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In vitro Antioxidant Activity of Essential Oil and Polar and Non-Polar Extracts of *Ocimum canun* from Mbuji-Mayi DR Congo

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ABSTRACT

DPPH radical scavenging activity of essential oil from fresh leaves of *Ocimum canum* Sims as well as methanolic, ethyl acetate, dichloromethane and n-hexane crude extracts, from dried leaves were analysed. The methanolic extract has the low IC50 so the strongest radical scavenging activity among extracts of *Ocimum canum* with IC50 values of 0.0234 mg.mL^{-1}. Ethyl acetate , Dichloromethane and essential oil showed respectively IC50 values of 0.0767, 0.1938 and 1.4217 mg.mL^{-1}. So, polar extracts exhibited stronger antioxidant activity than none-polar ones. None-polar solvents have provided fewer extraction yields than polar. The GC-MS analysis of the essential oil of *Ocimum canum* has been done and led to the identification of 1,8-Cineole as major compound

Keyword: *antioxidant activity, Ocimum canum, essential oil, polar and non-polar extracts*

INTRODUCTION:

Oxidation process is one of the most important roots for producing free radicals in food, drugs and even living systems. Reactive oxygen species may be the causative factor involved in many human degenerative diseases, and antioxidant compounds are known to have some degrees of preventive and therapeutic effects of these desorders [1].

Out of vitamins E and C, several molecules have antioxidant potential. However , their effectiveness is sometimes questionable . This is particularly the case of pro -oxidant properties assigned to them and which constitute one of the factors that restrict trade in food and cosmetic products of these compounds; which justifies the renewed attention given to this area [2].

In Africa, about 80 % of the population rely for reasons of poverty to plants for healing [3-5]. Indeed, plants produce a diverse range of secondary metabolites that can be therapeutic. It is in this framework that the research is undertaken in the hope of identifying effective active ingredients.

Our research team listed, a number of plants used in traditional medicine against sickle cell disease in Democratic Republic of Congo and tested their antisickling activity among which some species of *Ocimum* genius [3-20]. *Ocimum* , is a versatile aromatic genus (family-Lamiaceae) well known for medicinal properties and also for economically important essential oils. The genus is very variable and possesses wide range of intra- and inter-specific genetic diversity [21,22].

Ocimum canum is a small erect plant, with a strong aromatic flavor and is commonly cultivated for culinary purposes. The plant is used in the treatment of various diseases such as cold, fever, parasitic infestations on the body and inflammation of joints headaches. The plant acts as stimulant, carminative, and diaphoretic and leaves as bechic, febrifuge and are used in cold, bronchitis, externally in skin disease. The antifungal property is shown by the essential oil of Ocimum canum Sims. The seed is effective as a hypoglycaemic; also used in the treatment of leucorrhoea and other diseases of urinogenital system. The essential oil at the flowering stage contains citral as a major component along with methylheptenone, methylnonylketone and camphor. Leaves yield beta- sitosterol, betulinic acid and ursolic acid and flavanois, pectolinarigenin-7-methylether and nevadensin [23]

In our previous work, the antisickling activity of different extracts from *Ocimum canum* Sims was studied, but radical scavenging activity has not be done [6].

The aim of this study, to evaluate the antioxidant potential of essential oil and extracts from fresh and dried leaves of *Ocimum Canum* Sims using the reduction of DPPH radical as bioassay.

MATERIELS AND METHODES

Plant material

Plant samples used in this works (leaves of *Ocimum canun*) were harvested in the vicinity of Mbuji-mayi city in the central part of DRC on May 2013. The collected materials were identified and deposited at herbarium of the Faculty of Science, University of Kinshasa.

Oil distillation

Extraction of essential oil from leaves of *Ocimum Canum* has been done by hydrodistillation. A well-known amount (200g) of a fresh leaves were ground and immersed in a 500 mL round bottom flask of water and hydrodistilled. Water and essence are recovered in a decant bowl, anhydrous magnesium sulphate was used for drying trace of water and then stored in a dark glass bottle at 4 ºC, before submitting it to GC-MS analysis. The process is repeated more time for increasing amount of oil [24].

Gaz Chromatography-Mass Spectrometry analysis

The GC-MS analysis of the essential oil isolated from *Ocimum Canum* SIMS was performed by using a AGILENT gaz phase Chromatography and a coupled SHIMADZU GC-MS system. The GC was equiped with a 15m column containing 5% diphenyldimetylsiloxane with a FID detector. Mass spectra have been recorded on a 30m DBXLB column coupled to an electron ionisation (EI) system with electronic impact. LRMS data have been obtained on an UPLC/MS-TOF apparatus equipped with an ESI interface and C-18 (1.7µm, 2.1X50mm) column by using ammonium carbonate (0.005M)/methanol as mobile phase. The chemical identification of some compound is done by comparing with the Wiley 275.L Data base masse spectra.

Preparation of plant extracts

Plants extracts were obtained by increasing polarity extraction method. So, for obtaining good results , the DPPH radical test requires the methanol as a reaction solvent [25]. Thus, to make easier this test, plant metabolites that are soluble in methanol were extracted.

Since a multitude of compounds are extracted by methanol, a fractionation of the extract would be a significant step towards the isolation of pure products. Thus , a subsequent liquid-liquid methanolic extraction using n-hexane , dichloromethane , and ethyl acetate was done.

100 g of plant powder was macerated sequentially in n-hexane, dichloromethane, ethyl acetate and methanol as separate for 48 hours. The solution obtained is filtered , the organic extract filtra serve the following tests . The resulting fractions were evaporated to dryness on an evaporator apparatus.

These different extracts obtained are submitted to antioxidant test based on reducing of the 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical

Radical Scavenging activity using DPPH method

The DPPH free radical scavenging assay was carried out as previously reported [26]. Briefly, a 0.3 mM solution of DPPH radical in methanol was prepared. 3.5 mL of this solution added to 0.5 mL solution of essential oil or each of extract in methanol. Each bioactive essential oil and extract solutions are prepared at various concentrations in methanol in common ratio of a geometric progression. Six values of concentrations (19. 85, 39.69, 79.38, 158.75, 317.5 and 635 µg extract per mL) for extracts and five (582.75, 1165.50, 2331.00, 4662.00 and 9324.00 µg oil per mL) for oil were used. The mixture was shaken vigorously and the absorbances at 517 nm were recorded during 35 minutes considered as equilibrium time, using Uv-vis 320/safas monaco Spectrophotometer, in order to determine the DPPH radical scavenging of reactions and to calculate parameters of antioxidant activity for ascorbic acid and extracts (Percent of reduction, the IC50 index and effectiveness antioxidant 1/IC50)[27,28].

The reduction of DPPH free radical by ascorbic acid was also analyzed to the same concentrations for comparisons.

Percentage of reduction was calculated according to the following equation:

$$I\% = \frac{A_{blanck} - A_{sample}}{A_{blanck}} x100$$

where: A_{blank}= Absorbance of blank (containing all reagents except the tested compound) and

A_{sample}= Absorbance of the tested compound.

RESULTS AND DISCUSSIONS

Essential oil yield

From 360g of fresh leaves and inflorescences of *Ocimum canum* Sims 2.19 gr (0.607%) of essential oil were obtained after hydrodistillation. This yield seems to be higher than that of *Ocimum canum* Sims from Cameroon (0.44%) [29].

Chemical composition of essential oil

The major compound of *O. canum* essential oil obtained by comparing the obtained Mass spectrum with database spectra 1, 8-cineole. This composition is the same with that of this specie harvested in Burkina Faso [30].

Extracts yields

Figure 1 shows the yield of extraction of *O. canun* air dried leaves.

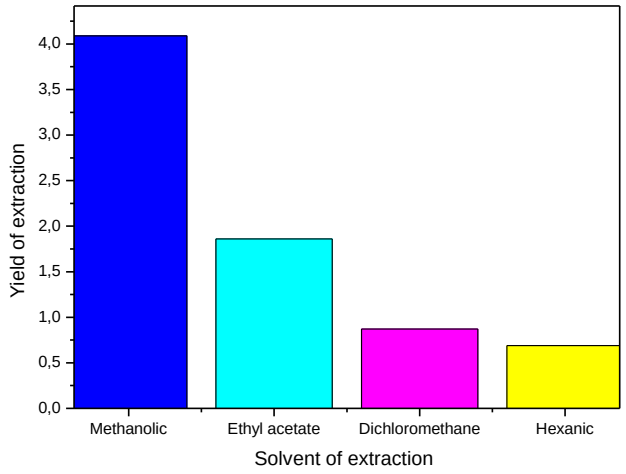


Figure 1 : Extraction yields (%) of O. canun in different solvents

It can be clearly noticed in this figure that the none-polar fractions have lower yields than polar ones. That indicates the majority of metabolites in the leaves of *Ocimun canun* Sims are those which pass easily into the polar solvents [6, 31,32].

DPPH radical reduction of essential oil

The percentages of reduction of the DPPH radical versus concentration values of essential oil of *O. canum* and the control (L-ascorbic acid) are shown in figure 2.

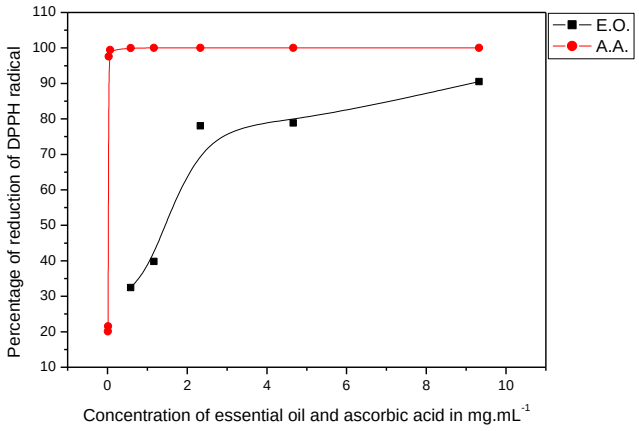


Figure 2 : DPPH radical reduction by the essential oil (E.O.) and ascorbic acid (A.A.)

Figure 2 indicates that the essential oil extracted from leaves of *O. canum* shows radical scavenging activity in presence of DPPH

radical. The reduction percentage of DDPH reaches 90 % at the concentration of 10 mg/mL of essential oil. But this activity in low compared to that of ascorbic acid used as positive control.

DPPH radical reduction of extracts

The percentages of reduction of the DPPH radical versus concentration values of various extracts of *O. canum* and the control L-ascorbic acid are shown in figure 3.

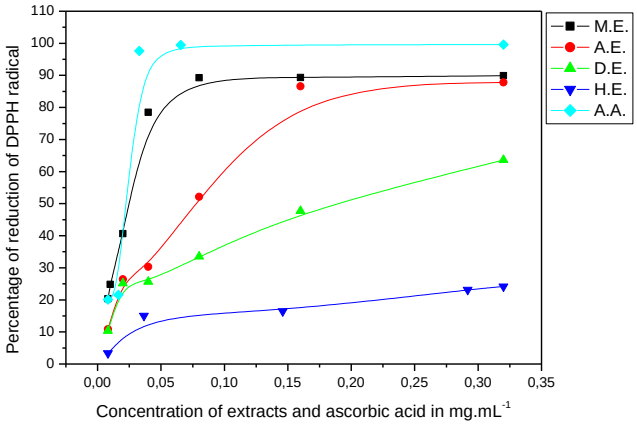


Figure 3: DPPH radical reduction by the four extracts (M.E. : methanolic, A.E. : ethyl acetate, D.E. : dichloromethane, H.E. : hexanic) and the ascorbic acid (A.A.)

This figure shows that methanolic extract has the highest DPPH radical reduction activity but this radical scavenging activity remain lower than that of L-ascorbic acid (positive control). The n-hexane extract has reduced the DPPH radical below 50 % in the range of concentrations used in this work and shows the lowest DPPH radical reduction activity.

Calculated radical scavenging activity

The IC50 values of and 1/IC50 values of extracts and essential oil are shown in (Table 1).

Table 1 : Antioxidant activity of ascorbic acid and the methanolic, ethyl acetate, dichloromethane and hexanic extracts.

Reducing agent		IC_{50} Values ($mg.mL^{-1}$)	Antioxidant activity (1/ IC_{50})
Control	Ascorbic acid	0.0220	45.45
Extracts	Methanolic	0.0234	42.73
	Ethyl acetate	0.0767	13.04
	Dichloromethane	0.1938	5.16
	Hexanic	-	-
Essential oil		1.4217	0.70

Among all extracts samples studied, the methanolic extract has the low IC50 so the strongest radical scavenging activity among extracts of *O.canum* with IC50 values of 0.0234 $mg.mL^{-1}$. But this activity is lower than that of positive controls (L-ascorbic acid with IC50 of 0.0220 $mg.mL^{-1}$). The ethyl acetate extract and the dichloromethane extract showed a good DPPH radical scavenging activity, while hexanic extract showed little activity, it's IC50 could not be calculated because the percentage of reduction of DPPH radical was under 50%. So, polar extracts exhibited stronger antioxidant activity than non-polar ones. In fact, compounds known for their radical scavenging activity like polyphenols are soluble in polar solvents [17,18].

The antioxidant activity can also be calculated as 1/ IC50. The values obtained confirm that of IC50. The essential oil from *O.canum* shows low antioxidant activity.

This plant species displayed promising radical scavenging effects in vitro. The ability of its extracts to display such pharmacological properties may represent a rational explanation for the use of this species by the traditional healers to treat quoted diseases.

CONCLUSION

The present study evaluated the *in vitro* DPPH radical antioxidant scavenging activity of essential oil from the fresh leaves and extracts from the dried leaves of *Ocimumi canun* Sims. Extracts have shown higher antioxidant scavenging activity than essential oil. The GC-MS analysis of the essential oil of *Ocimum canum* Sims has permit the identification of 1,8-Cineole as major compound.

The strong antioxidant activity indicates a possible use of extracts as a natural antioxidant in food and pharmaceutical industries. Fractioning of extracts in order to determine active molecule and elucidate molecular structure is undergoing.

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