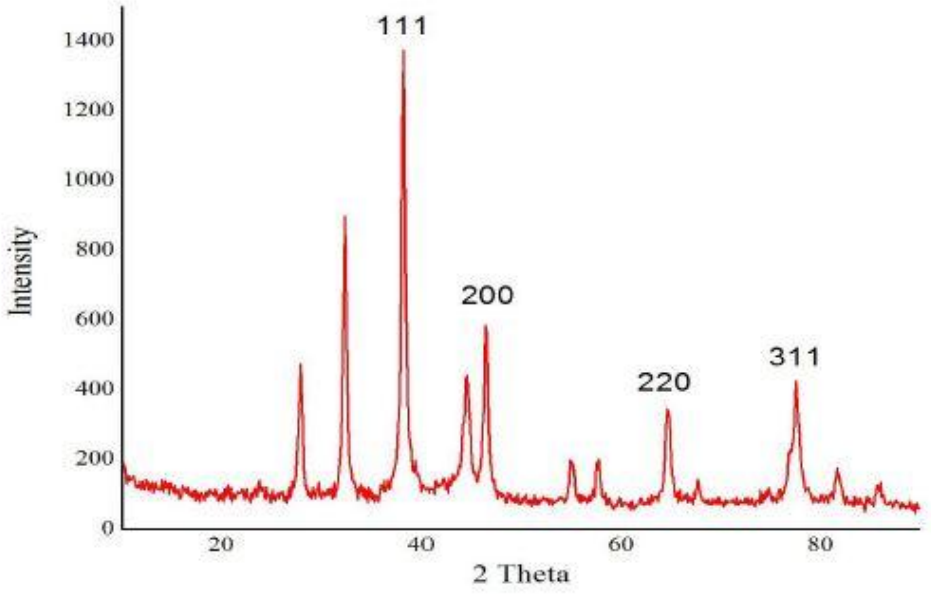

Fig : 6 XRD analysis of betel leaf mediated bioinspired AgNPs

SEM (Scanning Electron Microscope)

Scanning Electron Microscopy (SEM) image shows shape of the bioinspired synthesized silver nanoparticles *using betel* leaf aqueous extract with AgNO3. SEM analysis shows high-density silver nanoparticles synthesized by betel leaf extract. The particles shape distributions of the silver nanoparticles was observed at different magnifications (Fig. 7). It was shown that relatively spherical and a uniform silver nanoparticle with high agglomeration was noted. The large silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements (Preetha *et al*., 2013). Study obtained high density spherical in shape and uniform silver nanoparticles with diameter of study obtained high density spherical in shape and uniform silver nanoparticles with diameter of 13 to 61 nm synthesized by using cannonball leaf extract. The scanning electron microscopy image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the silver nanoparticles. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (Priya *et al*., 2011). In present study, betel leaf extract reduced AgNO3 to AgNPs shown that relatively spherical and uniform with high agglomeration.

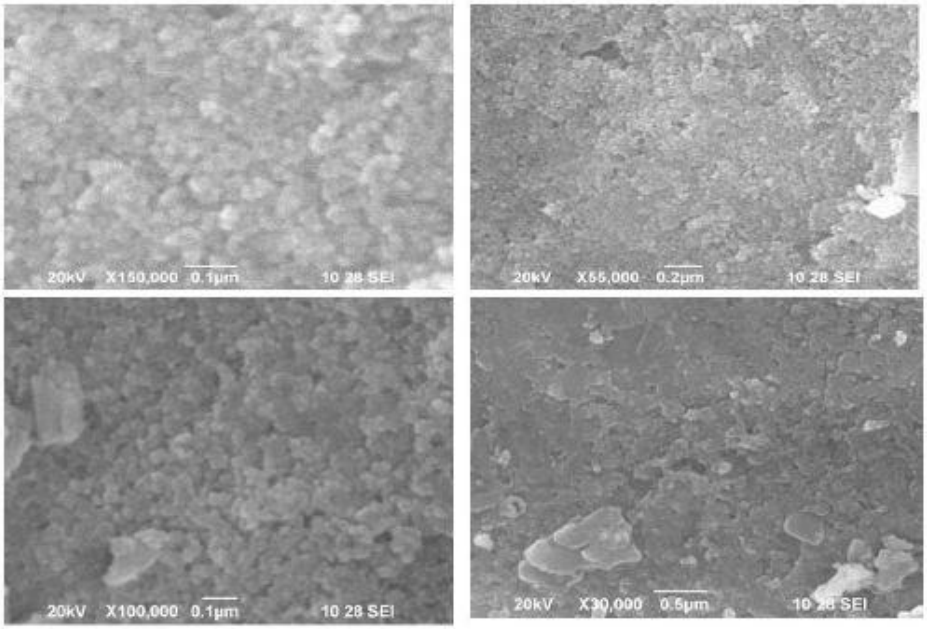

Fig: 7 SEM micrograph of silver nanoparticles formed after reaction of *betel* leaf extract with 1mM $AgNO_3$

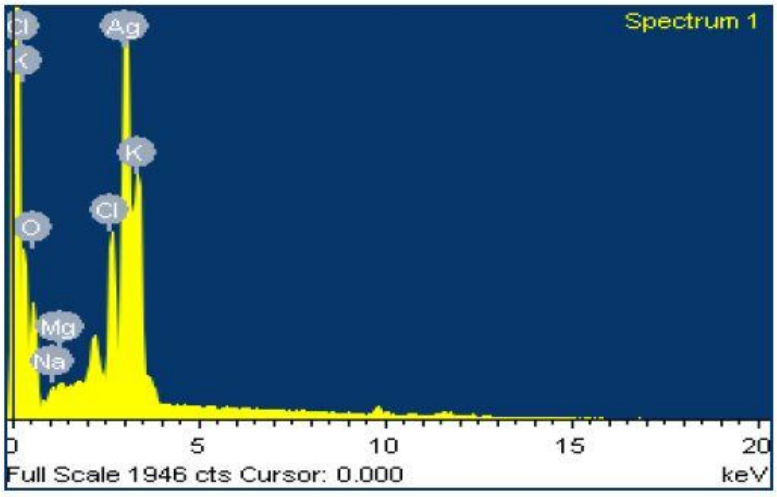

Fig .8 Energy dispersive X-ray spectrometers *betel* leaf

Energy dispersive X-ray spectrometers (EDX)

EDS analysis through Energy dispersive X-ray spectrometers (EDS) confirmed the presence of elemental silver signal of silver nanoparticles. The vertical axis displays the number of X-ray counts whilst the horizontal axis displays energy in keV. Identification lines for the major emission energies for silver (Ag) are displayed and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified All the peaks of Ag are observed and assigned. Reduction of silver ions to elemental silver by the aqueous extract of *betel* leaf was confirmed by energy dispersive spectroscopy (Fig. 8).

The silver nanocrystallites displayed an optical absorption band peak at approximately 3 KeV, which is typical of the absorption of metallic silver nanocrystallites due to surface plasmon resonance (Magudapathy *et al*., 2001). The *betel* leaf extract contains several biologically active compounds including chavibetol, chavicol, hydroxychavicol, estragole, eugenol, methyl eugenol, hydroxycatechol, caryophyllene, eugenolmethylthe, carvacrol, sesquiterpenes, cadinene, caryophyllene, dotriacontanoic acid, hentriacontane, pentatriacontane, stearic acid (Sripradha, 2014; Fawad *et al*., 2010) that may be play a significant role as reducing agents for silver ions. This result is consistent with the results reported by (Haytham, 2015; Afrah, 2015).

4. CONCLUSION

In the present work was undertaken with a preliminary phytochemical screening of *betel leaves* showed the presence of active compound in the aqueous extract. The aqueous extract of *betel leaves* showed the presence of preliminary phytochemical such as alkaloids flavonoids, Saponins, tannins terpenoids and glycoside substance. In the present work demonstrated an eco-friendly and convenient green method for the synthesis of silver nanoparticles using *betel leaf* aqueous extracts and found suitable reducing agent for the green synthesis at room temperature itself. Colour change occur due to surface plasmon resonance during the reaction with the ingredients present in the aqueous extract solutions resulting in the formation of silver nanoparticles, which is confirmed by UV-vis spectroscopy. FT-IR spectra of *betel leaf* mediated biosynthesized silver nanoparticles indicate various functional biomolecules groups present at different position.

The existence of these functional groups is responsible for the stabilization of synthesized silver nanoparticles and also acts as reducing and capping agent. The X-ray diffraction revealed that the structural properties of silver nanoparticles showed numbers of Different Bragg reflections clearly indicated the presence of face-centered cubic structure and crystalline in nature. *betel leaf* mediated silver nanoparticles found that the average size from XRD data and using the Debye-Scherrer equation was approximately 26 and 15 nm respectively SEM analysis shown obtain silver nanoparticles shows uniformly distributed of spherical in shape with high agglomeration on the surface of the cell. The composition of silver nanoparticles studied by using EDS analysis how the absence of any impurities in the prepared samples. The silver nanocrystallites displayed an optical absorption band peak at approximately 3 Key, which is typical of the absorption of metallic silver nanocrystallites due to surface plasmon resonance.

DISCLOSURE STATEMENT

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