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Research Article

Chemical Profiling, Antioxidant and Antibacterial Properties of *Cotinus coggygria* Essential Oil from Western Himalaya

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ABSTRACT

Cotinus coggygria is an important herbal medicine in western Himalaya which potentially contributes to the advancement of healthcare. The aim of present study was to access an extensive account of antibacterial and antioxidant properties of *Cotinus coggygria* essential oil in relation to chemical composition so that efficacy of the herbal drug can be reassured. Essential oil showed a strongest activity profile within the concentration range 25µl-75µl. The antioxidant profile of the sample was determined by two different test systems (DPPH and ABTS). The essential oil composition of *Cotinus coggygria* was analyzed by GC/MS and a total of 19 compounds were identified. The compounds characterizing major peaks were å -Phellandrene (7.83%), å-Myrcene (5.36%), (+)-2-Bornanone (14.52%), Caryophyllene (7.65%), (+)-epi-Bicyclosesquiphellandrene (5.59%), ç-Elemene (9.56%), Globulol (7.95%). The composition of monoterpene hydrocarbons was observed dominant over sesquiterpene hydrocarbons.

Keywords: *Cotinus coggygria*, essential oil, GC/MS, antibacterial, antioxidant

INTRODUCTION

Among diversified hot spots of the world an extensive biodiversity hot spot is represented by the Indian Himalayan Region (IHR)[1]. Western Himalaya located in the IHR contributes to about 50% of the medicinal plants which have been traditionally used in India and are exploited by pharmaceutical industries not only in India but abroad as well[2-5]. A sudden increase in the demand for herbal products in pharmaceuticals and nutraceuticals across the world has switched the attention of researchers towards various metabolites produced by medicinal plants. The growing interest in biologically active secondary metabolites has led to the development of valuable antioxidants and potent biocides[6]. Secondary metabolites such as essential oils are volatile in nature with characteristic smell and color[7-9]. They are complex natural products which safeguard the plant by showing antimicrobial properties and are also used for various industries like perfumery, pharmaceuticals, nutraceuticals cosmetics etc[10-11]. In contemporary era plant infusions have also been used for medicative purposes i.e., aromatherapy, which demonstrate the valuable effects of essential oils and other aromatic compounds[13-16]. *Cotinus coggygria* from family Anacardiaceae has shown its potential as traditional medicine since times immemorial. Among 800 members of the family, *Cotinus coggygria* is a valuable plant known for its medicinal activity, useful timber and landscape appeal. *C. coggygria* is a deciduous, polygamous shrub reaching a height upto 7 mt. It is commonly known as a "Smoke tree'', fustic or sumac. The leaves and young branches are utilized for the production of essential oil with a terpenic odour for use in perfumery[17]. *C. coggygria* has also been employed for its anti-inflammatory, antimicrobial and wound-healing properties[18]. Various ailments such as diarrhoea, paradontosis, gastric and duodenal ulcer etc. have been cured by this plant[19]. These therapeutic effects of this plant can be attributed to the essential oils and various antioxidants[20-23]. According to the literature, essential oil of *C. coggygria* consists of monoterpenes, gallic acid and flavonoids[24]. Free radical scavenging activity of the plant can be attributed to disulfuretin, sulfuretin and sulfurein[25]. Analyzing the fact that the secondary metabolites of *C. coggygria* show promising antimicrobial and antioxidant activity the aim of the present study is to highlight the pharmacological potential of the plant to design safer alternate medicines.

MATERIALS AND METHODS

Collection of Plant Material

Leaves of *Cotinus coggygria* were collected from Solan Distt of Himachal Pradesh. Collected leaves were surface sterilized using $HgCl_2$ (0.1%) and then rinsed with distilled water thrice.

Isolation of essential oil

Essential oil was extracted from fresh collected leaves of *C. coggygria*. The leaves were subjected to hydro distillation method employing Clevenger type apparatus. The extraction was carried out for six hours. The oil collected was dried over anhydrous sodium sulphate and stored in dark sealed vials kept at 4°C for further analysis.

GC-MS analysis

The GC-MS analysis of the oil was carried out using a TRACE 1300 GC, TSQ 8000 TRIPLE QUADRUPOLE

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Table 1: Chemical composition (%) of the essential oil of *Cotinus coggygria* leaves

S. No.	Compound Name	Rt	Composition (%)
1	3-Carene	5.04	2.63
2	å-Phellandrene	5.69	7.83
3	å-Myrcene	5.93	5.36
4	2-Carene	6.38	4.11
5	D-Limonene	6.58	4.59
6	Ç-Terpinene	7.07	3.81
7	(+)-4-Carene	7.55	1.13
8	(+)-2-Bornanone	8.51	14.52
9	Terpinen-4-ol	8.98	4.27
10	Copaene	11.83	1.69
11	Caryophyllene	12.46	7.65
12	Humulene	12.88	2.32
13	(+)-epi-Bicyclosesquiphellandrene	13.23	5.59
14	ç-Elemene	13.44	9.56
15	ç-Muurolene	13.70	2.56
16	Globulol	14.51	7.95
17	tau.Cadinol	15.13	2.36
18	a-Cadinol	15.30	1.49
19	Stigmasterol	33.92	3.66

Table 2: Mean diameter of the growth inhibition zones for the strain of bacteria

Concentration	Zone of inhibition
25µl	14 ±0.88
50 µl	18±1
75 µl	21±1

MS, fitted with a TG 5MS (30m X 0.25mm, 0.25µm) column. The column temperature was programmed from 60ºC to 210ºC at 3ºC/min using helium as a carrier gas at 1.0 ml/min. the injection temperature was 250˚C and injection volume was 1.0µL prepared in in *n*-hexane. MS transfer line temp was 280˚C.

Antioxidant activity

Evaluation of DPPH

The DPPH radical scavenging capacity of essential oil was measured by the method following the standardized method[26]. The essential oil (20 µl) was added to 0.5 ml of methanolic solution of DPPH and 0.48 ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the essential oil, served as the positive control. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer. The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity \%} = \frac{Abs_{(Contol)} - Abs_{(Sample)}}{Abs_{(Control)}} \times 100$$

Evaluation of ABTS

The antioxidant effect of the essential oil was studied using ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation assay[27]. ABTS radical cations ($ABTS^{+}$) were produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Aliquots (0.5 ml) of the essential oil were added to 0.3 ml of ABTS solution and the final volume was made up to 1ml with ethanol. The absorbance was read at 745 nm in a spectrophotometer and the per cent inhibition was calculated using the formula

$$\text{Inhibition (\%)} = \frac{Abs_{(Contol)} - Abs_{(Sample)}}{Abs_{(Control)}} \times 100$$

Antibacterial activity

The antibacterial activity of the essential oil was investigated by the agar well assay, also known as the hole plate diffusion method[28]. Prior to testing, the bacteria from the agar slants were inoculated in sterile Nutrient broth in universal bottles and incubated for 24 hours at 37 C. After incubation, the bottles lacking growth were discarded and new strains obtained. For the agar well assay, 0.1ml of the bacteria suspension was thoroughly mixed with 20ml of autoclave-sterilised Nutrient Agar in sterile Petri dishes. The agar was left to cool and set. Four holes were punched into the agar using a hole borer with diameter of 4mm and the agar was removed from the holes. If the bottom of the Petri dish was exposed, extra amount of agar was squirted in using a micropipette tip in order to avoid leakage of extract from the holes. The essential oil was aseptically put into three holes at amounts of 25µl, 50µl, 75µl for each well respectively and distilled water was put in the fourth well as negative control. The plates were left for an hour to allow diffusion and penetration to the agar. The test substances diffuse into the agar with decreasing concentration towards the periphery. The plates were put into the incubator at37°C and examined regularly for growth and inhibition.

Statistical Analysis

Results were expressed as mean ± SEM. The statistical analysis was done using GraphPad Prism® 5.2. The least significance difference (LSD) at 5% level was used to compare the means of different test parameters.

RESULTS

The essential oil of *Cotinus coggygria* leaves has been investigated for its chemical composition, antioxidant and antibacterial properties. The essential oil composition of the leaves analyzed by GC-MS and was characterized by a total of 19 compounds (Table 1). The compounds characterizing major peaks are å-Phellandrene (7.83%), å-Myrcene (5.36%), (+)-2-Bornanone (14.52%), Caryophyllene (7.65%), (+)-epi-Bicyclosesquiphellandrene (5.59%), ç Elemene (9.56%), Globulol (7.95%). The composition of monoterpene hydrocarbons was observed dominant over sesquiterpene hydrocarbons. The whole *C. coggygria* plant[29] and its leaves and flowers[30] have presented high antioxidant effectiveness. The essential oil showed an ability to scavenge the DPPH radical. The percent inhibition by ABTS activity was also studied for the essential oil. The DPPH activity was reported to be 7.6±0.2. (%) and ABTS activity was 55.43±0.4. According to the analysis the ABTS assay showed better results for expressing antioxidant property than DPPH assay because the ABTS assay is sensitive, requires a short reaction time, and can

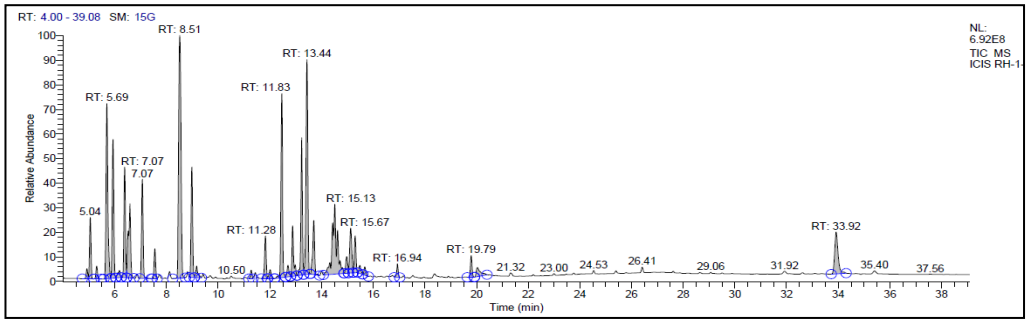

Figure 1: GC/MS graph showing peaks of different compounds at different RTs

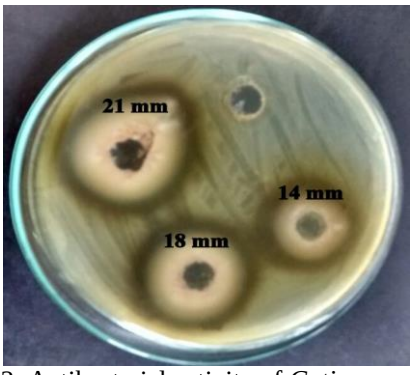

Figure 2: Antibacterial activity of *Cotinus coggygria* essential oil against *E.coli.*

be used in both organic and aqueous solvent systems. Therefore, the ABTS assay may be preferable over the DPPH assay for evaluating the total antioxidant capacity[31]. *Escherichia coli* strain showed varying susceptibilities towards the plant essential oil *C. coggygria.* The diameters of the growth inhibition zone for the strain of bacteria were determined to be in the range of 14 to 21 mm. (Fig. 2). The inhibition zones were increased when we increased the concentration of the essential oil. At the highest concentration of 75µl *C. coggygria* essential oil showed larger zone of inhibition (21mm). 75µl oil showed the best antibacterial activity against *E. coli* as compared to 25 µl concentration of essential oil. The zone of inhibition increased when we increased the concentration of oil. The experiments were performed thrice and the p value suggests that the data is significant and analyzed by graph pad prism. *p <0.05.

DISCUSSION

The GC/MS analysis of oil has revealed that the essential oil of *Cotinus coggygria* consists of many complex compounds with different characteristics. Limonene is valuable component of essential oil of *C. coggygria* which is a common flavoring and fragrance agent used commercially[25]. Terpineol is a monoterpene which has reported antibacterial and antiviral properties and has proved a potential anticancerous agent[32]. +-2-bornanone (14.52%) which is the major component of essential oil is used for its scent and as an important ingredient in cooking. å-Phellandrene which constitutes about 7.83% of essential oil having pleasing aromas so are used in fragrances. Caryophyllene (7.65%) has antinociceptive, neuroprotective, anxiolytic, antidepressant and anti-alcoholism activity. Globulol (7.95%) present in the essential oil of *C. coggygria* is main antibacterial compound. The essential oil of plant showed effective results for the inhibition of production of reactive oxygen species by DPPH and ABTS assay. These antioxidant assays give information about susceptibility of a potent antioxidant towards free radical. Several phenolic compounds are present in *C. coggygria*[33,34] and their antioxidant ability can be attributed to these polyphenols as the can scavenge free radicals[35,36]. The essential oil of *C. coggygria* exhibited strong antibacterial activity when tested with *E. coli*, a bacteria which can cause a wide range of ailments like minor skin infections to severe life threatening diseases such as meningitis, pneumonia etc.

CONCLUSION

The present study on profiling of chemical constituents, antioxidant and antibacterial properties of *Cotinus coggygria* from western Himalaya has contributed to further studies to reveal more pharmacological activities like hepatoprotective, anthelmintic, anti-inflammatory, anti-stress etc. The secondary metabolites of this plant can help in virtue of modern medicines.

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