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Phyto-chemical Evaluation of Aqueous, Methanolic and Hydro-Ethanolic Extracts of *Allium sativum* Bulbs and *Nigella sativa* Seeds

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ABSTRACT

Anthelmintic resistance is a global problem and researchers are attempting to find effective alternatives. One of these solutions is to use plant extracts and herbal-based products that have been shown to have anthelmintic properties. Because different herbs create a wide range of pharmacological activity, it is vital to understand their basic chemical makeup in order to correlate clinical consequences. Hence, the present study was carried out to quantitatively

assess the phytochemistry of aqueous, methanolic and hydro-ethanolic extracts of *Allium sativum* and *Nigella sativa*. The physical properties of *A. sativum* were yellowish to dark brown and solid, whereas *N. sativa*, dark black to brown and semi solid. The phyto-chemical study of both extract of *A. sativum* and *N. sativa* exhibited positive for constituents like alkaloids, glycosides, flavonoids, tannins, saponins, sterols and fixed oils and negative for proteins and anthraquinones. However, aqueous extracts of *A. sativum* does not contain tannins or fixed oils but *N. sativa* does. Nonetheless, variations were observed in the presence or absence of the phyto-chemical compounds between the extracts individually.

Keywords *Allium sativum*, *Nigella sativa*, Phyto-chemical analysis, Methanolic and Hydroethanolic.

INTRODUCTION

The discovery of medicinal herbs has always been vital to humanity since ancient times. The bioprospecting efforts are on-going to find new plant extracts and their derivatives that can overcome anthelmintic resistance (Cragg and Newman 2013). Bioactive compounds and their secondary metabolites found in plants may work to eliminate parasitic worms when consumed by the host and are currently being exploited for the development of miracle drugs in both traditional medicines and other areas of science (Dias *et al.* 2012). Every sickness has a remedy in the plant kingdom (Mintah *et al.* 2019). *A. sativum* (Garlic) plant is a member of the Liliaceae family and has antibacterial, antifungal, antiviral, anthelmintic, immune-stimulating, hepatoprotective, antioxidant and anticancer activities (Alam *et al.* 2016). As a bulb, garlic has many cloves that are grouped together and enclosed in whitish layers (Neeraj *et al.* 2014). When fresh garlic bulbs are chopped or crushed, the chemical alliin is transformed into the active ingredient allicin, which adds to its medicinal effects and aroma. Nonetheless, the produced allicin is unstable and is converted into a variety of active metabolites, including ajoene, allyl sulphides and diallyl sulphides (Borlinghaus *et al.* 2014). *N. sativa* is a Ranunculaceae family plant that has become one of mankind's most cherished medicinal plants. Besides black seed, black cumin, black caraway, roman coriander, kalonji, and fennel flower, there are also several other names by which it is known in Asian and European nations (Thippeswamy and Naidu 2005). The Arabs refer to it as "healing medicine". Additionally, this botanical wonder is shown to have anti-thyroid, anti-cancer, analgesic, antioxidant, bronchodilator, anti-asthma, spasmolytic and anti-inflammatory activities (Ahmad *et al.* 2013). Thymoquinone is one of the important phytonutrients of *Nigella sativa,* responsible for its potential pharmacological properties. Additionally, it contains thymol, carvacrol, dithymoquinone, thymohydroquinone, nigellicine, nigellidine, nigellimine-N-oxide and alpha-hederin compounds for which the pharmaco-therapeutic properties must be investigated (Zafar *et al.* 2016). The proper understanding of plants phytochemistry is imperative for developing novel therapeutic agents against major diseases (Egbuna *et al*. 2018). Therefore, the present study was carried out to assess the phyto-chemicals of *A. sativum* and *N. sativa* employing qualitative techniques and a comparative analysis was performed between the plant extracts.

MATERIALS AND METHODS

Collection and processing of plant material

Garlic cloves of medium to large size and *N. sativa* (Kalonji) seeds were selected and purchased from the nearby markets in Jabalpur. These herbs were manually cleaned of coarse impurities. The garlic cloves were initially peeled off and chopped into small pieces in order to trigger the bioactive substance present in it. The chopped garlic cloves were air dried at a well-ventilated place in laboratory for about four days. Further drying was done by transferring them into incubator at a precise temp of 27^{o}C for two days, as they are heat labile. The kalonji seeds were dried for about two days in incubator at temp of 27^{o}C. After ensuring that the herbs were thoroughly dried, they were crushed and ground in a mixer grinder, yielding crude powder forms. These powders were stored in air-sealed containers separately and used for preparation of aqueous, methanolic and hydro-ethanolic extracts.

Preparation of aqueous extract of *A. sativum* : One hundred grams of garlic powder was weighed

and then poured into a 1000 ml beaker with 600 ml of distilled water and stirred continuously for about 4 hr to ensure complete mixing. Then gentle mixing was done for 3-4 times at hourly intervals and left undisturbed for 24 hr at room temperature. Filtration was performed in a conical flask using Whatmann filter paper no. 4 and muslin cloth and the filtrate was transferred to Petri plates and placed in an incubator at 28ºC for evaporation. Room temperature was preferred in the incubator as excess temperature may act as constraint owing to the heat labile nature of active principle. The final extract obtained was taken out of the plates, the weight was measured (Kanojiya *et al.* 2015).

Preparation of aqueous extract of *N. sativa* : Fifty grams of kalonji powder was soaked in 400 ml distilled water and stirred at hourly intervals for 2-3 times initially followed by 12 hrs of undisturbed activity at room temperature. Filtration was carried out using whatmann filter paper no.4 in a conical flask and the obtained filtrate was concentrated in incubator at 28°C-30°C. The oil present after evaporation was separated carefully and final extract was scraped out from Petri plates and then weighed (Bendigeri *et al.* 2019).

Preparation of methanolic extract of *A. sativum* : One hundred grams of garlic powder was taken into a beaker with 400 ml methanol and tightly sealed with aluminium foil. It was mixed gently and then left undisturbed for 24 hours as described by Moazeni and Nazar (2010). It was stirred again and filtered using whatmann filter paper no.4. Concentration of extract was done by transferring the filtrate into incubator and the obtained final extract was scraped and weighed.

Preparation of methanolic extract of *N. sativa* : Fifty grams of kalonji powder was soaked in 500 ml of absolute methanol for 8 hrs by sealing the beaker with aluminium foil. Filtration was done and the filtrate was concentrated in incubator. The extract was scraped gently and weight measurement is done (Anjum *et al.* 2015).

Preparation of hydro-ethanolic extract of *A. sativum* and *N. sativa* : One hundred grams of each powder was added to the mixture solvent of 90 ml distilled water and 210 ml of ethanol and sealed with aluminium foil. Gentle stirring was done at hourly intervals initially and left undisturbed for 48 hrs at room temp. Filtration was done and the obtained filtrate was concentrated in incubator at 30°C. Final extracts obtained were scraped gently and weighed (Garcia *et al.* 2018).

Preservation of extracts

The extracts prepared were transferred to sterile airtight containers, labelled and stored at 4°C under refrigeration until further use.

Calculation of extraction percentage

The extraction percentage was calculated as per the formula given by Kanojiya *et al*. (2015).

$$\% \text{ Extractability} = \frac{W_1}{W_2} \times 100$$

Where, W_1 – Weight of the extract obtained after evaporation, W_2 – Weight of the plant powder taken for extract preparation.

Phyto-chemical analysis of plant extracts

Preliminary phyto-chemical screening of the prepared extracts was done to identify the presence of phyto-nutrients and other secondary metabolites through procedure followed by Bendigeri *et al.* (2019) with few modifications.

Test for alkaloids

Each extract of 0.5-0.6 g were taken into a test tube with 8 ml of 1% HCl, heated till dissolved and filtered. Two ml of each filtrate were treated separately with two reagents.

Dragendorff's test : Few drops of Dragendorff's reagent were added to 2 ml filtrate of each extract. Development of turbidity is considered positive for the presence of alkaloids.

Wagner's test : Few drops of Wagner's reagent were

Table 1. Extractability of aqueous, methanolic and hydro-ethanolic extracts of *Alium sativum* and *Nigella sativa*.

Sl. No.	Plant extracts	Weight of the plant powder taken	Weight of the extract	Extraction percentage
1.	Allium sativum			
	Aqueous extract	100 g	41.9 g	41.9 %
	Methanolic extract	100 g	3.53 g	3.53%
	Hydroethanolic extract	100g	42.8 g	42.8%
2.	Nigella sativa			
	Aqueous extract	50 g	1.56 g	2.52%
	Methanolic extract	50 g	2.10 g	4.2%
	Hydroethanolic extract	100 g	6.3 g	6.3%

added to 2 ml of each extract. A brown flocculent precipitate formation is indicated as positive for alkaloids.

Test for glycosides : Test extract solution was prepared by dissolving 0.5 g of extract in 10 ml of respective solvent, heated and cooled. Five ml of extract solution was taken into test tube and equal amounts of Benedict's reagent was added and boiled. Occurrence of brownish red precipitate indicates presence of glycosides.

Test for tannins

Three ml of methanol were added to each extract in separate test-tubes and heated, filtered through muslin cloth. Filtrate thus obtained was treated with different reagents.

Lead acetate test : Two to three drops of lead acetate solution were added to extract solution. The formation of precipitate indicated the presence of tannins.

Ferric chloride test : Few drops of ferric chloride were added to the extract solution. The formation of green color in the filtrate revealed the presence of tannins.

Test for saponins : One ml of extract solution was taken in a test tube with small amount of sodium bicarbonate, water and shaken vigorously. The presence of saponins was indicated by the formation of foam.

Test for sterols

Salkowski reaction : One gram of each extract was taken in 2 ml of chloroform. 2 ml of concentrated sulfuric acid was added by side of the tube and shaken carefully. The formation of a red color in the chloroform layer and a greenish yellow color in the upper layer indicated the presence of sterols.

Test for fixed oil : A small amount of extract residue was applied on a filter paper and the existence of an oil mark indicated the presence of fixed oil.

Test for protein

Biuret test : One gram of each extract was taken in separate test tube with 2 ml of water and 1 ml of 4% NaOH solution was added. The development of a violet pink color indicates that proteins were present.

Test for anthraquinones

Bontrager's test : A small amount of each extract was taken in test tubes with 5 ml of 10% H_2SO_4 and filtered through muslin cloth while still hot. After cooling the filtrates, 3 ml of benzene was added and vigorusly shaken. The benzene layer was then transferred to additional test tubes and mixed with

Table 2. Physical properties of aqueous, methanolic and hydro-ethanolic extracts of *Allium sativum* and *Nigella sativa*.

Sl. No.	Properties	*Allium sativum*			*Nigella sativa*		
		AQ	ME	HE	AQ	ME	HE
1.	Color	Yellowish red	Brown	Dark brown	Dark black	Blackish brown	Black
2.	Consistency	Solid	Solid	Solid	Semi solid	Semi solid	Semi solid

Table 3. Phytochemical analysis of aqueous, methanolic and hydro-ethanolic extracts of *Allium sativum*.

Sl. No.	Active principle	Test applied	Results		
			Aqueous	Methanolic	Hydro-ethanolic
1	Alkaloids	i) Dragendorff's test	Positive	Positive	Positive
		ii) Wagner's test	Positive	Positive	Positive
2	Glycosides	Benedict's test	Positive	Positive	Negtive
3	Tannins	i) Lead acetate test	Negative	Positive	Positive
		ii) Ferric chloride test	Negative	Negative	Negative
4	Saponins	Foam test	Positive	Positive	Positive
5	Sterols	Salkowski reaction	Positive	Positive	Negative
6	Fixed oils	Filter paper test	Negative	Positive	Positive
7	Proteins	Biuret test	Negative	Negative	Negative
8	Anthraquinones	Bontrager's test	Negative	Negative	Negative
9	Flavonoids	With diluted NaOH, diluted HCl	Positive	Positive	Positive

half of its volume of 10% ammonia. Pink coloration in the ammonical layer indicated the presence of anthraquinones.

Test for flavonoids : One ml of each extract solution was blended with five ml of 95 % ethanol and a few drops of diluted NaOH solution, yielding an intense yellow color. With the addition of a few drops of HCl, the colorless appearance of the tubes was found to be positive for flavonoids.

RESULTS AND DISCUSSION

The physical properties considered for characterizing the extracts here included color, consistency and the extractability. The aqueous extract of *A. sativum* acquired a yellowish red color and solid in consistency with an extractability of 41.9%. The methanolic extract obtained a brown color with solid consistency and 3.53% extractability. The hydro-ethanolic extract of the same plant material attained a dark brown color with solid consistency and extraction of 42.8%. The aqueous extract of *N. sativa* obtained a dark black colour with semi-solid consistency and its extractability was 2.52%. The methanolic extract appeared as blackish brown material with semi-solid consistency and its extractability was 4.2%. The hydro-ethanolic extract was black in color and semi-solid in nature with an extractability of 6.3% as depicted in the Tables 1–2. Tijani *et al.* (2019) reported that juice extract, ethanolic extract and aqueous extract of *A. sativum* exhibited extractabilities of 12.7%, 17.2%, and 14.7%, respectively. Chak *et al.* (2020) found that *A. sativum* extractability percentages in petroleum ether, chloroform, ethyl acetate and methanolic extract were 6.8%, 5.4%, 5.2% and 8.6%, respectively. The extractability of aqueous and methanolic extracts of *Delonix regia* was 2.3% and 3.1%, respectively (Pensalwar *et al.* 2018). The proportion of extracts obtained differs markedly between solvents. The discrepancies in percentage yields might be attributed to the polarity of the solvents, with less polar solvents such as ethanol giving more extracts than more polar solvents such as water. The physical parameters such as color and consistency of aqueous, methanolic and hydro-ethanolic extracts of *A. sativum* and *N. sativa* were yellowish red with solid, brown with solid and dark brown with solid and dark black with semi solid, blackish brown with solid and semi solid, respectively. Tijani *et al*. (2019) observed that the juice and ethanolic extracts of *A. sativum* were pale yellow, whereas the aqueous extract was brown. Orengo *et al.* (2016) assessed the color of *A. sativum* ethanolic extract as mid brown. According to Chak *et al.* (2020), the consistency of *A. sativum* extracts in petroleum ether, chloroform, ethyl acetate and methanolic extracts was semisolid, sticky, semisolid and sticky respectively. Pensalwar *et al.* (2018) observed that aqueous and methanolic extracts of *Cassia alata* yielded brown and greenish brown colors, respectively, whereas Bidkar *et al.* (2012) reported that aqueous extract of *Allium cepa* generated a dark brown color and ethanolic extract yielded a pale white color. The variation in color in-

Table 4. Phytochemical analysis of all extracts of *Nigella sativa.*

Sl. No.	Active principle	Test applied	Results Aqueous	Methanolic	Hydro-ethanolic
1	Alkaloids	i) Dragendorff's test	Positive	Positive	Positive
		ii) Wagner's test	Positive	Negative	Positive
2	Glycosides	Benedict's test	Positive	Negative	Positive
3	Tannins	i) Lead acetate test	Positive	Positive	Positive
		ii) Ferric chloride test	Negative	Positive	Positive
4	Saponins	Foam test	Positive	Positive	Positive
5	Sterols	Salkowski reaction	Positive	Positive	Positive
6	Fixed oils	Filter paper test	Positive	Positive	Positive
7	Proteins	Biuret test	Negative	Negative	Negative
8	Anthraquinones	Bontrager's test	Negative	Negative	Negative
9	Flavonoids	With diluted NaOH, dil HCl	Positive	Positive	Positive

tensity might be due to the use of differing detection methods, solvents and plant material origins.

Preliminary phyto-chemical screening of aqueous, methanolic and hydro-ethanolic extracts of *A. sativum* revealed positive for constituents such as alkaloids, saponins and flavonoids, but no proteins or anthraquinones were found. However, the aqueous extract lacked tannins and fixed oil, while the hydro-ethanolic extract lacked glycosides and sterols (Table 3). The existence of all of these chemical components in *A. sativum* has been demonstrated by Singh and Kumar (2017), Abraham *et al.* (2019), Nazir and Chauhan (2019) on phyto-chemical assessment and hence present results were consistent with their findings. Alkaloids were shown to be a major component of *A. sativum* in aqueous and methanolic extracts demonstrated by Abraham *et al.* (2019) and Nazir and Chauhan (2019) and Tijani *et al.* (2019) and our findings are in accordance with their observations. However, alkaloids and glycosides were not found in the methanolic and aqueous extract respectively (Singh and Kumar 2017, Arify *et al.* 2019). Tannins were not detected in any of the three extracts of *A. sativum* tested with ferric chloride. On the contrary, tannins were found using the lead acetate test in methanolic and hydro-ethanolic extracts. The negative result of our lead acetate test of aqueous extract agrees with findings of Singh and Kumar (2017). These disparities might be attributed to the different compounds utilized for detection. In the current study, fixed oils were found in both methanolic and hydro-ethanolic extracts, except for the aqueous extract, applying a filter paper test. The reason might be because oils are insoluble in polar solvents such as water. Arify *et al*. (2019) found the presence of protein, which differed from our findings, although the same author did not identify the presence of sterols or fixed oils in extract of *A. sativum*. This difference in results might be attributed to the adoption of different detection methods.

The phyto-chemical analysis of aqueous, methanolic and hydro-ethanolic extracts of *N. sativa* were found to be positive for alkaloids, saponins, sterols, fixed oils and flavonoids but negative for proteins and anthraquinones as depicted in Table 4. However, tannin was undetectable in the ferric chloride test, and glycosides in the Benedict's test and alkaloids in the Wagner's test in the aqueous and methanolic extracts, respectively. Ishtiaq *et al.* (2013) observed steroids, tannins, alkaloids, flavonoids, glycosides, and saponins in eight solvents (aqueous, methanol, ethanol, chloroform, butanol, diethyl ether, n-hexane and acetone) of *N. sativa* and our findings supported their findings. However, there was no evidence of sterols or glycosides in the aqueous extract. With the exception of proteins and glycosides, Reddy *et al.* (2018) demonstrated the presence of alkaloids, flavonoids, carboxylic acids, coumarins, tannins, saponins and sterols in *N. sativa* and our findings were comparable with theirs. Possible reason for these differences might be due to the variations in solvents used for extract preparation and the methods of extraction involved. Detection tests for fixed oils was done and positive confirmation was achieved, even in

case of aqueous extract, as the test subject were seeds and the oil content was proven to be higher in whole seeds comparatively. Khan *et al.* (2017) detected the presence of terpenoids and fixed oils in ethanolic and n-hexane extract of the *N. sativa* and could not demonstrate the presence of glycosides and sterols. Reddy *et al.* (2018) also demonstrated the presence of coumarins, carboxylic acids and sterols in aqueous, methanolic and other solvent extracts of *N. sativa*. These discrepancies in results can be attributed to various facts from point of seed collection, processing, storage methods, technique and solvents of extraction and methods of phyto-chemical evaluation. All these factors may or may not collectively contribute to the minor inconsistencies observed in our phyto-chemical analysis.

CONCLUSION

On phyto-chemical evaluation, both *A. sativum* and *N. sativa* exhibited positive for constituents like alkaloids, glycosides, flavonoids, tannins, saponins, sterols and fixed oils and negative for proteins and anthraquinones. However variations in the presence or absence of the phyto-chemical compounds were noticed between the extracts individually.

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