Phytochemical Analysis of Habbatus Sauda'

Azuin Mustafa[1], Hannis Fadzillah Mohsin[2]
& Ibtisam Abdul Wahab[3]*

[1,2,3]Department of Pharmacology & Chemistry, Faculty of Pharmacy,
Universiti Teknologi MARA Selangor, Puncak Alam Campus,
42300 Bandar Puncak Alam, Selangor Darul Ehsan

* Corresponding author
ibtisam@puncakalam.uitm.edu.my

ABSTRACT

This study aimed to highlight the Islamic prophetic medicinal herb called Habbatus Sauda' or black cumin. It is botanically known as *Nigella sativa* (plant family: Ranunculaceae). Historically, this herb is used for the treatment of a cold symptom, skin condition like eczema, cough, bronchitis, against indigestion, promote lactation, headaches, toothaches and to treat diarrhea. The medicinal use of this herb includes anti-diabetes, immunopotentiating, immunomodulating, presenting the analgesic effect, anti-microbes, anti-inflammatory agent, and antioxidant. The focus of this study is to review the phytochemicals of *Nigella sativa* from the scientific journals. From the literature, the extracts were analyzed in order to detect various potential compounds. It was anticipated that major constituents such as the phenolics, alkaloids, steroids, terpenoids and cardiac glycosides could be acquired from this miracle herb. In the methodology, the fractionation of a *Nigella* extract was carried out via liquid chromatographic technique. Finally, an aromatic aldehyde was suggested, following a preliminary spectroscopic analysis.

Keywords: cumin; Habbatus Sauda'; *Nigella*; phytochemistry; product.

1. Introductıon

Habbatus Sauda' is commonly known as the black cumin. It is scientifically referred as Nigella sativa (N. Sativa), which belongs to the family of Ranunculaceae. There are 20 species of annual herbs under the genus Nigella, but Nigella sativa is the most common species. Habbatus Sauda' is considered as a miracle herb due to its powerful healing capability. It is used in folk medicine since it contains more than hundreds of phytochemicals. From the literature, Habbatus Sauda' has

many different names. In Arabic, it is termed as *Habbah Sawda'* or *Habbat el Baraka* and translated as seeds of blessing. Meanwhile, in old Latin, it is known as Panacea, meaning cure all. In China, it is called as *Hak Jung Chou*, while in India it is referred as *Kalonji* (Aggarwal et al., 2008). In English, the herb is called as small fennel or black caraway, and *tsiyah daneh* in Persian.

Habbatus Sauda' has finely divided leaves and can attain heights up to 70 cm. It is grown in many countries, including Middle Eastern, Mediterranean region, Pakistan, South Europe, Turkey, Saudi Arabia, Syria, and India. It is harvested in a cool season, but it requires a frost-free growing because it is sensitive to frost. Therefore, its growth is limited in the Europe and high area. The herb can grow in the cold weather at the early stage of the growth, but during seed formation, the warm sunny weather is more desirable. This plant is acknowledged for its therapeutic use worldwide. The seeds are used for the therapy of different ailments and diseases. They are widely utilised in Middle Eastern countries and other parts of the world because the seeds show an extensive spectrum of pharmacological and biological activities, which include antihypertensive, diuretics, antidiabetic, analgesic and anticancer (Al-Jasass et al., 2012).

Habbatus Sauda' has a long history as an important herb. The record pertaining to black cumin is found in the oldest medical and religious text as a component of herbal medicine and culinary purposes. For example, in the first century, Greek physician named Dioscorides, recorded that the seeds were used to treat headaches, toothache, nasal congestion, and intestinal worms. According to one of the Prophetic hadith, Habbatus Sauda' is considered as one of the enormous forms of healing medicine among Muslims. Al-Bukhari mentioned about Abu Hurairah RA heard that Holy Prophet Muhammad SAW once said "Keep your treatment with Habbah-Al-Sawda', it can cure all illnesses except death" (Baasya, 2000). This special herb was also mentioned by Ibnu Sina in his greatest medical book *The Canon of Medicine*. He stated that Habbatus Sauda' stimulates body's energy and helps to recover from tiredness and fatigue. This study aimed to review the traditional use, its pharmacological activity and the phytoconstituent of Habbatus Sauda'. The advancement in biotechnological research on this herb is also discussed. These efforts lead to the manufacturing of Habbatus Sauda' products (Al-Saleh et al., 2006; Edris et al., 2016), available in community pharmacy.

2. Review on The Traditional Use and Pharmacological Activity of Habbatus Sauda'

Habbatus Sauda' has a wide variety of uses since the ancient times and it is regarded as an important remedy for many diseases. In the Southeast Asian and Middle Eastern countries, this seed has been used traditionally to cure diseases such as bronchitis, rheumatism, asthma and inflammatory-related disease. The seed is also used to fight against parasite infection, promote digestion and increase milk production in breastfeeding. The oil is useful in the treatment of cold symptom and skin condition such as eczema. Both seed and oil have been used in food as well as medicine for a long time in India and Arabian civilization. The seed is also used to improve digestion, flavouring, and produce warmth, especially for those people live in cold climate (Ansari & Satish, 2013).

The antimicrobial effect of Habbatus Sauda's extract and its constituent have been widely studied (Ishtiaq et al., 2013). The ether extract showed a strong antibacterial effect against gram-positive bacteria. Meanwhile, the methanol extract showed anti-plaque action by inhibiting *Streptococcus mutants.* An alcoholic extract of this seed showed an antimicrobial effect against *Micrococcus pyogenes var. aureus, Shigella dysenteriae, S. boydii, S. sonnei, Escherichia coli* and *Vibrio cholerae* (Gilani et al., 2004).

Habbatus Sauda' was stated to have a hypoglycemic effect by inhibiting the hepatic gluconeogenesis and can cause blood glucose to decrease. A study showed that elevated glucose level in alloxan-induced diabetic rabbits was decreased after two-months treatment with Habbatus Sauda's seed extracts when given orally (Meral et al., 2001). Another clinical study was done on human volunteers and showed that one gram of Habbatus Sauda's seeds taken orally twice daily can cause a significant decrease in blood glucose level after 2 weeks (Bamosa et al., 1997).

Habbatus Sauda' was reported to have an enhancement effect of the immune system, exhibit immunomodulating and interferon-like activities. This seed extract was found to inhibit cancer cells and endothelial cell progression *in vitro* (Medenica et al, 1997; Swamy & Tan, 2000). The essential oil also was tested against colon cancer and the result showed a significant effect of the antiproliferative effect, without damaging the other organ (Islam et al., 2004).

3. Review on The Natural Constituents of Habbatus Sauda'

The chemical investigation on Habbatus Sauda' seed was started ever since 1880 where the first report was published by Greenish (1880) in the presence of 4.1% ash and 37% oil in the seeds (Hussein El-Tahir et al., 2006). The seed contained numerous chemical compounds and yet, many active components have been isolated and identified from the seed. The most important active compounds are thymoquinone (TQ, Figure 1), *ρ*-cymene, carvacrol, *trans*-anethole, 4-terpineol, thymohydroquinone and longifoline (Ali & Blunden, 2003; Hussein El-Tahir et al., 2006). The chemical composition in Habbatus Sauda's seed was listed (Wajs et al., 2008; Rajsekhar & Kuldeep, 2011; Ahmad et al., 2013).

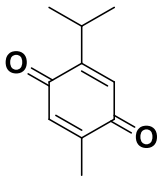

Figure 1: Thymoquinone (TQ), or 2-isopropyl-5-methyl-1,4-benzoquinone ($C_{10}H_{12}O_2$), the major component of Habbatus Sauda's essential oil.

Specific chemical studies were performed on the volatile oil since 1960's. These studies revealed various bioactive components that include TQ. Many reports mentioned on the pharmacological actions of Habbatus Sauda' is due to TQ. It is considered as a major component of Habbatus Sauda's essential oil. It was also tested against different treatments of arthritis and the result showed that TQ has effective treatment of arthritis in rats (Tekeoglu et. al., 2007). In addition, TQ is a potent anti-inflammatory agent, which used in a study that was carried out on a rat. This work involved intraperitoneal administration of TQ into the rats and it produced significant suppression of carrageenan-induced paw edema. The anti-inflammatory effect of TQ may be due to inhibition of cyclo-oxygenase (COX) and lipoxygenase enzymes. An analgesic effect of TQ is due to its ability to inhibit the enzyme prostaglandin COX. In another study, TQ was given orally to the rat model before giving alcohol. This study revealed that TQ protects the rats from alcohol-induced ulcers through an antioxidant mechanism, which involved an increase in superoxide dismutase availability and inhibition of reactive oxygen radicals (Hussein El-Tahir et al., 2006).

TQ also showed immunomudulatory effects by increasing natural killer cell-mediated and T cell immune response. Administration of TQ in rats through i.v. shows a decrease in heart rate and arterial blood pressure. In another study, pre-treatment on the rat hepatocytes cell *in vitro* showed that cells were protected from hepatotoxicity of t-butyl hydroperoxide. TQ also showed an antibacterial effect against *Mycobacterium tuberculosis, Staphylococcus aureus* and against fungi such as *Trichophyton mentagrophytes* and *Trichophyton rubrum.* This inhibitory effect is due to inhibition of protein and RNA synthesis (Hussein El-Tahir et al., 2006).

Habbatus Sauda's seeds contain both essential and fixed oils. However, the major composition was fixed oil while essential oil was 1.4% of the seed weight. The fixed oil is rich in unsaturated fatty acids, compared to saturated fatty acids. The fatty acids contained linolenic acid (omega-3) and linoleic acid (omega-6) which are not produced in the body, but important to sustain health. Minerals such as iron, calcium and phosphorus were also found in the Habbatus Sauda's seeds. These minerals are required by the human body in a small amount, as a cofactor in various enzyme functions (Nasaruddin, 2006). In addition, there are two types of alkaloids, which were found in this seed. The alkaloids include the isoquinolines, which were represented by nigellicimine and nigellicimin-*N*-oxide, whereas pyrazol includes nigellicine and nigellidine (Ahmad et al., 2013).

4. Methodology

The natural constituents and phytochemical analysis of Habbatus Sauda' were investigated. The plant material includes the herbal seeds, which were purchased from retail. The solvents include chloroform ($CHCl_3$), acetone and methanol (MeOH) (analytical reagent grade) that were purchased from Merck (Germany). For the maceration technique, 16.7 g of the seeds were soaked in the 30 ml of acetone in an amber bottle for five days, at room temperature. The extract was filtered and evaporated using a rotary evaporator to remove the excess solvent. The crude extract was transferred into a clean, dried amber vial.

The screening of natural compounds in the extract was performed using thin layer chromatography (TLC). The extract was separated using commercially produced analytical silica gel chromatographic plates (Merck, 60G F_{254}, 0.25 mm). The preparative thin layer chromatography

(PTLC) was used to purify the target compound. The solvent system was chloroform and methanol (95:5). The PTLC plates were put in the development tank.

After PTLC were developed, the bands were visualized under both short and long wavelength ultraviolet (UV) light. Bosch heat gun was used to heat the plate (250 ^{o}C) after spraying with the staining reagent (e.g. sulphuric anisaldehyde or ferric chloride) at the edge of the plate, to compare the colour of the PTLC band with the TLC spot. The target band (Rf = 0.5) was scrapped out from the plate. The silica from the band was filtered using the same solvent used during extraction (acetone). Then, it was dried using a rotatory evaporator to remove the excess solvent. The weight of the compound of interest was recorded (0.05 g). The isolated compound was subjected to the proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy (Bruker 500 MHz, CD_3COCD_3) to identify the hydrogen framework of the compound.

5. Results and Discussion on The Phytochemical Analysis of Habbatus Sauda'

Figure 2 shows the ^{1}H-NMR spectrum for the isolated compound of the acetone extract. The compound was dissolved in the deuterated acetone (CD_3COCD_3) as the NMR solvent and its chemical shift was at the δ_H = 2.1 ppm. Based on **Figure 2**, there were signals in the aliphatic regions, assignable to the fatty acid components (Maulidiani et al., 2015) of Habbatus Sauda's seed extract [δ_H = 5.37 ppm (m), 2.23 ppm (t, J = 7.5 Hz), 2.09 ppm (m), 1.87 ppm (m), 1.20 ppm (m), 0.90 ppm (s)]. Furthermore, the aromatic regions also display some peaks, however, they were too small since the sample concentration was low. Two peaks (two doublets) were detected at the region δ_H = 7 - 8 ppm. Both signals were doublets with a characteristic of an *ortho* coupling, J = 8.5 Hz. This indicates for four protons at the aromatic ring. An aldehyde group attached to an aromatic ring was also observed at the chemical shift of δ_H = 9.9 ppm. The hydroxyl group could be observed in the ^{1}H-NMR spectrum (δ_H = 4.93 ppm). Thus, the isolated compound from the acetone extract can be suggested as 4-hydroxybenzaldehyde or *para*-hydroxybenzaldehyde (Figure 3, Amina et al., 2013).

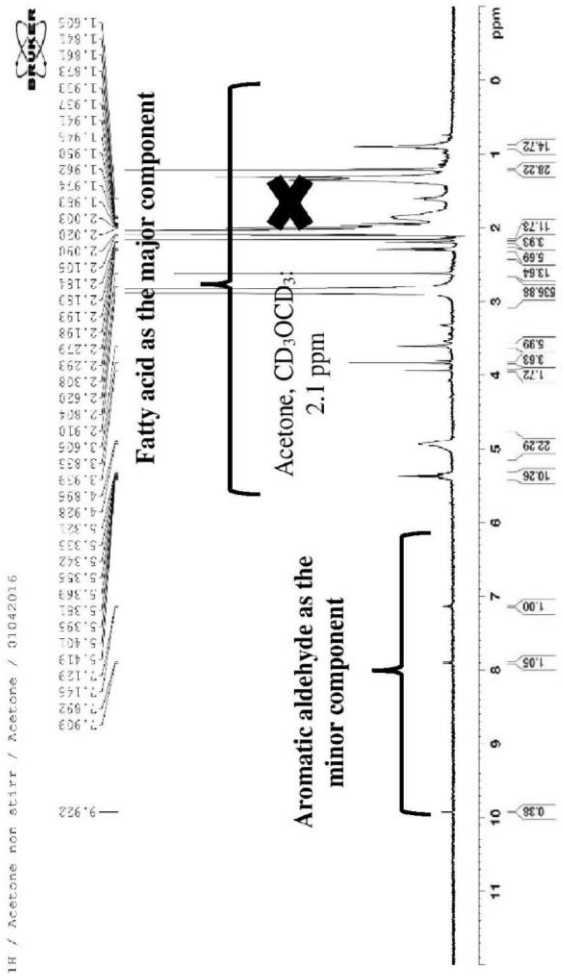

Figure 2: The ^{1}H-NMR spectrum (500 MHz, CD_3COCD_3) of Habbatus Sauda's acetone extract.

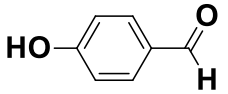

Figure 3: *Para*-hydroxybenzaldehyde is the aromatic aldehyde from Habbatus Sauda'.

This phenolic is detectable once it is isolated in its solid, residue form, when compared to constituents in other prophetic medicinal plant, such as pomegranate (Malešević et al., 2014). This is due to its existence as the insoluble-bound phenolic acids. Therefore, the high quantity of this compound is measurable via automated technique such as high-performance liquid chromatography.

6. Conclusion

The biotechnology research would lead to the production of Habbatus Sauda' as the health products for anti-diabetes, anti-microbes, anti-inflammatory agent, and antioxidant. On another note, the photodimerization of Habbatus Sauda's components could possibly occur, since most of the phytochemicals are thermolabile, photo reactive and unstable. Thus, certain precautions should be taken for future study.

7. Acknowledgements

The authors wish to thank Mr. Mohd Syukri Baharudin, Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA Selangor, Puncak Alam campus. Azuin Mustafa would also like to acknowledge the University for the support during undergraduate research.

8. References

Aggarwal, B. B., Kunnumakkara, A. B., Harikumar, K. B., Tharakan, S. T., Sung, B. & Anand, P. (2008). Potential of spice-derived phytochemicals for cancer prevention. *Planta Medica, 74*(13), 1560 - 1569.

Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A., Damanhouri, Z. A. & Anwar, F. (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine*, 3(5), 337-352.

Al-Jasass, F. M. & Al-Jasser, M. S. (2012). Chemical composition and fatty acid content of some spices and herbs under Saudi Arabia conditions. *The Scientific World Journal*, 859-892. http://doi.org/10.1100/2012/859892.

Al-Saleh, I. A., Billedo, G. & El-Doush, I. I. (2006). Levels of selenium, dl-αtocopherol, dl-γ-tocopherol, all-trans-retinol, thymoquinone and thymol in different brands of *Nigella sativa* seeds. *Journal of Food Composition and Analysis*, *19*(2-3), 167-175.

Ali, B. H. & Blunden, G. (2003). Pharmacological and toxicological properties of *Nigella sativa*. *Phytotherapy Research: PTR*, *17*(4), 299–305.

Amina, B. & Rachida, A. (2013). Molecular composition and antibacterial effect of essential oil of *Nigella sativa*. *African Journal of Biotechnology*, 12(20), 3006-3012.

Ansari, Z. & Satish, T. (2013). Traditional uses of *Nigella sativa*, in Malegaon region of Nashik - A Review. *Int. J. Pure App. Biosci*., 1(2), 19-23.

Baasya, H. S. (2000). Rahsia Perubatan Habbatus Sauda: Penyembuhan Dengan Habbatus Sauda' Antara Mukjizat Kenabian & Perubatan Moden. Al-Hidayah Publishers, Kuala Lumpur.

Bamosa, A. O., Ali, B. A. & Sowayan, S. A. (1997). Effect of oral ingestion *Nigella sativa* seeds on some blood parameters. *Saudi Pharmaceutical Journal*, 5, 126-129.

Edris, A. E., Kalemba, D., Adamiec, J. & Piątkowski, M. (2016). Microencapsulation of *Nigella sativa* oleoresin by spray drying for food and nutraceutical applications, *Food Chemistry*, 204, 326-333.

Gilani, A. H., Qaiser, J. & Khan, M. A. U. (2004). A review of medicinal uses and pharmacological activities of *Nigella sativa. Pakistan Journal of Biological Sciences*, 7(4), 441-451.

Greenish, H. G. (1880). Contribution to the Chemistry of *Nigella sativa*. *Pharmac J Trans*, 10, 909-911.

Hussein El-Tahir, K. E.-D. & Bakeet, D. M. (2006). The black seed (*Nigella sativa* Linnaeus)-a mine for multi cures: A plea for urgent clinical evaluation of its volatile oil. *Journal of Taibah University Medical Sciences*, 1, 1-19.

Ishtiaq, S., Ashraf, M., Hayat, M. Q. & Asrar. M. (2013). Phytochemical Analysis of *Nigella sativa* and its Antibacterial Activity against Clinical Isolates Identified by Ribotyping. *International Journal of Agriculture & Biology*, 15(6), 1151-1156.

Islam, N., Begum, P., Ahsan, T., Huque, S. & Ahsan, M. (2004). Immunosuppressive and cytotoxic properties of *Nigella sativa*. *Phytotherapy Research*, *18*(5), 395-398.

Malešević, V. K., Vaštag, Z., Popović, L., Popović, S. & Peričin-Starčevič, I. (2014). Characterisation of black cumin, pomegranate and flaxseed meals as sources of phenolic acids. *International Journal of Food Science and Technology*, 49, 210–216.

Maulidiani, M., Sheikh, B. Y., Mediani, A., Sze Wei, L., Ismail, I. S., Abas, F., Lajis, N. (2015). Differentiation of *Nigella sativa* seeds from four different origins and their bioactivity correlations based on NMR-metabolomics approach. *Phytochemistry Letters*, 13, 308 – 318.

Medenica, R., Janssens, J., Tarasenko, A., Lazovic, G., Corbitt, W., Powell, D. & Mujovic, V. (1997). Anti-angiogenic activity of *Nigella sativa* plant extracts in cancer therapy. In *Proc. Annual Meeting Am. Assoc. Cancer Res* (Vol. 38, p. A1377).

Meral, I., Yener, Z., Kahraman, T. & Mert, N. (2001). Effect of *Nigella sativa* on Glucose Concentration, Lipid Peroxidation, Anti-Oxidant Defence System and Liver Damage in Experimentally-Induced Diabetic Rabbits. *Journal of Veterinary Medicine Series A*, *48*(10), 593–599.

Nasaruddin, N. (2006). Extraction of pharmacologically active thymoquinone in *Nigella sativa* L. Bachelor of Technology (Chemical

Engineering and Natural Resource) thesis, Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang.

Rajsekhar, S. & Kuldeep, B. (2011). Pharmacognosy and pharmacology of *Nigella sativa* - A review. *Int Res J Pharm*, *2*(11), 36-9.

Swamy, S. M. K. & Tan, B. K. H. (2000). Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. *Journal of Ethnopharmacology*, *70*(1), 1-7.

Tekeoglu, I., Dogan, A., Ediz, L., Budancamanak, M. & Demirel, A. (2007). Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. *Phytotherapy Research*, *21*(9), 895–897.

Wajs, A., Bonikowski, R. & Kalemba, D. (2008). The composition of essential oil from seeds of *Nigella sativa* L. cultivated in Poland. *Flavour Fragr. J.*, 23, 126-132.