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Identification of active compounds in *Cupressus* through FTIR and GC-MS analysis

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Abstract
Medicinal plants have been an important source phytochemical, aromatic compounds etc. Gymnosperms used as ornamental purpose but nowadays most of the researchers are concentrate the isolation of active metabolites from many gymnosperms. In our present study to screen the phytochemicals and identify the active compounds through FTIR and GC-MS from the methanolic leaf extract of *Cupressus*. The active compounds were isolated from the leaf extract of *Cupressus* Geranyl tiglate, Oxirane, Caryophyllene, Humulene, β-copaene, Methanoazulene, Naphthalene, (+)-epi-Bicyclosesquiphellandrene, Cyclononene, 2-Piperidinone, acrylic acid, Phthalic acid, Androstan-17-ol, 1-Chloroeicosane, Tetratetracontane, Benzamide, Sandaracopimar-15-ene and Oxadiatzol. In the FTIR analysis to identified by the most important functional groups were noticed. There are 10 different functional groups were obtained such as esters, aromatic compounds, phenols, amines, aldehydes, ketons, halogens. Thus, the present study the proved that the *Cupressus* as a pool of secondary metabolites.

Keywords: *Cupressus*, GC-MS, gymnosperm, FTIR, secondary metabolites

Introduction

Natural products have been the most successful source of potential drug leads. However, their recent implementation in drug discovery and development efforts have somewhat demonstrated a decline in interest (Mishra and Tiwari, 2011) [11]. Nevertheless, natural products continue to provide unique structural diversity in comparison to standard combinatorial chemistry, which presents opportunities for discovering mainly novel low molecular weight lead compounds. Since less than 10% of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery with the challenge being how to access this natural chemical diversity (Cragg and Newman, 2007) [4]. Medicinal plants are the significant reservoir of novel bioactive secondary metabolites including antimicrobial, antiinsect, anticancer, antidiabetic, and immunosuppressant compounds with their great potential applications in agriculture, medicine, and food industry (Kharwar *et al*., 2011) [10]. These bioactive compounds could be mainly classified as alkaloids, terpenoids, steroids, quinones, isocoumarins, lignans, phenylpropanoids, phenols, and lactones (Strobel and Daisy, 2003) [14]. The most widely used breast cancer drug is paclitaxel (Cragg, 1998) [5], isolated from the bark of *Taxus brevifolia* (Pacific Yew). Most of the gymnosperms are antimicrobial, anticancer agents, biological control agents, immunosuppressive agents, and other bioactive compounds by their different functional roles. Despite this potential, a repertoire of medicinal plants remains to be studied regarding their endophytic composition, for example *C. torulosa* D. Don. This is a well-known medicinal plant whose leaves have been proven to have anti-inflammatory, anticonvulsant, antimicrobial and wound-healing properties (Almeida *et al*., 2013) [2]. Due to the medicinal properties of *C. torulosa*, this species was bioactive substances with antimicrobial activity and cytotoxic activity to produce bioactive agents with pharmaceutical potential, and may provide a new lead in the pursuit of new biological source of drug candidates.

Material and Methods

Collection and identification of plant sample

Fresh leaf samples of *Cupressus* were collected form ornamental garden at Sivakasi. All leaf samples were dried under shade and then ground into fine powder form by electrical mixture. Powdered sample of leaves stored in clean paper bags and preserved at 4°C for further analysis.

Preparation of plant extracts and leaf powder

Leaf extracts of *Cupressus* were prepared by using methanol. Dried leaf powder weighed carefully and used for extract preparation through soxhlet apparatus at respective temperature. The concentrated leaf extracts were stored and the dried organic extracts in air tight brown bottles. The extracts were filtered and stored for further studies.

Qualitative analysis of secondary phytochemicals

Extracts of leaves were evaluated for preliminary screening of secondary phytochemicals such as, alkaloids, flavonoids, Anthocyanin, Tannins, Steroids, Terpenoids and saponins (Ajayi *et al*., 2011) [1] following the reported methods with minor modifications.

GC-MS analysis

The GC-MS was performed by HP 5ms columns Agilent's 30-meter. Column diameter was 0.25 mm and the thickness of the film 0,25μm. The carrier gas is helium and ionizing used EL (70Ev). GC conditions: *Column oven temperature* 40°C, injector temperature 300°C by injection models are split with a ratio of 207.9. *Flow control mode* is *pressure* 12 kPa. Column flow 0. 56 ml/min, linear velocity 27.1 cm/sec. Mass spectrometry used under conditions Ion source temperature 250°C, interface temperature 300°C. Scan range 28-600m/z speed 1250, time scan starts at minute 0 to minute 50.

FTIR analysis
The IR spectra of the compound were measured on Perkinelmer FTIR- Spectrum- RX I Series instrument. The samples was grounded with IR grade potassium (KBr) (1: 10) pressed into discs under vacuum using spectra lab pelletizer. The IR spectrum was recorded in the region 4400 – 400 cm^{-1} and the typical stretching frequency of the bioactive substance was recorded.

Result and Discussion
In the present study to investigate the phytochemicals present in the leaf extract of *Cupressus* are the source of the secondary metabolites i.e. alkaloids, flavonoids, terpenoids, steroids, phenols and tannins etc., (Table 1). Plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgestic, anticancer, antiviral, antimalarial, antimicrobial activities of the medicinal plants are due to the presence of the above-mentioned secondary metabolites. Medicinal plants are used are discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. The previous phytochemical, present studied show nearly the similar results due to the presence of the phytochemical constituents. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for the treatment of various diseases. Thus the important phytochemical properties identified by our study in the local plant of modern will be helpful in the copping different disease of this particular region.

Table 1: The phytochemical screening of *Cupressus* leaves

Sl. No	Phytochemicals	Methanolic leaf extract
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	-
4	Flavonoids	+
5	Steroids	+
6	Phenols	+
7	Tannins	+

GC-MS analysis
The gymnosperm plants are include many medicinally important genera and species which are to be elucidated for the biological activity of crude extracts and active compounds from these plants (Digrak *et al*., 1999; Watanabe and Fukao, 2009) [7, 15]. In *Cupressus* methanolic leaf extract are analyzed 56 compounds (Table 2). Among the 56 compounds 20 active compounds were present in plenty. The essential oil of *C. lusitanica* presented a diversity of constituents that were identified by mass spectrometry and retention index based on the equation. (Adams, 2001). Among monoterpenes identified, stands out as major constituents b-pinene and b-(Z)-ocimene, besides the endo-fenchol and geraniol as oxygenated monoterpenes. Sesquiterpenes were also identified, being the major constituents' acoradiene, aamorphene, thujopsan-2a-ol and 7a-epi-selinene. The most abundant diterpenes were identified as abietadiene and totarol. The composition of essential oil from Brazilian *C. lusitanica* is quite different from those reported from some countries. Clearly there are many differences between the chemical composition of essential oils extracted from *C. lusitanica* in different regions and countries. A strong justification for this phenomenon could be related to different climate and soil conditions between the regions, which directly influence the metabolism of the plant (Cheraif *et al*., 2007), but also due the exposition to different biotic components.

Table 2: GC MS analysis of *Cupressus* leaf extract

Sl. No	Compound Name	Molecular formula	Nature of the compound
1	Geranyl tiglate	$C_{15}H_{24}O_2$	Aromatic
2	β - Caryophyllene	$C_{15}H_{24}$	Sesquieterpene
3	Humulene	$C_{15}H_{24}$	Sesquieterpene
4	Naphthalene,	$C_{10}H_8$	Aromatic hydrocarbon
5	Sandaracopimarene	$C_{22}H_{36}O_2$	Aromatic
6	Oxirane	$C_{19}H_{38}O_2$	Organic
7	Oxadiazoamine,	$C_2H_2N_2O$	Aromatic
8	Androstan-17-ol, 2,3-	$C_{19}H_{32}$	Steroid
9	Nonadecane, 2,3-dimethyl-	$CH_3(CH_2)_{17}CH_3$	Alkane
10	3 Cyclononene	C_9H_{16}	Cycloalkane
11	n-Heptadecanol-	$C_{17}H_{36}O$	Acidic
12	Hexanoic acid, decyl ester	$C_6H_{12}O_2$	Carboxylic acid
13	Benzenedicarboxylic acid,	$C_8H_6O_4$	Carboxylic acid
14	Phthalic acid	$C_8H_6O_4$	Carboxylic acid
15	2-Piperidinone	C_5H_9NO	Aromatic
16	1Naphthalenemethanol,	$C_{11}H_{10}O$	Aromatic
17	1-Chloroeicosane	$C_{20}H_{41}Cl$	Alkane
18	Tetratetracontane	$C_{14}H_{28}O_2$	Fatty acid
19	Benzamide, 3-methoxy-	$C_6H_5CONH_2$	Benzoic acid
20	3-Methylindole-2-car	C_9H_9N	Organic

FTIR analysis

The broad peak obtained at the 517.85 cm^{-1} is result from the stretching of the C-Br bond of alkyl halide groups and it indicates bonded halogen group. The more intense absorption peak at 3502.49cm^{-1} representing amines and N-H stretch functional groups. It gives strong peak between 3000-2311 cm-1 represent C-H stretching vibration generated by alkynes. The peak obtained at 2376.14 cm^{-1} indicated the presence of O-H (COOH) carboxylic acid. The peak observed at 1736.78 cm^{-1} –C-O stretch representing group of esters present in the leaf powder of *Cuppressus*. Aromatic functional groups were present as C-N stretch at the 1317.29 cm^{-1} and the strongest absorption band at 1154.23cm^{-1} could be due to stretching vibrations of aromatic hydrocarbons. The peak obtained at the 780.15 cm^{-1} which indicates the presence of Cl-Cl benzene. FTIR spectroscopic study of *Cuppressus* showed the hydroxyl (-OH), carboxyl (-C=O) and amine (groups of coumarins, alkaloids or tannins. identification of functional groups in *Cupressus* leaf powder and its characterization through Fourier transform infrared spectroscopy (FTIR), spectra of *Cupressus* leaf extract bands of absorption observed at around 517.85, 675.04, 1265.22 and 3738.75 cm^{-1} and these bands are matching to *Cupressus* L. leaf powder FTIR spectrum. In FTIR spectroscopic study Moses predicted the presence of O H, N-H, C-H, C=O, C-N, C=N, C=C stretching of the detected functional groups (Moses and Robert, 2013) [12]. Species of genus *Cupressus* are frequently attacked by fungi which cause them to develop the disease called "Cypress Canker" (Graniti, 1998; Muthuchelian *et al*., 2005), which depreciate its commercial value. This disease is most frequently caused by fungi of genera Sphaeropsis and Seiridium, and can spread world-wide pretty fast (Sparapano *et al*., 2004). *C. lusitanica* is a good terpenoid producer, including monoterpenes, sesquiterpenes and diterpenes (Adams *et al*., 1997). Some monoterpenes, such as b-thujaplicin, have being proved to act as phytoalexin in *C. lusitanica* (Sparapano *et al*., 2004). Therefore, it seems that terpenoid compounds plays important role in interactions between plants and pathogens.

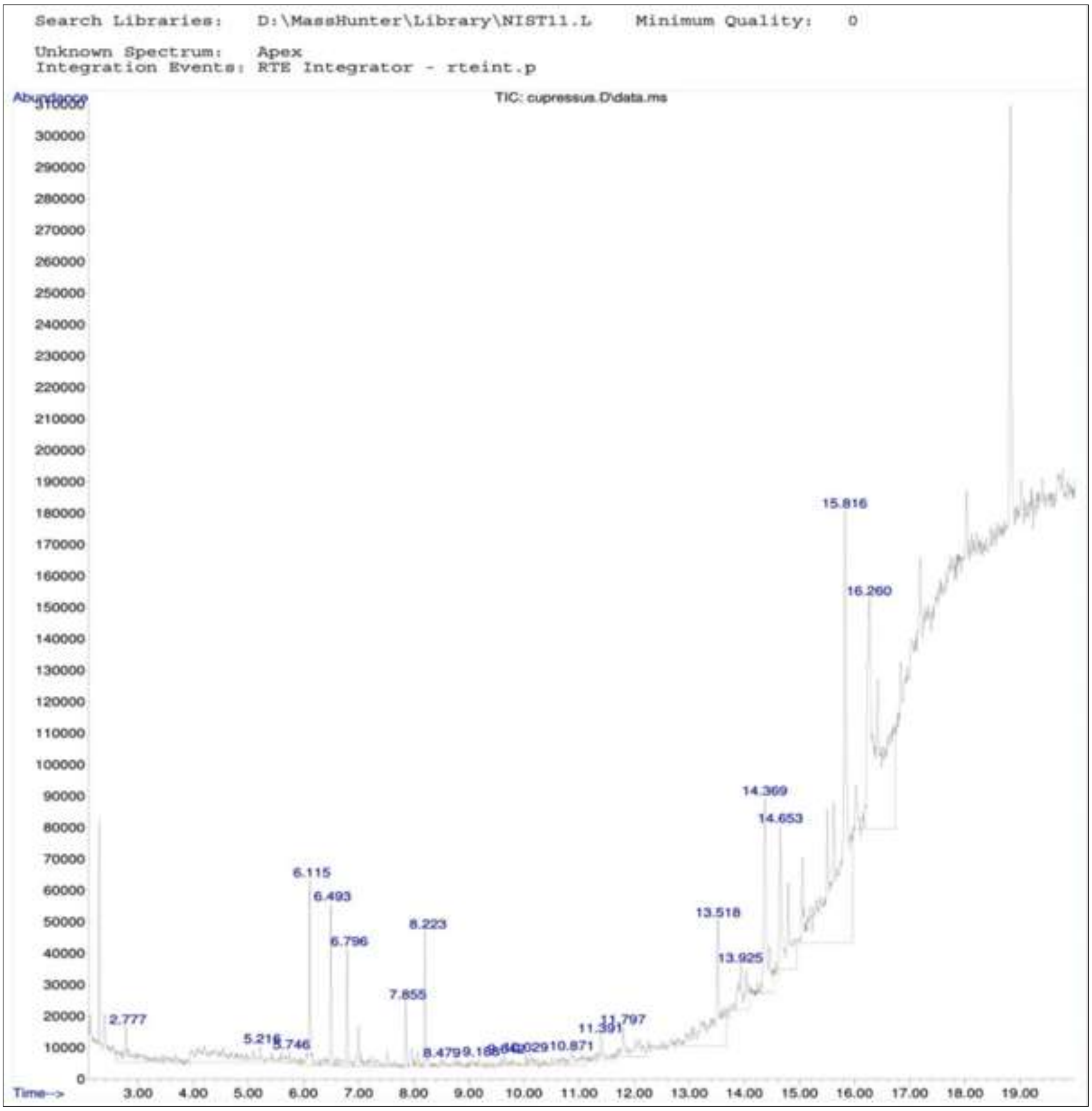

Fig 1: GC MS analysis of *Cupressus* leaf extract

Table 3: FTIR analysis of *Cupressus* leaf powder

Sl. No	Absorption cm^{1}	Class of compounds	Bond
1	517.85	Alkyl halide	C-Br Stretch
2	675.04	Alkyl halide	C-Cl Stretch
3	780.15	Substituted Benzene	C-Cl Stretch
4	1024.15	Ester	C-O Stretch
5	1146.6	Ester	C-O Stretch
6	1312.29	Aromatic	C-N Stretch
7	1394.44	Phenol	O-H Bending
8	1454.23	Alkane	C-H Bending
9	1511.12	Aromatic	C=C Bending
10	1623.95	Alkene	C=C Stretch
11	1680.85	Ketones	C=O Stretch
12	1743.53	Esters	C=O Stretch
13	2311.53	Carboxylic acid	CO_2 Stretch
14	2852.52	Aldehydes	C-H Stretch of CHO
15	3360.73	Alcohols	O-H Stretch
16	3502.49	Amines	N-H Stretch
17	3738.75	Amide	N-H Stretch

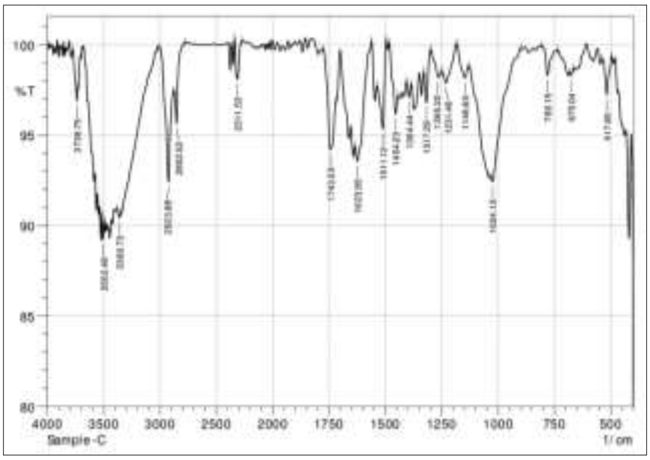

Fig 2: FTIR analysis of *Cupressus* leaf powder

Conclusion

Identification of bioactive compounds by GC-MS there are 56 types of compound with area 0.35% to 30.24% Compounds containing more than Hexyl octyl ether 0.35%, Caryophyllene 2.64%, Geranyl tiglate 4.56%, 3-Methylindole-2-carboxylic acid 5.84%, Androstan 7.79%, Oxadiazol 23.47%, Sandaracopimar 30.24%. FTIR analysis is useful for the identification and to elucidate the structure of bioactive compounds. Therefore, the presence of the important or potential bioactive constituents in gymnosperm *Cupressus* may attribute health benefits by providing the protective effects during the progressive stages of chronic disorders.

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