ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 12, pp.43393-43401, December, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

A PROMISING SOURCE OF POTENTIAL BIOACTIVE METABOLITES FROM THE ROOT EXTRACTS OF *CASUARINA JUNGHUHNIANA* MIQ

Chathurdevi, G. and *Uma Gowrie, S.

Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women, Chennai-600008, Tamil Nadu, India

ARTICLE INFO

Article History:
Received 15th September, 2016
Received in revised form
28th October, 2016
Accepted 15th November, 2016
Published online 30th December, 2016

Key words:

Casuarina junghuhniana,
Root,
Phytochemicals,
Antifungal,
TLC,
HPLC,
GC-MS.

ABSTRACT

Casuarina junghuhniana is one of the most important members of nitrogen fixing, woody, non leguminous tree in the family of Casuarinaceae. The roots form a symbiotic association with *Frankia*- an actinomycete that fixes atmospheric nitrogen enabling the tree to tolerate varied environmental constrains. It is a multipurpose tree which is widely used in soil reclamation, agro forestry systems, dune stabilization and in wind breaks. It has also end uses such as pulp wood, fuel wood and as poles for constructions. Increasing work has been reported on genetic improvement and yield of this species, not much work has been documented on the phytochemistry of the tree. Therefore, the present research work focuses on the presence of potent secondary metabolites of *Casuarina junghuhniana* root. *Casuarina junghuhniana* root samples were collected from the State Forest Research Institute, Kolappakam, Chennai. Different extracts of root samples were evaluated for the presence of phytochemicals by qualitative and quantitative analysis. Anti fungal activity was tested against different phytopathogens. The maximum zone of inhibition was found against *Fusarium oxysporum* (20±0.2mm). FT-IR analysis revealed the presence of functional groups such as alkyl halides, aliphatic amines, aromatic amines, nitro compounds, amides, alcohols and phenols. The chromatographic studies such as TLC, HPLC, GC-MS revealed the presence of a rich source of phenols and flavonoids in root extract. The secondary metabolites present in the root may also play an important role in plant growth and resistance to their pathogens and herbivores. Further, this study may also help us to understand the compatibility of *Casuarina sp* cultivation with different intercrops and to design and promote the suitable intercropping system in agro forestry.

Citation: Chathurdevi, G. and Uma Gowrie, S., 2016. "A Promising Source of Potential Bioactive Metabolites from the root extracts of *Casuarina junghuhniana* Miq.", *International Journal of Current Research*, 8, (12), 43393-43401.

INTRODUCTION

Plants produce a variety of compounds which can be divided into primary and secondary metabolites. The secondary metabolites of plants are known as secondary products or natural products which are considered to have no direct function in plant's growth and development (Lincoln taiz *et al.,* 2015). These compounds have other alternate functions such as protecting plants against pest and pathogens, repellence to herbivores, defense against abiotic stress and also for the communication of the plants with other organisms (Schafer *et al* 2009). Such plants are said to be more resistant, may be due to the presence of higher concentrations of secondary metabolites (Simms, 1992). The important secondary metabolites of plants include phenols, terpenoids, alkaloids, saponins etc., Some of these compounds are found to be present in one plant species or taxonomically related group of species, whereas primary metabolites are found throughout the plant kingdom (Lincoln taiz *et al.,* 2015). These secondary metabolites are biosynthesized in specific pathway and the sites of synthesis are specific to each compound and to the plant species (Yazdani *et al., 2011*). Plant secondary metabolites have wider important applications in the field of medicine, industry, and agriculture and food science. *Casuarina junghuhniana* Miq (named after Friedrich Franz Wilhelm Junghuhn, 1809-1864, a German Botanist) is a woody, non leguminous, nitrogen fixing actinorhizal tree, has a symbiotic association with an actinomycete *Frankia* belongs to the family Casuarinaceae. This tree species is distributed naturally in the climax montane rainforest of Bali, as an associate species with *Podocarpus imbricatus*, *Eugenia sp*, *Laplacea sp*, *Ficus sp*. (Robinson *et al.,* 1982). This tree grows on the slopes of volcanoes at an altitude of 1500-3100m but also at lower altitudes in Wetar and Timor, where it occurs from near sea level to 1500m altitude. In the Lesser Sundas, it grows as a major forest tree crop at an elevation from 2100 to 2700m above the mixed montane forests (Jayaraj, 2010). *Casuarina junghuhniana* is an exotic species, systematically

Corresponding author: Uma Gowrie, S.
Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women, Chennai-600008, Tamil Nadu, India.

introduced into India in 1996. It normally grows up to 25 to 35 m tall and 50 to 80 cm in diameter (Luechanimitchit *et al.,* 2016). In recent years, *C.junghuhniana* proved to be more preferred species than widely cultivated *Casuarina equisetifolia* for its good coppicing ability, it can readily root and can quickly establish in plantations after planting. The excellent coppicing ability makes it responsive for clonal forestry (Nicodemus *et al.,*2016). It has recorded faster growth, with a short rotation period of 3-4 years both in coastal and inland areas (Varghese *et al.,* 2011). The desirable characters of *Casuarina junghuhniana* such as drought resistant, adaptability to varied soil conditions, resistance to blister bark disease and its growth rate, cultivation practices and marketability made it popular among the south Indian farmers. It has various end uses such as pulp wood, fuel wood, as poles in constructions, used as shelterbelts, widely planted to improve soil fertility and as windbreaks in agroforestry system (Sureshkumar *et al.,* 2016). The presence of rich and potential secondary metabolites of *Casuarina* sps might be responsible for the tree to be healthy and resistant against pests and pathogens. In the recent year research works, attention has been given to the improvement of yield and quality of *Casuarina junghuhniana* but not much work has been documented on secondary metabolites and its significance. Since the documentation about the phyto chemistry of the plant is important, the present research work focuses on the study of bioactive compounds in *Casuarina junghuhniana* root extract and its importance. The objective of the present research work is to screen the qualitative and quantitative phytochemicals and to study its antimicrobial potential. FT-IR, TLC, HPLC and GC-MS analysis were also carried out to characterize the potential bioactive compounds present in the root extract.

MATERIALS AND METHODS

Collection of Plant Material

The plus trees of *Casuarina junghuhniana* were identified from State Forest Research Institute, Kolapakkam, Chennai. Healthy root samples were collected from 4 year old plantation site. Identification (authentication) of the plant sample was confirmed at Botanical Survey of India (BSI), Coimbatore, Tamil Nadu.

Preparation of plant extracts

The collected roots were thoroughly washed, shade dried and finely powdered. Powdered root material was extracted with different solvents using cold percolation method. The extract was then concentrated and dried under reduced pressure and preserved at 5^0C until further use.

Qualitative and Quantitative Phytochemical analysis

Preliminary screening of qualitative phytochemicals were carried out using different solvents, Maximum positive results were obtained with Methanolic root extractsbased on polarity, hence, methanolic root extract was used for the following analysis. Qualitative phytochemical analysis was carried out to check the presence of alkaloids, phenols, tannins, flavonoids, terpenoids, proteins and amino acids, carbohydrates, steroids, phlobatanin saponin, Gum and mucilage using standard procedures (Raaman, 2006 and Harborne, 1998). Quantitative analysis such as Estimation of Total Phenol Content, Total Flavonoid Content (Samidha Kamtekar *et al., 2014*). and Terpenoid content (Tejavathi and Jayashree., 2013) was carried out using standard procedures. Total phenolic content was calculated as Gallic acid equivalents (mg of GAE/g of extract). Total flavonoids content was calculated as Quercetin equivalents (mg of QAE/g of extract). Total Terpenoids was calculated using the formula:

$$\text{Terpenoid content (\%)} = \frac{\text{Weight of terpenoid extract (g)}}{\text{Weight of sample (g)}} \times 100$$

Antifungal activity

The ethanol, methanol and aqueous root extract of 50µg & 100µg were assayed for its antifungal activity by well diffusion method using Potato Dextrose Agar. The extracts were tested against *Alternaria alternata, Curvularia lunata*, *Fusarium oxysporum, Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani.* Carbendazim (100µg) was used as positive control. The plates were incubated at room temperature. Zone of inhibition around the well was observed after 48 to 72 hrs for antifungal assay. Triplicates were maintained.

Fourier Transform Infrared Spectrophotometric analysis

For the FT-IR study, Spectrum FT-IR system (Shimadzu, IR Affinity 1, Japan), equipped with a DLATGS detector with a mirror speed of 2.8mm/sec. scan range: from 400-4000cm^{-1} with a resolution of 4cm^{-1} was used. The ethanol, methanol and aqueous extracts of root sample were used. The extract was evaporated by flash evaporator and it was pelletized using KBr salt in ratio 1:100. Using the thin pellet, Infrared spectra were recorded at the range of 4000 - 500cm^{-1} (John Coates, 2000).

Thin Layer Chromatography analysis

Different extracts (ethanol, methanol & aqueous) of root were used for TLC analysis. TLC was performed for the detection of Phenolic compounds after screening with different solvent systems. Solvent system A Ethyl acetate: Formic acid :acetic acid : water (20:11:11:26) was finally selected to identify the presence of gallic acid in root sample, 2% ferric chloride was used as spraying reagent and Solvent system B Chloroform: Ethyl acetate: Formic acid (5:4:1) was used to detect the presence of quercetin in root sample.1% ferric chloride was used as spraying reagent. The samples were run along with the standards. The spots were noted and Rf value was calculated and compared with standard (Radomir *et al.,*2004).

HPLC Studies

Sample preparation: The methanol extract of the root sample was filtered through Whattman No. 1 filter paper and the filtrate was evaporated to dryness. Dried sample was resuspended in 1ml HPLC graded methanol by vortexing. The samples were further filtered through 0.45mm membrane (Millipore) and stored at 4^{o}C for further use. Standards such as Gallic acid, Quercetin were prepared using HPLC Graded methanol and used for HPLC analysis. Chromatographic conditions: HPLC analysis was carried out using SHIMADZU LC 20 AD with UV detector. Stationary phase: C-18 column, Reverse phase. Mobile phase used was Methanol: HPLC grade distilled water: 1% Acetic acid (80:20:1**),** flow rate: 1ml/min, wave length: 280nm, 20 microlitres of the sample was introduced with a Rheodyne valve equipped with 20µl external loop. Phenolic compounds present in the sample were detected

on the basis of comparison with the retention time (Rt) of the sample with that of the Rt of the individual standards at similar chromatographic conditions (Gupta Mradu *et al.,*2012).

GC MS studies

The methanol root extract was subjected to GC MS analysis to identify the bioactive compounds. GC-MS analysis was performed at the SAIF, IIT-Madras, Chennai, Tamil Nadu. The sample was subjected to GC and MS JEOL GC mate equipped with secondary electron multiplier. JEOL GCMATE II GC-MS (Agilent Technologies 6890N Network GC system for Gas chromatography). The column (HP5) was fused silica 50 m X 0.25 mm I.D. The experimental conditions were 20 min at 100 °C, column temperature: 235°C for 3 min; injector temperature: 240 °C; carrier gas: helium; and split ratio: 5:4. 1µl of the sample was evaporated in a split less injector at 300 °C and the run time was 40 min. The active phytochemical components were identified by gas chromatography coupled with mass spectrometry. The spectrum of GC-MS was analyzed using the database of NIST having more than 62,000 patterns.

RESULTS AND DISCUSSION

Casuarina junghuhniana Miq. roots were collected from State Forest Research Institute, Kolapakkam. The roots were dried and powdered.

Qualitative and Quantitative Phytochemical analysis

The preliminary qualitative phytochemical screening of Methanolic root extract of *Casuarina junghuhniana* showed the presence of diverse phytochemicals. It showed the presence of phenol, flavonoids, tannin and terpenoids, carbohydrates and proteins. In the present study, the total phenol content of *Casuarina junghuhniana* root determined by Folin-ciocalteu method was reported as Gallic acid equivalent (Standard Curve equation Y=0.027x +0.176, R^2=0.994). The total flavonoid content determined by aluminium chloride method was reported as Quercetin equivalent (Standard Curve equation Y=0.009x +0.136, R^2=0.997). From the results, the Total phenol content of 68.5 ± 0.2 mg/g, total flavonoids content of 34.3 ± 0.4 mg/g and total Terpenoid content of 3.9% were observed in the methanolic root extract. The term phytochemical is used to describe the large number of secondary metabolic compounds that are derived from plants. Phytochemical screening assay is simple, quick and inexpensive method which gives immediate answer to various types of phytochemicals present in the sample (Sasidharan *et al.,* 2011). Roots are rich source of natural compounds that contribute to the competitiveness of invasive plant species (Inderjit and Duke, 2003). Some secondary metabolites can be produced by all tissues whereas some compounds are tissue or cell-specific (Yazdani *et al.,* 2011). The secondary metabolites in the plants play vital role either as local or systematic resistance factors in protecting plants against various pathogens (Redman *et al.,* 2003). It has been reported that root secreted compounds belonging to phenolics and terpenoid classes have strong external antibacterial and antifungal activities (Lanoue *et al*., 2010 and Wurst *et al*., 2010). The presence of phytochemicals such as alkaloids, flavonoids, phenols, tannins and terpenoids from the root of related *Casuarina* species (*Casuarina equisetifolia)* has been reported (Gopichand *et al.,* 2015).

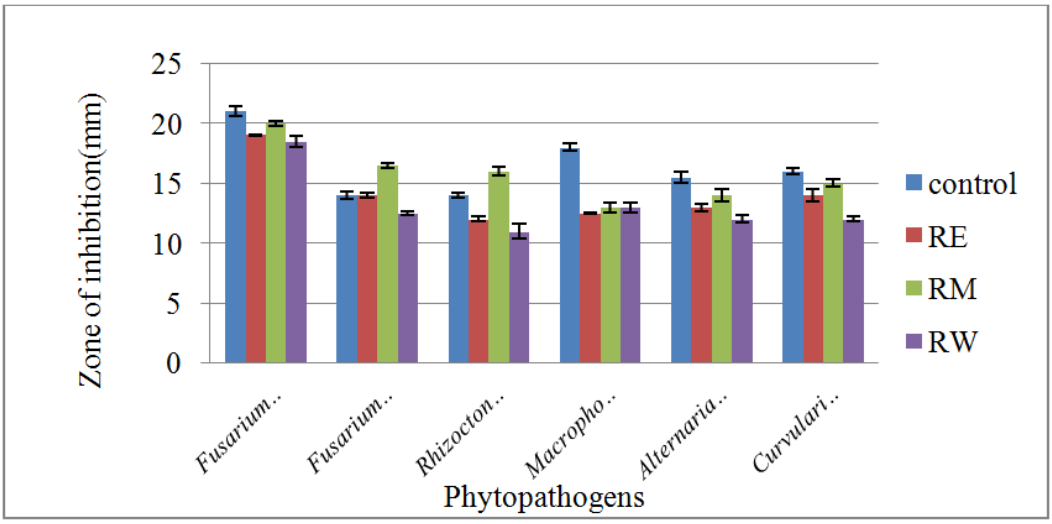
Phenols are the major groups of secondary metabolites in plants. They are chemically heterogenous group of nearly 10,000 individual compounds. Some are soluble only in organic solvents, some are water soluble and others are large and insoluble polymers (Lincoln Taiz *et al.,* 2015). Phenolic compounds released from the root or residue decomposition can act against soil borne pathogens, root feeding insects and in turn these compounds may also play an important role in the induction of resistance in plants (Ndakidemi *et al.,* 2003). Flavonoids are one of the largest groups of plant phenolics. In plants, flavonoids are involved in diverse functions such as UV protection, plant pigmentation, stimulation of nitrogen-fixing nodules and also lead a defensive role in the management of pathogens (Koes *et al.,* 1994). The terpenes or terpenoids constitute largest class of secondary metabolites. They are synthesised from acetyl-CoA or its glycolytic intermediates (Lincoln Taiz *et al.,* 2015). Terpenoid structure ranges from linear to polycyclic molecules (Mahmoud and Croteau, 2002). These compounds are reported to be important factors in resistance against several insect pests and pathogens. The insecticidal activity of terpenes might be due to their action as antifeedants (Harbone 1988).

Several terpenoids have their role in plant defense against biotic and abiotic stresses. Some of the diterpene and sesquiterpene act as phytoalexins, which are low molecular weight compound, that are produced as a part of plant defense system (Bharat singh and Ram.A.Sharma, 2015). The presence of polyphenols such as flavonoids and phenolic compounds in higher level indicates the effective role of these secondary metabolites in host defense mechanism. Being a non leguminous nitrogen fixing tree species, the presence of polyphenols also induces root nodule formation and enhance the nitrogen fixing ability of this tree species thus leading to remarkable growth and yield with shorter period of rotation.

Antifungal activity

In the present study, the methanol extract showed maximum zone of inhibition at 100µg concentration against *Fusarium oxysporum* (20±0.2mm) followed by *Rhizoctonia solani* (16.5±0.3mm), *Fusarium solani* (16±0.2mm), *Curvularia lunata* (15±0.4mm) *Alternaria alternata* (14±0.2mm) and *Macrophomina phaseolina* (13±0.3mm). The ethanol extract showed maximum zone of inhibition at 100µg concentration against *Fusarium oxysporum* (19±0.1mm) followed by *Fusarium solani* (14±0.2mm), *Curvularia lunata* (14±0.4mm) *Alternaria alternata* (13±0.2mm), *Macrophomina phaseolina* (12.5±0.3mm) and *Rhizoctonia solani* (12±0.3mm). The aqueous extract showed maximum zone of inhibition at 100µg concentration against *Fusarium oxysporum* (18.5±0.2mm) followed by *Macrophomina phaseolina* (13±0.3mm), *Fusarium solani* (12.5±0.2mm), *Curvularia lunata* (12±0.1mm) *Alternaria alternata* (12±0.3mm) and *Rhizoctonia solani* (11±0.3mm).

Among all the phytopathogens screened *Fusarium oxysporum* was showing maximum susceptibility at 100µg concentration of all the root extracts. 50 µg concentration of all the root extracts (Ethanol, Methanol and aqueous extracts) showed minimal zone of inhibition and also was found to be variable against all the phytopathogens screened. (Fig 1). From the present findings, the potent antifungal activity of the methanolic root extract may be attributed to the presence of rich phenolic compounds in *Casuarina junghuhniana* root.



(RE: Ethanol root extract, RM: Methanol root extract, RW: Aqueous root extract). Values are mean inhibition zone (mm) ±SD of three replicates.

Fig. 1. Antifungal activity of *Casuarina junghuhniana* root (100µg concentration) against phytopathogens

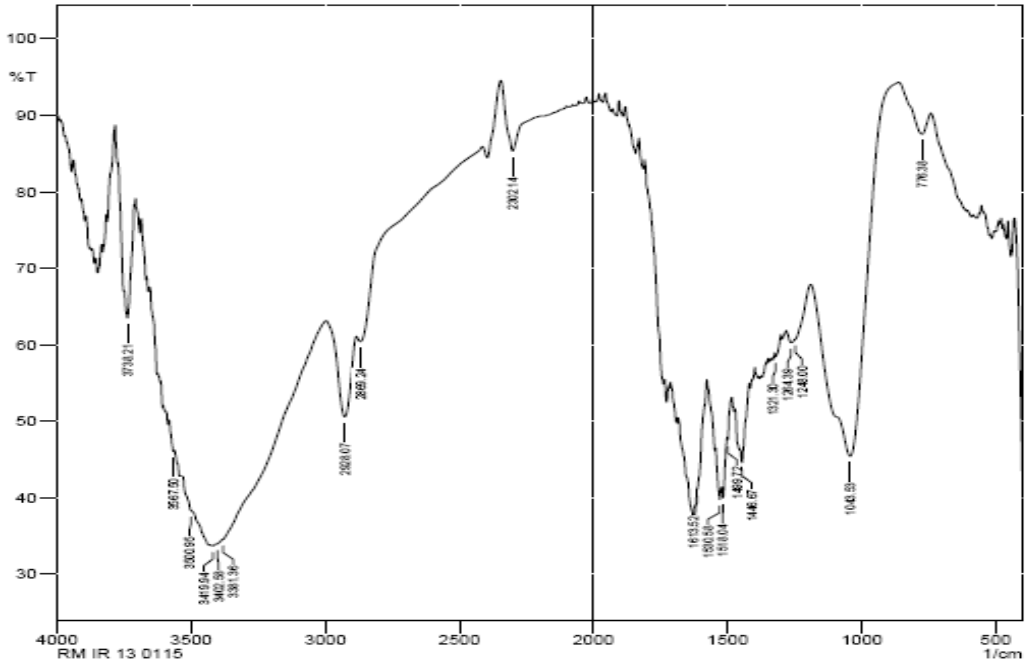


Fig. 2. FTIR spectrum of methanol extract of root of *Casuarina junghuhniana*

The susceptibility of fungal pathogens may be due to the interference of the secondary metabolites with molecular target including Bio membrane, proteins and nucleic acids in their organs, tissues or cells (Yazdani *et al.,* 2011). Most of the identified phyto compounds which are active against microorganisms are aromatic or saturated organic compounds, which are most often, obtained using initial ethanol or methanol extraction. (Cowan, 1999). Several researchers have used the plant's crude extract instead of specific fraction for antifungal activities. Advantages of using crude plant extract are the additive or synergistic effect of compounds, which in turn increases the antimicrobial spectrum of the extract and the decreased risk of pathogen resistance to mixture (Yazdani *et al.,* 2011). Several studies reported that plant defence against soil borne pests and pathogens are based on the release or accumulation of phenolic compounds in soil (Dakora, 1995). The activity of phenolic compounds depends on their structural diversity. Several phenolic acids possess antifungal activities (Nicholson and Hammerschmidt, 1992.) and these phytocompounds can be an alternative to chemical control of pathogens on agricultural crops (Dakora and Phillips, 1996). Accumulation of these secondary phyto compounds at the challenging site also reinforces cell wall which is accompanied by localised production of reactive oxygen species driving cell wall cross linking, antimicrobial activity and defence signalling (Field *et al.,* 2006).

FTIR Analysis

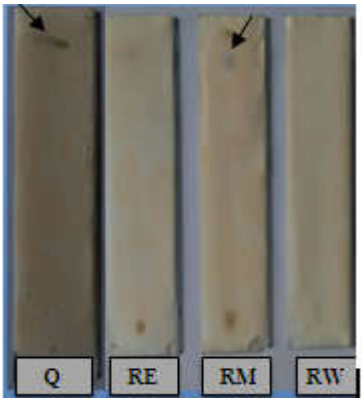
FTIR spectrum of methanol root extract showed the presence of sharp peaks and different functional groups. Sharp peaks at 1043.53 (C-N Stretch), 1530.58 (Nitro compounds), 1613.52 (C=C stretch alkenes), 2928.07 (CH alkanes), 3402.58 and 3419.94 (OH Stretch alcohols) were observed. Ethanol and aqueous extract did not reveal much of the prominent peaks in the FTIR analysis. (Fig 2). FT-IR has proven to be a valuable tool for the characterization and identification of compounds or functional groups present in an unknown mixture of plant

extracts (Eberhardt *et al*., 2007). FT-IR spectra of pure compounds are usually so unique that they are like molecular "fingerprint". The spectrum of most common plant compounds can be identified by comparing to a library of known compounds. In the present study, FT-IR of the methanol extract revealed the distribution of functional groups within organic fractions. The presence of various functional groups may be attributed to the existence of variety of potential phytochemicals. The multiple functional groups reflect either the complex structure or it indicates the nature of sample as mixture.(John Coates 2000).

Thin layer chromatographic analysis

Three different extracts of root were subjected to chromatographic analysis for the detection of phenolic compound. Fig 3 shows the TLC run for sample along with Gallic acid standard. Gallic acid and Methanol extract showed blue spot after spraying with 2% $FeCl_3$. The Rf value of methanol root extract was 0.6 and it was found to be similar with standard Gallic acid Rf value. This indicated the presence of Gallic acid in the root sample. Fig 4 shows the TLC run for root sample along with Quercetin standard. In this the methanol extract, showed a prominent chromatogram after spraying 1% $FeCl_3$. The Rf value of methanol root extract was 0.8 and it was found to be similar with standard Quercetin Rf value. This indicated the presence of Quercetin in the root sample. Ethanol and aqueous extract did not show prominent spot after spraying. TLC readily provides qualitative information and it separates small amounts of compounds. TLC has many advantages such as low cost, short time analysis, the possibility of multiple detection and specific derivatization on the same plate (Jamuna 2014).

Fig. 3. TLC run with gallic acid standard



G: Gallic acid and Q: Quercetin

Fig. 4. TLC run with Quercetin standard

In the present work, TLC study revealed the presence of phenolic compounds such as gallic acid and quercetin in the root extract in comparison with standard gallic acid and quercetin Rf value. The phenolic compounds could be an important part of the plants defense system against pests and diseases including root parasitic nematodes (Radomir *et al.,* 2004).

HPLC Analysis

To confirm the presence of phytochemicals in the root extract HPLC analysis was carried out. In the present study, HPLC analysis of methanol extract of root revealed 3 different peaks at different retention time (Rt) .Among the three peaks, two peaks were identified as Gallic acid(GA) (Rt 2.9min) and Quercetin (Rt 3.3min) Presence of these compounds was confirmed by comparing its retention time with that of its standards under similar chromatographic conditions. (Fig 5,6 &7).

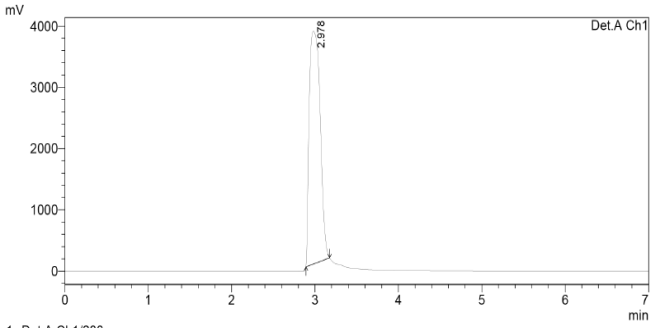


Fig. 5. HPLC analysis of standard Gallic acid

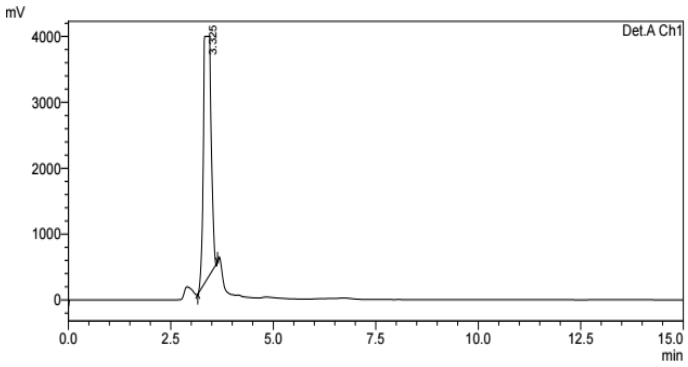


Fig. 6. HPLC analysis of standard Quercetin

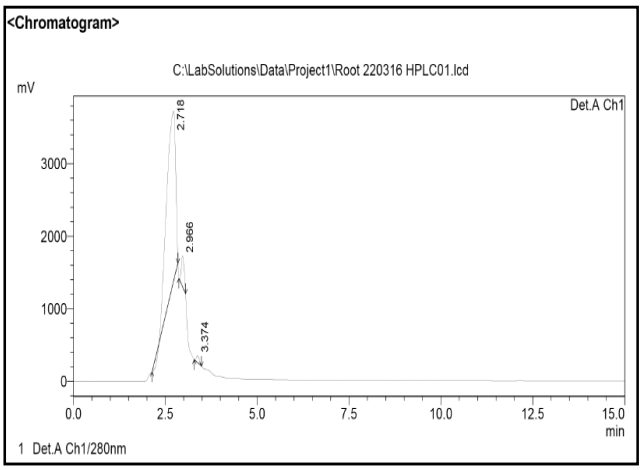


Fig. 7. HPLC Analysis for Methanol Extract of *C.junghuhniana* Root

Table 1. Gas Chromatography -Mass Spectrometry of phytochemicals present in Methanolic extract of *Casuarina junghuhniana* root

Compound name	Retention time	Molecular formula	Molecular weight	Peak area %	Compound nature	Biological activity
Phenol 3,5 bis (1,1 dimethylethyl)	12.77	$C_{14}H_{22}O$	206.3239	16.9	an organic compound in which an –OH group is attached to a carbon atom as part of aromatic carbon ring system	-antiseptic and disinfectant properties. -flavorant and antibacterial properties -ingredient in mouth wash formulation. (Igwe *et al.,* 2015)
4,5,7 trihydroxy iso flavones	17.07	$C_{15}H_{10}O_5$	270.24	59.9	isoflavones	-Antioxidant, anthelmintic, anticancer
4H-1 Benzopyran 4 one 2 (3,4 dimethoxy phenyl)7 hydroxy	19.05	$C_{17}H_{14}O_5$	298.29006	65.6	Flavones group	-Antibacterial activity, Insecticide activity
Oleic acid	19.75	$C_{18}H_{34}O_2$	282.4614	100	fatty acid ester formed (9- octadecenoic acid)	-used as a solvent for pharmaceutical drug by the condensation of preparations involving lipophilic substances oleic acid and ethanol such as steroids. - used as a lubricant and a plasticizer.(Igwe *et al.,* 2015)
Ethanol, 2 (90ctadecenyl oxy)z	25.68	$C_{20}H_{40}O_2$	312.53	6.3	-	-
Palmitic acid	17.88	$C_{16}H_{32}O_2$	256.43	84.7	a saturated fatty acid that is the major fat in meat and dairy products	-Lubricant, Antiandrogenic, Flavor, Hemolytic, Antioxidant, Nematicide, Pesticide (Igwe *et al.,* 2015)
3a 9 dimethyl dodecahydrocyclo hepta (d) inden-3-one	13.92	$C_{16}H_{26}O$	234.37704	13.1	-	-
Phytol	18.82	$C_{20}H_{40}O$	296.54	59.6	acyclic diterpene	-used in the manufacture of Vitamin E and K. Antimicrobial Anti-inflammatory Anti cancer Diuretic (Rajeswari *et al.,*2012)
4 methoxy 5,7 dihydroxyisoflavone	19.87	$C_{16}H_{12}O_5$	284.26	84	Flavonoids group	-Natural aromatase inhibitor
Flavone	13.47	$C_{15}H_{10}O_2$	222.2387	13.2	Flavonoids group	-The pharmacological properties of different flavonoids include - diuretic, anti-inflammatory, antiseptic, antispasmodic and also anti-tumor. (Nan Jiang *et al.,* 2016*)*

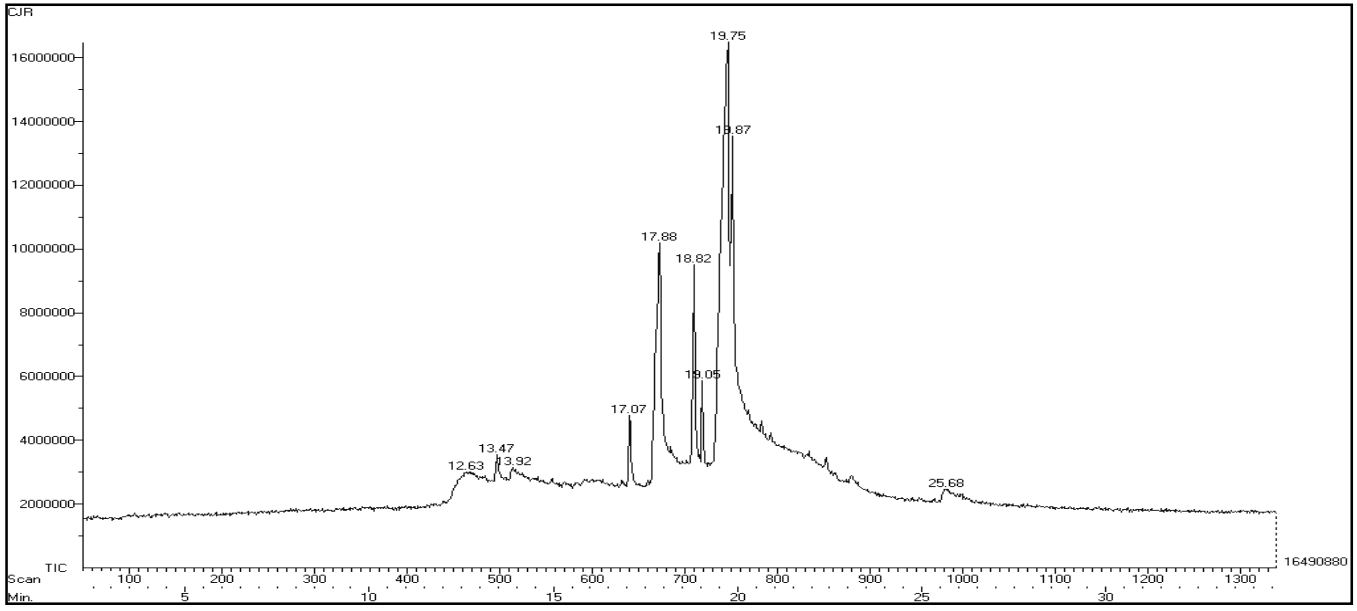


Fig.8. GC-MS spectrum of methanol extract of C.*junghuhniana* root

HPLC is a versatile, robust and widely used technique for the isolation of natural products (Cannel 1998). The phenolic acids and their derivatives are more common among plant phenols. Phenolic acids are also known as hydroxybenzoates (Gross, 1992). Gallic acid is pre eminent among other phenolic acids which is enlisted in plant tissues in ester form (Haslam and Haworth 1964). Gallic acid is the base unit of gallotannins, a polyhydroxy phenolic compound widely distributed in various plants.GA and its derivatives were found to be strong antioxidant which are able to scavenge reactive oxygen species. Gallic acid also has anti viral, anti microbial and anti oxidative properties (Vijayalakshmi and Ravindhran, 2012). It was reported that flavonols (Quercetin and kaempferol) in black alder (*Alnus glutinosa*) root exudates are able to enhance the level of nodulation (Van Ghelue *et al.,* 1997). The presence of flavonoids in plant has become popular because of their health benefits such as anti-allergy, anticancer, antioxidant, anti-inflammatory and anti viral. The flavonoids quercetin is known for its ability to relieve high fever, eczema, asthma and sinusitis (Guardia *et al.,* 2001 and Hertog *et al.,*1995).

GC-MS Study

In the present work, Several peaks were obtained in GC MS analysis of methanol extract of *C.junghuhniana* root which indicates the presence of various distinct secondary metabolites (Fig 8). These bioactive compounds were identified using NIST database on comparison with actual mass spectral obtained. The chromatogram revealed the presence of phenol 3,5 bis (1,1 dimethylethyl), 4,5,7-Trihydroxy isoflavone, 4H-1-Benzopyran 4-one,2-(3,4-dimethoxy phenyl-7-hydroxy), Oleic acid, Ethanol 2 (9 octadecenyloxy), Palmitic acid, 3a 9 dimethyl dodecahydrocyclo hepta(d) inden-3-one, Phytol, 4methoxy 5,7 dihydroxyisoflavone, flavone. Molecular formula, Molecular weight, peak area retention time and biological activities of all these compounds are discussed in Table 1. In addition to the biological activities of each compounds listed in the table, the potential compounds are important to the plant for its various functions. Phytol is one among the compounds that is present in the extract. Phytol is one of the simplest and most important diterpene,a reduced form of geranylgeraniol, which forms the lipophilic side chain of the chlorophylls (Vetter & Schröder, 2011). Most other compounds from the GC-MS spectrum of *Casuarina junghuhniana* root can be grouped under phenolic and flavonoids group. In the GC MS spectrum, compounds such as 4,5,7- Trihydroxy isoflavone, 4H-1-Benzopyran 4-one,2-(3,4-dimethoxy phenyl-7-hydroxy), 4methoxy 5,7 dihydroxyisoflavone, flavone can be grouped under flavonoid, isoflavones and flavones group. It was observed that the plant flavonoids act as a signal to microbial symbiont and induces the nodule formation in legumes (Phillips, 1972) and can alter the nitrogen fixing symbiosis. The strain specificity in the *Myricaceae-Frankia* symbiosis was found to be correlated with the plant root phenolics (Popovici *et al.,* 2010). Leguminous plants secrete a variety of phenolic compounds from roots such as flavones, flavonols, isoflavonoids and vanillin (Zawoznik *et al.,* 2000). The host root secretes phenolic compounds that act as a signaling molecule during the expression of various symbiotic plasmid encoded *nod* (nodulating) gene. Some of these *nod* gene encode enzymes to synthesise a special class of glycolipids. The non reducing end of these signal molecules, which contain N-acyl long chain of fatty acid, is bioactive in plant host, triggereing root hair deformation and cortical cell divisions within root leading to nodule formation (Santi Mandal *et al.,* 2010). Similar phenomenon may also be responsible in the nodule formation of non leguminous plants. GC MS spectrum revealed that *C.junghuhniana* root has a rich source of phenolic compounds and the presence of flavonoids helps in formation of nodules in the root which inturn can perform effective nitrogen fixation in soil. The isoflavones are a group of flavonoids in which the position of one aromatic ring is shifted. They are reported to be present mostly in legumes and also in multiple other plant species with different biological activities (Lapcik, 2007). They are effectively used as insecticides, pesticides and piscicides. It was reported that isoflavones have become best known for their role as phytoalexins (Lincoln Taiz *et al.,* 2015). Similar observation such as derivatives of isoflavonoid, phytoalexin pisatin from *Pisum sativum* with potent antimicrobial properties in legumes was recorded. Flavones have very close structural relationship with flavonols. Presence of flavones makes the plant highly adaptable to a terrestrial environment which includes, protection against UV radiation, oxidative stress, interspecies interaction (resistance against pathogens, symbiosis, protection from herbivores and allelopathy), in plant pigmentation and in lignifications (Nan Jiang *et al.,* 2016). The presence of flavone in the plant root would be the reason for the distribution of this tree crop in varied edaphic and exhibits tolerance towards adverse environments.

Conclusion

Casuarina junghuhniana root extracts revealed the presence of rich phytochemicals possessing antimicrobial activity against phytopathogens. The chromatographic studies revealed the presence of rich phenolic and flavonoids content in root extracts. The potential phytoconstituents might be the reason for the luxuriant growth, its establishment in extreme soil condition and also its resistance to plant pathogens. The bio active compounds from the plant can be further isolated and evaluated as a bio protectant for the management of plant pathogens. This work also lays an emphasis on the cultivation of *Casuarina* species, along with the other commercially important plant communities as an intercropping system for their better performance in terms of growth and biomass and in-addition resistance to their pathogens and herbivores.

Acknowledgement

The authors thank Mrs. Prema Sampathkumar, Associate professor and Head, the Faculty members and non teaching staff of the Department of Plant Biology and Plant Biotechnology, Dr. Mrs A. Nirmala Principal Ethiraj College for Women, (autonomous) Chennai 600008, for their valuable support and encouragement throughout the entire period of research. We would also like to express our thanks for the facilities extended by the Central Instrumentation Centre of Ethiraj College for Women. Sincere thanks to State Forest Research Institute, Kolapakkam, Chennai-48 and IIT Madras for extension of facilities.

REFERENCES

Bharat Singh and Ram A. Sharma, 2015. Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. *Biotech*., 3. 5:129–151

Cannell, R.J.P. 1998. Natural Products Isolation. Human Press Inc. New Jersey, pp. 165-208.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.,* 12(4): 564-581

Dakora, F.D. 1995. Plant flavonoids: biological molecules for useful exploitation. *Aust J Plant Physiol*., 22:87-99.

Dakora, F.D., Phillips, D.A. 1996 Diverse functions of isoflavonoids in legumes transcend ant-microbial definitions of phytoalexins. *Physiol Mol Plant Pathol*., 49:1-20.

Eberhardt, T.L., Li, X., Shupe, T.F. and Hse, C.Y. 2007. Chinese TallowTree (*Sapium Sebiferum*) utilization: Characterization of extractives and cell-wall chemistry. *Wood Fiber Sci.,* 39: 319-324.

Field, B., Jordon, F., Osbourn, 2006. First encounters-deployment of defence related natural products by plants. *New Phytol.,* 172:193-207.

Gopichand Ch, V., Anjani, A., Himaja, A., Shilpa, U., Ravi Kumar, A., Chinna Eswaraiah, M. 2015. Phytochemical evaluation of *Lantana camara*, *Casuarina equisetifolia*, *Michella nilagirica.* Indian *Journal of Research in Pharmacy and Biotechnology,* 3(6): 461-463.

Gross, G.G. 1992. Enzymes in the biosynthesis of hydrolysable tannins. In R.W.Heminway, P.E. Laksand S.J. Branham (eds), *Plant Polyphenols*. Plenum Press, New York, 43–60.

Guardia, T., Rotelli, A. E., Juarez, A. O., and Pelzer, L. E. 2001. "Anti-inflammatory Properties of Plant Flavonoids. Effects of Rutin, Quercetin and Hesperidin on Adjuvant Arthritis in Rat." Farmaco. 56 (9): 683-7.

Gupta Mradu, Sasmal Saumyakanti, Majumdar Sohini, Mukherjee Arup, 2012. HPLC Profiles of Standard Phenolic Compounds Present in Medicinal Plants. *International Journal of Pharmacognosy and Phytochemical Research,* 4(3); 162-167.

Haslam, E. and Haworth, R. D. 1964. Progr. org. *Chem*. 6, 1.

Hertog, M. G., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., and Fidanza, F. 1995. "Flavonoid Intake and Long-Term Risk of Coronary Heart Disease and Cancer in the Seven Countries Study." *Archives of Internal Medicine,* 155 (4): 381-6.

Igwe, K.K., P.N. Okafor and I.I. Ijeh, 2015. GC-MS analysis of phytocomponents in *Vernonia amygdalina.* Del leaves and its contractile potential in mammary tissue in female albino Wistar rats. *IOSR Journal of Agriculture and Veterinary Science,* 8, (11).25-30

Inderjit, Duke, S.O. 2003. Ecophysiological aspects of allelopathy. *Planta*; 217:529-39.

Jayaraj, R.S.C. 2010. *Casuarina junghuhniana* (Casuarinaceae) in India. *Australian Journal of Botany*, 58(2), 149-156.

John Coates, 2000. Interpretation of infrared spectra; A practical approach in encyclopedia of analytical chemistry. Meyers RA (ED). John wiley and Sons Ltd, Chinchester., 10815-10837.

Koes, R.E., Quattrocchio, F. and Mol, J.N.M. 1994. The flavonoid biosynthetic pathway in plants: function and evolution. *BioEssays*, 16: 123–132.

Lanoue, A. et al 2010. De novo biosynthesis of defense root exudates in response to *Fusarium* attack in barley. *New Phytol.,* 185, 577–588.

Lapcik, O. 2007. Isoflavonoids in non-leguminous taxa: A rarity or a rule? *Phytochemistry*, 68, 2909–2916.

Lincoln Taiz, Eduardo zeiger, Ian Max Moller and Angus Murphy, 2015. Plant Physiology and Development. Sixth edition. A41-A417.

Luechanimitchit, P., Luangviriyasaeng, V., Laosakul, S. and Pinyopusarerk, K. 2016. Clone Trial of *Casuarina junghuhniana* in Thailand. In: Nicodemus, A., Pinyopusarerk, K., Zhong, C.L., Franche, C. (Editors). Casuarina improvement for securing rural livelihoods. Proceedings of Fifth International Casuarina Workshop. 85-89.

Mahmoud, S.S. and Croteau, R.B. 2002. Strategies for transgenic manipulation of monoterpene biosynthesis in plants. *Trends in Plant Science*, 7: 366-373.

Ndakidemi, P.A., Dakora, F.D. 2003. Legume seed flavonoids and nitrogenous metabolites as signals and protectants in early seedling development. *Rev Funct Plant Biol.,* 30:729-45.

Nicholson, R.L., Hammerschmidt, R. 1992. Phenolic compounds and their role in disease resistance. *Annu Rev Phytopathol*., 30:369-89.

Nicodemus, A., Kannan, K., Sagariya, Y.C., Vipin, P., Durai, A., Singh, B.G. and Pinyopusarerk, K. 2016. Clonal evaluation of *Casuarina junghuhniana* for growth and stem form in south India. In: Nicodemus, A., Pinyopusarerk, K., Zhong, C.L., Franche, C. (Editors). Casuarina improvement for securing rural livelihoods. Proceedings of Fifth International Casuarina Workshop. 99-103.

Phillips, D.A., Torrey, J.G. 1972. Studies on cytokinin production by Rhizobium. *Plant Physiol*., 49:11-5.

Popovici, J., Comte, G., Bagnarol, E., Alloisio, N., Fournier, P., Bellvert, F., et al. 2010. Differential effects of rare specific flavonoids on compatible and incompatible strains in the *Myrica gale-Frankia* actinorhizal symbiosis. *Appl Environ Microbiol.,* 76:2451- 60.

Radomir V. Malbasa, Eva, S. Loncar and Ljiljana A. Kolarov, 2004. TLC analysis of some phenolic compounds in Kombucha Beverage. *Acta Periodica Technologica*, 35, 199-205.

Rajeswari, M Murugan and V.R. Mohan, 2012. GC-MS analysis of bioactive components of *Hugonia mystax L.* (Linaceae). *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 3 (4): 301-308

Redman, R.S., Freeman, S., Clifton, D.R., Morrel, J., Brown, G., Rodriguez, R.J. 2003. Biochemical analysis of plant protection afforded by a nonpathogenic endophytic mutant of *Colletotrichum magna*. *Plant Physiol*., 119:795-804.

Robinson, A.H., Beudelsr, C., Kurnianto, A. 1982. Nature reserves of Bali. UNDP/FAO Field report No.48.FAO, Bogor Indonesia.

Samidha Kamtekar, Vrushali Keer and Vijaya Patil, 2014. Estimation of phenolic content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. *Journal of Applied Pharmaceutical science*, 4(09):61-65.

Santi M. Mandal, Dipjyoti Chakraborty and Satyahari Dey. 2010. Phenolic acids act as signaling molecules in plant-microbe symbioses. *Plant Signaling and Behavior,* 5:4, 359-368.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M. and Yoga Latha, L. 2011. Extraction, isolation and characterization of bioactive compounds from plants extracts. *Afr J Tradit Complement Altern Med*., 8(1):1-10.

Schafer, H., Wink, M. 2009. Medicinally important secondary metabolites in recombinant microorganisms or plants: progress in alkaloid biosynthesis. *Biotechnology Journal,* 4(12): 1684-1703.

Simms, E.L., 1992. Costs of plant resistance to herbivory. In Plant resistance to herbivores and pathogens. eds. Ecology,

evolution and genetics, Fritz RS and Simms EL, Chicago: University of Chicago Press, 392-425.

Sureshkumar, G., Seenivasan, R. and Chezhian, P. Mini-cutting technique for large scale clonal propagation of *Casuarina junghuhniana.* In: Nicodemus, A., Pinyopusarerk, K., Zhong, C.L., Franche, C. (Editors). Casuarina improvement for securing rural livelihoods. Proceedings of Fifth International Casuarina Workshop. 2016; p.118-120.

Tejavathi, D.H. and Jayashree, D.R. 2013. Phytochemical screening of selected medicinal herbs inoculated with arbuscular mycorrhizal fungi. *International Journal of Biology,Pharmacy and Allied sciences,* 2(11): 2090-2106.

Van Ghelue, M., Lovaas, E., Ringo, E., Solheim, B. 1997. Early interactions between *Alnus glutinosa* and F*rankia strain ArI3*. Production and specificity of root hair deformation factor(s). *Physiol Plant*., 99:579-87.

Varghese, M., R. Kamalakannan, A. Nicodemus and K. Pinyopusarerk, 2011. Fertility and progeny performance in seedling seed orchards of C*asuarina equisetifolia* and *C. junghuhniana.* In: Zhong, C., K. Pinyopusarerk, A. Kalinganire and C. Franche (Eds.). Improving Smallholder livelihoods through improved Casuarina Productivity: Proceedings of the Fourth International Casuarina Workshop, Haikou, China, 21-25 March 2010. China Forestry Publishing House, Beijing. 75-81.

Vetter, W. and Schröder, M. 2011. Phytanic acid–a tetramethyl branched fatty acid in food. *Lipid Technology*, 23: 175-178.

Vijayalakshmi R and Ravindhran R. 2012. HPTLC Method for quantitative determination of Gallic Acid in ethanolic root extract of *Diospyrus Ferrea (Willd.) Bakh And Aerva Lanata* (L.) Juss. Ex Schultes –A Potent Indian Medicinal Plants. *Asian Journal of Pharmaceutical and Clinical Research*, 5(4), 170- 174.

Wurst, S. et al. 2010. Microorganisms and nematodes increase levels of secondary metabolites in roots and root exudates of Plantago lanceolata. *Plant Soil,* 329, 117–126.

Yazdani, D., Tan, Y. H., Zainal Abidin, M. A. and Jaganath, I. B. 2011. A review on bioactive compounds isolated from plants against plant pathogenic fungi. *Journal of Medicinal Plants Research,* Vol. 5(30), 6584-6589.

Zawoznik, M.S., Garrido L.M., del Pero Martinez, M.A., Tomaro, M.L. 2000. Effect of vanillin on growth and symbiotic ability of *Bradyrhizobium sp.* (*Arachis*) strain. Proc Int Plant Growth-promoting Rhizobacteria. (http:/ www.ag.auburn.edu/argentina).

****