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# Biological insights of Aromatic Plant Extract from *Zingiber ottensii* (Valeton) Leaf Against the Phytopathogenic Organisms

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***Abstract:*** *The present work was planned to frame the objectives are as follows initially to investigated the antibacterial efficiency of two leaf solvent extracts from Zingiber ottensii* (Valeton). *Then analysed the silver nano particle production in an experimental plant extracts through SEM and XRD techniques also identification of potential bioactive compounds performed by GCMS method. Antibacterial effect has been studied against the phytopathogenic bacterial strains by the disc diffusion method. Maximum and minimum zone of inhibition (1.9±0.07&1.6±0.004) has been noticed against yellow and pink coloured tested organisms showed on both water and ethanol extracts respectively. It was significantly maximum antibacterial activity when compared with the ethanol Z. ottensiileaf extract. Moreover, SEM revealed the presence of polydisperse silver nanoparticles with an average size of 3.0 to 6.2nm range respectively. Hence the current study was clearly indicated that when the leaf aqueous extract of Z. ottensii subjected with AgNO3 produced silver nanoparticles were typically possessed the potentially antibacterial property against the experimental phytopathogenic organism. Similarly GC-MS chromatogram observed in ethanol extract totally possessed nine components supremely Isogeraniol, compound noticed in peak level (71.56), followed by 1-2di β-Myrene, present in second level peak bio-compound (56.34), subsequently, other seven other bioactive compounds were identified as minimum to optimum level Correspondingly Z. ottensi water extract also been possessed the peak compounds named as Bornyl acetate biocompound in addition minimum and optimum level other more bioactive compounds like Asaraldehyde, β-Elemene and Cubenolalso identified. From the present result clearly indicated among the two extract, maximum potential bioactivity (phytopathogenicity) perceived in water extract because this extract contains above noticed antibacterial efficiency of bioconstituents compared with ethanolic leaf. extract of Z. ottensi plant.Hence the current research clearly depicted Z. ottensi leaf water extract possessed the potential antibacterial agent against the phytopathogenic bacterial species in legume plants (diseased Leaf).*

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## I. Introduction

*Zingiber ottensii* (Valeton) is a member of the Zingiberaceae family. The rhizome is dark purple on cross-section. The leaf stalks are close together and can reach up to 1.5m tall with many leaves. Zingiberaceae is a large family with 53 genera and over 1200 species distributed throughout Asia, Africa and America (Karnchanatat *et al*., 2011). Plants in the genus are known for their aromatic constituents and medicinal properties. Zingiberaceae plant species contain many essential oils including terpenes, alcohols, ketones, flavanoids, carotenoids and phytosterogens (Bartley and Jacobs, 2000; Supreetha *et al*., 2011). Zingiberaceae plants which have been reported for their biological activities in antifungal, antioxidants, insecticidal and inflammatory activities (Gold and Moellering, 1996) are particularly vital for these applications. Previously, Karnchanatat *et al*., 2011 published *Zingiber ottensii* (Valeton) with antiproliferative activities against fungi and human malignant cell lines. It was also called as 'The Great Medicament" in Ayurvedic medicines (Tan and Vanitha, 2004). It belongs to family *Zingiberaceae* and is a perennial plant with thick tuberous rhizomes as well as leaves, which are the medicinally useful part of this plant. The medicinal history of ginger has been extensively searched throughout the world and found to possess anti-inflammatory, cholesterol-lowering, and antithrombotic properties (Zaika, 1975). The bioperspective spectrum of *Z. ottensii* plant leaf for the antimicrobial activity and chemical analysis of this plant has been investigated (Singh *et al*., 2008).According to Chandan *et al*., (2011) Silvernano particles study was mainly deals with the production of nanoparticles having various shapes, sizes and organization of their biological and physiological parameters for further use in scientific society especially human welfares. Among the different Zingiber family plant in this study has been a

selected traditionally important medicinal valuable plant such as *Z. Ottensii* plant leaf. Silver nanoparticles are having good history in the field of antimicrobial properties. Previously, Vanaja *et al.*, (2013) the silver nanoparticles are vigorously involved in the antimicrobial activity against a lot of disease causing food borne and water borne pathogenic bacteria and fungus. So far, no one work was has been done like this kind of research, Keeping this view in mind the importance and scope of this experimental plant of *Z. ottensii* was selected. Thus the present study was aimed to the following objectives such as investigation ofthe leaf extract (water and ethanol) of *Z. ottensii* against the phytopathogenic organisms from the legume plant of *V. radiate* leaf. Secondly, analysed the silver nano particles from the experimental plant leaf extract through SEM and XRD techniques. Along with this identified the potential bioactive compounds from plant extract of leaf in *Z. oittensii* by GCMS.

## II. Materials And Methods

### COLLECTION OF EXPERIMENTAL SAMPLES

The Zingiber species of *Zingiber ottensi* is used here as the study material. It was collected from Thirparappu area in K.K.District. Sample was confirmed by Plant Genetic Resource Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institution, Palode, Trivandrum, Kerala, India

**Sample extraction**

The leaf and rhizome samples are dried under shady condition then grind well using a mortar and pestle. The rhizome sample was washed with tap water and it again with sterile water. Then it is grind well and preserved under cold condition.

**Collection of diseased plant pathogens**

The diseased leaf legume (*V. radiata*) family plant was collected from place. The diseased part (leaf) is separated on the basis of colour (brown, yellow and dried green). Brown, yellow and dried green coloured leaf samples were washed thoroughly for surface sterilization then grinded separately with distilled water in mortar and pestle. The extract is collected and centrifuged for 10000rpm for 15 minutes. The supernatant which contains pathogenic organism are collected and pellet is discarded. Then the supernatant was incubated for further experiments especially antibacterial test.

**Antibacterial assay**

Antibacterial activities of the extracts were studied by the disc diffusion method. Lawns of each organism were prepared on nutrient agar plates. The plant extract was concentrated by evaporation and was added to sterile filter discs and allowed the solvent to evaporate after each addition. The discs were then placed on air dried surface of the medium. The plates were then incubated for 24hrs at $37^{o}$C. After incubation the degree of sensitivity was determined by measuring the zone of inhibition of growth around the discs. Antibacterial activity of disease caused microorganisms were analyzed by plating the diseased part extracts in agar plates and more dominant microbial colonies formed were inoculated in nutrient broth and used for further antibacterial analysis.

### BIOSYNTHESIS OF NANOPARTICLES

The leaf extract (1ml) was added to 50ml of $10^{-3}$ $AgNO_3$ aqueous solution and kept at room temperature. The time of addition was considered as the start of the reaction. Under continuous stirring conditions, after 10 minute, the light yellow color of $AgNO_3$ solution gradually changes to brownish yellow color indicates the formation of silver nanoparticles. The bioreduction of $AgNO_3$ ion in solution was monitored by periodic sampling of aliquots (0.1ml) of aqueous component and measuring UV-Vis spectra of the solution. The nanoparticles were characterized and conformed by SEM and XRD analysis (Chandran *et al*., 2006).

**Characterization of silver nanoparticles**

To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken 300–540 nm using a UV–vis spectrophotometer. The de-ionized water was used as the blank. The samples from the maximum time point of production of silver nanoparticles were air-dried and allowed to characterize by Atomic Force Microscopy for its detail size, morphology and agglomeration of silver. AFM Image was taken with silicon cantilevers with force constant 0.02–0.77 N/m, tip height 10–15 nm, contact mode. To check phase formation and purity, XRD patterns were recorded using powder X-ray diffractometer. The samples from the maximum time point of production of silver nanoparticles were mounted on specimen stubs with double-sided adhesive tape and coated with gold in a sputter coater to avoid charging and examined under SEM.

**Scanning Electron Microscopy (TEM) analysis**

TEM samples of the gold and silver nanoparticles synthesized using ginger rhizome extract were prepared by placing a drop of nanoparticle solutions on carbon coated copper grids and allowing water to evaporate. SEM measurements were performed on a Morgagni 268(D), which was operated at accelerating voltage of 100 kV.

**GCMS Analysis**

Gas chromatography study includes the important optimization process such as i) introduction of sample extract onto the GC column, ii) separation of its components on an analytical column and iii) detection of target analysis by using mass spectrometry (MS) detector. 5ml of ethanol extract was evaporated to dryness and reconstituted into 2ml methanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was carried out with instrument GC-MS-QP 2010 (SHIMADZU instrument) with Db 30.0 column (0.25 µm diameter × 0.25 µm thickness). The oven temperature was programmed from 70°C (isothermal for 5 minutes), with an increase of 10°C/min. up to 200°C, then 5°C/min. up to 280°C and ending with 35 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; scan interval of 0.5 seconds and scan range from 40–1000 m/z. Helium was used as the carrier gas at 99.99 % pressure with flow rate of 1.0 ml/min. and electronic pressure control on Samples were dissolved in methanol and injected automatically.

**Statistical Analysis**

The resulted data of antibacterial effect was expressed as mean ± SD. Analysis of variance (ANOVA) was used to test for differences in the groups and multiple range test of significance was used.

## III. Result

**Antibacterial effect of in *Z. ottensii* leaf ethanol and control extracts on Phytopathogenic bacterial strain**

The in vitro antimicrobial activity of the two extracts against the tested Phytopathogenic bacterial organism like pink and white bacteria and it was assessed on both cases of presence and absence of inhibition zone diameters. Antibacterial activity of *Z. ottensii* leaf extract on these tested organisms showed a maximum zone of inhibition (1.9±0.07) was noticed against yellow coloured colored in ethanol extract followed by maximum antibacterial zone of inhibition effect noticed against pink coloured colonies on both water (1.7±0.05) and ethanol (1.6±0.004) extracts respectively. It was significantly maximum when compared with the control experiment. Moreover similar kind of zone of inhibition 0.5±0.003 has been observed in both extract of water and ethanol against white colored colonies (Table-1).

**Table-1: Effect of $AgNO_3$ treated *Z. ottensii* extract on the Experimental Phytopathogenic bacterial strains**

| Experimental Phytopathogenic Colonie(s) | Water (mm) | Ethanol (mm) | Ethanol+ $H_2O$ (mm) |
|---|---|---|---|
| PPO (black) | 0.5±0.001 | - | $0.3±0.01^{IS}$ |
| PPO (yellow) | 0.9±0.00 | 1.9±0.07** | 0.8±0.021 |
| PPO (white) | 0.5±0.003 | 0.5±0.002 | 0.6±0.013 |
| PPO (pink) | 1.7±0.05** | 1.6±0.004 | $0.8±0.0061^{**}$ |
| PPO (Intense) | 0.3±0.07 | - | 1.0±0.00 |

PPO--- Phytopathogenic Organisms

*- Denotes statistically significant at P< 0.05% level

**- Stands for P<0.005% Level of Highly significant

Table-2 shows that the total antibacterial efficiency against the current study selected pytopathogenic organisms such as brown and yellow coloured as well as burned apperance given bacterial strain to the affected legume plant. Mainly disease causative yellow colored dominant colony was significantly (2.8±0.02) suppressed by the ethanol extract of leaf in *Z. ottensii*. Subsequently brown coloured phytopathogenic agent also been observed the second most antibacterial effect same leaf extract. Meanwhile, maximum (2.1±0.006) and minimum (0.5±0.00) zone of inhibition was noticed on brown and yellow coloured phytopathogenic agents in the control extract generated nanoparticles. Consequently, mixture of water and ethanol extract based nanoparticles were denoted minimum (0.3±0.00) to (2.3±0.06) maximum zone of inhibition noticed on brown and yellow colored phytopathogenic colonies respectively (Fig-1).

**Table-2:-Antibacterial effect of leaf in *Z. ottensii* ethanol and control extracts on Phytopathogenic bacterial strain diseased leaf from *V.radiata* plant**

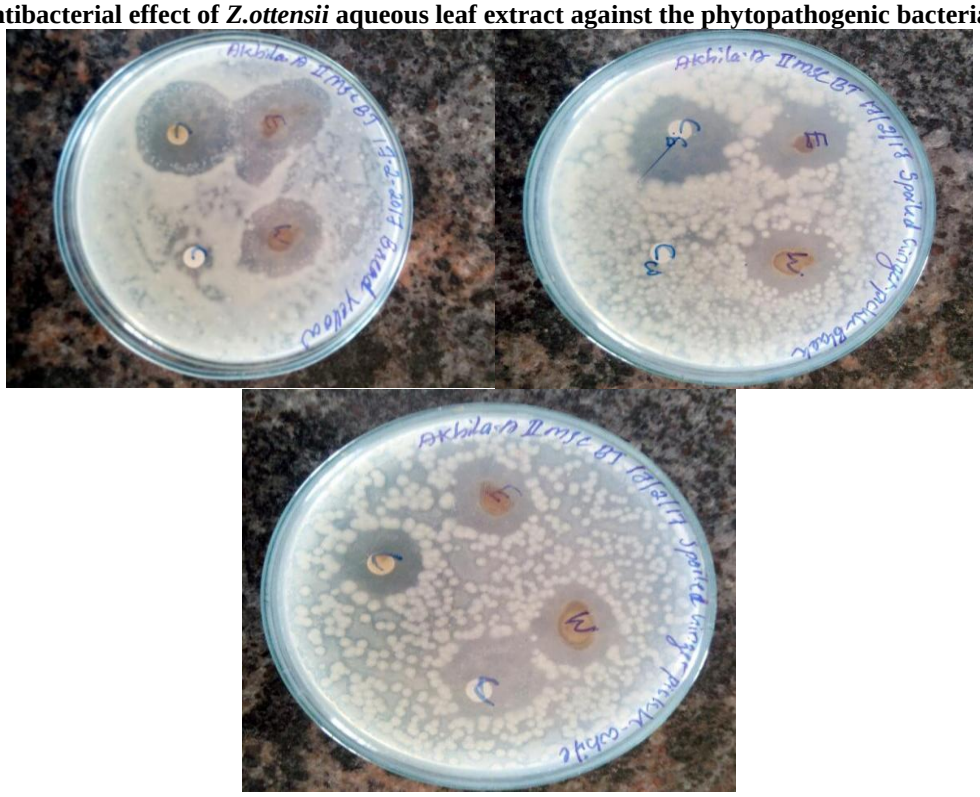
| Name of the sample identity | Ethanol (mm) | Water (mm) | Control + ethanol (mm) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Diseased leaf(brown) | 1.7±0.001* | 1.4±0.00 | 0.3±0.00 |
| Diseased plant (yellow) | 2.8±0.02** | 2.1±0.006 | 0.5±0.001 |
| Diseased plant (green) | 0.3±0.001 | $0.5\pm0.00^{IN}$ | 1±0.03* |
| Control | 2.2±0.04 | 3.9±0.4 | 2.3±0.06 |

*- Denotes statistically significant at P< 0.05% level

**- Stands for P<0.005% Level of Highly significant

**Fig:1- Antibacterial effect of *Z.ottensii* aqueous leaf extract against the phytopathogenic bacterial strains**



**Production of *Z. ottensii*rhizome based 1N$AgNO_3$ nanoparticles and its bioactivity**

After the addition of ethanol and $AgNO_3$ the colours starts to change. After the short interval of time, the colour changes in first test tube is dark greenish yellow colour, in second test tube colour changes light greenish yellow colour and in fourth test tube colour changes to light yellow colour are shown in Fig-2a and b**.** After one day of incubation the colour change in first test tube was dark greenish colour, in second test tube colour changes to light reddish colour, in third test tube colour changes to dark greenish colour and in fourth test tube color changes to greenish yellow colour. After the addition sample with water and silver nitrate solution the colour of first test tube changes to light yellowish solution and in second test tube the colour changes to dark yellowish solution. The first test tube contains sample, water and silver nitrate shows brownish red particles which are settled under the tubes. The second tube contains sample, ethanol and silver nitrate shows dark yellow colour and the third tube contains sample, water rhizome and silver nitrate shows light purple grey colour solution which are shown in the figure Fig-2b.

**Fig:2- Nano particle production of *Z. ottensii*Leaf(A) water and (B) ethanol extract**

**(A)** **(B)**

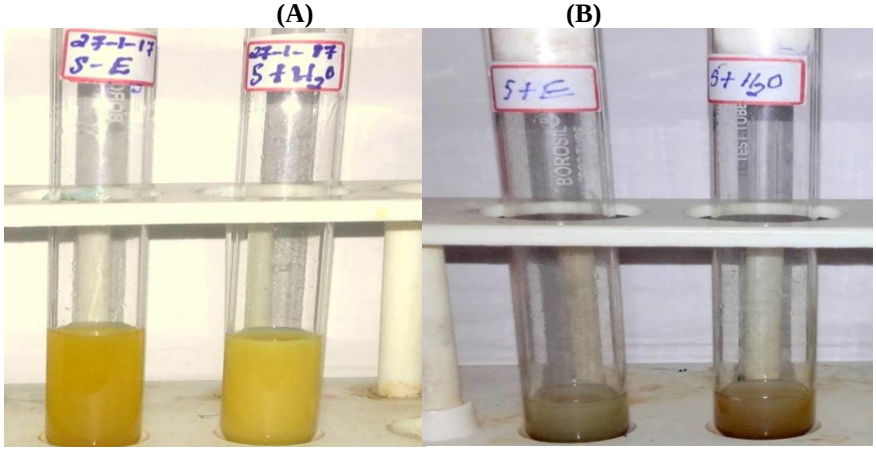
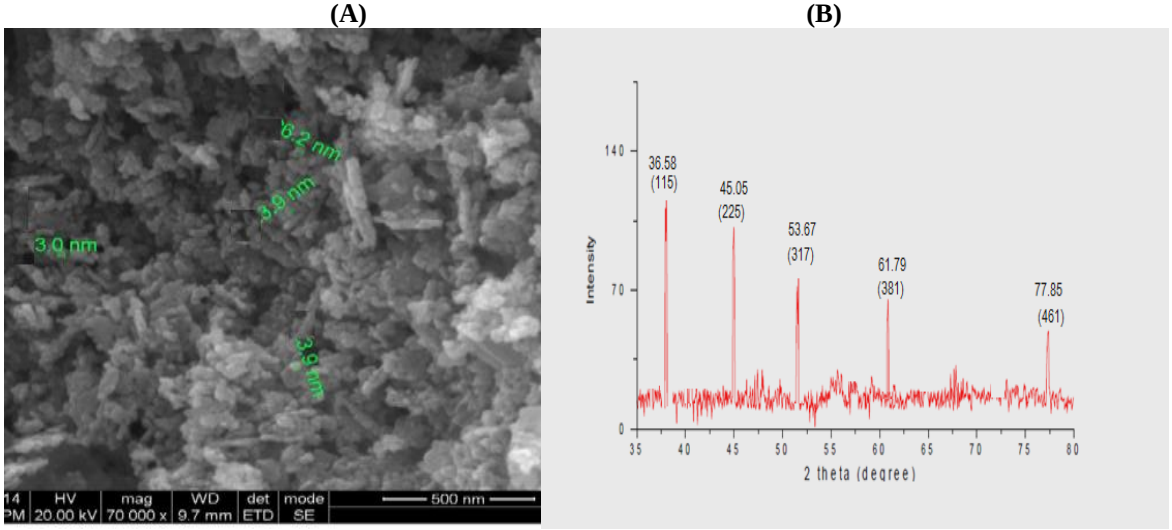



**Fig 2:- Sem view (A) of *Z. ottensii* leaf extract with 1N AgNO3 and its XRD analysis (B)**

**(A)** **(B)**




SEM view of the current research sample of *Z. ottensii* leaf water extract based nanoparticle view was clearly expressed the range between 3.0 to 6.2nm range of the nanoparticles. The present nanoparticle study was clearly showed that the X-ray diffraction (XRD) pattern showed the number of Bragg's reflections at diffraction (2$\theta$) angles 35.58°, 45.05°, 53.67°, 61.79° and 77.85°, corresponding to planes 115, 225, 317, 381 and 461 respectively, which indicated that spherical silver nanoparticles are crystalline in nature with face-centered cubic structure represent the fig-2. Hence, the current study was indicated that when the *Z. ittensii* leaf water extract subjected with $AgNO_3$silver nanoparticles were typically possessed thepotentially antibacterial property against the experimental phytopathogenic organism from legume plant of *V.radiata* leaf.

**Fig-3: GCMS chromatogram view of *Z. ottensii* (Roxb.) ethanol extract of leaf**

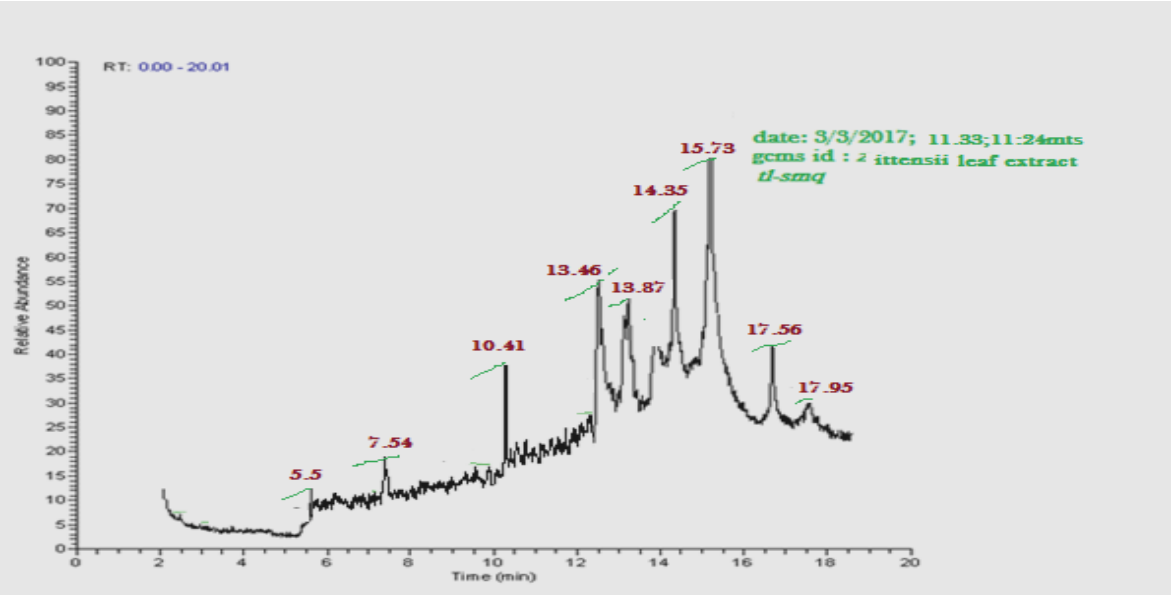



**Table-7: Bio-Compounds elucidation of ethanol leaf extract from Z. *ottenssi* (V.) by GCMS Analysis**

| S.No | Phytoconstituent(s) | Relative abundance | Retention time |
|---|---|---|---|
| 1. | 5-Nonaol,-5-methyl | 12.43 | 5.5 |
| 2. | tau-Muurolol | 17.16 | 7.54 |
| 3. | Caryophyllene | 3.20 | 10.41 |
| 4. | 1-2 di β-Myrcene | 56.34 | 13.46 |
| 5. | 7-Oxabicyclo (2.2.1) hept-5-en-2-one | 52.71 | 13.87 |
| 6. | Isogeraniol | 71.56 | 14.35 |
| 7. | Aromadendrene oxide | 4.05 | 15.73 |
| 8. | Cyclohexanone, 3-ethenyl | 41.83 | 17.56 |
| 9. | Terpinen-4-ol | 29.64 | 17.95 |

The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in [Figure 3]. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. GC-MS chromatogram for *Z. ottensi* ethanolic leaf extract showed the following result, such as totally 9 components has been identified initially Isogeraniol, compound noticed in peak level (71.56), followed by 1-2di β-Myrene, present in second level peak bio-compound(56.34), subsequently, 7-Oxabicyclo (2.2.1) Hept-5- en-2-1, present in peak value (52.71), Cyclo Hexanone, 3- Ethyl, bioconstituents present in other peak value (41.83), Terpinen-4-ol bioconstituents present in peak value (29.64), Tau-Muurolol, bioconstituents present in peak value (17.60), 5-nonaol, -5- methyl, bioconstituent present in peak value (12.43), Aromadendrene Oxide, bioconstituents present in peak value (4.05), Caryophyllene, bioconstituents present in peak value (3.20)

**Fig-4: GCMS view of control leaf extract of *Z. ottensi* plant**

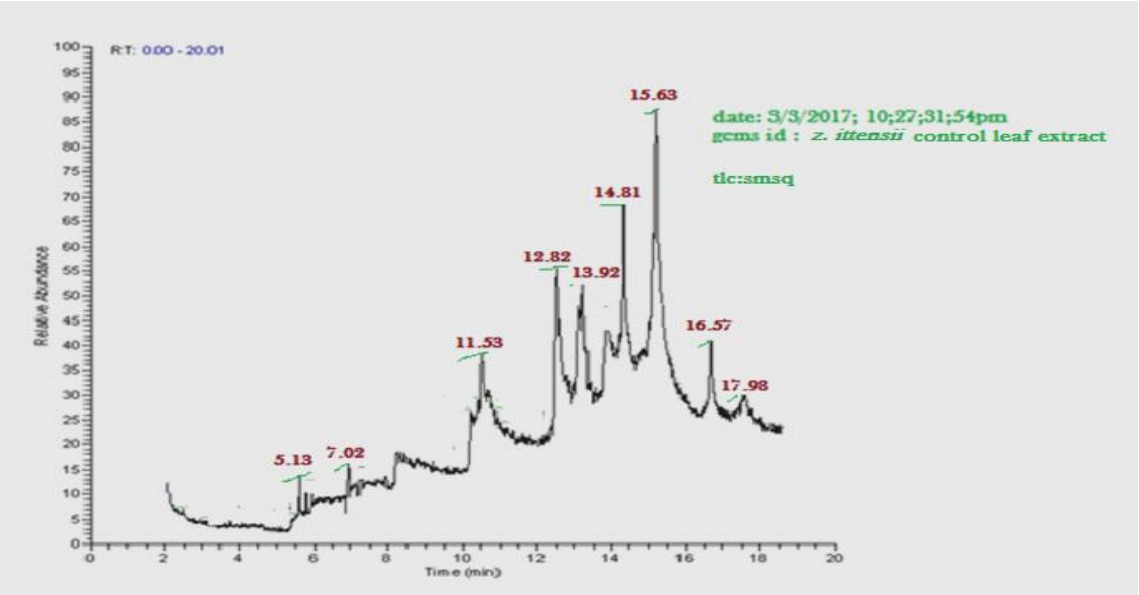



**Table-8: Bio-Compounds elucidation of control leaf extract from *Z. ottensi*(Roxb.) by GCMS Analysis**

| S.No | Phytoconstituent(s) | Relative abundance | Retention time |
|---|---|---|---|
| **1.** | 5-Nonaol Caryophyllene | 15.03 | 5.13 |
| **2.** | β-Elemene | 16.26 | 7.02 |
| **3.** | Cyclohexanone, 3-ethenyl Pinocarvone | 40.15 | 11.53 |
| **4.** | Cubenol | 56.86 | 12.82 |
| **5.** | Asaraldehyde | 52.07 | 13.92 |
| **6.** | 1-dimethyl-3-methyleneβ-Myrcene | 70.24 | 14.81 |
| **7.** | Bornyl acetate | 92.01 | 15.63 |
| **8.** | 3,4,5-Trimethoxybenzylchloride | 42.74 | 16.57 |
| **9.** | 6-(1-methylethyl) Cedrene | 31.27 | 17.98 |

According to figure-4and table-8 showed GC-MS chromatogram for *Z. ottensi*control leaf extract. It was showed the following result, such as totally 9 components. In addition, Bornyl acetate bioconstituent present in peak value(92.01), 1-dimethyl-3-methyleneβ-Myrcene, bioconstituents present in peak value (70.24), Cubenol (56.86), Asaraldehydebioconstituents present in peak value (52.07), 3,4,5-Trimethoxybenzylchloride, bioconstituents present in peak value (42.74), Cyclohexanone, 3-ethenyl Pinocarvonebioconstituents present in peak value (40.15), 6-(1-methylethyl) Cedrene, bioconstituents present in peak value (31.27), β-Elemene, bioconstituent present in peak value (16.26), 5-Nonaol Caryophyllene , bioconstituents present in peak value (15.03). Finally least level of compounds wereobserved named as 6-(1-methylethyl) Cedrene along with its percentage of abundance 31.27% and retention time 17.98.

## IV. Conclusion

Conclusion of this present study was clearly noted that the aqueous extractof *Z. ottensi* leaf possessed better antibacterial potential against the dominant diseased causative agent of phytopathogenic organism in legume crop of *V.radiata* plant. It was significantly maximum response compared with ethanol extract. While, in this study successfully demonstrated that this experimental plant leaf extract has the ability to synthesize the nanoparticles it was mainly highest potential against the any pathogenic organism. Spectroscopic analysis from GC–MS studies shows that the major antibacterial characteristic compounds such as Cubenol, Asaraldehyde and 5-Nonaol Caryophyllene present in aqueous extract of *Z. ottensi* leaf than the ethanol extract. Hence, it can be concluded that antimicrobial activity of especially against the pathogenic bacteria shows its medicinal value and supports the widespread use of the plant as well as crop specific remedy for a variety of ailments of various leaf blight diseases in legume crop plant.

## V. Discussion

Most of rural people even today depend on plants for medicines as a leaf from aromatic plants. In India, 95% of the traditional system prescriptions ofunani, ayurveda, Homeopathy and Siddha are plant based chemicals especially derived from aromatic plants (Zielińskaand Matkowski, 2014). The plant based chemical compounds are classified into two classes; primary and secondary metabolites based on their chemical, biosynthetic origin and functional groups(Akiyama*et al*., 2006). Primary metabolites are involved in growth and development and secondary metabolites are involved in defense mechanism against harmful pests and infectious agents. The later class exhibit medicinal properties. Plant derived chemicals such as terpenoids, phenolics, alkaloids, flavonoids, glycosides, diterpenes, triterpenes and minor chemicals are having better compatibility with the human body. It is estimated that 30% of the worldwide sales of drugs is based on plant products

Medicinal plants have been used as a source of medicine in all cultures since times immemorial (Gottlieb *et al*., 2012). Initially plants were used by the people to meet their nutritional requirements. The natural flora became a very useful source for health improvement and to cure many diseases across various human communities and a variety of plants species are offered which are still in use in many parts of the world such as Asia (Ghulam *et al*., 2017), for remedies against several diseases. Furthermore, the gingerols, including 6-gingerol, a phenolic compound present in ginger root, have been shown to have chemopreventive effects that are associated with their antioxidative and anti-inflammatory activities (Surh*et al.,* 2002; Habsah *et al*., 2000). Many efforts have been made to discover new antimicrobial compounds from various kinds of sources of zingiber family particularly, the present experimental plant leaf of *Z. ottensii* similar opinion also already agreed by Karnchanatat *et al*., (2011); Chantaranothai *et al*. (2013). A large classes of bioactive compounds like flavonoids, tannins possessed in leaf so clinical pathogens are arrested them growth, hence it have long been utilized as a source of therapeutic agents worldwide this kind of result was agreed by Gottlieb *et al*., (2002); Gibbons, (2005); Akiyama *et al*. (2006); Khan *et al*., (2009). Recently, herbal medicines have increasingly been used to treat many diseases including several infections. Plants produce certain chemicals which are naturally toxic to bacteria (Singh and Bhat, 2003) and many plants have been investigated for the development of novel drugs with therapeutic properties (Tomoko *et al*., 2002;Bode *et al*., 2007); Yusha *et al*., 2008) Most of the studies that test the medicinal values of zingiber family leaf extracts of both water and ethanolic extract possessed pronounced remarkable antibacterial activity this kind of similar opinion was already depicted by several authors (Akoachere *et al*., 2002; Thubthimthed *et al*., 2006; Adeshina *et al*., 2011). Previous study HAS BEEN MADE BY (Atai *et al*., 2009; Poeloengan, 2011) has shown that ethanolic extract of ginger has anti-fungal activity. The similar kind of result has been noticed in *Z. ittensii* rhizome extract (Elechiguerra *et al*., 2005; Atai *et al*., 2009). This result is comparable with other studies which have shown that ginger has broad as well as pronounced inhibitory activities against *C. albicans*. antibacterial activity opined by several authors (Ficker, 2003; Chairgulprasert *et al*., 2005; Chen *et al*., 2008; Adeshina *et al*., 2011).The phytochemical constituents that are playing a significantrole in medicines can be identified using crudeextracts/drugs of the medicinal plantsuch same concept was agreed by number of authors (Abad *et al*., 2007; Jigna and Sumitra, 2007; Tiengburanatamc *et al*., 2010). Structural and crystalline nature of the silver nanoparticles has been performed using XRD analysis. Figure 6 shows that the biosynthesized silver nanostructure by using *Z. ittensii* leaf extract was demonstrated and confirmed by the four characteristic peaks observed in the XRD image at 2θ values ranging from 35.58 to 77.85. The five intense peaks are 38.13°, 46.2°, 64.44°, and 77.36° corresponding to the planes of 115, 225, 317, 381 and 461 respectively. These lattice planes were observed which may be indexed based on the face-centered crystal structure of silver The XRD pattern thus clearly showed that the Ag-NPs are crystalline in nature. It has been makes use of environmental friendly, nontoxic and safe materials similar result was already been reported by several researchers (Mukherjee *et al*., 2001; Sharma *et al*., 2009; Paulkumar*et al*., 2014) like plant leaf extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles that offer numerous benefits of being eco-friendly and compatible for pharmaceutical and other biomedical applications (Gull *et al*., 2012). Mechanism of action regarding with silvernano particle study on antimicrobial activities mainly depends it their high aspect ratio of nanoparticle production it has been reported Erdogrul (2002). The nano particles interfere with cellular processes once entering the microbes opined by Sharma *et al*. (2013). Also, the nano particles surface adhesion with the microbial cell surface leads to its immobilization Antibacterial activity of $AgNO_3$ nanoparticles similar opinions were already been reported by Chantaranothai *et al*. (2013). In addition (Prabhu *et al*., 2010) reported on the basis of the oxygen species released on the surface of $AgNO_3$ which cause fatal damage to microorganisms. They react with hydrogen ions to produce molecules of $H_2O_2$ which results in more oxygen species on the surface and the higher antibacterial activity of the smaller nanoparticles (Aruoma *et al*., 1997; Sunanda *et al.,* 1998; Chandan *et al*., 2011).

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