AJPS, 2014, Vol. 14, No.1

Antibacterial Activity of Two Iraqi Plants from the Family Pinaceae

Rasha Eldalawy*, Widad Alani* and Mohammed Al-Araji**
Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, Al-Mustansiriyah University
****Clinical laboratory science department, college of pharmacy, Al-Mustansiriyah University.***

Abstract:

Pine is regarded as a remedy of wounds in traditional medicine. Terpene acids like abietic and isopimaric acid are the active constituents which poses antibacterial activities.

The hexane fraction of both *Pinus halepensis* and *Cedrus libani* was assayed against eight different bacteria of G+ve and G-ve using agar diffusion method. The zones of inhibition were determined and compared with the wide spectrum antibacterial ciprofloxacin as a positive control.

***Key words**: terpen acid, abietic acid, Pinus halepensis, Cedrus libani, antibacterial activity*

الخلاصة:

اشجار الصنوبر استعملت منذ قديم الزمان كمعقمات للجروح حيث تحتوي هذه الاشجار في اوراقها الابرية وسيقانها ومخاريطها على تربينات ذات خواص مضادة للبكتريا. تم استخلاص التربينات من اشجار الصنوبر العراقية وأجريت له فحص كمضاد للبكتريا باستعمال ثمانية انواع من البكتريا المعزولة. اثبتت هذه الدراسة فعالية المستخلصات ضد البكتريا بالمقارنة مع المضاد واسع الطيف سبروفلوكساسين.

Introduction:

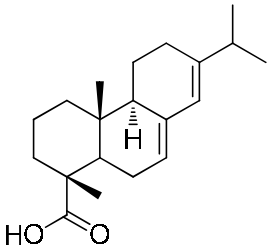
The emergence of new infectious diseases, the resurgence of several infections that appeared to have been controlled and the increase of bacterial resistance have created the need for studies directly towards the development of new antimicrobials [1].This situation is considering because of the emergence of strain of microorganism's antibiotic resistant, efflux of bacteria to many antibiotics and the emergence of uncommon infections that compromise treatment with existing drugs [2].

Faced with those many challenges posed by using of antibacterial agents available, it is necessary to search for new antibacterial substance that characterized more effective with broad spectrum of action. One strategy for this research is to explore the plants used in traditional medicine. Medicinal plants are a rich source of antimicrobial agents [3]. Many plant secondary metabolites are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated by tissue damage or pathogen attack [4]. Currently, majority of the pharmaceutically important secondary metabolites are isolated from wild or cultivated plants as their chemical synthesis is not economically feasible [5]. Major groups of antimicrobial compounds from plants include simple phenols and phenolic acids, quinones, flavones, flavonoids and flavonols, tannins, coumarins, alkaloids, terpenoids and essential oils, lactin,and polypeptides [6]. Antimicrobial compounds identified have shown promising activity *in vitro* [7], and differ *in vitro* methods were used for determining antimicrobial susceptibility include broth dilution assay, disc diffusion assay and well diffusion assay[8].

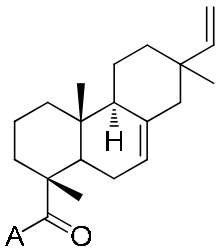
Pinaceae is the largest and most economically important family of conifers, with 11 genera and approximately 220 specie including Cedrus, Pinus, Picea, and Abies[9]. The Pinaceae contain a diversity of terpenoid compounds in the bark, wood, leaves, and cones, particularly in the characteristic oleoresins of the resin canals or vesicles. The pines are trees, evergreen

dense clusters of leaves at apex, the bulk of the volatile portion of the stem and leaf oleoresins are usually a complex mixture of monoterpenes. These impart much of the characteristic fragrance associated with Pinaceae. Terpenoid and hydrocarbon profiles of the oleoresins or turpentines (the steam-distillable portion) often show significant differences among species and have been widely used in chemosystematics of the Pinaceae [10].

Diterpene resin acids are important defense compounds of conifers against potential herbivores and pathogens [11].The biological activity of natural abietane- acids has been reviewd antimicrobial, antiulcer, and cardiovascular activities are the most representative for this class of diterpenoids. [12] Abietic acid and isopimaric acid has shown antiallergic[13]. Anti-inflammatory [14], phytoalexin-like[15], and anticonvulsant activities[16]. Isopimaric acid **1** and abietic acid **2** has shown antibacterial activity against multidrug resistant and methecillin resistance *staphylococcus aureus* [17].



abietic acid **1**



isopimaric acid **2**

The present study investigate the antibacterial activity of terpene extracted from *Pinus halepensis* and *Cedrus libani*`.

Material and Methods:

Plant material

Medicinal plants were collected from the north of Iraq and identified by Iraqi National Herbium (Abu Ghraib), the research were done in College of Pharmacy/ Al-Mustansiriyah University and take about six months to be done from September 2012 to February 2013 . The wood of the plant were washed thoroughly by tap water to remove dust and dirt, dried by placing the clean plant material in a shade for a period of seven days at 25ºC., and chopped into small pieces.

Extraction of terpene:

A sample (10 g) of stems were placed in thumble and subjected to extraction in a Soxhlet extractor using hexane (200 ml) for 24 hour.

The extract was filtered using Whatman filter paper No. 1 concentrated using rotary evaporator to a smaller volume by removing hexane from the solution below 45ºC under reduced pressure. The hexane fraction was analyzed by Shimadzu GC/MS apparatus. Carrier gas was helium; progress temperature was 100-300ºC, rate of flow 12.5/min.

Also the extract was analyzed by TLC, using benzene/methanol 9/1 as a solvent system and then sprayed with Halphen hicked (Ccl4/phenol 2:1) then allow to dry [18].

Collection of test organism and preparation of stock culture:

Test organisms were received from Almustansyria University/College of Science/ department of microbiology and confirmed by gram staining and culturing in appropriate selective media.

Microorganism used in the experiments:

*Gram positive bacteria: *Staphylococcus aureus, Streptococcus pneumonia*

*Gram negative bacteria: *Proteus vulgaris, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Acnitobacter spp.*

Estimation of antibacterial activity:

The extract were dissolved in methanol to obtain final concentration of (100, 50, 25) mg/ml and sterilized by filtration through a 0.2 *M*m membrane filter.

The agar well diffusion method was used to determine antibacterial activity of extract. All microbial stock cultures were freshened by streaking using a sterile inoculation loop on nutrient agar medium plates in a laminar flow hood, then incubated at 37ºC for 24 hrs. After 24 hrs the inoculate diluted in sterile saline solution to a final concentration of 10^6 colony forming units (cfu)/ml (adjusted according to the turbidity of 0.5 mcfarland scale tube). The diluted bacteria then spread on a muller-hinton agar, six diameter wells were punched into the muller-hinton agar and filled with (100, 50, 25) mg/ml of extract, solvent (methanol) was used as a negative control while ciprofloxacin (5*m*g/disc) was used as a positive conrol.

Plates were incubated at 37°c for 18-24 hr, after overnight incubation the diameter of the zone of inhibition around the well was measured in mm and recorded for *Pinus halepensis* and *Cedrus libani.*

Results and discussion:

In this study hexane extract showed an efficient antibacterial activity against most of the bacteria used except *Proteus vulgaris*. This bacterium showed resistance to all concentration used. The most sensitive microorganism was *Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphy lococcus aureus* (inhibition zone, 20, 20 and 19 mm respectively). *Escherichia coli* were sensitive to terpene fraction while resistant to many antibiotics (Figure-1).

The result of the current study revealed that terpene fraction of *P*inus *halpensis* has antibacterial properties (Table-1). *Cedrus libani* terpene fraction also exhibit antibacterial activity. *Proteus vulgaris* was the most sensitive bacteria (inhibition zone 17 mm). *E. coli* was sensitive only in high concentration 100 mg/mL (Table-2).

The antibacterial activity of the plants from the family pinaceae is attributed to the presence of the terpene acids. GC/MS analysis of hexane fraction revealed the presence of abietic acid M^+ 302 as a terpene which may be responsible for this anti bacterial activity in both plants. Retention time was 20 mins for each extract indicate the ocuurence of the same terpene acid in both plants (Figure 2 and 3).

The mechanism of action of the antimicrobial is due to the toxic effect or may be impair variety of enzyme systems including those involved in energy production and structural component synthesis [19].

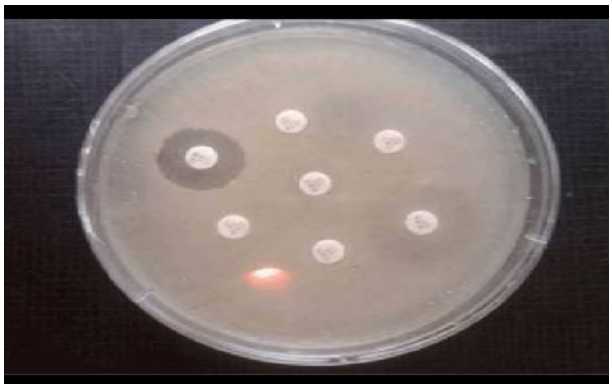


Figure -1: E coli exhibits positive to extract and resistant to antibiotics.

Table-1: Zone of inhibition of *Pinus halepensis* by disc diffusion method in mm.

microorganism	100mg/ml	50mg/ml	25mg/ml	methanol	ciprofloxacine
Staphylococcus aureus	19	12	11	negative	24
Streptococcus pneumoniae	17	14	12	negative	35
Proteus vulgaris	negative	negative	negative	negative	27
Escherichia coli	13	12	10	negative	negative
Klebsiella pneumoniae	20	19	14	negative	24
Pseudomonas aeruginosa	20	13	12	negative	35
Salmonella typhi	11	15	15	negative	22
Acinetobacter spp	10	20	16	negative	26

Table-2: Zone of inhibition of *Cedrus libani* by disc diffusion method in mm.

microorganism	100mg/ml	50mg/ml	25mg/ml	methanol	Ciprofloxacine
Staphylococcus aureus	14	13	12	negative	24
Streptococcus pneumoniae	negative	12	11	negative	35
Proteus vulgaris	17	16	15	negative	27
Escherichia coli	7	6	negative	negative	negative
Klebsiella pneumoniae	negative	12	10	negative	24
Pseudomonas aeruginosa	16	12	10	negative	35
Salmonella typhi	11	12	10	negative	22
Acinetobacter spp	17	15	13	negative	26

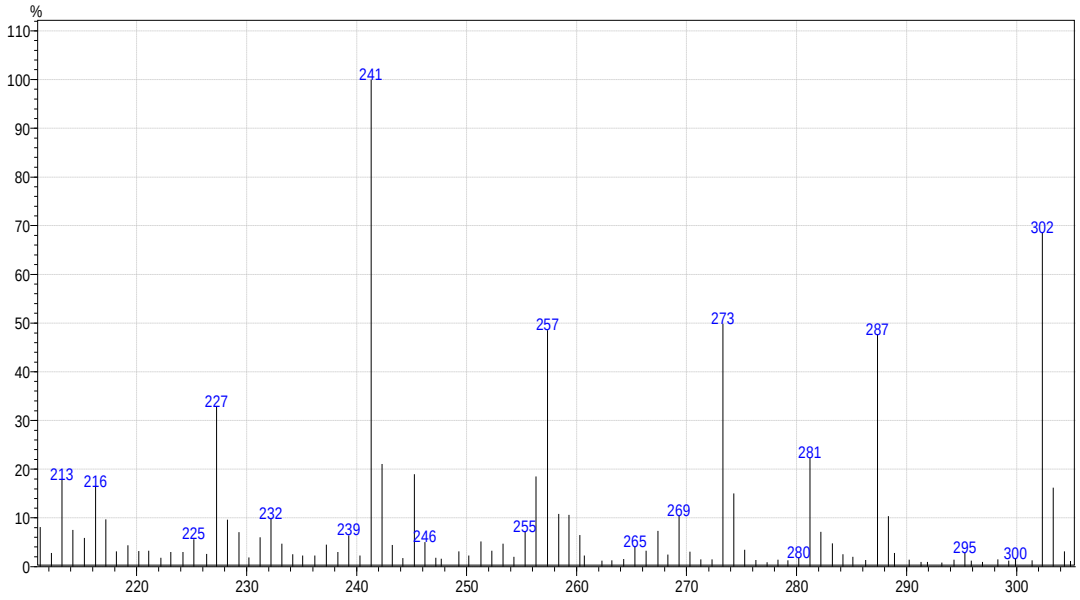


Figure- 2: GC/MS of *Cedrus libani* hexane fraction.

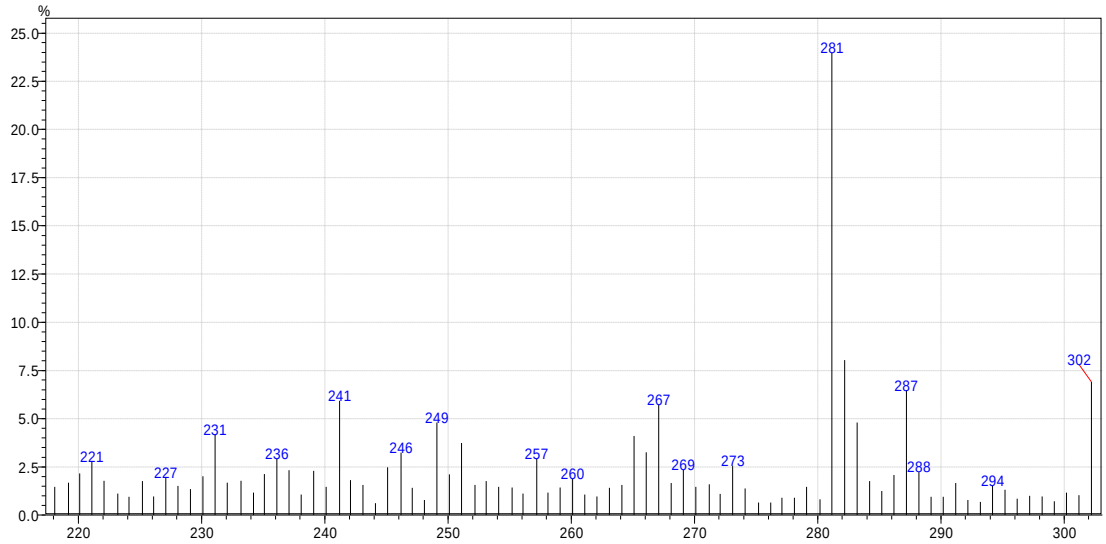


Figure- 3: GC/MS for hexane fraction of *pinus halepensis.*

Fragmentation pattern support this ion peak 302 as compared with literature (Figure-4)[20]

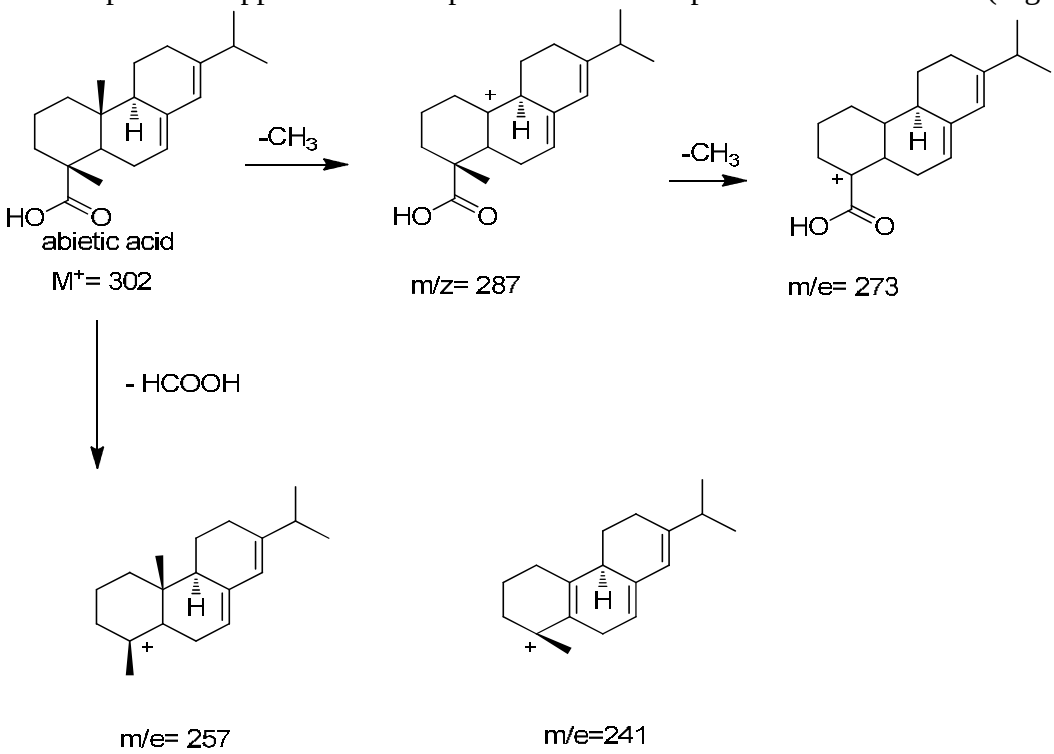


Figure- 4: Fragmentation pattern of abietic acid from hexane fraction [19].

TLC of the extract show a blue spot at 0.72 which referred to abietic acid as shown in (Figure5).

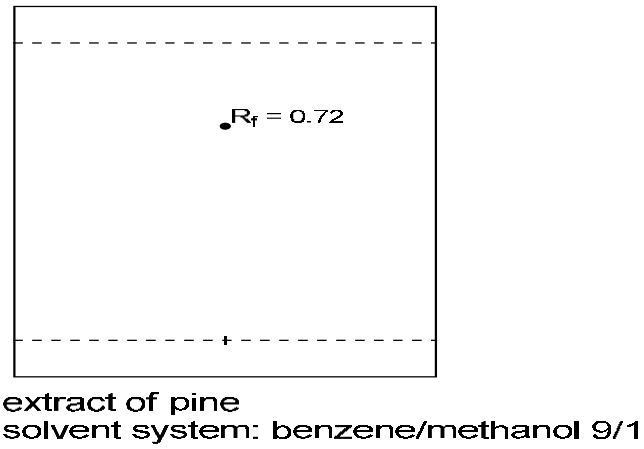


Figure- 5: TLC for the hexane extract of the plant

Cedrus also contain ion M^+ 137 which is not found in pinus indicate the presence of the antibacterial volatile oil pinene as compared with the literature (Figure-6)[21].

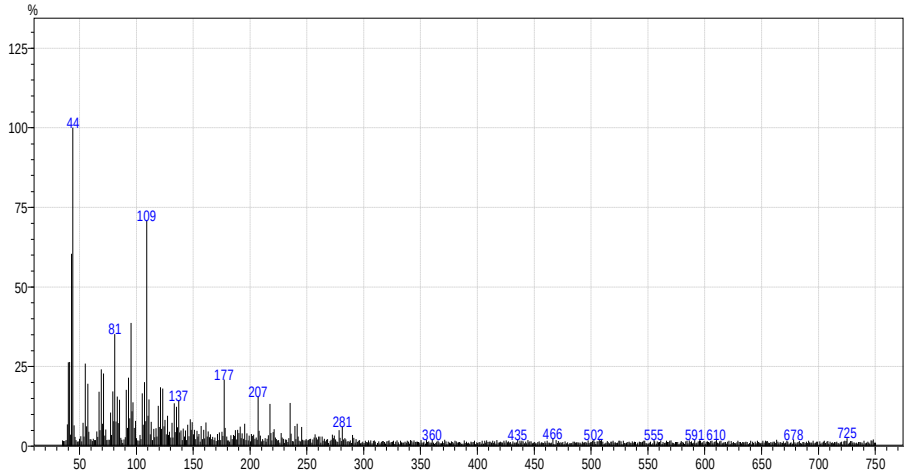


Figure- 6: GC/MS of cedrus showing pinene volatile oil.

Fragment of this oil is shown in Figure-7.

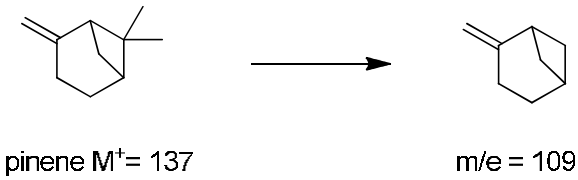


Figure-7: Fragmentation of pinene from cedrus.

References:

1- Recio, M.C. and Rios, J.L. A review of some antibacterial compound isolated from medicinal plants reported in the literature 1978-1988. Phytoter.Res.1989. Vol. 3. Pp: 117-115.

2- Vanden,B. D. A. and Vlietlnck, A. J. Screening methods for antibacterial and antiviral agent from higher plants. Academic Press. 1991. Vol. 6. Pp: 47-58.

3- Kubmarawa, D. G.; Ajoku, A.; Enweram, N. M. and Okorie, D. A. Preliminary phytochemical and antimicrobial

screening of 50 medicinal plants from Nigeria. Afric. J. Biotechnol.2007.Vol. 64. Pp: 1600-1696.

4- Osbourne, A. E. Performed antimicrobial compounds and plant defense against fungal attack.The plant cell.1996.Vol. 8. Pp: 1821-1831.

5- Caldenty K. M. O. and Inze, D. Plant cell factories in the post genomic era. New ways to produce designer secondary metabolites. Trends in plant sci. 2004. Vol.9. Pp: 433- 440.

6- Cowan, M. M. Plant products as antimicrobial agents. Clinical microbial review. 1999. Vol. 12. Pp: 564- 582.

7- Zahin, M. F.; Aqil , M. S. A. Khan and Ahmed, I. Ethnomedicinal plants derived antibacterial and their prospects. Ethnomedicine: Asource of comple mentary therapeutics, (E.d.). Chatatt opadhyay. Research signpost, India. 2010. Pp: 149-178.

8- Jiang, L.; Wang, F. and Han, F. Prinyawiwa tkul, W. No HK, Ge, B. Evaluation of diffusion and dilution methods to determine the anti-microbial activity of water-soluble chitosan derivatives. J. Appl Microbiol. Apr 2013. Vol. 114 (4). Pp: 956-63.

9- Song, ke, Fu; Liguo, Li Nan and Robert, R. Mill. Pinaceae. Flora of china. 1999. Vol. 4. Pp: 11–52.

10- Robert, A. price. Pinaceae Review from 1989 of the reproductive morphology of all Pinaceae in relation to all the other conifers Journal of the Arnold Arboretum. 1989. Vol. 70 (2). Pp: 247-305.

11- Martin, D.; Tholl, D.; Gershenzon, J. and Bohlmann, J. Methyl Jasmonate Induces Traumatic Resin Ducts, Terpenoid Resin Biosynthesis, and Terpenoid Accumu-lation in Develo-ping Xylem of Norway Spruce Stems. Plant Physiol. 2002. Vol. 129. Pp: 1003–1018.

12- San Feliciano, A.; Gordaliza, M.; Salinero, M. A. and Miguel del Corral, J. M. Abietane Acids: Sources, Biological Activities, and Therapeutic Uses. Planta Med. 1993. Vol. 59. Pp: 485–490.

13- Ulusu, N. N.; Ercil, D. Sakar, M. K. and Tezcan, E. F. Abietic acid inhibits lipoxy-genase activity. Phytother. Res. 2002. Vol. 16. Pp: 88–90.

14- Nam-Ho Kim, Yong Son, Sun-Oh Jeong, Jong Moon Hur, Han Soo Bang, Ki-Nam Lee, Eun-Cheol Kim, Hun-Taeg Chung, and Hyun-Ock Pae; Tetrahydroabietic Acid, a Reduced Abietic Acid, Inhibits the Production of Inflammatory Mediators in R AW 264.7 Macrophages Activated with Lipopoly saccharide. Clin Biochem Nutr. March; 2010.Vol. 46 (2). Pp: 119–125.

15- Spessard, G. O.; Matthews, D. R. Nelson, M. D. Rajtora, T. C. Fossum, M. J. and Giannini, J. L. Phytoalexin-like Activity of Abietic Acid and Its Derivatives. J. Agric. Food Chem. 1995. Vol. 43. Pp: 1690–1694.

16- Talevi, A.; Cravero, M. S.; Castro, E. A. and Bruno-Blanch, L. E. Bioorg. Discovery of anticonvulsant activity of abietic acid through application of linear discriminant analysis**.** Med. Chem. 2007. Vol. 17. Pp: 1684–1690.

17- Eileen Smith, Elizabeth Williamson, Mire Zloh and Simon Gibbons. Isopimaric acid from *Pinus nigra* shows activity against multidrug-resistant and EMRSA strains of *Staphylococcus aureus***.**Phytother. Res. 2005. Vol. 19. Pp: 538–542.

18- Pruthi, J. S. Quality assurance in species and spice products. 1999. Ch. 6. Pp: 202.

19- Conner, D. E. and Beuchat, B. Sensitivity of heat stressed yeasts to essential oils of plants, Applied Environ. Microbiol. 1984. Vol. 47. Pp: 229.

20- Mario, V. Russo, and Pasquale Avino Characterization and Identification of Natural Terpenic Resins employed in "*Madonna con Bambino e Angeli*" by *Antonello da Messina* using Gas Chromatography– Mass. Chemistry central journal. 2012. Vol. 6. Pp: 59.

21- Digrak et.al,. Antimicrobial activities of several parts of Pinus brutia, Juniperus oxycedrus, Abies cilicia, Cedrus libani and Pinus nigra, Phytotherapy Research. 1999. Vol. 13. Pp: 587.