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Research paper

Comparative phytochemical analysis and antioxidant activities of two herbal powdered drugs used in the treatment of uterine fibroids

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Abstract*: Nigella sativa* Linn (Family: Ranunculaceae) and *Anethum sowa* Linn (Family: Apiaceae) are commonly used herbs in Ayurveda medicine from ancient times highlighting its' use in the treatment of uterine fibroids throughout the island in Sri Lanka. Uterine fibroids are the most common benign tumor of the female reproductive tract and occur in about 25% of all women of reproductive age and up to 30 – 40% of women over age 40. *N. sativa* and *A. sowa* are used in Sri Lanka for the treatment of uterine fibroids and other common gynecological disorders from ancient days. The phytochemical analysis was carried out by Thin Layer Chromatography and Gas Chromatography analysis. Besides, DPPH scavenging ability, total phenol, and total flavonoid contents were compared. The TLC fingerprints of *N. sativa* and *A. sowa* were prominent and can be used as standards for the authentication of these plants. The DPPH free radical scavenging activity was significantly higher in *A. sowa* than that of *N. sativa*. Moreover, total polyphenol and flavonoid contents were significantly higher in *A. sowa* than that of *N. sativa. A. sowa* showed comparatively more antioxidant activities than that of *N. sativa* and may use for treating uterine fibroids due to the presence of higher polyphenols and flavonoid types of compounds.

Keywords: Phytochemical Analysis, Antioxidant Activities, *Nigella sativa* L., *Anethum sowa* L.

INTRODUCTION

Uterine fibroid is seen during the reproductive life of a female irrespective of the age, which may result in various menstrual problems such as dysmenorrhea, menorrhagia, and irregular periods, by disturbing anatomical as well as physiological integrity. Medical management of this problem is popular based on the Ayurveda system of medicine. Some of these Ayurveda medicines are used single ingredient medicinal plant materials as drugs including *Saraca asoca*, *Emblica officinalis*, (Gautam, et.

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al. 2017), *Asparagus racemosus* (Ramchandani, 2019), *Withania somnifera* (Nair & Praveen 2019). Among these single ingredients plant materials, *Nigella sativa* Linn and *Anethum sowa* Linn (Figure 1) are two commonly used herbs in Sri Lankan Ayurveda medicine for the treatment of uterine fibroids and other common gynecological disorders from ancient days.

Figure 1. (a) seeds of *Nigella sativa* and (b) seeds of *Anethum sowa*

N. sativa is indicated to having the ability to cleanse the uterus, tumors in the abdominal region (Gulma) (Sitaram, 2014). Its effectiveness on ovarian follicular growth, breast milk production was also proven (Akour et al., 2016). Also, the therapeutic potential on dysmenorrhea, amenorrhoea, follicular maturity, galactogenesis of *A. sowa* was mentioned in Ayurveda's authentic text of KashyapaSamhita (Gandhi et al., 2016) and indicated both these herbs provide similar benefits. Clinical utilities of *N. sativa* and *A. sowa* on gynecological conditions, polycystic ovarian syndrome were scientifically evaluated (Shirani et al. 2016; Katakdound, 2017; Kumarapeli et al., 2018, Jazani, et al., 2019). In Sri Lanka, dried seed powder of *N. sativa* and *A. sowa* included as Ayurveda single ingredients drugs (Ayurveda Pharmacopoeia, 1974). These plant materials are included in the drug list of many government Ayurveda hospitals and dispensaries including the National Ayurveda Teaching Hospital in Sri Lanka. These two drugs are widely available at the local market by the names; Satakuppa (*A. sowa*) and Kaluduru (*N. sativa*) as dried seeds. Apart from the medicinal purpose, these two herbs have been used for culinary purposes mainly as seed spices since ancient times. Both these seeds spices influence various systems of the body including the reproductive system resulting in diverse metabolic and physiologic actions through natural phytochemicals (Lal and Meena, 2018). The purpose of this present study was to conduct a comparative study on the above two herbs which show many similarities in clinically. Therefore, the phytochemical analysis was carried out by Thin Layer Chromatography and Gas Chromatography analysis. Besides, DPPH scavenging ability, total phenol, and total flavonoid contents were compared.

MATERIAL AND METHODS

Collection of seeds: N. sativa seeds (500 g) and *A. sowa* seeds (500 g) were purchased from an Ayurvedic drug supplier at Colombo district, Sri Lanka. The plant materials were identified, washed, dried and packed separately.

Thin Layer Chromatography (TLC) fingerprint profiles of Nigella sativa and Anethum sowa: Each (50 g) seed sample was crushed and taken into an around bottom flask containing 150 ml of dichloromethane. Then refluxed for 1 h, filtered and the filtrate was subjected to concentrate (up to 10 ml) using a rotary evaporator. The TLC plate was prepared according to the standard laboratory practice. The solvent system is used containing dichloromethane, cyclohexane, and methanol in a ratio of 2:2:0.1 (v/v). Then the developed TLC plate was placed under UV 254 nm and 366 nm. The

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spots were marked and the plate was sprayed with Vanillin sulphuric acid, heated on a hot plate at 100 °C for 5 minutes. Subsequently, Rf values were calculated.

Sample preparation: Each (50 g) seed sample was crushed and taken into a round bottom flask containing 150 ml water, refluxed for 1 h, filtered and the filtrate was subjected to concentrate using a rotary evaporator. Then each extract was freeze dried at -40 °C for further study.

*DPPH free radical scavenging activity:*The DPPH free radical scavenging activity was determined according to the method of Blois (1958) with some modifications. Freeze-dried extracts were dissolved in methanol and tested at the assay concentrations of 10, 50, and 200 µg/ml. Extracts (50 µl) were incubated with DPPH solution (200 µg/ml, 60 µl) and methanol (90 µl)in 96 well microplates for 10 min in dark at room temperature. Absorbance was recorded at λ=517 nm. The DPPH free radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = [(\text{Abs. control} - \text{Abs. sample}) / \text{Abs. control}] \times 100.$$

control: without the plant extract
sample: with the plant extract

Quantification of total polyphenol content and total flavonoid content: The flavonoid content was calculated using the calibration curve of quercetin and results were expressed in terms of mg quercetin equivalent/g of extract (Meda et al., 2005). Total polyphenol content was calculated using the calibration curve of gallic acid and the results were expressed in terms of mg gallic acid equivalents/g of extract (Singleton et al., 1999).

Extraction of essential Oil: Each *N. sativa* seeds (200 g) and *A. sowa* seeds (200 g) were added separately to a round bottom flask, fixed with a Clevenger-type apparatus and essential oil was obtained separately by hydrodistillation. The extracted volatile oil was dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C until analysis. The yield of the oil was calculated based on the dry weight of seed samples.

Analysis of essential oil: The essential oil was analyzed by using Shimadzu 2010 gas chromatograph equipped with FID using capillary column Rtx –wax, gas: Argon (1ml/min), Temperature program (60oC -225oC at 5oC/min), Injector temperature (230 oC), and Detector temperature (240 oC).

RESULTS AND DISCUSSION

TLC fingerprint profiles of *N. sativa* and *A. sowa* were observed under both 254 nm and 366 nm (Figure 2). Compared to the TLC fingerprint profile of *N. sativa* more spots were present in the TLC fingerprint profile of *A. sowa*. Furthermore, TLC fingerprints of *N. sativa* and *A. sowa* can be used as standards for the authentication of these plants. DPPH is characterized as a stable free radical and the effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Huang et al., 2005). In the DPPH assay, the violet color DPPH solution is reduced to a yellow color product, diphenylpicryl hydrazine, by the addition of the extract in a concentration-dependent manner. Radical scavenging activities of plants are very important to prevent the different diseases, including cancer, diabetes, inflammation cardiovascular disease, Alzheimer's disease, Down's syndrome, Parkinson's

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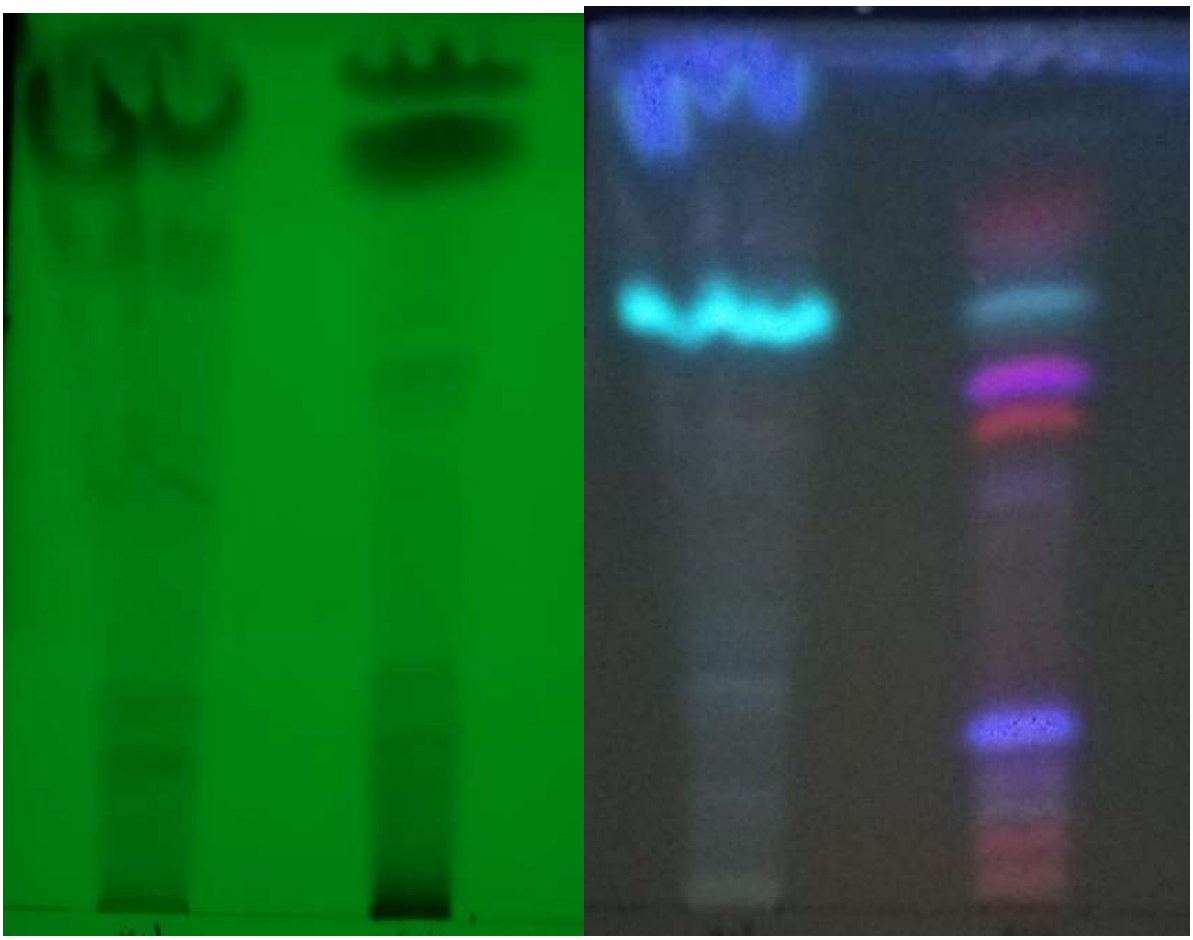
disease, etc (Matteo and Esposito, 2003; Willcox et al., 2004). DPPH radical scavenging activity was significantly higher in *A. sowa* than that of *N. sativa* (Table 1).

Free radical scavenging activity was not demonstrated in low concentrations of *Nigella sativa* seeds. Phenolic compounds and flavonoids are reported to have antioxidant and free-radical scavenging activity (Huan-xiaet al.2014; Kainama, 2020). Total polyphenol and flavonoid contents were significantly higher in *A. sowa* than that of *N. sativa* (Table 2). Scientific investigations have shown that antioxidants play a major role in the inhibition of uterine fibroids (Chen et al., 2019). Therefore, the antioxidant properties of both *A. sowa* and *N. sativa* may play a major role in gynecology conditions and polycystic ovarian syndrome. The essential oil composition of *A. sowa* and *N. sativa* is given in Table 3 and Table 4 respectively.



Nigella sativa/*Anethum sowa* at 254 nm | *Nigella sativa*/*Anethum sowa* at 366 nm

Figure 2. Thin Layer Fingerprints of *Nigella sativa* and *Anethum sowa*at(a) 254 nm and (b) 366 nm

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Table 1. Antioxidant activity of hot ethanol extracts of *Nigella sativa* and *Anethum sowa* seeds

Extract	Test concentration (µg/mL)	Free radical scavenging activity (%)
Nigella sativa	10	NA
	50	NA
	200	6.25±0.34
Anethum sowa	10	3.82±0.27
	50	15.64±0.70
	200	49.23±3.04

Results are given as mean ± SEM; n=3, NA= No activity

Table 2. Total polyphenol content (TPC) and Total flavonoid contents (TFC) hot ethanol extracts of *Nigella sativa*) and *Anethum sowa* seeds

Extract	TPC (mg GAE/g)	TFC (mg QE/g)
Nigella sativa	3.12±0.38	0.29±0.02
Anethum sowa	38.95±0.45*	7.02±0.07*

Results are given as mean ± SEM; n=4; GAE=Gallic acid equivalents; QE=Quercetin equivalents

Table 3. Chemical composition of essential oil of *Anethum sowa*

Compound	Retention time (min)	Area (%)
D-Limonene	4.60	20.63
gamma-Terpinene	5.34	0.69
p-Cymene	5.79	1.33
Styrene,3,4-dimethyl	9.12	0.14
cis-Dihydrocarvone	13.03	2.39
trans-Dihydrocarvone	13.45	4.44
Estragole	14.29	0.16
Carvone	15.80	31.47
1,6-Dihydrocarveol	16.20	0.14
Thymol	24.73	3.39

The principle compounds detected in *A. sowa* seed oil include Carvone (31.47%), D-Limonene (20.63%), trans-Dihydrocarvone (4.44%), Thymol (3.39%), cis-Dihydrocarvone (2.39%), and p-Cymene (1.33%). However, the principle compounds detected in *N. sativa* seed oil include p-Cymene (15.06%), cis-Dihydrocarvone (5.57%), 1,6-Dihydrocarveol (5.14%), Camphor (3.18%), Carvone (2.78%), Terpinen-4-ol (1.72%), and Carene (1.42%). Our findings were in the range of the published research data of *A.sowa* and *N. sativa* chemical profiles (Venkatachallam et al., 2010; Singh, 2012)

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CONCLUSION

In conclusion our finding suggested that total polyphenol and flavonoid contents were significantly higher in *A. sowa* than that of *N. sativa.* Further free radical scavenging activity was not demonstrated in low concentrations of *N. sativa* seeds. Antioxidant properties of both *A. sowa* and *N. sativa* may play a major role in gynecology conditions including uterine fibroids.

Table 4. Chemical composition of essential oil of *Nigella sativa*

Compound	Retention time (min)	Area (%)
3-Carene	2.67	1.42
3-Carene	3.92	0.53
D-limonene	4.61	0.70
Terpinene	5.35	0.69
p-cymene	5.79	15.06
Cumin	6.00	0.59
cis-Thujopsene	9.74	0.40
Camphor	10.99	3.18
Caryophyllene	12.61	0.60
Terpinen-4-ol	12.93	1.72
cis-Dihydrocarvone	13.03	5.57
Carvone	15.79	2.78
1,6-Dihydrocarveol	16.21	5.14

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CONFLICTS OF INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Akour A, Kasabri V, Afifi FU, Bulatova N (2016) The use of medicinal herbs in gynecological and pregnancy-related disorders by Jordanian women: a review of folkloric practice vs. evidence-based pharmacology, Pharmaceutical Biology, 54(9):1901-1918.

Chen HY, Lin PH, Shih YH, Wang KL, Hong YH, Shieh TM, Huang TC, Hsia SM (2019) Natural antioxidant resveratrol suppresses uterine fibroid cell growth and extracellular matrix formation in vitro and in vivo. Antioxidants. 8(4):99.

Department of Ayurveda. (1994) Ayurveda Aushada Samgraha, Part Two. Second Edition, Volume I, Colombo, Sri Lanka.

Gandhi AJ, Tavhare S, Bedarkar P, Patgiri BJ (2016) Herbs indicated in the management of infertility w.s.r.Vandyatwa and Klaibya: a review. (5) 6,www.wjpps.com

Gautam P, Bairwa O, Dwivedi D, Kumar S, Singh SK (2017) A progressive step towards classicle uses of medicinal plants in gynecological disorders. J Ayurveda Integrated Med Sci 3: 225-236.

Hewageegana HGSP, Arawwawala LDAM (2018) Development of Thin Layer fingerprint profiles for Leptadenia reticulate (Rez) Wight and Arn and its substitutes. 3 (5):1-4.

ASIAN JOURNAL OF PHARMACOGNOSY *Research paper*
Asian J. Pharmacogn 4(3): 30-36

eISSN-0128-1119

Huang D, Ou B, Prior RI. (2005) The chemistry behind antioxidant capacity assays. J Agric Food Chem. 53:1841–56.

Huan-xia Z, Hai-sheng Z, Shu-fang Y (2014) Phenolic compounds and its antioxidant activities in ethanolic extracts from seven cultivars of Chinese jujube, Food Science and Human Wellness. 3:189–194.

Jaiswal S, Yadav DS, Mishra MK, Gupta AK (2016) Detection of adulteration in spices through chemical method and Thin Layer Chromatography for forensic consideration. International Journal of Development Research. 6 (8): 8824 - 8827.

Jazani M, Azgomi AND, Azgomi HND. et al. (2019) A comprehensive review of clinical studies with herbal medicine on polycystic ovary syndrome (PCOS). DARU J Pharm Sci 27:863–877

Kainama H, Fatmawati S, Santoso M. et al. (2020) The Relationship of Free Radical Scavenging and Total Phenolic and Flavonoid Contents of Garcinialasoar PAM. Pharm Chem J 53:1151–1157.

Karunaratne TDN, Sugataratana K, Ariyawansa HAS, Silva HAD, Samarasingha K, Arawwawala LDAM (2015) Standardization of SarasvathaChoorna: Used as a remedy for dementia. American Journal of Clinical and Experimental Medicine. 3:288-292.

Katakdound SD (2017) Ayurvedic Management of Recurrent Abortions due to Uterine Fibroid. Ancient Science of Life. 36 ; 159 - 162.

Kulathunga RDH, Gunarathna EDTP, Jayawardhana NDN, De Silava RHSK, Ranasinghe RLDS, Faisulhaq MH, Samarasinghe UKA, Arawwawala LDAM (2019). A comparative analytical study on two types of Sharibadi decoctions: An Ayurveda preparation. Journal of Complementary and Alternative Medical Research. 8(3):1-8.

Kumarapeli M, Karunagoda KPKR, Perera PK (2018) A randomized clinical trial to evaluate the efficacy of satapushpa-shatavari powdered drug with satapushpa-shatavarigrita for the management of polycystic ovary syndrome (PCOS). Int J Pharm Sci Res. 9(6): 2494-99.

Lal G, Meena SS (2018) Medicinal and Therapeutic Potential of Seed Spices. Biomedical Journal of Science & Technical Research 5(4)

Majid S, Sheida S, Mahnaz Y (2016) A systematic review of Iranian medicinal plants effective on female infertility. Journal of Global Pharma Technology, 10 (8):44-49.

Matteo D, Esposito E (2003) Biochemical and therapeutic effects of the antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, Current Drug Targets – CNS & Neurological Disorders.(2) 2;95–107.

Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG, (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honeys as well as their radical scavenging activity. Food Chem.91:571–577.

Nair A, Praveen N (2019) Biochemical and phytochemical variations during the growth phase of Withaniasomnifera (L.). Journal of Pharmacognosy and Phytochemistry. 8(3): 1930-1937 phytojournal.com

Nupearachchi CN, Perera VPS, Samarasingha, KA, Arawwawala, LDAM (2019) Secondary metabolic analysis of the pith of selected local banana varieties as a constituents in bio-batteries. Research Journal of Chemical Sciences. 9(4): 46 -49.

Ramchandani P (2019) A clinical study on the effect of Shatavarichurna and ShatavariTailaUttarabasti in Atyartava. International Journal of Green Pharmacy 12(4): 937-945.

Sharma V, Chaudhary A K (2015) Ayurvedic pharmacology and herbal medicine. International Journal of Green Pharmacy. 9 (4): 190-198

Singh S (2012) Chemical Constituents of Essential Oil from Anethum Sowa Kurz. Seed Journal of Chemical and Pharmaceutical Research, 4(9):4156-4160

Singleton VL, Orthofer R, Lamuela-Raventos, RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods in Enzymology. 299: 152-178.

Sitaram B (Ed.). (2014) BhavaPrakasha, Vol.IIMadyamaKhanda, Chapter 3, verse 44, Chaukambhaorientalia, India ; 388

Venkatachallam SKT ,Pattekhan,H & Divakar S, Kadimi JUS (2010) Chemical composition of Nigella sativa L. seed extracts obtained by supercritical carbon dioxide. Food Sci Technol 47(6):598–605

Verbitski SM, Gourdin GT, Ikenouye LM, McChesney J.D., Hildreth, J (2008) Detection of Actaearacemosa adulteration by Thin Layer Chromatography and combined Thin Layer Chromatography bioluminescence. Journal of AOAC International. 91(2): 268 – 275.

Willcox JK, Ash SL, Catignani GL (2004) Antioxidants and prevention of chronic disease, Critical Reviews in Food Science and Nutrition. 44 (4): 275–295.