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Screening of Larvicidal and Pupicidal Activity Of Biogenic Silver Nanoparticles (AgNPs) of Bidens biternata(L) against the Dengue & Chikungunya Vector, Aedes Aegypti

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Abstract: Mosquitoes are one of the most medically noteworthy groups of vectors, having an capacity to spread parasites and pathogens that can have devastating impacts on humans. The development of reliable and ecofriendly method for the synthesis of metallic nanoparticles is an key step in the field of application of nanotechnology [7]. In this study, we address the biosynthesis of silver nanoparticles (AgNPs) using leaf extract of Bidens biternata (L), and evaluate its lethal concentration (LC50) values against fourth instar larvae and pupae of the mosquito vectors, Aedes aegypti. The nanoparticles were characterized by UV-Vis spectrum, XRD Analysis, scanning electron microscopy, EDAX, and Fourier-transformed infrared spectroscopy analysis. Larvae and pupae were exposed to different concentrations of aqueous extracts of biogenic AgNPs for 24. The larval mortality was increased from 30-100% with increasing concentration from 1-10 ppm. The total mortality of IV Instars larvae of Ae. aegypti was increased from 20-100% with increasing concentration o aqueous leaf extract of Bidens biternata (L) from 10-100 ppm. The LC50 of the AgNPs synthesized from the leaf extract of Bidens biternata (L) against Ae. aegypti was 5.261 ppm.
Keywords: silver nanoparticles, larval mortality, leaf extract, Aedes aegypti, Bidens biternata (L).

I. INTRODUCTION

Mosquitoes are responsible for many vector borne diseases which affect human beings and animals. Dengue has been known in India since 1945 and the classical dengue fever was mainly associated with febrile illness and joint pains. The severe form of infection manifests as dengue haemorrhagic fever (DHF) and Dengue shock syndrome (DSS); 44% of these cases can be fatal. In India, the first outbreak of DHF/DSS was documented in Delhi in 1988 [1].

Chikungunya is caused by the chikungunya virus, recently emerged as an important public health problem in the Indian Ocean islands and India. In 2006, as estimated 1. 38 million people across southern and central India developed symptomatic diseases. The disease is a self limiting febrile illness and treatment is symptomatic [2].

Plants possess rich source of secondary metabolites, inclusive of mosquitocidal properties apart from to their biodegradable capacity they are considerable good candidates for controlling mosquitoes. The experimental plant Bidens biternata, is a member of Compositae family and is commonly known as black jack which is commonly found in India. Extract from this herb is applied in leprosy and skin diseases. Leaf juice is applied to heal ulcers. Dried powder of leaves of black jack is used to cure toothache, flowers are used to treat diarrhea and seeds are used to cure helminthes problems in animals.

Nanoparticles have a main challenge due to the extremely small size, high surface energy and high surface area. These characteristics could comprise possible advantages as faster dissolution, improved penetration, reduced active amount or better distribution of small amounts of active substances over larger area [3]. Biogenic nanoparticles has received increased concentration due to a increasing need to develop environmentally benign technologies in material synthesis. The nanoparticle synthesis is current interest due to their wide variety of function in fields such as electronics, photonics, catalysis, medicine etc [4].

Many researchers have reported on the efficiency of plant extracts against mosquito larvae. In the present study, to evaluate the larvicidal and pupicidal activity of AgNPs synthesized using B. biiternata, leaf extract was assessed under laboratory conditions against the target species of Ae. aegypti.

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II. MATERIALS AND METHODS

A. Collection of Plants And Preparation of Extracts

B. biternata plants were collected from in and around areas of Sivakasi, Virudhunagar dist. The *B. biternata* leaves washed thoroughly in tap water, pat dried with paper towel and shade dried at room temperature (35±1°). These dried leaves were powdered using electrical mixer. The aqueous extract was prepared by mixing 10g of dried leaf powder in 100ml o double distilled water. This suspension was mixed well and left for 5 hour without disturbance, then filtered through Whatmann filter paper (No.1) and the filtrate was used to find out the larvicidal and pupicidal activity against the target vector.

B. Collection and Maintenance of Target Vector

Different larval instars and pupa of *Ae. aegypti* were collected from the Indian Council for Medical Research, Madurai and were brought to the laboratory safely without disturbance. These larvae and pupae were maintained in enamel trays containing deionized water and allowed to feed on Brewer's yeast, dog biscuits and sucrose in 3:1:1 ratio in the laboratory at room temperature for 24 hours, before the experiment begins.

C. Synthesis of Silver Nanoparticles From Leaf Extract

Aqueous leaf extract *B. biternata* was prepared by placing 10g of chopped fresh leaves in a 250ml Erlenmeyar flask and boiled with 100ml of sterile double distilled water upto 60 minutes at 60°C in a water bath. The crude extract was passed through Whatmann filter paper (No.1) and the filterate (=aqueous leaf extract) were stored at 4°C and used within 3 days. 10mm of aqueous leaf extract was treated with 90ml of prepared 1mm aqueous $AgNO_3$ solution in an Erlenmayer flask and incubated in dark at room temperature. The aqueous solution of 1mm of $AgNO_3$ was greatly reduced from Ag^+ to Ag^0 by aqueous leaf extract leading to change of pale yellow to dark brown resulting in synthesis of AgNPs.

D. Characterization of Synthesized Silver Nanoparticles

The bio-reduction of silver ions to silver was monitored by measuring the UV-Vis spectrum of the reaction mixture at a wavelength of 300-800 nm using a UV-2450 Shimadzu spectrophotometer at 1 nm resolution. An aliquot of this filtrate including silver nanoparticles was used for scanning electron microscopy (SEM), EDAX and Fourier transformed infra red spectroscopic (FTIR) studies. The structure and composition of purified silver particles was analysed by using a FEI Quanta 200 scanning electron microscope at accelerating voltage of 20 kV. The functional groups of the nanoparticles were qualitatively confirmed by using FTIR spectroscopy. The presence of synthesised silver nanoparticles in leaf extract was analysed by EDAX.

E. Larvicidal and Pupicidal Activity

The larvicidal and pupicidal activity was evaluated using WHO method (1996) with slight notifications. Different test concentrations of leaf extract and AgNPs in 200ml deionized water were prepared. Bio-efficacy analysis was conducted against the larvae and pupa of target vector at ten different concentrations of aqueous leaf extract (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10%), synthesized AgNPs and 10 larvae were exposed to each test solution. Mortality rate was recorded after 24h of exposure period.

F. Statistical analysis

The dose response mortality data were concerned to probit analysis for finding the LC_{50}, standard deviation and mean separation statistical tools are employed. Upper and lower confidence limit at 95% confidence and values determined using the software (SPSS, 2007).

III. RESULTS AND DISCUSSION

The results of the larvicidal and pupicidal mortality caused by leaf extracts of B. biternata and synthesized AgNPs against Ae. aegypti are presented in (Table 2,3). LC_{50} value of B. biternata and synthesized AgNPs against larvae and pupae of Ae. aegypti is 45.135ppm and 5.261 respectively.(Table 1). All the larvae and pupae experimented confirm support of a lethal effect, and mortality was absolutely dose-dependent against mosquito vectors Ae. aegypti.

Larvicides play a essential role in controlling mosquitoes in their breeding sites. The present study was carried out to establish the larvicidal activities of biosynthesized silver nanoparticles (AgNPs) against An. stephensi responsible for diseases of public health importance. The use of natural product chemistry with nanotechnology together that reduces mosquito populations in the larval stage can provide many associated benefits to vector control. Since silver nanoparticles are considered to be prospective agents for various biological applications including antimicrobials, their application as a mosquito larvicide was investigated. Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest control agents. The botanical extracts from the plant leaves, roots, seeds, flowers and bark in their crude form have been used as conventional insecticides for centuries. Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality to target organisms to be effective [5].

The potential uses and benefits of nanotechnology are numerous. These include agricultural productivity enhancement involving nanoporous zeolites for slow release and efficient dosage of water and fertilizer, nanocapsules for herbicide delivery, vector and pest management, and nanosensors for pest detection. The utilization of biological systems for nanoparticle synthesis is a notable alternative for the advancement of this multifaceted approach. Biological systems have revealed the skill to interact with metal ions and reduce them to form metallic nanoparticles. Plants are widely used for nanoparticle synthesis, and the use of plants for the fabrication of nanoparticles is a rapid, low-cost, eco-friendly single step method for the biosynthesis process [6].

The present study evident that the leaf extract of B. biternata has the potential to reduce silver nitrate ions into silver nanoparticles. The colour of the reaction mixture is changed slowly from yellow to brown colour after bioreduction. Similar findings were reported by Sivapriyajothi et. al., (2014) in the plant extract of Leucas aspera. The UV-Vis spectroscopy can be used to examine size and shape of nanoparticles in aqueous suspensions. In the present study, UV-Vis absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 539.00nm (Fig. 1). Similar phenomenon was reported in Ficus racemosa leaf extract, broadening peaks in the reaction media has absorbance peak at 425nm [7].

In the present study, SEM reports revealed that the average size of synthesized silver nanoparticles was 7nm and the shape of nanoparticles proved to be spherical in nature (Fig. 2). Similar shape of nanoparticles was synthesized in Mushbaibisiana [8]. The rough morphology of the AgNPs provides excellent larvicidal activity of the synthesized nanoparticles.

The EDAX results provided chemical analysis of field of view and as well as the spot analysis of minute particles and confirmed the presence of specific elements [9]. In the present study, EDAX pattern showed that the photographs of derived silver nanoparticles (Fig. 3). The peaks of silver are observed and are assigned. The signal was observed at 3 kV, which is typically for silver nanoparticles due to the surface Plasmon resonance. The other special signals such as Ca, C and O were also noticed in the EDAX spectrum may arise from the organic content of faecal pellets extract that are already bound with the surface of silver nanoparticles.

The FTIR spectra of aqueous silver nanoparticles prepared from the B. bitternata leaf extract show transmittance peaks (Fig. 4). Further, the results of the FTIR values of synthesized silver nanoparticles showed the presence of various functional groups such as alkane groups, methylene groups, alkene groups, amine groups and carboxylic acids. These functional groups are the key groups in many of the chemical groups, which have been formerly proven to have probable as reducing agents in the synthesis of silver nanoparticles. Studies indicate that the carboxyl (–C=O), hydroxyl (–OH) and amine (–NH) groups in leaf extracts are the ones mainly involved in the fabrication of silver nanoparticles, and for which bands were reported [10].

In the present study, the LC_{50} value of aqueous leaf extract of B. biternata is 5.261 ppm. It indicates the toxicity found in B. biternata. Similar findings were reported for the leaf extract Hippophae rhamnoides against the mosquitoes of Ae. aegypti and An. Stephensi [11]. Results of the present study are consistent with the earlier findings of [12] who observed that the LC50 values of ethyl acetate extract of L. aspera were 75.40, 93.09, 132.20, and 138.60 ppm against the first, second, third, and fourth larval instars, respectively of C. quinquefasciatus. Mwangi. R.W and H. Remboldh, (1998) reported that the leaf extracts of L. aspera exhibited high mortality, especially during the moulting process or the subsequent process of melanization and tanning.

In the present study, the larvicidal activity of aqueous leaf extract of B. biternata was observed. The larval mortality was increased from 30-100% with increasing concentration from 1-10 ppm (Table 2 and 3). Similar findings were observed in the leaf extract of Rhizophora mucronata against the IV instar larvae of Ae. aegypti and C. quinquefaciatus [14]. In the present study the pupal mortality for the aqueous leaf extract on Ae. aegypti was observed. The pupal mortality was observed for 20% in 1,3,5,6 ppm and 10% in 2,4,7,8 ppm (Table 2 and 3). It may be due to the toxic effect of the B. biternata. Similar findings were observed for the leaf extract of Morinda tinctoria against the pupal of Ae. aegypti mosquito [15].

IV. CONCLUSIONS

The present study confirmed that the silver nanoparticles synthesized from the leaf extract of B. biternata has larvicidal, pupicidal and adulticidal activity against the mosquitoes Ae. aegypti. The viewpoint of developing natural products for synthesizing silver nanoparticles and testing their efficiency in controlling mosquitoes larvae is a edvanced technique make possible the development of a more potent and environmentally safe biopesticide. Thus the present study concludes that the leaf extract of B. biternata has the otential to produce silver nanoparticles. Further the AgNPs of B. biternata be used as larvicidal, pupicidal and adulticidal tool against Ae. aegypti. Hence the AgNPs synthesized from leaves of B. biternata extract can be used as an ecofriendly larvicidal, pupicidal and adulticidal agent in the vector management programme.

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Table 1: LC50 values of the test solutions for aqueous leaf extract and silver nanoparticles synthesized leaf extract of Bidens biternata against IV instar larvae of the mosquito Aedes aegypti.

Types of leaf extract	Test solution	LC50 values in ppm
Aqueous leaf extract	Aqueous leaf extract of *Bidens biternata*	45.135 ppm
Silver nanoparticles synthesized leaf extract	Silver nanoparticles synthesized from extract of leaf	5.261 ppm

Table 2: Effect of different concentrations of aqueous leaf extract of Bidens biternata on the larval and pupal period, pupal and adult mortality, percentage of total mortality and adult emergence on the IV instar larvae of Aedes aegypti.

S.No	Parametres	Control	Concentration of aqueous leaf extract in ppm									
			10	20	30	40	50	60	70	80	90	100
1	Larval period in days	4	4	4	4	4	4	4	4	4	4	4
2	pupal period in days	2	2	2	2	2	2	2	2	2	2	2
3	Larval mortality	0	1	2	4	6	7	9	9	9	10	10
4	Pupal mortality	0	3	4	1	1	0	0	0	0	0	0
5	Adult mortality	0	0	0	0	0	0	0	0	0	0	0
6	Total mortality %	0	30	40	60	80	90	90	90	90	100	100
7	Adult emergence %	100	70	60	40	20	10	10	10	10	0	0

Table 3: Effect of different concentrations of silver nanoparticles synthesized from aqueous leaf extract of Bidens biternata on the larval and pupal period, pupal and adult mortality, percentage of total mortality and adult emergence on the IV instar larvae of Aedes aegypti.

S.No	Parametres	Control	Concentration of silver nanoparticles synthesized from aqueous leaf extract in ppm									
			10	20	30	40	50	60	70	80	90	100
1	Larval period in days	4	4	4	4	4	4	4	4	4	4	4
2	pupal period in days	2	2	2	2	2	2	2	2	2	2	2
3	Larval mortality	0	1	2	5	5	4	4	8	9	10	10
4	Pupal mortality	0	3	2	3	2	0	0	0	0	0	0
5	Adult mortality	0	0	0	0	0	0	0	0	0	0	0
6	Total mortality %	0	30	40	70	70	60	60	80	90	100	100
7	Adult emergence %	100	70	60	30	30	40	40	20	10	0	0

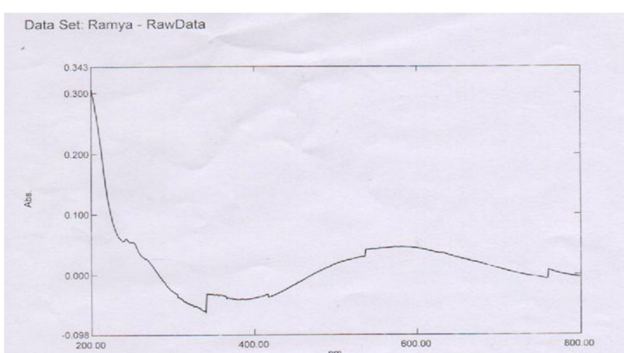


Fig. 1 UV-Visible spectrum of silver nanoparticles synthesized from leaf extract

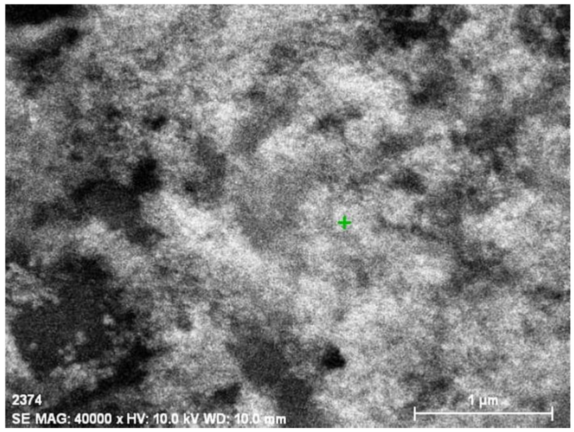


Fig. 2 SEM image of synthesized silver nanoparticles using *Bidens biternata* leaf extract

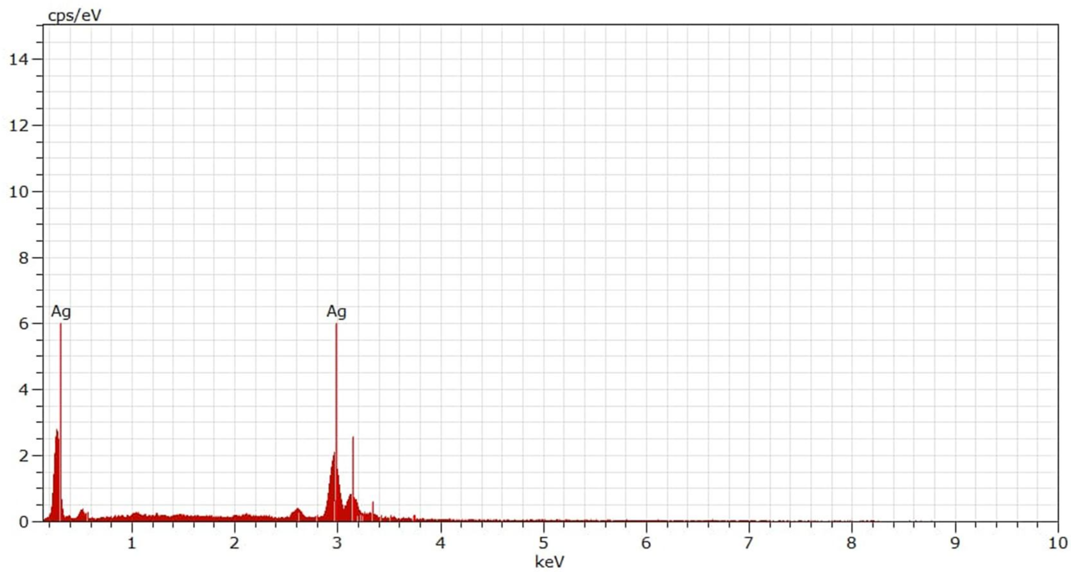


Fig. 3 EDAX Spectrum of synthesized silver nanoparticles using Bidens biternata leaf extract

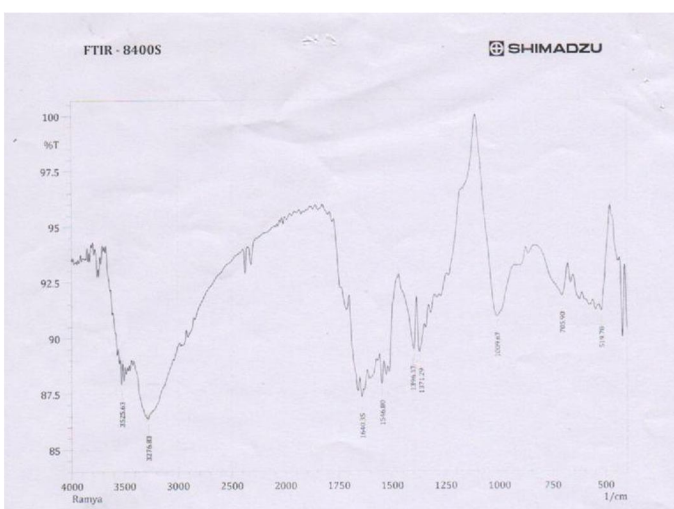
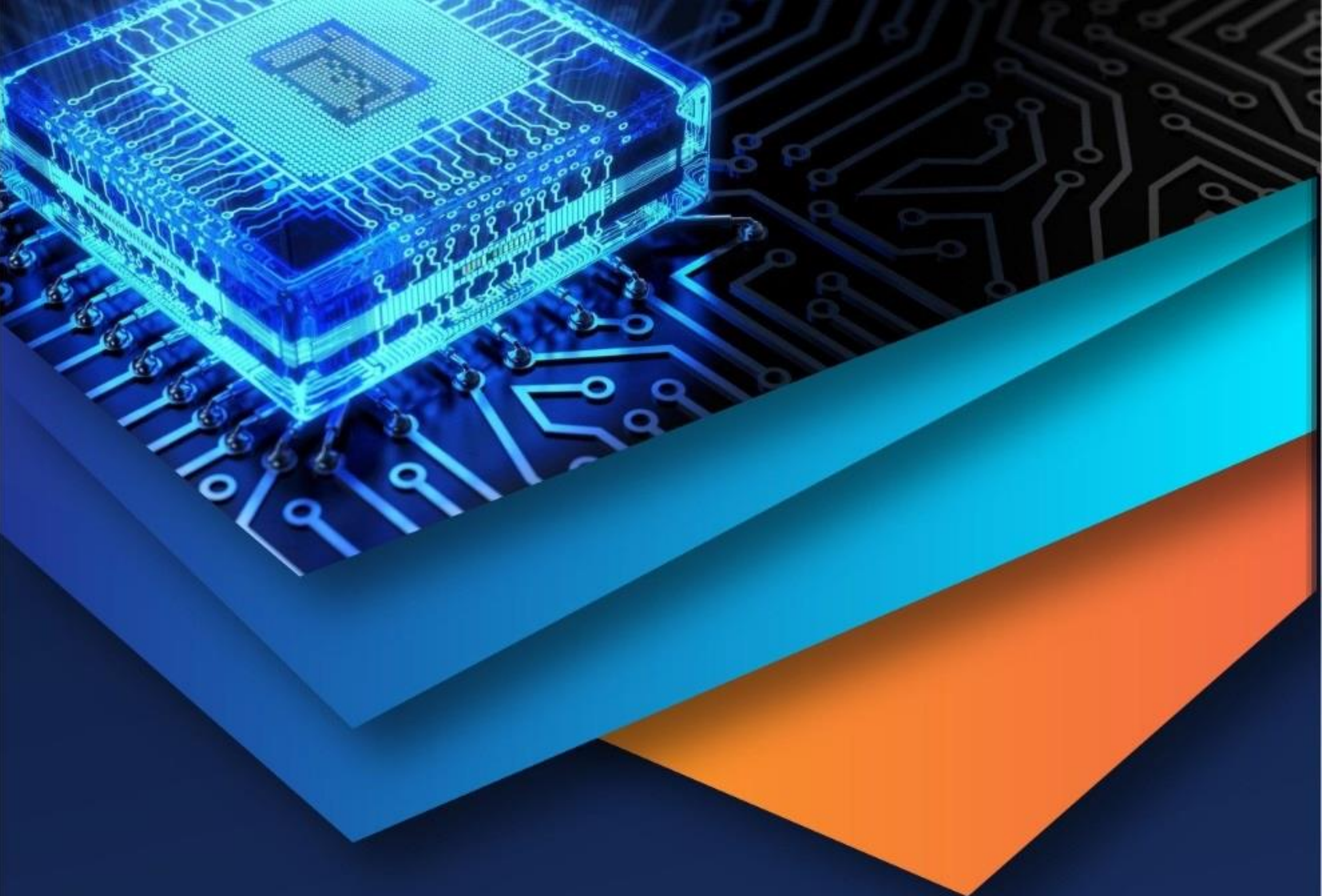


Fig. 4 FTIR of synthesized silver nanoparticles using *Bidens biternata* leaf extract



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