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A STUDY ON PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF CRYPTOMERIA JAPONICA

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ABSTRACT

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Essential oils (EO) from the Japanese cedar, *Cryptomeria japonica*, has various medicinal properties, including antibacterial and antioxidant activity. The plant *C. japonica* leaves extracted by hydro distillation method for 6 hours using Clevenger apparatus. The oil was analyzed by Gas Chromatography-Mass spectrophotometry (GC-MS). Total fifty one compounds were identified constituting 98.7% of the total oil. The main compounds were α-Kaurene (27.52%), α-pinene (12.41%), Sabinene (11.06%), Cadin-4-en-10-ol (6.02%), L-4-terpineneol (5.73%), Elemol (5.00%), Myrcene (4.34%), Limonene (3.50%) Camphene (1.75%) and Bornyl acetate (1.44%). The samples showed antioxidation activity and have IC_{50} value of 51.36 in *C. japonica* extract. The results obtained in the present study suggest that an essential oil possesses strong medicinal activities can be utilized for the treatment of various diseases.

KEYWORDS: *Cryptomeria japonica*, phytochemical analysis, essential oil, GC-MS.

INTRODUCTION

The plant kingdom is a treasure house of potential drugs and in the recent years after COVID-19 there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. The aromatic oils from plant leaves are used as pharmaceutical raw material in the formulation of many drugs.[1]

Cryptomeria japonica (Thunb. ex L.f.) D. Don (Cupressaceae), commonly called a Japanese cedar or sugi, is a forest tree endemic to Japan, and widely distributed in warm and cool temperate climates.[2] Cryptomeria is a monotypic genus that includes only one species with two recognized varieties: *C. japonica* var. japonica and *C. japonica* var. sinensis, the latter being native to China.[3]

Essential oils (EOs) are generally complex mixtures of volatile, lipophilic, and odiferous, secondary metabolites synthesized and emitted by several plants to facilitate their growth and survival.[4] Research indicates that C. japonica exhibits antifungal, antipathogenic, and protective effects against insects. The leaves of C. japonica contain bioactive compounds associated with essential oil and flavones.[5] Terpinen-4-ol identified as the component responsible for the antibacterial activity of tea tree oil.[6] However, myrsene is critical for the activity of tea tree oil, even though its concentration is very low.[7]

In the present study, therefore, we examined the relationship between the antioxidant activity of essential oil obtained from *C. japonica* and the ratio of its constituents. The present paper deals with the phytochemical analysis of essential oil obtained from leaves of *Cryptomeria japonica* and their antioxidant activity.

MATERIALS AND METHODS

Plant material

The leaves of *Cryptomeria japonica* were collected in the month of January 2020 from Thalkedar near Pithoragarh, India in the Kumaon Himalayas. The plant was identified by the expert in the Department of Botany, Kumaun University, Nainital. The collected plant material was first washed with cold water to remove the soil particles and then shade dried.

Chemicals

Isolation of essential oil

The leaves of *C. japonica* extracted by hydro-distillation method for 6 hours using Clevenger apparatus. The oil was dried over anhydrous sodium sulphate and stored at room temperature in a sealed vial until analysis was performed. The percentage oil yield was calculated based on the dry weight of the plant. The oil yield was (0.09%).

GC and GC/MS Analyses and Identification

Essential oil analyses were performed by GC-MS and GC-FID on a Shimadzu QP-2010 instrument, equipped with FID, in the same conditions. The percentage composition of the oil sample was computed from the GC peak areas without using correction for response factors. The oil was analyzed using a Shimadzu GC/MS Model QP 2010 Plus, equipped with Rtx-5MS (30 m × 0.25 mm; 0.25 mm film thickness) fused silica capillary column. Helium (99.99%) was used as a carrier gas adjusted to 1.21 ml/min at 69.0 K Pa, spitless injection of 1 mL, of a hexane solution injector and interface temperature was 270^0C, oven temperature programmed was 50–280^0C at 3 ^{0}C/min. Mass spectra was recorded at 70 eV. Ion source temperature was 230^0C.

The identification of the chemical constituents was assigned on the basis of comparison of their retention indices and mass spectra with those given in the literature.[8] Retention indices (RI) were determined with reference to a homologous series of normal alkanes, by using the following formula (Kovats, 1958).

$$KI = 100\,[n + (N-n)\ X \quad \frac{\log t^1_R(\text{unknown}) - \log t^1_R(C_n)}{\log t^1_R(C_N) - \log t^1_R(C_n)}$$

t^1_R – the net retention time ($t_R - t_0$)

t_0 – the retention time of solvent (dead time)

t_R – the retention time of the compound.

C_N – number of carbons in longer chain of alkane

C_n– number of carbons in shorter chain of alkane

n - is the number of carbon atoms in the smaller alkane

N - is the number of carbon atoms in the larger alkane

Determination of biological activity

Antioxidation test

The DPPH free radical scavenging ability of essential oil was determined by following method with slight modifications.[9] In brief, the crude oil was three fold diluted in methanol, and 2.4mg DPPH was prepared in 25ml of methanol. 100 µl Different concentrations of oils were taken and equal volume of DPPH solution was added to each concentrations and final volume of 200µl was makeup using methanol in ELISA plate. After 30 min of incubation, the absorbance of the reaction mixture was measured at 517 nm. Ascorbic acid was used as the standard. The DPPH radical scavenging ability of a sample was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs of control}} * 100$$

Where, Control is DPPH solution+ethanol; Sample is DPPH solution+oil samples

The oil samples scavenging activity was determined by IC50 value. IC50 value is the concentration of extracts at which DPPH radicals are scavenged by 50%.

RESULTS AND DISCUSSION

The GC and GC-MS analyses of essential oil of *C. japonica* resulted in the identification of 51 compounds (table-1). The oil yield was (0.09%) by raw material weight. Both, the major as well as minor constituents were identified by their retention indices and comparison of their mass spectra. Total 51 compounds were identified constituting 98.7% of the total oil. The main compounds were α-Kaurene (27.52%), α-pinene (12.41%), Sabinene (11.06%), Cadin-4-en-10-ol (6.02%), L-4-terpineneol (5.73%), Elemol (5.00%), Myrcene (4.34%), Limonene (3.50%) Camphene (1.75%) and Bornyl acetate (1.44%). The main minor compounds were β-Ocimene (0.02%), cis-Piperitol (0.03%), (E)-Nerolidol (0.04%), T-Muurolol (0.10%), α-Terpinyl acetate (0.11%), Linalyl acetate (0.12%) and Tricyclene (0.13%). The presence of 27.52% α-Kaurene show good source of natural α-Kaurene. α-

Kaurene good source of natural fragrance. There is great need to do further work on this plants like separation of essential oil of the plants.

Table 1: Essential oil composition of *cryptomeriya japonica.*

SN	Compounds	Area%	Mol. Formula	Mol. Wt.	RI	Mode of Identification
1	Tricyclene	0.13	$C_{10}H_{16}$	136	920	a,b
2	α-Thujene	1.88	$C_{10}H_{16}$	136	927	a,b
3	α-pinene	12.41	$C_{10}H_{16}$	136	929	a,b
4	CAMPHENE	1.75	$C_{10}H_{16}$	136	940	a,b
5	Sabinene	11.06	$C_{10}H_{16}$	136	955	a,b
6	β-Pinene	0.75	$C_{10}H_{16}$	136	975	a,b
7	Myrcene	4.34	$C_{10}H_{16}$	136	989	a,b
8	(+)-3-CAREN	1.76	$C_{10}H_{16}$	136	1000	a,b
9	α-Terpinene	1.51	$C_{10}H_{16}$	136	1015	a,b
10	para-Cymene	0.15	$C_{10}H_{14}$	134	1024	a,b
11	Limonene	3.50	$C_{10}H_{16}$	136	1025	a,b
12	β-Ocimene	0.02	$C_{10}H_{16}$	136	1030	a,b
13	γ-Terpinene	2.32	$C_{10}H_{16}$	136	1046	a,b
15	Terpinolene	0.67	$C_{10}H_{18}O$	154	1080	a,b
19	2-p-Menthen-1-ol	0.24	$C_{10}H_{18}O$	154	1106	a,b
20	L-4-terpineneol	5.73	$C_{10}H_{18}O$	154	1130	a,b
22	β-Terpineol	0.24	$C_{10}H_{18}O$	154	1137	a,b
23	cis-Piperitol	0.03	$C_{10}H_{18}O$	154	1170	a,b
25	Linalyl acetate	0.12	$C_{12}H_{20}O_2$	196	1245	a,b
26	Bornyl acetate	1.44	$C_{12}H_{20}O_2$	196	1250	a,b
27	α-Terpinyl acetate	0.11	$C_{12}H_{20}O_2$	196	1350	a,b
30	δ-Cadinene	0.30	$C_{15}H_{24}$	204	1505	a,b
31	Elemol	5.00	$C_{15}H_{26}O$	222	1522	a,b
32	(E)-Nerolidol	0.04	$C_{15}H_{26}O$	222	1560	a,b
33	Germacren D-4-ol	0.21	$C_{15}H_{26}O$	222	1570	a,b
37	γ-Eudesmol	1.00	$C_{15}H_{26}O$	222	1632	a,b
36	Agarospirol	0.33	$C_{15}H_{26}O$	222	1643	a,b
39	T-Muurolol	0.10	$C_{15}H_{26}O$	222	1646	a,b
40	Cadin-4-en-10-ol	6.02	$C_{15}H_{26}O$	222	1660	a,b
43	Oplopanonyl acetate	0.92	$C_{17}H_{28}O_3$	280	1893	a,b
44	Cembrene	0.14	$C_{20}H_{32}$	272	1940	a,b
47	Pimaradiene	2.70	$C_{20}H_{32}$	272	1953	a,b
46	Sclarene	2.97	$C_{20}H_{32}$	272	1976	a,b
49	α-Kaurene	27.52	$C_{20}H_{32}$	272	2040	a,b
51	Nezukol	1.29	$C_{20}H_{34}O$	290	2132	a,b
		98.7				

a=Retention Index (RI), b=MS (GC-MS)

Antioxidation assay

In the current study, DPPH assay have been used to estimate antioxidation activity of EO extracted from *Cryptomeriya japonica* and ascorbic acid was used as standard. The oil sample of *Cryptomeriya japonica* showed antioxidation activity. The percent inhibition of *Cryptomeriya japonica* oil was 66.89% with 51.36 IC_{50} against ascorbic acid (standard) (38.03 IC_{50} and 76.33% inhibition).

CONCLUSIONS

Our study conclude that the oil extract has good antioxidation property. The essential oil from *C. japonica* showed a qualitative and quantitative make-up of constituents. Clinically, this plants leaves can be a good source of herbal medicine for the treatment of diseases indigenously. The study will also help to generate a database of species which can be exploited scientifically and judiciously in the future by local people and so that ecological balance is maintained. The results obtained in the present study suggest that the essential oil of *C. japonica* possesses medicinally active compounds.

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