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GC/MS PROFILING AND ANTIOXIDANT ACTIVITY OF *NIGELLA SATIVA* ESSENTIAL OIL FROM SUDAN

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ABSTRACT

Background: The objectives of this work to determine the chemical composition of the essential oil of *Nigella sativa* from fixed oil of seeds and to evaluate the antioxidant activity of essential oil in vitro. **Method:** The essential oil was extracted from fixed oil by distillation, then the essential oil was chemically analyzed and identified by Gas Chromatography-Mass spectrometry (GC/MS). The essential oil was tested for a possible antioxidant activity by using DPPH method. **Results:** The principal components of the essential oil was as follows: thymoquinone (57.99%), p-cymene (28.90%), alpha-phellandrene (6.331%), alpha- terpineol (2.706%) and limonene (1.353%). **Conclusion:** Thymoquinone the major constituents of the Sudanese commercial oil sample, (57.99%) has not recorded in any previous literature in dominant quantities among the major compounds from the oils of *N. sativa*. The observed compositional difference between N. sativa found in Sudanese commercial sample and other area from the globe could be due to environmental and genetic factors or chemo-types, which can influence essential oil composition.

KEYWORDS: Nigella sativa, Essential oil, GC/MS analysis, Antioxidant activity and Thymoquinone.

INTRODUCTION

The use of the herbal drug as complementary medicines is become common and popular, most of the drugs in the market either derived directly from the plant or chemically modified natural product. Nigella sativa L. (family Ranunculaceae), sometimes known as black cumin, has been used as a spice and to treat a variety of diseases in many places of the world since ancient times. The seeds are currently used in a variety of culinary, medicinal, and cosmetic purposes (Hosseinia et al., 2019). It is an annual flowering plant which grows to 20-90 cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike. The flowers are delicate, and usually colored white, yellow, pink, pale blue or pale purple, with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds (Ahmed and Husian *et al*,2013).

The seeds of N sativa are used as a spice in bread, yogurt, marinades, sauces, and salads. In Islamic culture, also is known as "El Habba Saouda" and used in traditional medicine with reference to a proverb stating that "a drug for all diseases except death." These words remained a mystery, until science was able to determine its therapeutic properties (Al-Sheikh et. al., 1999). Considering the richness of its biological heritage, it is possible that the seed extracts could contain one or more active ingredients that specifically target each disease. Acually, the diversity of secondary metabolite explain this variety of therapeutic uses (Saleh et. al.2017, Grech and Pietrosiuk, 2012).

The most components in N. sativa seeds are: Thymoquinone, Thymohydroquinone, Dithymoquione, P-cymene, Carvacrol, 4-Terpinol, trans-anethole, Sesquterpene longifolene, Alpha-pinene and thymol, Alkaloidal compounds (nigellicimine,nigellicimine-N-oxide, pyrazole alkaloids as ligellidine and nigellicin. Alpha-hedrine), triterpine and saponin. (Ahmed and Husian *et al*, 2013).

The seeds are mostly used in Sudan as a spice, to protect food from pathogenic and spoilage germs. Also were used in traditional medicine to alleviate articulation pain, stomachache, jaundice, diabetes, headaches, and hypertension (Issa et al., 2018). Recently, it is believed that the seeds can be used to treat coronavirus. On the other hand, N. sativa, is not cultivated in Sudan, but the seeds are primarily imported from Ethiopia and India. The seed and its oils have several pharmacological properties. Numerous studies indicated many biological activities like anticancer, antiviral, antibacterial,

antipyretic, galactagogue, carminative, antidiabetic, and antioxidant activities (Darakhshan et al., 2015).

The seed oil or extract is found to have therapeutic properties and is considered as one of the newer sources of edible oils (Cheikh-Rouhou et al. 2007). Both seeds and oils are often used as nutritional supplement due to its various health properties as they have been reported to possess antitumour activity and antioxidant activity (Burits & Bucar 2000), anti-inflammatory activity, antibacterial activity and a stimulatory effect on the immune system (Salem & Hossain 2000).

Several studies have shown that plant derived antioxidant nutraceuticals scavenge free radicals and modulate oxidative stress-related degenerative effects (Joseph et. al, 1999). Free radicals have been implicated in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging (Halliwell et. al, 1999). Previous research reports suggest that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular diseases and cancer (Thatte et. al, 2000). N. sativa is consider as a traditional and natural source of antioxidants (Schwartz et.al. 2008). In fact, N. sativa is capable of free radical inhibition also significantly can reduce oxidative stress (Kumar et. al., 2018).

The importance of the antioxidant constituents of plant materials is related to maintenance of health and protection from coronary heart disease and cancer. Also the interest raising among scientists, food manufacturers, and consumers as a general trend in the future toward functional food with specific health effects (Lo¨-liger, 1991). Hence the study aimed to evaluate the antioxidant activity of the essential oil and identify the main constituent of it by using GC/MS analysis.

MATERIALS AND METHODS

Oil Material

Fixed oil was purchased from Omdurman market, Khartoum., Sudan.

Extraction

100 ml of the fixed oil added to 400 ml distilled water in conical flask ,then the heater was degenerate until obtained 1 ml of essential oil material.

GC/MS analysis

Analysis were performed on An Ultra model GC-MS-QP2010 Ultra shimadzu Japan for mass spectral identification of the GC component at (MS) ionization voltage of (Rtx-5MS…Length (30m).. diameter(0.25 mm)..Thickness (0.025µl).the linear velocity of the helium carrier gas was 47.2cm/sec. the injector temperatures was 300.00^{0}c, the oven temperatures was 50.0^{0}C/ to 300.0^{0}C/min and held for 30 min.

Antioxidant method

The antioxidant activity was done for the essential oil by the following method: The DPPH• radical scavenging activity was estimated by measuring the decrease in the absorbance of metabolic solution of DPPH. In 96-wells plate, extracts (5-10mg/ml) were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as (300µM). The test samples & DPPH were dissolved in methanol. After incubation, decrease in absorbance was measured at 517nm using Thermo Scientific Multiscan spectrophotometer. Percentage radical scavenging activity (%RSA) by samples was determined in comparison with a methanol treated control group. All tests and analysis were run in triplicate. Parallel to examination of the antioxidant activity of plant extracts, the values for standard compound was obtained and compared to the values of the antioxidant plants extracts (Irina, 2002).

RESULTS AND DISCUSSION

GC/MS analysis

The chemical composition of the essential oil obtained from a commercial fixed oil of *N. sativa* seeds was determined by GC/MS. The hydro-distillation of the comirical fixed oil gave a yellowish essential oil with a characteristic odor. Eleven compounds, representing 99.731% of the oil were identified. GC/MS analysis of essential oil showed 13 peaks indicating the presence of 13 compounds in the oil (Table 1). The principal components of the oil as follows: thymoquinone (57.99%), p-cymene (28.90%), alpha-phellandrene (6.331%), alpha-terpineol (2.706%) and limonene (1.353%). The compounds that represented divided into three main group, the first one is the monoterpene hydrocarbons (p-cymene 28.90% and alpha-phellandrene 6.331%), the second one is the oxygenated monoterpenes (alpha-terpineol 2.706%) and the third one is sesquiterpene component (valencene 0.1417%). The result is disagreement with Tahir et. al., (2022) who indicated low percentage of thymoquinone (18.7%) and high percentage of p-cymene (36.76%). Moreover TLC screening methods for the essential oils, exihbited thymoquinone, carvacrol, t-anethole and 4-terpineol (Mejía et. al.,2000).

On the other hand the oil sample from Sudan represented high content of polyunsaturated fatty acid (PUFA) (65.13±5.45%) (Haron,2014). While the volatile oil showed 31 compounds by GC-MS analysis included two new chemical compounds 2(1H)- Naphthalenone (C11H18O) and Uvdin (C15H24O3) (Gerige,2009).

Regarding comparison of the oil compounds from different geographical origins indicated some qualitative and quantitative variation in their essential oil constituents (Nickvar *et al.*,2003)**,** (Burits and Bucar, 2000) and (Gerige *et al.*, 2009) (Table 2). P-cymene and thymoquinone were found to be the major compounds

identified in high concentrations (Sudan commercial sample). Interestingly, the major constituents of the Sudanese commercial oil sample, thymoquinone (57.99%) has not been reported in the previous available literature with dominant quantities among the major compounds from the oils of *N. sativa*. According to comparison between the essential oils from Iran and India, the result that we found indicated high presence for thymoquinone and p-cymene, while Nickavar (2003) and Gerige *et al.* (2009) reported weak presence for thymoquinone (.60 and 11.8%), and p- cymene (14.8 and 9.0 %). The observed compositional difference between Sudanese commercial sample and the rest of the sample from different area (Burits and Bucar, 2000), (Nickavar, 2003) and (Gerige et. al., 2009) may be due to environmental and genetic factors or chemo-types, which can influence essential oil composition.

Table 1: Chemical composition of essential oil obtained from N.sativa fixed oil analyzed by GC/MS.

Peak No.	Retention Time	Compound	Molecular formula	%
1	11.24	Alpha-phellandrene	C10H16	6.331
2	11.53	3-carene	C10H16	0.9525
3	13.48	Gamma-terpinene	C10H16	0.2652
4	13.69	Alpha-pinene	C10H16	0.8797
5	16.31	p-cymene	C10H14	28.90
6	16.38	Limonene	C10H16	1.353
7	19.59	Unidentified	C10H18O	0.1713
8	20.78	Alpha-terpineol	C10H18O	2.706
9	23.11	Trans-2-caren-4-ol	C10H16O	0.0498
10	23.97	Unidentified	C10H18O	0.0922
11	24.87	1,3,4-trimethyl-3-cyclohexene-1-carboxaldehyde	C10H16O	0.1621
12	27.80	Thymoquinone	C10H12O2	57.99
13	34.02	Valencene	C15H24	0.1417

Table 2: Comparison of major constituents of *N.sativa* oils from different countries.

Constituents	Iran	Austria	India	India	Present study
Thymoquinone	0.6	+	11.8	3.4	57.99
p-cymene	14.8	-	9.0	31.4	28.90
Limonene	4.3	-	4.3	1.0	1.353
trans-anethole	38.3	+	27.1	-	-
Carvone	4.0	-	2.0	Trace	-
Alpha- thujene	2.4	-	2.4	5.6	-
Carvacrol	-	+	3.7	1.4	-
Longiflene	-	-	5.7	-	-
Myristicin	-	-	1.4	-	-

Antioxidant activity

The present result showed that essential oil of N.sativa exhibited high antioxidant activity as compared to the reference n-propyl gallate (Table 3), this may attributed to the presence of thymoquinone which is very useful in controlling the injurious effect of free radicals causing the induction and / or amplification of several pathologies (Woo et. al., 2011) Also it is the major active monoterpene derived from N. sativa, it has been the subject of many recent studies due to its pharmacological properties such as anti-inflammatory (Jyothi 2009), antidiabetic, neuroprotective, hepatoprotective and anti-arthritic (Saptha et. al., 2009). The result is in agreement with what was reported by Haron (2014) who represented the essential oil of N. sativa has the highest antioxidant activity (IC50 = 4.48 mg/mL). While this is contravened with what was reported by Khither (2018) who indicated less effective of thymoquinone as a scavenger of both DPPH and ABTS radicals.

A further possible explanation for the increased antioxidant activity of the oil might be as a result of the presence of p-cymene that has been suggested as an in vivo antioxidant compound due to its ability to reduce the formation of oxygen and nitrogen reactive species, also it is acting as a potential neuroprotective agent in the brain. Therefore, it could be involved in the treatment of oxidative stress related diseases (De Oliveira, 2015).

The differences between the antioxidant activity of whole oil and their isolated compounds as thymoquinone may be due to the synergistic effect which was reported by Azaizah et al. (2003) who stated that medicinal plants typically contain several different pharmacological active compounds that may act individually, additively or synergistically to improve health.

Table 3: Relative scavenging activity (RSA) of Nigella sativa sees oil.

Essential oil	%RSA ± SD
N sativa oil	91.30±0.017
n-Propyl gallate	92.83±0.006

CONCLUSION

The essential oil of N. sativa which is rich in thymoquinone and p-cymene possess significant antioxidant activity in vitro behavior. The results could provide useful data for utilization of the oil in pharmaceutical products. Further work should be made to isolate the active constituents in essential oil and clarifying their mode of action.

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