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Comparative antioxidant activity of essential oil of leaves of *Citrus limettioides* and *Citrus pseudolimon* of Nainital District

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ABSTRACT

Air dried leaves essential oil was extracted by hydrodistillation. Quantitative Phytochemical analysis of volatile compounds viz. Limonin, Citronellol, linalool and terpineol was done by Gas chromatography, Limonin, linalool, terpineol (36.9%, 2.12%, 4.19%) was found higher extent in *Citrus limettioides* as compared to *Citrus pseudo limon* (10.6%, 1.7%, 2.59%). Citronellol content was found higher extent in *Citrus pseudolimon* (62.30%) than *Citrus limettioides* (5.11%). Antioxidant activity of leaves essential oil was done by using 1, 1, Diphenyl 2Picryl Hydrazine (DPPH) free radical scavenging assay. IC₅₀ of both plants leaves essential oil was calculated. Comparative study shows that *Citrus limettioides* shows higher antioxidant potential (IC₅₀=15.35 ul) as compared to *Citrus pseudolimon* (IC₅₀=18.43 ul) against DPPH free radicals.

Keywords: *Citrus limettioides*, *Citrus pseudolimon*, Essential oil, Antioxidant activity, DPPH.

1. Introduction

Oxidation of biomolecules can cause generation of free radical in the body. They can be responsible for various disorders in human like atherosclerosis, gastritis, cancer, arthritis, ischemia and reperfusion injury of many tissues and AIDS [1]. Free radicals can also affect food quality; reducing its nutritional content and promoting the development of food deterioration [2]. Antioxidant inhibits the oxidation and prevents the body from various disorders. Moreover, *Citrus* essential oils have been recognised as safe due to their wide spectrum of biological activities such as antimicrobial, antioxidant anti-inflammatory and anxiolytic [3-5]. *Citrus* crop is an ancient crop humans cultivate this since 2100 BC [6]. *Essential oil* has been found in various parts of fruits, leaves, major volatile content of essential oil are dl-limonene, β-myrcene, α-pinene, sabinene, Δ-3-carene, α-terpinolene and other elements are that major aromatic compounds of many *Citrus* species [7]. These aromatic compounds are abundance with application in flavor and food industries [5]. *Citrus limettioides* locally known as Limba nimbu and *Citrus pseudo limon* locally known as Galgal, both plants species belongs to family Rutaceae [8], both plants leaves essential oil chosen for their antioxidant potential.

2. Materials and Methods

2.1 Collection and Authentication

Fresh leaves of *Citrus limettioides* and *Citrus pseudolimon* collected on September from National Bureau of Plant and Genetic Resources (NBPGR) Bhowali Nainital Uttarakhand. Authentication of plants species done by Dr. S. K. Verma as per their IC code and both plants species have been maintained at their gene bank.

2.2 Processing of Plant materials

Both Plants processed properly at National Botanical Research Institute Lucknow. Fresh leaves were allowed to air dry at room temperature until two consecutive weights were found to be constant.

2.3 Extraction and Evaluation of essential oil.

The essential oil of air dried leaves was extracted by hydrodistillation with the help of

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Clevenger apparatus. Analysis of volatile compounds was done by *Perkin Elmer clarus 500*. Were GC fitted with a silicone DB-5 capillary column, carrier gas Nitrogen, flow rate 1.5 ml/min, run time 50min, split mode temperature programmed 70 –280 °C at 30 C/min. Injector temperature and detector temperature were 250 °C and 300 °C respectively. Compound detected by using selected markers. Injector volume for all samples was 0.1 ul.

2.4. Antioxidant activity of essential oil.

In Vitro DPPH free radical scavenging activity of essential oil of the air dried leaves was done by using reference procedure [8].

3. Results

3.1 GC analysis

Table 1: Some major compounds found in leaves essential oil.

Compound Name	Retention Time	% Conc. <i>Citrus pseudo limon</i>	% Conc. In <i>Citrus limettioides</i>
Limonin	13.7	10.6	36.9
Citronellol	14.07	62.30	5.11
Linalool	15.2	1.7	2.12
Terpineol	19.03	2.59	4.19

Percentage content of limonin, linalool, terpineol was found higher extent in *Citrus limettioides* as compared to *Citrus pseudolimon*, while Citronellol was found higher extent in *Citrus pseudolimon*.

3.2 Antioxidant Activity of essential oil

Table 2: Inhibition control of *Citrus limettioides* (Limba nimbu) against DPPH at various concentrations.

concentration ug/ml	% Inhibition
5	23.27
10	40.85
20	59.85

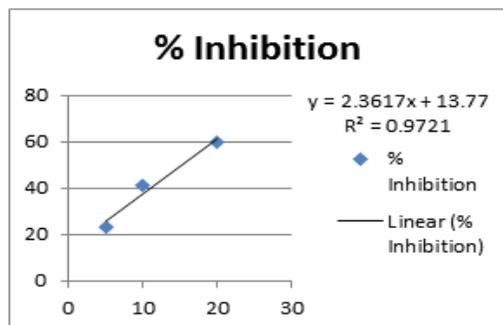


Fig 1: *Citrus limettioides* (Limba Nimbu) DPPH % free radical inhibition Vs. Concentration ul/ml. IC₅₀= 15.35ul.

Table 3: Inhibition control *Citrus pseudo limon* (Galgal) against DPPH at various concentrations.

Concentration ug/ml	% Inhibition
10	25.35
20	62.2
30	73.92

Inhibition control of essential oil of *Citrus limettioides* was and *Citrus pseudo limon* was calculated by using Ascorbic acid as a standard. It was found to be that *Citrus limettioides* IC₅₀ at 11.69 ul while *Citrus pseudo limon* shows IC₅₀ at 18.43 ul (Table 4)

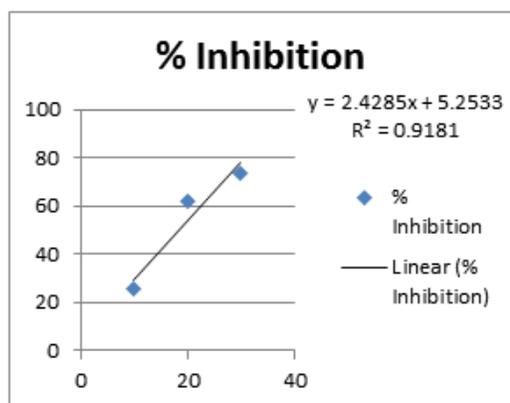


Fig 2: *Citrus pseudo limon* (Galgal) DPPH % free radical inhibition Vs. Concentration ul/ml, IC₅₀= 18.43 ul

Table 4

Sample	Inhibition control 50% (IC ₅₀)
<i>Citrus limettioides</i>	15.35 ul
<i>Citrus pseudo limon</i>	18.43 ul

4. Discussion

Comparative Phytochemical analysis of air dried leaves essential oil shown (Fig. 3) that *Citrus limettioides* contain higher extent of limonin, terpineol and linalool while *Citrus pseudo limon* contain higher extent of Citronellol.

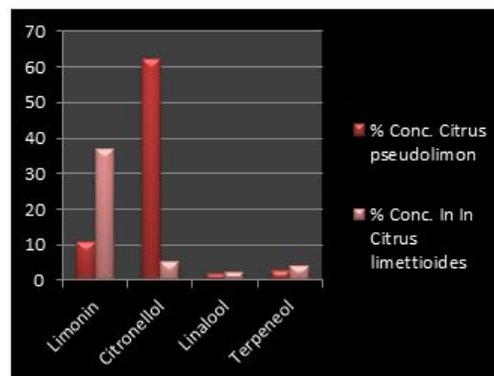


Fig 3: Shows comparison of Concentration of some active essential oil constituents in between *Citrus limettioides* and *Citrus pseudo limon*.

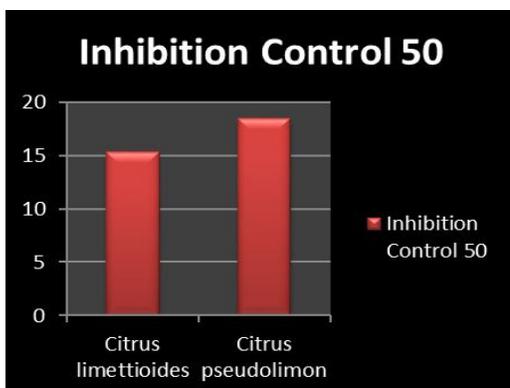


Fig 4: comparison of concentration at which potential of essential oil *Citrus limettioides* and *Citrus pseudo limon* shows antioxidant potential.

It is reported that limonin reduced DABA induced buccal pouch

epidermoid carcinoma in female Syrian hamsters and neoplasia in experimental mice¹⁹⁻¹⁰. Thus *Citrus limettioides* essential oil could be help to prevent DABA induce carcinoma as compared with *Citrus pseudolimon* leaves essential oil. DPPH is stable radical of purple colour which reduces to yellow-coloured diphenylpicrylhydrazine when it reacts by antioxidant compound which can donate hydrogen¹⁷. Comparison of antioxidant activity against DPPH Free radical scavenging shows (Fig. 4) that *Citrus limettioides* is potent antioxidant (IC₅₀=15.35 ul) as compared to *Citrus pseudo limon* (IC₅₀=18.43 ul).

5. Conclusion

Study concludes that essential oil of *Citrus limettioides* shows higher concentration of limonin, linalool, terpineol and higher antioxidant potential as compared to essential oil of *Citrus pseudo limon*.

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